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Poster Presentations

Track 1: Infectious and Neglected Diseases

1.1 Malaria

1.1-001

Measurement of some Th1, Th2 cytokines and white cell count in childhood haemoglobinopathies with uncomplicated malaria infection

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BACKGROUND AND OBJECTIVE The relative balance between Th1 and Th2 cytokines appears crucial in the outcome of infections . We assessed the levels of some proinflammatory Th1 cytokines, interleukin-2 (IL-2) and gamma interferon (IFNγ), and anti-inflammatory Th2 cytokines, IL-4 and IL-10 in homozygous haemoglobin (Hb) AA, heterozygous AS genotyped and sickle cell (SS) individuals with uncomplicated *P. falciparum* malaria.

METHODS Levels of Th1 and Th2 cytokines of 111 children aged 1–5 years with uncomplicated malaria and 89 healthy controls were determined by Enzyme Linked Immunosorbent Assay and haematological parameters were estimated using the automated Swelab counter (Boule Medical Stockholm, Sweden).

RESULTS Th1 and Th2 cytokine levels were significantly higher in HbAA, HbAS genotyped patients than their respective healthy controls ($P < 0.05$). IFNγ, IL-2 and IL-10 were significantly higher in HbAA than HbAS and HbSS subjects ($P < 0.05$). The mean haematological parameters (total white cell count, monocyte) of HbSS infected children were significantly higher than that of HbAA and HbAS subjects ($P < 0.05$), however, their mean packed cell volume was significantly lower compared to others ($P < 0.05$).

CONCLUSION Our results of revealed some stronger cytokine response in HbAA than HbAS and HbSS individuals, this may suggest an immunocompetence of the HbAA individuals.

1.1-002

Prevalence of malaria parasitaemia among infants in Uganda and its association with breastfeeding, peer counselling and vitamin a supplementation

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BACKGROUND Malaria is the second highest contributor to the disease burden in Africa and there is a need to identify low cost prevention strategies . The objectives of this paper were to estimate the prevalence of malaria parasitaemia among infants and to assess the effect of peer counselling for exclusive breastfeeding, vitamin A supplementation and anthropometric status on malaria parasitaemia.

METHODS A cluster randomized intervention trial was conducted between 2006 and 2008. Twenty-four clusters, each comprising one to two villages, in Eastern Uganda were randomised to the intervention, receiving peer counselling for exclusive breastfeeding or to control, receiving standard care. Pregnant women were recruited in these villages and followed up. In the intervention clusters, five home based peer counselling visits were scheduled, one in the third trimester and four postpartum. Blood was drawn for malaria parasitaemia from 483 infants between 3 and 12 months.

RESULTS The prevalence of malaria parasitaemia was 11% in the intervention areas and 10% in the control areas. Children in intervention areas were as likely as children in control areas to have malaria (RR 1.7; 95% CI: 0.9, 3.0). After controlling for potential confounders, infants not supplemented with Vitamin A had a higher risk for malaria than those who had been supplemented (RR 6.1; 95% CI: 2.1, 17.7). Among children supplemented with vitamin A, every unit increase in length-for-age Z (LAZ) scores was associated with a reduced risk in malaria (RR 0.5; 95% CI:0.4, 0.6). There was no association between LAZ scores and malaria among children that had not been supplemented.

CONCLUSION There was no association between peer counselling for exclusive breastfeeding and malaria parasitaemia. Children that had not received Vitamin A supplementation had a higher risk of malaria than children that had been supplemented.

1.1-004

Measurement of underreporting of cases of malaria in ten municipalities of Nariño pacific coast: the problem of sub-typing in 2009

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BACKGROUND In Colombia the extent of under-reporting of malaria cases is unknown. This study aims to estimate the errors through the use of individual notification sheet in 10 municipalities of Nariño in 2009.

MATERIALS AND METHODS Between June and July 2010, we conducted a cross-sectional study in 10% of the 486 areas of diagnosis and treatment of malaria in 10 municipalities of Nariño.

RESULTS Of 27,203 collected cards 2816 were for positive cases. Of these 2156 (76.6%) were due to *P. falciparum*, 560 (19.9%) to *P. vivax*, 8 (0.3%) to *P. malariae*, 2 (0.1%) were mixed infections and in 90 (3.2%) cases we could not identify the parasite species. 65.6% of cases were older than 14 years of age, 59.1% male, 61.4% went to the health post in the first 3 days of onset of symptoms, and 44.1% came from rural area. 60% did not report municipality of residence, county of origin and occupation. In the comparison 'case by case' 845 (30.0%) records were not admitted to SIVIGILA. Of the 2816 cases, 8.1% of those who were not on the table SIVIGILA compared with 21.8% of those who if they were under 15 years ($2 = 4.85$, $P = 0.03$) and 11.3% of those who were not in the SIVIGILA compared with 26.3% of those who if they were women ($2 = 0.00$, $P = 0.98$).

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CONCLUSION The design of this study not allow inferences, because it can only be applied for the posts of diagnosis and treatment of the municipalities that were included in the investigation.

1.1-005

Introducing the EU funded consortium to tackle multi-drug resistance in malaria under combination therapy and to develop innovative simplified diagnostics: Malactres

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Accurate diagnosis and effective treatment of cases are crucial to successful malaria control. Laboratory methods to confirm malaria infection lack sensitivity and specificity and are hard to perform in resource poor settings; improved diagnostic tools to support the clinical suspicion of malaria are urgently needed. Furthermore, the treatment of malaria is shifting from older drugs, such as chloroquine, which have lost their effectiveness in most parts of the world due to resistance, to more expensive artemisinin-based combination therapies (ACTs). There is however a growing risk that these ACTs will also eventually fail due to the emergence of resistance. This process could be exacerbated by the overtreatment of fever cases with ACTs in the absence of a reliable laboratory diagnosis. The Malactres project addresses both challenges in a new multidisciplinary consortium. Our aim is to assess specific genetic markers for ACT resistance and to develop innovative, rapid and simple diagnostics for malaria. The consortium comprises partners from Belgium, Burkina Faso, Nigeria, Tanzania, Netherlands and UK. The work now underway will move our knowledge of ACT resistance forward in two complementary ways: (i) We will use our collection of parasite DNA and RNA from ACT-treated individuals to identify and validate genetic markers for selective changes induced by ACT action; (ii) We will develop and validate simple tests in new formats, applicable in the field, not only for these markers but also for already established markers of parasite resistance to ACT partner drugs, for which current detection methods are slow, laborious and technically demanding. The work is a balanced blend of clinical field work, laboratory research and test development and is linked to ongoing EU initiatives such as the European Developing Countries Clinical Trial Partnership (EDCTP). Details of current and planned studies of ACT resistance and diagnostic test development will be presented.

1.1-006

Development and evaluation of simplified molecular diagnostic devices for malaria

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BACKGROUND Microscopy is the 'gold-standard' for malaria diagnosis, but at low parasitaemia it becomes less sensitive, and time consuming. Molecular tools allow for specific/sensitive diagnosis but current formats, such as PCR with gel-electrophoresis, are difficult to implement in resource poor settings. Therefore, a simple, fast, sensitive and specific detection system, Nucleic Acid Lateral Flow Immunoassay (NALFIA) to detect amplified PCR products of Pan-Plasmodium and of Human GAPDH (internal control) was developed.

METHODS A laboratory evaluation was performed with classified diagnostic specimens (70 negative samples and 30 microscopically confirmed specimens, parasitemia from 600 to 70,000 parasites/μl) and control samples with known levels of parasites (spiked blood samples with 0, 1, 10, 100 or 10,000 parasites/μl blood and water control). Furthermore, a ring trial was initiated in which the diagnostic device was evaluated by five independent laboratories [Netherlands (3), Belgium (1), and United Kingdom (1)] to determine sensitivity, specificity and inter laboratory variability.

RESULTS Analytical sensitivity/specificity of PCR-NALFIA system in single laboratory was >95% and able to detect 1 parasite/μl blood. All laboratories in the ring trial reported ease of use of the technique and all were able to successfully perform the protocol. When the results of the detection of Pan-Plasmodium are considered, the overall laboratory inter variability was low and the agreement of reported results was high. The overall kappa value was 0.89 (95% CI 0.83–0.94; $P < 0.001$). The overall sensitivity and specificity of the tests in the different laboratories was over 95% with very small confidence intervals.

CONCLUSION PCR-NALFIA for malaria diagnosis conducted well in all laboratories and further evaluation of this test under real conditions in disease endemic countries (formal phase 2 evaluation) is justified. Work funded by: European Commission grant 201889 Multi-drug resistance in malaria under combination therapy: Assessment of specific markers and development of innovative, rapid and simple diagnostics (MALACTRES).

1.1-007

Efficacy of methylene blue monotherapy in semi-immune adults with uncomplicated falciparum malaria: a controlled trial in Burkina Faso

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OBJECTIVE To assess the efficacy of methylene blue (MB) monotherapy in semi-immune adults with uncomplicated malaria in Burkina Faso.

METHODS In an open-label controlled phase II study with 60 semi-immune adults with uncomplicated falciparum malaria in Nouna, north-western Burkina Faso, MB monotherapy (390 mg twice daily) was given sequentially to groups of 20 adults for 7 days (MB7), 5 days (MB5) and 3 days (MB3), respectively. The primary outcome was the rate of adequate clinical and parasitological response (ACPR) on day 28 of follow-up.

RESULTS Of the study population, 27 of 58 (47%) and 5 of 51 (10%) patients still had parasites on days 2 and 3, respectively, of follow-up resulting in 9 of 58 (16%) early treatment failures. By day 14, no recrudescence was observed but in 4 of 19 (MB5) and 2 of 20 (MB3) individuals by day 28. The PCR corrected rate of ACPR was 72%, 58% and 85% in groups 7, 5 and 3, respectively, by per protocol analysis. Self-limiting dysuria was the most frequent adverse event.

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CONCLUSIONS MB acts slowly against the blood stages of *P. falciparum*. MB alone needs to be given for at least 7 days to be efficacious in the treatment of falciparum malaria but should be used in combination with a fast acting antimalarial.

1.1-009

Malaria surveillance in Tunisia

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BACKGROUND Tunisia became malaria free in 1979 and the majority of reported cases of malaria diagnosed each year are imported from endemic countries. The aim of this study was to estimate the completeness of malaria notification to the public healthcare system (PHCS) and to describe retrospectively data of malaria cases in Tunisia.

METHODS We conducted a retrospective epidemiological survey using a standardized questionnaire for all cases of malaria reported to PHCS and those diagnosed in a parasitological laboratory or infectious disease service between January 2002 and December 2007. To estimate the total number of cases, we used a two sources capture-recapture analysis.

RESULTS After record-linkage and cross-validation, 317 cases of malaria were identified, of whom 231 were notified, resulting in an under-notification of 17%. The estimated number of malaria cases using capture-recapture analysis was 366 [95%CI (336–397)] for the period of study with a completeness of 63.1% which increased from 44.8% for 2002 to 78.7% for 2007. One hundred and sixty-two patients (51.1%) had been born in sub-Saharan Africa, 113 (35.6%) in Tunisia, 35(11.0%) in North Africa and 7 (2.2%) in Europe with predominance of men (87.1% of all cases). The median age was 25.0 years (21–30) for sub-Saharan Africans, 38.0 years (23.5–45.5) for North Africans, 38.5 years (30.75–38.5) for Tunisians and 39.0 (26–43) for Europeans ($P < 10^{-3}$). The predominant malaria species was *P. falciparum* with 216 cases (72.5%), and the most frequent area of acquisition was sub-Saharan Africa. Information on compliance with malaria prophylaxis was only sporadically available and 34% of Tunisian infected individuals had not used any chemoprophylaxis.

CONCLUSION Our survey marked variety in the type and availability of key data and showed an underreporting of malaria cases. It demonstrates that both sources of malaria registration are substantially incomplete.

1.1-010

Effect of malaria on pregnancy outcome in a malaria endemic population of Assam, Northeast India

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The northeastern region of India is burdened by falciparum malaria which is an important cause of morbidity and mortality of the people of Assam. We aimed to see the effect of malaria on pregnancy outcome in malaria endemic areas of Assam. Of the total 1233 enrolled participants from four locations, 1143 could be followed till the outcome of the pregnancy. Among 1076 malaria negative participants, 891 deliveries were normal (82.81%), 133 low birth weight (12.36%), 24 stillbirths (2.23%), 13 miscarriages (1.21%), 10 abortions (0.93%), and five premature deliveries (0.46%). Among the malaria positive participants ($n = 67$), *P. falciparum* contributed 58 (86.57%), *P. vivax* 7 (10.45%) and mix infections (Pf & Pv) 2 (2.98%). Among the falciparum group ($n = 58$), outcome of pregnancies were 28 normal (48.28%), 16 low birth weight (27.59%), six stillbirths (10.34%), three miscarriages (5.17%), three abortions (5.17%), one premature delivery (1.72%) and one death of mother and baby (1.72%). Among the malaria negative group ($n = 1076$), abnormal outcome of pregnancy is much less common than in those with malaria ($n = 67$); the difference highly significant ($P < 0.0001$). Among malaria positive group, trimester-wise, normal outcome of pregnancy were in the 1st trimester ($n = 16$), 11 (68.81%), in the 2nd trimester ($n = 38$), 19 (50.02%) and in the 3rd trimester ($n = 13$), 6 (46.25%). Low birth weight was more common in the 2nd trimester group compared to 1st and 3nd trimester. Abnormal outcomes among the participants of first and 2nd gravidae were more frequent than in multigravidae. Parasite load (density) was directly related to abnormal outcome. Malaria during pregnancy has significant adverse effects on pregnancy outcome and warrants concerted efforts to tackle this problem in this region.

1.1-011

The importance of hemoglobin level at enrollment on subsequent malaria risk: results from a pediatric cohort in Mali, West Africa

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INTRODUCTION WHO estimates around 60% of people (<5 years old) living in sub-Saharan Africa where malaria is endemic, suffer from iron-deficiency anemia. Several studies evaluated the effect of iron supplementation on malaria risk, but the results are controversial. Further, the impact of baseline hemoglobin (Hb) level on subsequent risk of malaria infection has not been well documented.

METHODS AND MATERIALS We initiated a 5-year longitudinal cohort study in three villages in rural Mali. From June 2008 to December 2009, we enrolled 1419 children aged 6 months to 17 years. Enrollment occurred just prior to the malaria transmission season, and data collected included: age, ethnicity, village, Hb level, blood type (ABO and Rh) and hemoglobinopathy (HbS, HbC, alpha-thalassemia, G6PD deficiency). The relative risk (RR) for each factor was calculated by a Poisson regression model. In the model, we categorized the children into three groups based on Hb level (Hb <8.5, 8.5–12 and >12 g/dl).

RESULTS In the 1356 subjects with complete data, we identified 1933 malaria episodes and more than 90% were uncomplicated malaria. As expected, the older children had a lower risk than younger children. Interestingly, while there was no difference of RR between children with Hb <8.5 g/dl and with 8.5–12 g/dl at enrollment, children with Hb >12 g/dl showed significantly lower risk (RR 0.81, 95%CI 0.69–0.95, P = 0.008) than those with 8.5–12 g/dl Hb when all other factors were considered. HbS was the only other factor associated with a significant change of RR.

CONCLUSION Although the mechanism of protection in the children with higher Hb is not clear, this study suggests that Hb levels could potentially be a major confounder and thus an important co-variate in studies with malaria incidence as a primary outcome.

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1.1-013

Current clinical efficacy of chloroquine in the treatment of vivax malaria in Thailand

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BACKGROUND Thailand has equal proportions of falciparum and vivax malaria. Chloroquine (CQ) is the drug of choice for the treatment of *Plasmodium vivax* infection in the country. CQ resistant *P. vivax* (CRPv) has not started to challenge the efficacy of the drug. The present study was conducted to assess the current response of *P. vivax* to chloroquine in Thailand.

METHODS A 28-day *in vivo* therapeutic efficacy test was conducted from June 2009 to December 2010. Recurrence of parasitaemia and the clinical condition of patients were assessed on each visit during the follow-up. Recurrence patients' blood drug levels were measured using HPLC. Data were analyzed using WHO's 2008 program for the analysis of *in vivo* tests.

RESULTS Of the total 212 patients included in the study, 201 completed their 28-day follow-up, 10 of whom were excluded for different reasons. In five patients, parasitaemia reappeared within the 28-dayfollow-up in spite of absence of malaria symptoms. In one patient, on the day of recurrence of parasitaemia the levels of chloroquine-desethylchloroquine (CQ-DCQ) were above the minimum effective concentration (≥100 ng/ml) but lower in four cases.

CONCLUSION Reappearance of the parasite within the 28 days of follow-up in one of five is due to parasite resistance to CQ. The 2.5% prevalence of CQ treatment failure in *P. vivax* malaria in the study area signals the need for launching monitoring activities for CQ resistant *P. vivax* and survey on CRPv malaria should be made in *P. vivax* endemic areas in order to estimate the level of burden across the country.

1.1-014

Relationship between incidence of malaria and altitude in Ethiopia

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Malaria, in Ethiopia is caused by *Plasmodium falciparum* or *Plasmodium vivax*. The epidemiological model of malaria transmission there is generally unstable and seasonal, the level of transmission varying from one place to another, as a result of different altitudes and rainfall across the country. The instability is particularly found in the high mountain ranges, where weather conditions are favourable to it. The disease mostly occurs below 2000 m altitude a.s.l., but cases have been documented in areas above 2400 m. This study was performed to determine the relationship between malaria incidence and altitude in different regions of southeast Ethiopia, located at 2000 and 2500 m. We assayed the presence of the parasite in 368 samples, 60% belonging to the adult group and 40% to children under 15 years. Blood samples were assayed by nested-PCR, according to the protocol of Rubio *et al*. 1999, for detection of *Plasmodium*. The percentage of positive samples detected at 2000 m was 6.5% in the adult group and 13.3% in children. At 2500 m, the percentage of positive samples was 7.5% in adults and 7.4% in children. We can conclude that in adults there is no difference with altitude, but in children there is an increased incidence of *Plasmodium* at 2000 m altitude, and there is no difference in the incidence at 2500 m between adults and children, indicating that adults have the same degree of protection as children. Maria A. Santana-Morales was supported by an International SEMTSI 2009 fellowship.

1.1-015

Mass screening and treatment for *P. falciparum* malaria: how effective is it likely to be?

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INTRODUCTION Mass screening and treatment (MSAT) is being considered in some places as part of strategies to locally eliminate malaria. MSAT could detect asymptomatic infections that would be missed by passive case detection, but has yet to be tested in the field. We simulated, across settings of varying vulnerability, receptivity and health systems, the effects of several different mass screening and treatment schedules on *P. falciparum* malaria disease burden and transmission.

METHODS AND MATERIALS Simulations were carried out with a dynamic, individual-based simulation model of *P. falciparum* malaria epidemiology. We considered 10,000 humans exposed to seasonal transmission based on a Tanzanian setting. Four mass distributions of insecticide-treated nets (ITNs) were conducted at 3-year intervals starting at the beginning of the simulation. Mass screen and treatment at 85% population coverage was carried out at five times of the year, either annually or every 3 years from years 5 to 12. We varied the initial EIR, infection importation rate, case management coverage and ITN coverage and assessed the effects on number of simulated disease episodes.

RESULTS Initial results suggest that MSAT leads to on average about a 10% reduction in the number of uncomplicated and severe episodes over the 15 years of the simulations since the start of MSAT, compared to scenarios with no MSAT. Timing of MSAT did not seem to make a substantial difference. Increased case management coverage and ITNs could potentially be more effective in reducing malaria in these settings.

CONCLUSIONS Under certain conditions, MSAT appears to have limited effectiveness according to our simulations. An economic evaluation of MSAT needs to be conducted alongside investigations of effectiveness in order to determine the role for MSAT in malaria programmes.

1.1-016

A randomised study to compare a fixed dose combination of artesunate plus amodiaquine vs. a fixed dose combination of artemether plus lumefantrine in treatment of repeated uncomplicated *Plasmodium falciparum* malaria attacks occurring during 2 years in children in Uganda

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Although in high-endemic areas artemisinin combination therapy (ACT) is used repeatedly by patients, very few studies document the safety of multiple ACT administrations. We designed a study

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to assess the safety and efficacy of repeated administrations of the fixed-dose combination artesunate + amodiaquine (ASAQ) in comparison with artemether–lumefantrine (AL) in consecutive episodes of uncomplicated *Plasmodium falciparum* malaria in children. This randomized, investigator-blinded, comparative study was conducted in a rural community of Eastern Uganda from June 2008 to June 2010. Patients under 5 years of age with uncomplicated *P. falciparum* malaria were randomized to receive either ASAQ once daily, or AL twice daily for 3 days for each malaria episode occurring over a period of 2 years. Treatment intake was supervised only for first episodes. All attacks were monitored until D42. A total of 413 patients were randomized in the two arms (208 ASAQ, 205 AL). During the study period, a total of 6032 malaria episodes were treated. The medians of episodes were 16 and 15 in ASAQ and AL groups respectively. Treatment-emergent AEs were reported during follow-up in 59.8% of the patients without significant differences between the two groups; only one AE in each treatment group was considered as related to treatment. Adverse events of special interest (AESI) were observed in 28 patients; abnormalities in liver function tests were reported in 23 patients (11 ASAQ, 12 AL), and neutropenia in six patients (four ASAQ, two AL). All AESI were reversible. Serious adverse events were reported in 25 patients without any difference between the two treatment groups. Incidence of adverse events did not increase with the repetition of treatment, in either group. Efficacy analysis is ongoing. These results confirmed the satisfactory safety profile of ASAQ in comparison with AL, with no issue related to repeated administration.

1.1-017

Cardiac involvement during and after severe malaria in patients from Northwestern Rajasthan, India

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INTRODUCTION AND OBJECTIVES There is paucity of information regarding the cardiac involvement in severe malaria patients. Therefore, we evaluated for cardiac involvement during and after severe malaria in adult age group.

MATERIAL AND METHODS Cardiac involvement was assessed by clinical examination, chest X-ray, standard 12-lead electrocardiogram (ECG), high resolution transthorasic echocardiograms and cardiac markers (troponin-I and CK-MB), in 100 patients with severe complicated malaria [*Plasmodium falciparum* (PF) 60, *Plasmodium vivax* (PV) 28 and 12 mixed (PF + PV)].

RESULTS Cardiac involvements were observed in 17% of complicated malaria patients. Circulatory failure was observed in 11%, congestive heart failure in 9%, and pulmonary edema in 2% patients. Cardiac involvements were more common among PF as compared to PV or mixed malaria (x^2 = 13.68; P = 0.001). In ECG, non specific ST-T changes in 2%, and sinus tachycardia was observed in 12%; normal ECG findings in 4%. No significant findings were revealed by echocardiography cardiac markers, both Troponin-I and CK-MB were increased in 14% patients. Among severe malaria cases, parasite density (PD) was higher in patients with cardiac involvement (mean PD = 23870/μl) than in those without cardiac involvement (mean PD = 10284/μl). Mortality with circulatory failure (45.45%) was higher than any other manifestation without circulatory failure.

CONCLUSIONS Patients with severe complicated malaria had cardiac involvement. Significant ECG changes and increased levels of cardiac markers (troponin-I, CK-MB) indicate myocardial involvement in severe malaria patients. These cardiovascular manifestations are associated with high morbidity and mortality as compared to other complications.

1.1-018

A study of pulmonary manifestations in patients with malaria from Northwestern Rajasthan, India

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BACKGROUND AND OBJECTIVE *Plasmodium falciparum* (PF) and *Plasmodium vivax* (PV) are responsible for most of the global burden of malaria. With the changing spectrum of clinical presentation in malaria, pulmonary system involvement has always been under diagnosed. Therefore, we planned to estimate the pulmonary manifestation in patients with malaria.

MATERIAL AND METHODS Pulmonary manifestations were estimated in 200 adult (145 males and 55 females) cases of severe malaria [PF, PV and mixed (PF + PV)]. The diagnosis of malaria was confirmed by demonstrating asexual form of parasites in peripheral blood smear. All patients were treated with artesunate 2.4 mg/kg over 10 min followed by 2.4 mg/kg every 24 h or I.V. quinine 20 mg/kg loading dose for 4 h in 500 ml D 10% and then 10 mg/kg every 8 h.

RESULTS Pulmonary involvement was observed in 60 (30%) malaria patients. Among the respiratory involvement cough 48 (80%), dyspnea 24 (40%), acute respiratory distress syndrome (ARDS) 14 (23%), bronchitis 6 (10%), and pneumonia 3 (5%) were the major clinical pulmonary manifestations. Metabolic acidosis and low oxygen saturation was observed in 14 (7%) patients. Chest X-ray abnormality was seen in 23 (11.5%) patients, 14 (7%) had bilateral infiltrates, 3 (1.5%) had inflammatory patch and 6 (3%) had findings suggestive of bronchitis. Spirometry findings showed that 34 (17%) patients had early small airway obstruction. All patients with ARDS had a poor outcome.

CONCLUSION Pulmonary manifestations are frequently observed in malaria patients. Delayed diagnosis can alter the outcome and prognosis of the disease. Therefore, early diagnosis of malaria induced ARDS can significantly affect the outcome and may reduce the associated mortality rate.

1.1-019

Impact of combining intermittent preventive treatment with home management of malaria in children under 10 years, in a rural area of Senegal

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INTRODUCTION Current malaria control strategies recommend (i) Early case detection using rapid diagnostic tests (RDT) and treatment with Artemisinin Combination Therapy (ACT); (ii) Intermittent preventive treatment; (iii) impregnated bed nets. However, these individual malaria control interventions provide only partial protection in most epidemiological situations. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions in reducing malaria prevalence and morbidity.

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METHODS We conducted a cluster randomized trial to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home based management of malaria (HMM) by community health workers (CHWs) in Senegal. Eight CHWs in eight villages covered by the Bonconto health post, (South Eastern part of Senegal) were trained to diagnose malaria using RDT and provide prompt treatment with Coartem™ to children under 10 years. Four CHWs were randomised to also administer monthly IPTc with single dose of Sulfadoxine-Pyrimethamine (SP) plus three doses of Amodiaquine (AQ) in October and November 2010. A total of 1010 children in the eight study villages were assigned to a weekly home visit by CHWs during 2 months. During each visit, an RDT was performed by CHWs for febrile children.

RESULTS The incidence of clinical malaria episodes was 7.1/100 child months (95% CI (3.7–13.7)) at risk in communities with IPTc + HMM compared to 35.6/100 child months [95% CI (26.7–47.4)] at risk in communities with only HMM (OR = 0.20 95% CI 0.09–0.41, P = 0.0001). A survey conducted at the end of the transmission season showed that malaria parasite prevalence was lower in communities with IPTc + HMM (2.05% vs. 4.6% P = 0.03). Adjusted for age groups, sex, *P. falciparum* carriage, prevalence of malnutrition, IPTc + HMM showed a significant protective effect against anaemia also (aOR = 0.59 95% CI 0.42–0.82 P = 0.02).

CONCLUSION Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of malaria as well as anaemia among children in Senegal.

1.1-020

Persistent hemolysis after parasite clearance in *P. vivax* malaria

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INTRODUCTION The hemolytic anemia that accompanies infection with *Plasmodium vivax* is sometimes persistent after appropriate antimalarial treatment. It has been speculated that immunological mechanisms might account for malaria induced hemolysis involving non-parasitized erythrocytes.

METHODS AND MATERIAL From July through December 2010, all patients presenting with sustained moderate anemia, defined as hemoglobin level <8 g/dl 1 month after clearance of peripheral parasitaemia, were investigated. Case-patients were thoroughly evaluated and standard hematological, biochemical, parasitological and immunological tests were performed. An individual who had splenomegaly of at least 10 cm, a serum IgM >3.1 g/l and a malaria serology titer >640 was considered a case of hyper-reactive malarial splenomegaly (HMS).

RESULTS Among a cohort of 1213 cases treated for *P. vivax* malaria from an isolated, Papua New Guinean population, 232 patients presented moderate anemia and in 29 cases anemia persisted 1 month after elimination of the parasite. Seventy-five per cent (22/29) of these cases had a non-inflammatory plausible explanation for their continuing anemia, including malaria relapse or re-infection, gastrointestinal or menstrual blood loss and megaloblastic anemia. The remaining seven adults received a diagnosis of hemolytic crisis and all of them fulfilled the criteria for HMS. Laboratory findings included 2/7 cases with IgG warm antibodies in the direct anti-globulin test (DAT). Hereditary hemolytic anemia was excluded in 5/5 patients. In four cases associated severe thrombocytopenia, with negative result for antibodies antiplatelets, was indentified. The administration of a high dose of prednisone with anti-malarial chemoprophylaxis, in all treated patients, resulted in dramatic improvement of all parameters of hemolysis and a rise in hemoglobin.

CONCLUSIONS The persistent severe episodes of hemolysis occasionally seen after *P. vivax* malaria appear to be associated with an auto-immune response intimately related to HMS which is mostly DAT negative. Both the initial administration of a course of steroids and anti-malarial chemoprophylaxis should be recommended.

1.1-021

A-436C>A polymorphism in the human Fas gene promoter associated with severe childhood malaria

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Human genetics and immune responses are considered to critically influence the outcome of malaria infections including life-threatening syndromes caused by *Plasmodium falciparum*. An important role in immune regulation is assigned to the apoptosis-signaling cell surface receptor CD95 (Fas, APO-1), encoded by the gene FAS. Here, a candidate-gene association study including variant discovery at the FAS gene locus was carried out in a case-control group comprising 1195 pediatric cases of severe falciparum malaria and 769 unaffected controls from a region highly endemic for malaria in Ghana, West Africa. We found the A allele of c. −436C>A (rs9658676) located in the promoter region of FAS to be significantly associated with protection from severe childhood malaria (odds ratio 0.71, 95% confidence interval 0.58–0.88, $p_{empirical}$ = 0.02) and confirmed this finding in a replication group of 1412 additional severe malaria cases and 2659 community controls from the same geographic area. The combined analysis resulted in an odds ratio of 0.71 (95% confidence interval 0.62–0.80, P = 1.8×10^{-7}, n = 6035). The association applied to c. −436AA homozygotes (odds ratio 0.47, 95% confidence interval 0.36–0.60) and to a lesser extent to c. −436AC heterozygotes (odds ratio 0.73, 95% confidence interval 0.63–0.84) and also to all phenotypic subgroups studied, including severe malaria anemia, cerebral malaria, and other malaria complications. Quantitative FACS analyses assessing CD95 surface expression of peripheral blood mononuclear cells of naïve donors showed a significantly higher proportion of CD69+CD95+ cells among persons homozygous for the protective A allele compared to AC heterozygotes and CC homozygotes, indicating a functional role of the associated CD95 variant, possibly in supporting lymphocyte apoptosis.

1.1-022

Efficacy of artemether–lumefantrine and artesunate–amodiaquine for the treatment of uncomplicated falciparum malaria in Tanzania

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INTRODUCTION Tanzania mainland introduced artemisinin combination therapy (ACT) with artemether/lumefantrine (ALu) as

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first-line treatment for uncomplicated malaria in 2006 following high levels of *Plasmodium falciparum* resistance to sulfadoxine/pyrimethamine (SP). There is growing evidence suggesting that malaria cases and entomological inoculation rates (EIR) have declined over the past 3 years in most parts of Tanzania. Despite good malaria control achievements, there is a threat of ACT drug tolerance/resistance as evidenced by recent reports of emerging drug tolerance/resistance to ACT along the Thai-Cambodia border. It is, therefore, critical for our region to monitor the development and spread of ACT drug resistance which will potentially occur due to wide use of the drugs.

METHODS We set up to conduct a study at four National Malaria Control Programme (NMCP) sentinel sites in May–August 2011 to monitor the *in vivo* efficacy of ALu and amodiaquine-artesunate both of which are first-line anti-malarials in mainland Tanzania and Zanzibar respectively. Participants will include febrile patients aged 6–59 months with *P. falciparum* mono-infection, meeting other inclusion criteria as per WHO protocol, presenting at the health facility to be treated with one of the two drugs. The recruited patients will be followed-up for 28 days. Treatment response will be corrected by PCR genotyping of msp1, msp2 and glurp genes to differentiate recrudescent from new infections.

RESULTS Preliminary results of this study will be out by the time of 7th European Congress of Tropical Medicine and International Health in October 2011.

CONCLUSION This study will provide data for monitoring the evolution, spread and intensification of ACT tolerance/resistance in Tanzania. Results from this study will be used to guide the Ministry of Health to assess and review National treatment guidelines for uncomplicated malaria.

1.1-023

Profile of IL 10 throughout human gestation and pregnancy outcomes in Kinshasa a high level of transmission area for malaria

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In endemic areas of Africa, pregnant women are a risk group for malaria. The symptoms and complications of malaria during pregnancy are related to the level of acquired immunity. In areas of high malaria transmission, *P. falciparum* infection during pregnancy does not usually result in clinical symptoms. In these areas, malaria is characterised by anaemia and placental malaria (PM) that leads to poor pregnancy outcomes. Diagnosis of placental malaria is difficult and no tools exist that can predict poor pregnancy outcomes. Several studies suggest that cytokine trajectories may vary by outcome, and patients at risk for adverse outcomes could be identify by immunologic profiling during the course of pregnancy. Among those cytokines, IL 10 appears to be a useful tool for monitoring pregnancy outcomes and detection of PM. Aim of the study: to analyse the profile of IL 10 in the peripheral blood, over the course of the human pregnancy, and to assess relationships to antenatal parasitemia, and to pregnancy outcomes. This longitudinal study will be held in Kinshasa (DRC). Peripheral blood will be obtained at 8–14, 18–22, and 28–32 weeks gestation. IL 10 levels in peripheral blood, will be detected by assays, antenatal parasitemia by peripheral blood smear, and at the delivery the placental levels of IL 10 will be detected by quantitative PCR. Postpartum microscopy of placental blood films and placental histology will be used to diagnose placental malaria infection. The results of this will provide more informations about IL 10 as biomaker that can predict poor pregnancy outcomes due to PM. This will help to potentially identify interventions to modify the pregnancy outcomes for at risk populations.

1.1-024

Antimalarial drug safety information generated through routine monitoring in a rural health centre in South-Western Senegal

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INTRODUCTION Safety profiles of antimalarials are derived from clinical trials which may not represent the spectrum of real patients. Scanty information exists from practice mostly due to inherent practical difficulties with safety monitoring. The question is whether a routine system for safety follow-up that is both informative and feasible can be set-up.

METHODS During 2001–2009 out-patients attending the dispensary of Mlomp, South-western Senegal who received supervised artesunate–amodiaquine treatment (ASAQ) for confirmed malaria were actively followed-up on Days 0–3 and 28 for the occurrence of adverse events by local health staff trained for the purpose.

RESULTS Safety information was collected on 3629 patients (91% of all those receiving ASAQ at the facility) – 55% male, aged 16 years ± 12.7, weighing 36.3 kg ± 19. Treatment was with loose (28.7%), co-blistered (37.4%) and fixed (33.9%) ASAQ products. Total target dose was 12 mg/kg of AS and 30 mg/kg of AQ over 3 days. Dosing was based on weight (60%) or age (40%). On Day 0 (after the first dose), 857 patients (of whom 38%, 35%, 27% on loose, co-blister and fixed ASAQ; frequency was 35%, 37% and 50% per group, respectively) reported 3657 events (37.4% on co-blister, 28.7% loose and 33.9% on fixed ASAQ). Headache, vertigo, vomiting, asthenia, anorexia, nausea were each >10% of all signs/symptoms. On Day 3, 47 events were reported by 34 patients (0%, 73%, 27% on loose, co-blister and fixed ASAQ). White cell counts, haematocrit, alanine and aspartic aminotransferases, bilirubin and creatinine were measured in a fraction of patients. No serious events occurred.

CONCLUSIONS ASAQ was generally safe and well-tolerated. Monitoring of safety is feasible in rural dispensaries. Baseline signs/symptoms should be investigated systematically to distinguish between malaria- and drug-related events. Laboratory investigations are expensive and feasible only in a fraction of patients.

1.1-025

Impact of IPTi-sp in protective acquired immunity in infant living in Kinshasa a hyper endemic level of transmission area for malaria

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Malaria is one of the most murderous diseases in the world and more than 80% of the cases are located in sub-Saharan Africa. The highest burden of the disease is held by pregnant women and children below 5 years of age. Intermittent preventive treatment in infant (IPTi) is an efficient strategy that has proved to reduce morbidity and mortality due to malaria in infant especially in moderate to high transmission area. The aim of the study is to assess in a hyper endemic area for malaria the relationship to IPTi

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to specific measure of immunity for malaria and to outcome, by detecting the level of IFN gamma, Ig against PfEMP1, the parasitemia and by checking the apparition of mild or severe episodes of malaria during the study. It is a randomized clinical trial with two arms including infant aging of 3 months. One arm will receive IPTi during EPI with Sulphadoxine-Pyrimethamine and the other will receive a placebo. Samples will be collected every month during 12 months. While some studies detected a rebound effect after IPTi, others suggest the protective effect of the strategy afterward. This study will give more information to understand the impact IPTi-SP on acquisition of protective immunity in infant resident in a hyper endemic area for malaria. The level of IFN gamma will give an estimation of immunity against pre erythrocyte stage of malaria; Ig against PfEMP1 will show the level of protective immunity knowing that PfEMP1 is responsible for the phenomenon of roseting involved in severe malaria. Outcomes will be the level of parasiteamia and the number of mild and severe clinical case of malaria.

1.1-026

Falciparum malaria precipitated myocardial infarction complicated by comorbid severe sepsis

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A 57 year-old Indonesian male patient was transferred from a peripheral hospital with falcifarum malaria, dyspnea and chest pains in need of ICU facilities. On adminission he was diagnosed as complicated falciparum malaria as he had increased liver transaminases and creatinine besides a very low thrombocyte count. He already received arthemether/lumefrantine and an ECG taken early showed myocardial ischemia. The repeated chest X-ray in contrast to the previous one showed bilateral pleuropneumonia with possible pulmonary edema. Subsequently he developed a full blown acute myocardial infarction confirmed by ECG, echocardiography and laboratory tests. Laboratory test at peak of illness: Hb 8.9 g/dl (dropping from 15.3 g/dl, leuc 12.210/l, thrombocytes 33 000/l, *Plasmodium falciparum* 18 sporozoites/200 leuc, blood urea 70 mg/dl, creatinine 2.3 mg/dl, creatinine kinase 772 U/l, CK-MB 79.1 U/l, SGOT 228 U/l, SGPT 452 U/l, PCT 10.800 ng/ml (range of severe sepsis), NT Pro BNP 15126 pg/ml (cardiac failure), troponin T 1.30 g/l (40× normal), troponin M 339 ng/l. Treatment given consisted of standard ACT, antipyretics, carbapenem 2 g/TID, dobutamine, lanoxin, lasix, thromboaspirin, clopidogrel, enoxaparin, ramipril, trimetazidine, isosorbide dinitrate, *N*-acetylcysteine, pantoprazole, sucralfate, ondansetron, herbal hepatoprotector and alprazolam. Half of the drugs were given parenterally. He was also administered nasal oxygen, human albumin, infusion for calories and electrolytes. Clinical assessment: malaria sporozoits cleared after 3 days, fever persisted for 10 days as the patients also suffered from severe sepsis. Antibiotics for sepsis of pulmonary origin was switched to oral levofloxacin on day 11 completing a 21 day period of treatment partly after discharge. Chest X-rays showed significant improvements. Impaired laboratory values improved day by day. Patient was transferred from the ICU to the ward after 6 days and discharged 10 days later with total recovery. Various mechanisms for malaria falciparum precipitating myocardial infarction are proposed.

1.1-027

Identification of antimalarial folate biosynthesis inhibitors using a functional screening

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Folate metabolism has long been recognized as a proven source of antimalarial targets, as it is essential for the survival of *P. falciparum*. Several antifolates, such as pyrimethamine or sulfadoxine, that inhibit key enzymes of the folate pathway (DHFR and DHPS respectively), have been used in antimalarial treatments. Inhibition of these enzymes led to a deficiency of dTMP that causes inhibition of cell growth. However, there is still a poor knowledge about other enzymes of this pathway that could be also good antimalarial targets. Using these antifolates as tool compounds we have set up a whole cell assay to select compounds that inhibit this pathway. The procedure is based on the different sensitivity of *P. falciparum* growth to antifolates depending on the concentration of folinic acid present in the culture media. We have used this method to perform a functional screening of the recently published Tres Cantos AntiMalarial Set (TCAMS) in order to identify inhibitors of the folate biosynthesis pathway. The goal was to identify new chemotypes of known antifolates and to find compounds acting in enzymes different from DHFR and DHPS. 519 compounds were detected as inhibitors of this metabolic pathway, of which 497 showed chemotypes resembling known antifolates. Only 22 compounds appeared with novel structures although 18 are likely to hit DHFR as are not active against pyrimethamine-resistant strains; the remaining four compounds do not show cross-resistance against pyrimethamine-resistant strains, so they could be targeting DHFR with a different mechanism than pyrimethamine or inhibiting a different enzyme of the pathway. These compounds are under study in order to characterize its biochemical target and although display a moderate activity against *P. falciparum*, could be a promising starting point to develop a novel generation of antifolates.

1.1-029

A nanovector with complete specificity for targeted delivery into *Plasmodium*-infected red blood cells *in vitro*

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INTRODUCTION Current administration methods of antimalarial drugs deliver the free compound in the blood stream, where it can be unspecifically taken by all of Barcelona cells, and not only by Plasmodium-infected red blood cells (pRBCs). Drug delivery represents a promising approach with the aim of increasing bioavailability and selectivity of drugs and minimizing the appearance of resistance. Liposomes encapsulating antimalarials have been widely used against murine malaria, but there are few data on liposomes targeting to RBCs infected with human-specific strains and a lack of studies on liposomal specific cell recognition, efficacy of cargo delivery and subcellular targeting.

METHODS AND MATERIALS We used fluorescence microscopy to assess *in vitro* the efficiency of liposomal nanocarriers for the delivery of their contents. Quantum dot-loaded liposomes were covalently functionalized with oriented, specific half-antibodies against *P. falciparum* late form-infected pRBCs.

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RESULTS In less than 90 min, liposomes dock to pRBC membranes and release their cargo, even when other cell types are present. One hundred percent of late form-containing pRBCs and 0% of non-infected RBCs are recognized and permeated by the content of targeted immunoliposomes *in vitro*. In preliminary assays, the antimalarial drug chloroquine at a concentration of 2 nM; 10 times below its IC50 in solution, cleared 26.7 ± 1.8% of pRBCs when delivered inside targeted immunoliposomes.

CONCLUSIONS Liposomal nanovectors are adequate for parenteral delivery, indicated in cases of complicated malaria, those at risk of developing severe disease, or if the patient is vomiting and unable to take oral antimalarials. Notwithstanding formulations adequate for oral intake would be a valuable contribution to treating malaria now in endemic areas. Our next objective is the development of new highly specific pRBC targeting nanovectors adequate for oral administration suitable to enter preclinical assays. This research was supported by grants BIO2008-01184, CSD2006-00012, and 2009SGR-760 and by PFIS grant from ISCIII.

1.1-030

Laboratory and field efficacy of two insecticide paints against malaria vectors and pest mosquitoes in Benin, West Africa

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INTRODUCTION There is a need for new products and strategies against malaria vectors. Two insecticide paints were tested in laboratory and field settings in Benin, West Africa. Inesfly-PYR contained two pyrethroids and pyriproxyfen, and Inesfly-OP contained two organophosphates and pyriproxyfen.

METHODS Laboratory WHO bioassays were done using pyrethroid-susceptible and resistant Anopheles gambiae on Inesfly-PYR and organophosphate-susceptible and resistant Culex quinquefasciatus on Inesfly-OP. Paint was applied at 1 kg/6 m^2 on porous and non-porous surfaces. In the field, treatment was applied on porous cement huts at 1 kg/6 m^2 in one or two layers on walls or walls and ceiling. Inesfly-PYR was tested on local mosquito populations susceptible to pyrethoids and organophosphates. Inesfly-OP on local mosquito populations susceptible to organophosphates but resistant to pyrethoids. Four entomological criteria were evaluated: deterrence, excito-repellence, blood-feeding inhibition and mortality. Mortality at distances of 1 m was evaluated using susceptible *An. gambiae* exposed overnight.

RESULTS Laboratory tests on Inesfly-PYR yielded mortalities of 90–100% on all surfaces for 12 months against susceptible *An. gambiae*, but only 13–39% against resistant *An. gambiae* 0 months after treatment. Inesfly-OP yielded mortalities of 93–100% against OP-resistant *Cx. quinquefasciatus* on non-porous surfaces 12 months after treatment. On porous surfaces mortality was low regardless of the resistance status. In the field, a 12-month efficacy was observed on Inesfly-PYR, and 9 months on Inesfly-OP in cement huts. Mortality at distances of 1 m was 84–100% for up to 12 months on both paints.

CONCLUSIONS Inesfly-PYR had a 12-month residual efficacy against susceptible mosquitoes but not pyrethroid-resistant strains on any surface. Inesfly-OP succeeded in killing pyrethroid and organophosphate-resistant strains for 12 months on non-porous surfaces. Cement huts treated with two layers performed better. The question of porosity is to be addressed. The social acceptance and epidemiological impact of Inesfly OP will be evaluated in Phase III studies.

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1.1-031

Detection of high levels of mutations involved in antimalarial drug resistance in *Plasmodium falciparum* and *Plasmodium vivax* at a rural hospital in Southern Ethiopia

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BACKGROUND In Ethiopia, malaria is caused by *P. falciparum* and *P. vivax*. Antimalarial drug resistance is the most pressing problem confronting control of the disease. Since co-infection by both species of parasite is common and sulphadoxine-pyrimethamine has been intensively used, resistance to these drugs has appeared in both species. This study was conducted to assess the prevalence of antimalarial drug resistance in *P. falciparum* and *P. vivax* isolates collected at a rural hospital in southern Ethiopia.

METHODS One thousand one hundred and forty-seven samples were collected across 2007–2009. Pfdhfr and pfdhps mutations and pvdhfr polymorphisms associated with resistance to sulphadoxine-pyrimethamine, as well as pfcrt and pfmdr1 mutations conferring chloroquine resistance were assessed.

RESULTS PCR-diagnosis showed that 125 of the 1147 patients had malaria. Of these, 52.8% and 37.6% of cases were due to P. *falciparum* and *P. vivax* respectively. Ten cases (8%) showed co-infection by both species and two cases (1.6%) by *P. ovale*. Pfdhfr triple mutation and pfdhfr/pfdhps quintuple mutation occurred in 90.8% [95% confidence interval (CI): 82.2–95.5%] and 82.9% (95% CI: 72.9–89.7%) of *P. falciparum* isolates, respectively. Pfcrt T76 was observed in all cases and pfmdr1 Y86 and pfmdr1 Y1246 in 32.9% (95% CI: 23.4–44.15%) and 17.1% (95% CI: 10.3–27.1%), respectively. The pvdhfr core mutations, N117 and R58, were present in 98.2% (95% CI: 89.4–99.9%) and 91.2% (95% CI: 80.0–96.7%) respectively.

CONCLUSION Molecular data show a high frequency of drug-resistance mutations in both *P. falciparum* and *P. vivax* in southern Ethiopia. Urgent surveillance of the emergence and spread of resistance is thus called for. The level of resistance indicates the need for implementation of entire population access to the new first-line treatment with artemeter–lumefantrine, with government monitoring to prevent the emergence of resistance to this treatment.

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1.1-032

Comparison of immunogenicity among vivax malaria proteins expressed in small-scale cell-free systems by suspension array assays

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In vitro cell-free systems for protein expression with extracts from prokaryotic (*E. coli*) or eukaryotic (wheat germ) cells coupled to solid matrices have offered a valid approach for antigen discovery in malaria research. However, no comparative analysis of both

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systems is presently available nor the usage of suspension array technologies which offer nearly solution phase kinetics.

METHODS Five *Plasmodium vivax* antigens representing leading vaccine candidates were expressed in the *E. coli* and wheat germ cell-free systems at a 50 µl scale. Products were affinity purified in a single-step and coupled to luminex beads to measure antibody reactivity of human immune sera.

RESULTS Both systems readily produced detectable proteins; proteins produced in wheat germ, however, were mostly soluble and intact as opposed to proteins produced in *E. coli* which remained mostly insoluble and highly degraded. Noticeably, wheat germ proteins were recognized in significant higher numbers by sera of *P. vivax* patients than identical proteins produced in *E. coli*.

CONCLUSIONS The wheat germ cell-free system offers the possibility of expressing soluble *P. vivax* proteins in a small-scale for antigen discovery and immuno-epidemiological studies using suspension array technology.

1.1-034

Public-private partnerships to maximize the delivery of anti-malarial medicines: confronting lessons learned from the ASAQ Winthrop experience

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BACKGROUND This case study describes the lessons learned by DNDi and by Sanofi, the two partners that developed, registered and launched a new anti-malarial combination, Artesunate Amodiaquine Winthrop (ASAQ Winthrop).

CASE DESCRIPTION A public and a private partner, DNDi and Sanofi, joined forces in 2004 to develop a non-patented, affordable fixed-dose combination of artesunate and amodiaquine adapted to the needs of patients, in particular, those of children. The partners had to address several issues that required confronting diverging viewpoints, in particular: the terms of collaboration, the response to safety issues reported with related compounds, the adaptation of dosage strengths and packaging to African patients' needs, the registration strategy and the development of a Risk Management Plan.

RESULTS Confronting diverging viewpoints on some critical issues has enabled both partners to reach decisions that led to the development, registration in African endemic countries and WHO pre-qualification of ASAQ Winthrop in only 4 years (2004–2008). As a result of the multiple collaborations established by the two partners, ASAQ Winthrop is registered in 30 sub-Saharan African countries and in India, with over 80 million treatments distributed in 21 countries since launch in 2007. Ten clinical studies, involving 3432 patients with ASAQ Winthrop were completed to document efficacy and safety issues identified in the Risk Management Plan.

CONCLUSIONS The speed at which ASAQ Winthrop was adopted in the field shows that this drug fits the needs of patients and health authorities. It also demonstrates the power of partnerships that combine different sets of strengths and skills, and that evolve to address new challenges.

1.1-035

Low *Plasmodium falciparum* specific antibodies and imbalanced pro-inflammatory cytokines are associated with severe malaria in Mozambican children

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INTRODUCTION Factors involved in progression from *Plasmodium falciparum* (Pf) infection to severe malaria (SM) are incompletely understood. Altered antibody and cellular immune response against Pf might increase the risk of developing SM.

METHODS AND MATERIALS A sex- and age-matched case-control study was carried out in 134 Mozambican children with severe or uncomplicated malaria (UM). IgG and IgM against Pf lysate, merozoite antigens, a Duffy binding like (DBL)-£\rosetting domain and antigens on the surface of infected erythrocytes, as well as levels of 12 plasma cytokines and chemokines, were measured by ELISA or flow cytometry.

RESULTS Compared to UM, matched children with SM had reduced levels of IgG against DBL£\ $P < 0.001$), IgM against MSP-1 and AMA-1 ($P < 0.049$ for both) and TGF-£] ($P < 0.001$), whereas levels of IgG against Pf lysate and IL-6 were increased ($P = 0.007$ and $P = 0.049$, respectively). Parasite density decreased with increasing anti-DBL£\IgG among SM children ($P = 0.004$).

CONCLUSIONS SM is characterized by low anti-merozoite IgM and low IgG against a DBL*f*Ñ domain involved in rosetting, together with an exacerbated pro-inflammatory cytokine response. These differences are not attributable to lack of previous exposure to Pf in children with SM. Results are relevant for the design of strategies to control life-threatening malaria.

1.1-036

Modeling the effects of ITNS and IRS in reducing malaria transmission and disease

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Malaria interventions are usually prioritized using efficacy estimates from intervention trials, without considering the context of existing intervention packages or long term dynamics. We used numerical simulation of an ensemble of mathematical models of malaria in humans and mosquitoes to provide robust quantitative predictions of effectiveness of different strategies in reducing transmission, morbidity and mortality. We can simulate indoor residual spraying (IRS) and insecticide-treated nets (ITNs), used singly and in combination. We can estimate reductions in the entomological inoculation rate, clinical cases, prevalence, and malaria deaths from simulations of different coverage levels of ITNs and IRS with different properties. The analysis of such simulation studies can be challenging because they can produce very large numbers of outputs, especially if the focus is on how different control interventions act in combination. We developed a web-based platform to be able to efficiently design, run and analyze simulation experiments. Here we present an overview of the architecture of the platform. Our results suggest that sustained coverage of one or two vector control interventions reduces malaria prevalence in 2–3 years but does not lead to further gains. However, in some settings, even with sustained coverage, clinical incidence of malaria increases as the population loses its naturally

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acquired immunity. In some low to medium transmission settings, our simulations suggest that high coverage of both interventions can lead to interruption of transmission. We can simultaneously capture in mathematical models the dynamics of mosquito ecology, malaria epidemiology, human demography, health systems effects, and control interventions. By integrating costing data, we can also assess the comparative cost-effectiveness of control interventions. Fitting an ensemble of models to data leads to plausible quantitative predictions, with accompanying uncertainty ranges, of the effects of a comprehensive set of different interventions in reducing and potentially interrupting transmission.

1.1-037

Prevalence and risk factors for malaria infection in a highland region of South-Central Ethiopia: a prospective community-based study in the Butajira area

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INTRODUCTION Malaria is one of the diseases of public health significance showing variation in space and time mainly at highland areas of Ethiopia. Thus, malaria outbreaks are the commonest events with catastrophic effects in these areas. However, there has been limited information on malaria epidemiology at high altitudes especially with the warming climate already underway. The objective of the present study is to determine malaria prevalence and risk of malaria infection in Butajira area, southern-central Ethiopian highlands.

METHODS AND MATERIALS A prospective community-based survey was conducted from October 2008 to June 2010. It was repeated seasonally each year: after major rainy, during dry, and after minor rainy seasons.

RESULTS AND CONCLUSION Overall, 178 (0.93%) people were malaria positive of the total 19,207 smears examined. *Plasmodium vivax* was the predominant species (0.80%, *n* = 154/178). The highest parasite rate was found during October–November 2009 survey (2.24%, *n* = 72/178). Variation of malaria prevalence of about 20-folds was determined between the high and low prevalence villages. Children below 10 years were the most affected compared to all age groups. Locally contacted malaria infection was detected in areas >2000 m including children below 5 years. Logistic regression analysis showed statistically significant association of greatest risk malaria infection and low altitude [OR 4.85 (3.28–7.13)] and October-November 2009 [OR5.30 (2.92–9.61)] and June–July 2010 [OR 2.60 (1.37–4.94)]. In conclusion, the present study not only confirmed the occurrence of malaria at high altitude areas beyond its presumed threshold 2000 m but also identified highly significant variation between villages including adjacent ones age groups and seasons of the year. This finding is considered to have practical implications on malaria control with emphasis to applying targeted interventions.

1.1-038

A cost benefit analysis of malaria rapid diagnostic tests for adults and children in Burkina Faso

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INTRODUCTION Malaria rapid diagnostic tests (RDTs) have generally been considered reliable and cost effective. In Burkina Faso the adherence of prescribers to negative test results was found to be poor. Moreover, the test accuracy for malaria atributable fever (MAF) is not the same as for malaria infection. We aimed at determining the costs and benefits of two competing strategies for the management of MAF: presumptive treatment for all or use of RDTs.

METHODS A cost benefit analysis was carried out using a decision tree, based on data obtained from a randomised controlled trial (RCT) recruiting 852 and 1317 febrile patients in the dry and rainy season, respectively. Cost and benefit were first calculated using the real adherence, then assuming an ideal adherence. A sensitivity analysis was performed.

RESULTS At real adherence, the test based strategy was dominated. Assuming ideal adherence, at the value of 525 € for a death averted, the total cost of managing 1000 febrile children was 1747 vs. 1862 € in the dry season and 1372 vs. 2138 in the rainy season for the presumptive vs. the test based strategy. For adults it was 2728 € vs. 1983 and 2604 vs. 2225, respectively. At the subsidized policy adopted locally, assuming ideal adherence, RDTs would be the winning strategy for adults. At sensitivity analysis, the choice of the better strategy was most influenced by the value assigned to a death averted and the proportion of potentially severe NMFI treated with antibiotics following false positive RDT results.

CONCLUSIONS RDTs appear advantageous for adults if adherence is satisfactory. For children the presumptive strategy remains the best choice for most scenarios and always so in the rainy season. A positive RDT should not influence the decision on antibiotic treatment. The full article has been submitted to Malaria Journal

1.1-039

Earth observation in malaria vector control and management

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Geographical Information Systems (GIS), Earth Observation (EO), Spatial Modeling and Statistical Analysis are increasingly being recognized as valuable tools for the management and planning of malaria vector control programmes. The EC 7th Framework Programme project MALAREO compiles a mixed European-African consortium combining years of experience in malaria control with GMES (Global Monitoring for Environment and Security) capacity. The project's main objective is the development and implementation of EO products and capacities within malaria vector control and management programmes in southern Africa. The project site is the cross-border region of Mozambique, Swaziland and South Africa. The region is largely undeveloped, this being exacerbated because it falls within a malarious area. Increased anti-malarial drug resistance and inadequate malaria

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control measures in the past (mainly in south Mozambique) have contributed to this impeded development. MALAREO aims at (i) the identification and to prioritize of the current capacity needs in the project area, (ii) the development of EO methodologies in response to the needs, (iii) local EO capacity development and (iv) the dissemination and promotion of developed methodologies. A gap analysis survey is carried out in the National Malaria Control Programmes of South-Africa, Mozambique and Swaziland to analyze the local use and capacity of EO methodologies for malaria vector control and management. As response to the user requirement survey, relevant and sustainable EO-products will be developed by the use of suitable sensor systems. Examples of such products are land use/cover classifications (indicate conditions for the vector ecology), high-resolution mapping of house distribution (supports the in-house spraying (IRS) campaigns) or the identification of permanent and transient water bodies (reflects favorable vector larval breeding sites) etc. The project results will form the building blocks of a malaria EO monitoring cell that can deliver real, sustainable and affordable EO services supporting malaria control programmes.

1.1-040

Entomological survey in a case of autochtonuos malaria in 2010 in Huesca, Spain

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INTRODUCTION Malaria was officially declared eradicated in Spain in 1964 but this parasitic disease is not rare, with upwards of 500 imported cases seen annually. At the beginning of October 2010 the Department of Public Health of the Government of Aragón informed of a patient from Huesca province, with no travel history, confirmed to have a *Plasmodium vivax* infection. As a result of this case an immediate need was seen to perform an entomological survey to assess the potential risk of an outbreak of this disease as well as to identify the possible vectors.

METHODS AND MATERIALS Before carrying out in-situ sampling, the area affected was studied using specific maps to locate the potential breeding sites of mosquitoes as well as the different land usages, to optimize the subsequent study. Field work consisted of examining water sites in search of larvae using the dipping technique, as well as sampling adult culicidae in their resting places with the aid of a umbrella-type drop-net and capturing them with dry-ice baited CDC traps.

RESULTS Numerous larval habitats were found and the species *Culex pipiens, Cx. hortensis, Cx. modestus, Culiseta longiareolata* and *An. maculipennis* s.s were identified. With the drop-net method *An. maculipennis* s.s. females were detected inside farms, whilst using CDC traps, the most abundant species found was *Ochlerotatus caspius* followed by *An. maculipennis s.s., Cx. theileri and Culiseta subochcrea*.

CONCLUSIONS *Anopheles maculipennis* s.s. is responsible for malaria transmission in limited regions of Europe where a shortage of domestic animals occurs. Cultivated land created in Monegros in the past decades through water management campaigns and the use of extensive agriculture techniques makes this place suitable for the presence of anopheline mosquitoes, competent vectors for malaria, therefore the reoccurrence of such an event in the future cannot be excluded.

1.1-041

Leishmania infantum infection induces in its later stage severe nuclear degeneration of the host cell

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INTRODUCTION A number of works have reported on the encapsulation in liposomes of drugs against *Leishmania* as a strategy to increase efficacy and decrease unspecific toxicity, but a few knowledge gaps still obscure some of the subcellular processes underlying this effect.

METHODS AND MATERIALS Peritoneal macrophages were obtained after stimulation of female Swiss mice with sodium thioglycolate, seeded in a chamber slide system and incubated for 24 h. The medium was then removed and a late stationary phase promastigote culture of the *L. infantum* strain MCAN/ES/92/BCN503 was added in RPMI medium. The culture was incubated for a further 24 h and then washed to eliminate free promastigotes. After addition of liposomes containing quantum dots (QDs) followed by 1-h incubation, confocal microscopy was used to observe liposomes, lisosomes, nuclei, Leishmania, and phagosomes.

RESULTS QD fluorescence could be observed inside the cells as whole internalized liposomes 30 min after the addition of liposomes to macrophages. Colocalization analysis of QDs and lisosomes revealed that internalized liposomes fuse with lisosomes. In the early stages of infection single promastigotes can be observed inside the phagosome, whereas lisosomes are still scattered throughout the cell. In those macrophages where *Leishmania* has replicated, lisosomes are absent from the cytosol and their specific fluorescence is strongly localized inside the phagosome where the parasite has already divided, but not in those containing only one amastigote. In later stages, when the parasite has multiplied several times, we have observed strong staining of the macrophage nucleus with dansyl-cadaverine, a phagosome marker.

CONCLUSIONS Lisosomes can be used to target liposomal anti-*Leishmania* drugs towards the parasite-containing phagosomes. Only after intraphagosomal replication of *L. infantum*, phagolysosomal fusion proceeds. *Leishmania* infection induces in its later stages severe nuclear degeneration and possibly apoptosis of the host cell. This research was supported by grants BIO2008-01184, CSD2006-00012, and 2009SGR-760.

1.1-042

Predicting and taking into account malaria transmission at local scale in cohort follow-up through environmental factors: a prospective study in Benin

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Malaria remains a major endemic disease in tropical areas, especially in Africa. Among the factors that could influence the evaluation of new tools and the enhancement of host-parasite interaction's knowledge, the precise assessment of the environmental risk of transmission especially at a local scale is essential. The aim of this study was to assess how the local variability of malaria transmission can be predicted by climatic and environmental factors. A follow up of a cohort of 650 newborns was carried out from June 2007 to February 2010 in nine villages of Tori-Bossito district in southern Benin. The overall aim of the follow-up was to survey the appearance of biological and clinical signs of first malaria infection up to the age of 18 months. Human Landing Catches were conducted to assess the density of malaria vectors and the intensity of transmission. Climatic factors as well as household characteristics were recorded throughout the study. The statistical association between the *Anopheles* density and environmental and climatic factors was tested through a three-level Poisson mixed regression model. The results showed a temporal (seasonal and rainfall-dependent) variability of the vector's density, and a spatial variability, at the village and house levels, significantly explained by several characteristics related to house's neighbourhood such as type of soil, vegetation index, proximity of a watercourse. Based on these results, a predictive regression model based on a leave-one-out method was developed to apprehend the spatiotemporal variability of malaria transmission in the nine villages. This study highlighted the importance of local environmental factors in the risk of malaria transmission and emphasized the need to develop predictive model to estimate the risk of malaria transmission to which each child can be subjected, depending on his own environmental and behavioural characteristics and both his date and location of birth.

1.1-043

Assessing the environmental factors of malaria transmission: a local-scale geographical study enhancing the importance of the territory at local scale

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The climate of African sub-Saharan countries like Benin is subtropical: warm and humid. Those climatic characteristics allow the existence of an endemic malaria transmission. However, at a micro-scale, people are exposed unequally to this health risk. Therefore, we wanted to investigate which environmental characteristics (in a broad sense) determine malaria transmission at this very fine scale. We conducted a study in a rural district of southern Benin, Tori-Bossito, composed of nine villages. A territorial diagnosis, combined with an epidemiological survey of a cohort of 600 new-born, and with catches of *Anopheles* vectors during the 3 years of the study (2007–2010), allowed the identification of (i) the areas where the vector was predominant, and (ii) the vulnerability factors of people. The field work consisted in collecting multi-scale environmental indicators (continental, regional, local), and conducting a human geography analysis concerning bed nets and insecticides utilization practices, structure of habitat, and soil occupation close to the life places. By modeling these environmental variables into a GIS (Geographic Information System), we were able to show that a 'territorialisation' of the risk in Tori-Bossito was present, associated with ethnic and socio-economic conditions. A spatial segregation linked to life-conditions questions the vulnerability of people in a context of rural-urban migrations.

1.1-044

A descriptive study in acute uncomplicated *Plasmodium falciparum* malaria in infants <5 kg body weight in four Sub-Saharan African countries

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INTRODUCTION Published reports on malaria in younger infants are scanty, leaving a significant knowledge gap about the pattern and outcome of malaria in this sub-population. Artemisinin-based combination therapy (ACT) is recommended as first-line treatment for infants 5 kg of body weight (BW) with uncomplicated falciparum malaria, but no ACTs are indicated in the population <5 kg. Epidemiological data to inform trial design in this age-group is a priority.

METHODS Hospital charts from four countries from sub-Saharan Africa (Bénin, Democratic Republic of Congo, Nigeria, and Togo) were retrospectively reviewed for the period between 2006 and 2010 for inpatient neonates and infants <5 kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria. Clinical features, age group, treatment, and outcome were collected.

RESULTS The annual incidence ranged from <20 to >90 cases across hospitals and calendar years. The proportion of cases varied by age 28 days vs. >28 days): the proportion of infants in the older group was generally higher, but the younger group represented from <2% at one hospital in the Democratic Republic of Congo to >70% at another in Togo. The most frequent clinical presentation was fever, followed by dyspnea, crying, or vomiting. Whenever results were available, parasite load was generally low; <10% of the infants presented with parasitemia >5000/μl. The majority of the infants were treated with oral quinine, except at two hospitals in Bénin and Togo, where AL and intramuscular artemether were administered, respectively.

CONCLUSIONS Although infrequent, malaria in neonates and infants <5 kg of BW does exist in certain endemic countries and calls for appropriate treatment. Further clinical evidence regarding the use of ACTs in this population is warranted.

1.1-045

Evaluation of the efficacy, safety, and PK of artemether–lumefantrine dispersible tablet in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in infants weighing <5 kg

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INTRODUCTION WHO recommends artemisinin-based combination therapy (ACT) as first-line therapy for infants with uncomplicated *P. falciparum* malaria with abody weight (BW) ≥5 kg. However, no ACTs are indicated in infants <5 kg. The poor safety profile of the current standard of care, quinine, limits its usage. Coartem (20 mg artemether–120 mg lumefantrine, AL), with an available pediatric formulation, has the largest clinical trial and postmarketing safety experience in infants ≥5 kg to-date.

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METHODS In this open-label, single-arm, multicenter study in Sub-Saharan Africa inpatient neonates and infants of <5 kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria will be enrolled in two sequential cohorts of 15 infants each: term age >28 days (cohort 1) and term age ¡Ü28 days (cohort 2) to minimize any theoretical risk. A joint data monitoring committee will review efficacy, safety, and pharmacokinetic (PK) data from cohort 1 and recommend whether to proceed to cohort 2, with or without dose adjustment. The primary objectives are to evaluate the efficacy and safety of AL dispersible tablet administered as one tablet bid over 3 days (to adjust if required), and to determine plasma levels of artemether, its active metabolite dihydroartemisinin, and lumefantrine. Exclusion criteria include severe malaria, signs and symptoms of a critical condition, hepatic or renal abnormality, and major neurological malformation. Neurodevelopment status follow-up of patients is planned until day 42 and at 3, 6 and 12 months. Primary endpoint is PCR-corrected parasitological cure at day 7. Secondary endpoints include reduction in parasite density at 24 h; PK assessments; PCR-corrected parasitological cure at days 14, 28, and 42; time to parasite, fever and gametocyte clearance; and safety and tolerability assessments. Appropriate use of antipyretics and quinine as a rescue medication will be permitted.

RESULTS Study results are expected in 2014.

1.1-047

Use of mobile phones for pharmacovigilance associated with artemether–lumefantrine trial in a community setting in Africa

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INTRODUCTION Malaria pharmacovigilance in developing countries is essential but challenging . Use of mobile phones for safety monitoring during a two-arm, community-based study for Coartem [artemether–lumefantrine (AL)] administered for asymptomatic [following systematic screening by rapid diagnostic test (RDT)] or symptomatic falciparum malaria in a rural district of Burkina Faso is described.

METHODS AND MATERIALS Pharmacovigilance procedures required close collaboration among study personnel, local health care facility (LHF) team, and district hospital staff involved with the study. Mobile teams periodically screened half of the population (intervention arm) by RDT. Asymptomatic carriers were treated with AL or alternative medication, with follow-up at Day 7. Subsequently every fever case was assessed by RDT at the LHF for diagnosis of symptomatic malaria episode, and if positive treated similarly with a follow-up at Day 7. Severe cases were transferred to the district hospital. Every serious adverse event (SAE) was notified to the Principal Investigator team by the LHF staff (usually a head nurse) using a mobile phone. Subsequently a study physician was dispatched to the LHF to collect data and transcribe them onto a SAE form in English within the required timeframe.

RESULTS Training on GCP and safety reporting performed at study onset emphasized the requirement to report SAEs in all subjects throughout the study duration, and AEs within 7 days following treatment with AL. Despite that, quality of SAE reporting was inconsistent, diagnosis of severe malaria was suboptimal, and no pregnancies were reported over 3 months. In order to improve reporting, five training sessions tailored to different study personnel, LHF and district hospital staff, were conducted in March 2011. Channels for detection of SAEs were identified and implemented.

CONCLUSIONS Retraining proved to be necessary to increase awareness of study procedures at the district hospital, improve fever case management at the LHF, and ensure pregnancy reporting.

1.1-048

Acceptability and comparative efficacy of artemether–lumefantrine vs. dihydroartemisinin/piperaquine in Kenyan children with uncomplicated falciparum malaria

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INTRODUCTION Artemisinin-based combination therapies (ACTs) are the cornerstone for the treatment of uncomplicated falciparum malaria worldwide. In Africa, artemether–lumefantrine (AL) and artesunate–amodiaquine are the most widely used ACTs. AL has been the first-line treatment for uncomplicated malaria in Kenya since 2006. Despite not yet receiving World Health Organization (WHO) prequalification, dihydroartemisinin–piperaquine (DP) has recently been adopted as a second-line treatment in Kenya.

METHODS This was an open-label, randomized, comparative trial in children aged 6–59 months with uncomplicated falciparum malaria conducted in Western Kenya. Parasite clearance rate, sensitivity to and acceptability of AL and DP were monitored. In total, 466 children were enrolled in the study; they were hospitalized for 3 days for observed treatment and 72-h parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Hemoglobin levels were assessed on Days 0, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. The study drugs were administered by the parent/guardian under the observation of a study team member. At discharge from hospital, a questionnaire on the acceptability of the study drug was administered to the parent/guardian.

RESULTS The parasite clearance rate, sensitivity to and acceptability of AL and DP in the treatment of uncomplicated malaria in Western Kenya will be discussed following completion of data analysis.

1.1-049

In vitro effect of artemether, dihydroartemisinin, and lumefantrine on drug metabolizing enzymes and metabolism of oral contraceptives

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INTRODUCTION This *in vitro* study evaluated the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartemisinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives.

METHODS AND MATERIALS The experiments were conducted according to the FDA drug-drug interaction guidance. The assess-

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ment was done *in vitro* in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 μM, and phenobarbital at 1000 μM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; â-napthoflavone at 10 μM was included as positive control for AhR-mediated induction of genes like CYP1A.

RESULT Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine.

CONCLUSION Based on the levels of mRNA or activity at least <2-fold, with respect to the vehicle control, and/or <40% of the maximal positive control induction response per FDA criteria, these results indicate that Coartem is a non-inducer *in vitro*.

1.1-050

Clearance of *Plasmodium falciparum* as assessed with microscopy, RDT and PCR after anti-malarial treatment in Tanzanian children

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INTRODUCTION Rapid Diagnostic Tests (RDT) are important tools for confirmatory malaria diagnosis in endemic areas like Tanzania. Until recently the tests have mostly been based on Histidine Rich Protein 2 (HRP2), a sensitive and stable marker for *Plasmodium falciparum* (Pf) malaria. HRP2 based RDTs, however, remain positive for a considerable period of time after successful treatment, whereas RDTs based on Pf-specific Lactate Dehydrogenase (PfLDH) appear to only detect live parasites. We assessed the clearance time of these two parasite antigens (HRP2 and LDH) after Artemisinin Combination Therapy (ACT) in comparison with clearance of microscopy detectable parasites and parasite DNA.

METHODS AND MATERIALS We included 50 children <5 years with a positive blood slide of Pf infection in rural Tanzania. Children were followed up on nine occasions up to day 42 after treatment. At each visit finger-prick blood samples were collected for the two RDTs, parasite DNA detection from filter samples, as well as Giemsa and Acridine-orange (AO) stained blood slides for microscopy. Clearance times at the different timepoints were calculated and sensitivity, specificity and predictive values of the two RDTs against microscopy and PCR were determined.

RESULTS Preliminary data show that the median clearance time of HRP2-based RDT was 28 days (range 7–42) and the pLDH based 7 days (2–14). Clearance time for DNA was 2 (1–7) days, Giemsa stained parasites 2 (1–3) days and AO stained parasites 2 (2) days. Antigen clearance as assessed with the two RDTs was thus significantly longer than parasite detection by microscopy and PCR.

CONCLUSION LDH-based RDTs may be a better tool for clinical management of fever patients, particularly in high endemic areas where recurrent malaria infections are common.

1.1-051

Researching antiplasmodial action mechanisms of new molecules

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Malaria caused by *Plasmodium falciparum* (Pf) is responsible for 800 000 deaths per year worldwide. The lack of efficient vaccines and the widespread resistance of the parasite to many existing antiplasmodial drugs drive the need to develop new and effective compounds with an original mode of action especially with respect to unexploited target types to prevent cross-resistance. In this work, after a selective chemical and biological screening of more than 300 neosynthesized candidates, we explored their parasitic targets in the aim to define a new bioactive chemotype, highlighting a new antiplasmodial mechanism of action. Among the large diversity of chemical structures tested, the 4-anilino-2-trichloromethylquinazoline series seemed to be the most relevant one, including both the most active and less toxic molecules. Furthermore, these molecules displayed no activity towards the apicomplexan parasite *Toxoplasma gondii* and were not mutagenic. Then, we focused on several derivatives with interesting biological profile compared to antiplasmodial reference drugs such as chloroquine, atovaquone and doxycycline. They were evaluated with regard to well-known mechanisms of action: heme crystallization, PfDHFR inhibition, modification of the mitochondrial membrane potential. Few compounds were found to mediate their potent antiplasmodial activity through a non classical mechanism of action, yet to describe. Thus we are currently exploring parasite targets, focusing on plasmodial kinases involved during the erythrocytic cycle of the parasite.

1.1-052

Glycosaminoglycans as targeting agents for antimalarial drug delivery

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INTRODUCTION Current delivery methods for antimalarial drugs require high doses that can trigger undesirable side-effects, a problem that can be averted with the use of targeted delivery strategies. Therefore, our main objective is the development of highly specific targeting agents for *Plasmodium*-infected erythrocytes (pRBCs) adequate for the functionalization of nanovectors, with a special emphasis on molecules and structures small enough and non-immunogenic that could be formulated for oral intake. We will explore the capacity of glycosaminoglycans as targeting agents for antimalarial drug delivery, using as a proof of concept the heparin-mediated targeting towards pRBCs of liposome and polymeric nanoparticles. As a second objective, we will investigate the interaction between biological membranes and cationic antimicrobial peptides potentially toxic for *Plasmodium*.

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METHODS AND MATERIALS Liposomes were prepared by the lipid film hydration method and purified by molecular exclusion, finally determining their size by dynamic light scattering. Positively-charged liposomes were examined for their capacity to bind heparin, and the specific targeting of heparin-coated liposomes towards pRBCs was explored by fluorescence confocal microscopy. Additionally, we prepared supported lipid bilayers through the fusion of lipid vesicles method on mica supports. Nanobiotechnological techniques such as atomic force microscopy were applied to study the dynamic processes implicated in the interaction between biological membranes and cationic antimicrobial peptides.

1.1-053

Haematological parameters in paediatric uncomplicated falciparum malaria in Sub-Saharan Africa

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INTRODUCTION Knowledge of haematological changes in acute uncomplicated and convalescent falciparum malaria is poor, particularly in children – which limits our understanding of malaria patho-physiology and haematological toxicity of anti-malarials.

METHODS Individual patient data on white blood cells total counts (WCC), neutrophil and lymphocyte counts, haemoglobin or haematocrit and platelet counts from 3044 African children <5 treated for uncomplicated falciparum malaria in seven randomized controlled trials at 14 sites.

RESULTS In acute malaria, the higher the parasitaemia, the higher WCC and neutrophils in all children; low lymphocyte counts occurred only in older children with higher parasitaemia. Baseline haemoglobin and platelets were independent of parasitaemia. Using paired analysis between Day 28 and baseline in patients without parasitological failure as a proxy for malaria-induced effects, we found that acute *P. falciparum* induced (i) a statistically significant but clinically modest increase in WCC (+5%) resulting from a large increase in neutrophils (+43%) and a smaller decrease in lymphocytes (−16%) and (ii) significant reductions in haemoglobin (-13%) and platelets (−49%). Multivariate random effects analysis showed that post-treatment (i) WCC decreased through Day 28 (neutrophils decrease >lymphocytes increase; all related to the baseline parasitaemia; and (ii) increased haemoglobin and platelets (unrelated to baseline parasitaemia). The risk of neutropaenia increased with follow-up time independent of treatment outcome, and was lower with older age, higher baseline parasitaemia and artemisinin combination treatment.

CONCLUSIONS These analyses help filling knowledge gaps on how acute uncomplicated falciparum malaria affects haematological parameters, and how these change post-treatment. More data are needed to verify these analyses on a larger database. Establishing normal laboratory values especially in pre-schoolers in sub-Saharan Africa is a priority.

1.1-054

Genetic characteristics of the apical membrane antigen-1 (AMA-1) in *Plasmodium vivax* isolates from Iran

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Malaria is a global public health problem in some 90 countries worldwide including Iran and estimated to be responsible directly for about 3000 deaths a day worldwide. Most at risk are young children living in malaria-endemic regions. As the burden of disease, the need for an effective vaccine has also assumed greater importance and the apical membrane antigen-1 is one of the most promising malaria vaccine candidates. Apical membrane antigen 1 (AMA-1) is an immunogenic type 1 integral membrane protein, of apicomplexan parasites that appears to be essential during the invasion of host cells. This protein is synthesized during the erythrocytic phase and suggesting as potential vaccine component. The regions chosen for this study were Sistan and Baluchistan and Hormosgan. Samples were collected from Vivax malaria patients with their informed consent. In current study 44 *Plasmodium vivax* isolates obtained from southeastern parts of Iran were analyzed for apical membrane antigen 1. One thousand base pairs region of this protein was sequenced and 880 base pair region (covering partial domains I and II) was considered for analyses. Non-synonymous mutations were found more frequently at domain I compared to domain II (16 at domain I and 11 at domain II) where almost all of them were dimorphic (26 dimorphic and one tetramorphic). The difference between synonymous and non-synonymous mutations for both domain I and domain II as well as entire 880 bp region were negative which suggests the role of purifying (negative) selection in this molecule. Our results show useful information about the nature of *Plasmodium vivax* parasite population that circulating in the southeast of Iran and also useful information for malaria vaccine development based on apical membrane protein.

1.1-055

Microsatellite analysis of *Plasmodium vivax* isolates from Central Sudan

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INTRODUCTION Polymorphic genetic markers and especially microsatellites can be used to investigate multiple aspects of the biology of *Plasmodium* species . Recently, 14 polymorphic microsatellites were described and used to analyse genetic and geographic variability in *P. vivax*. In the current study, we characterized seven polymorphic microsatellites in 56 Sudanese vivax isolates, with the aim of determining the genetic diversity and population structure of *P. vivax* isolates from Sudan.

MATERIALS AND METHODS In 2007, a total of 56 *P. vivax* isolates were collected from patients attending New Halfa Hospital in East Sudan and from Wad Medani district in Gezira-Central Sudan. PCR was used to amplify the microsatellite (MS) markers MS1,

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MS2, MS3, MS7, MS8, MS10 and MS20. PCR products were analysed on an automated DNA sequencer Ceq8000 (Beckman Coulter), and their lengths were determined. We calculated the expected heterozygosity (HE), and the overall multilocus linkage disequilibrium using a standardized index of association (ISA). Analyses were performed using LIAN 3.5 software.

RESULTS The number of alleles varies across loci from 3 (MS7) to 29 (MS8), with an average of 12.4 alleles. The genetic diversity of the studied population, calculated by the virtual HE of each locus, was considerably high, ranging from 0.43 (MS7) to 0.96 (MS20; 0.78 mean HE). This value is similar to the one previously reported in vivax population from Thailand (average, 0.77). Moreover, we found a significant multilocus linkage disequilibrium with a ISA value of 0.0838, $P < 0.0001$.

DISCUSSION Little is known about *P. vivax* population structure in the African endemic settings. The average of HE value estimated by this study indicated that *P. vivax* population from Sudan is consistent with a conspicuous level of genetic diversity

1.1-056

Impact of artemether–lumefantrine as first line treatment policy on malaria transmission and under five mortality in a rural area with high insecticide-treated net coverage in Tanzania

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INTRODUCTION Vector control measures in addition to systematic use of artemisinin-based combination therapy (ACT) are recommended to fight malaria. The ALIVE [Artemether–Lumefantrine (AL) In Vulnerable patients: Exploring health impact] project assesses the impact of AL as first line treatment for uncomplicated malaria on transmission and <5 years (U5) mortality in Tanzania.

METHODS AND MATERIALS Parasite prevalence was obtained by repeated cross-sectional surveys in two rural districts during two separate periods of first line anti-malarial therapy [2004–2006: sulfadoxine-pyrimethamine (SP) and 2008–2010: AL]. Mortality rates were obtained through a demographic surveillance system. Changes in community malaria parasitaemia and U5 mortality between both periods were compared taking into account the contribution of malaria interventions and contextual factors such as rainfall and rice yields using linear and Poisson regression models.

RESULTS Overall, asymptomatic parasite prevalence progressively declined from 25.0% in 2004 to 3.9% in 2010. There was no significant effect of AL on parasitaemia while ITN coverage was responsible for a significant annual reduction in community parasitaemia. U5 mortality rate decreased by 33% over the entire period from 27.0/1000 person-years in 2005 to 17.0 in 2009. The introduction of AL was associated with an 11% decrease in U5 mortality when adjusted for other key malaria interventions and contextual factors [incidence rate ratio (IRR) = 0.89; 95% CI = 0.79–1.01]. One unit (ton of rice/ha) annual increase in rice yields, was associated with a 36% reduction in annual U5 mortality (IRR = 0.64; 0.54–0.75). On the contrary, ITN coverage was not responsible for a significant reduction in U5 mortality but net usage rates were already very high in 2005.

CONCLUSIONS AL implementation in Tanzania together with other major malaria control programmes was associated with a considerable decline in malaria and U5 mortality. Food security with other key malaria interventions is crucial to support malaria control hence elimination.

1.1-057

Heterogenous decrease in malaria prevalence in children over a 6-year period in South-Western Uganda

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INTRODUCTION Malaria is a major public health problem in children though recent reports suggest its decline. The aim of this study was to assess the change in the prevalence of malaria infection among children below 5 years between 2004 and 2010 in a mesoendemic area of Uganda and to analyse the risk factors of malaria infection.

METHODS Two cross-sectional surveys were conducted in 2004 and in 2010 at the end of the rainy and dry seasons. Rapid diagnostic tests and blood smears were used to diagnose malaria infection. In 2010, sampling was stratified by urban and rural areas. In each selected household, information on malaria and bed net use was collected.

RESULTS In 2004 and 2010, respectively, a total of 527 and 2320 (999 in the urban area and 1321 in rural areas) children under 5 years old were enrolled. Prevalence of malaria infection declined from 43% (95% CI: 34–52) in 2004, to 23% (95% CI: 17–30) in rural areas in 2010 and 3% (95% CI: 2–5) in the urban area in 2010. From the rainy to dry season in 2010, prevalence decreased from 23% to 10% (95% CI: 6–14) in rural areas ($P = 0.001$) and remained stable from 3% to 4% (95% CI: 1–7) in the urban area ($P = 0.9$). The proportion of households having at least one bed net increased from 22.9% in 2004 to 64.7% in the urban area and 44.5% in rural areas in 2010 ($P < 0.001$). In 2010, the risk of malaria infection was associated with child age and household wealth.

CONCLUSIONS There is a significant drop in malaria prevalence among children below 5 years, paralleled by an uptake in bed-net use. However, prevalence remains unacceptably high in rural areas and is strongly associated with poverty.

1.1-058

Mapping of mutations in the *Plasmodium falciparum* sarcoplasmic/endoplasmic reticulum Ca2+-atpase (pfATP6) gene in samples from equatorial guinea (Central Africa) unexposed to artemisinin-based combination therapy

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INTRODUCTION The resistance of *Plasmodium falciparum* to anti-malarial drugs has become the main obstacle to malaria control. The current treatment strategy to avoid the emergence of resistance is based on the use of artemisinin derivatives in combination with other drugs. It is thought that resistance to artemisinin derivatives may be related to mutations in the pfATP6 gene. The aim of this study was to determine the prevalence of point mutations (SNPs) in pfatp6 in different regions of Equatorial Guinea (EG) and then correlate them to possible resistance to artemisinin derivatives.

METHODS We analyzed a total of 55 samples, 23 came from Bata, 17 from Malabo and 15 of the island of Annobon. The study consisted of PCR amplification of pfatp6 in 10 overlapping fragments and sequencing of each of the fragments to identify all possible mutations in the gene. The sequence analysis was performed using Sequencing Analysis 5.3.1 software.

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RESULTS A total of 20 SNPs have been detected in EG, including eight SNPs that have never been described previously: K481R in Malabo and R801H in Annobon, and the synonymous SNPs a141t, c1788t, a2211g, t2739g, a2760c and g2836a. We did not find polymorphism at codons L263E or S769N; these residues have been described to alter the susceptibility to artemisinin derivatives.

CONCLUSIONS In view of these results, we cannot conclude that mutations in these genes have a direct relationship with any case of resistance. However, we note that the study of these mutations is a good epidemiological tool, which allows monitoring the emergence and spread of mutant parasites, and to be alert to possible resistance cases to the new treatment introduced as first line intention in EG, which is artesunate/amodiaquine.

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1.1-059

Can a static cohort model predict a vaccine effectiveness against malaria of children in different transmission areas? comparing the results with a dynamic population model

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INTRODUCTION Dynamic malaria models are used to predict the impact of various interventions on malaria transmission and on disease burden . Dynamic models consider populations instead of cohorts, whereas a longitudinal cohort perspective is more convenient for economic assessment. We previously developed a static, deterministic Markov cohort model in TreeAge ProTM, simulating the impact of malaria vaccination in newborns until the age of 5 years. Model structure and data were derived from a published dynamic model. Our model approximates the morbidity/mortality incidence rates of the dynamic model under low/medium transmission settings. However, underlying assumption of non concurrent infections used for low/medium transmission areas had to be relaxed for high transmission settings. We explore new modeling techniques to accommodate for concurrent infections.

METHODS AND MATERIALS The cohort model was transformed into a stochastic program (first-order Monte Carlo) to allow multiple infections evolving simultaneously in one individual within the cohort, using two interacting components: (i) a 'child' component simulating the occurrence of the different malaria events, (ii) an 'infections' component simulating the parasite densities and immunity acquisition/loss to generate event probabilities feeding each cycle of the 'child' component. Incidence rates of uncomplicated, severe malaria episodes and deaths were compared with those of the dynamic model.

RESULTS After calibration, the Markov microsimulation cohort model is able to reproduce the morbidity/mortality in children for low/medium/high transmission settings. When introducing vaccination in an Expanded Program on Immunization schedule, similar reductions in morbidity/mortality were obtained with the dynamic model in all transmission settings.

CONCLUSION When the potential effect of vaccination on malaria transmission is not considered, a static cohort model can approximate a dynamic population model to estimate its impact on the malaria disease burden under different transmission settings.

1.1-060

Impact of control activities in malaria transmission by *Anopheles funestus* in Southern Mozambique (Manhiça district)

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INTRODUCTION Malaria transmission in Manhiça is perennial with substantial seasonality . An entomological surveillance programme to monitor the intensity of malaria transmission and the impact of control activities implemented in the study area was launched in 2006.

METHODS AND MATERIALS Mosquito sampling was carried out with light traps in 80 households randomly selected from four neighborhoods (Ilha Josina and Taninga areas). Each household was sampled bimonthly during five consecutive nights. The collected anophelines were identified to species or complex level, recording sex and feeding status and stored dry in silica gel for subsequent molecular processing. Household data as number of inhabitants and children, nets using, number of indoor residual spraying (IRS) or type of building materials were also collected.

RESULTS Twenty thousand four hundred and ninety-six (91.4% females) and 506 (95.1% females) *An. funestus* s.l were captured in 2006 and 2007, respectively. The majority of mosquitoes processed were *An. funestus* s.s. (98%). In 2006, the annual *Plasmodium falciparum* entomological inoculation rate (EIR) for *An. funestus* ranged from 100 infective bites per person in Ilha Josina to 133 in Taninga, both areas with marked seasonal differences. In 2007, the annual EIR decreased dramatically. A multivariate analysis indicated that the sampling location and the use of bed-nets were associated with *An. funestus* human biting rate in 2006, while IRS was strongly related with the absence of these mosquito species in 2007. The effect of IRS appeared influenced by the type of building materials used.

CONCLUSION New or refocused malaria initiatives have placed their efforts on scaling up control interventions, including sleeping under insecticide-treated nets and IRS. However, the same control measures do not have the same effects in any endemic malaria region. Ongoing collection of high-quality data that longitudinally measures the impact of the scaling up control interventions on malaria transmission is essential to monitor their progress.

1.1-061

Experience with a monthly 'rolling' malaria indicator survey (RMIS) in Chikwawa district, Malawi: a potential district-level malaria monitoring and evaluation (M&E) tool

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Recent changes in the malaria burden in sub-Saharan Africa have been attributed to rapidly expanding malaria control efforts. Reductions in malaria transmission, and subsequently malaria burden, are likely to result in control programmes targeting persisting malaria hotspots. Therefore, to complement the current 'gold standard' national Malaria Indicator Survey (MIS), novel M&E tools are required to provide timely, accurate, sub-national and district level burden estimates that could guide focused, sub-national control initiatives. To evaluate potential district level malaria M&E tool, a monthly 'rolling' MIS (rMIS), was launched

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in May 2010 involving 51 villages in Chikwawa district, Malawi. This district experiences perennial malaria transmission, and is a focus district for upscaling of national efforts with an antenatal care-based insecticide treated nets distribution and district wide indoor residual spraying in January–February 2011. Using the rMIS, we estimated the seasonal variation of the malaria burden and the progress of malaria control efforts over a one-year period. As various malaria control interventions are simultaneously developing, the effects of malaria control intervention efforts were determined. Based on a probability proportional to (village) size, approximately 1200 households were randomly selected in the first year. Each month, approximately 100 households were sampled, and approximately 60 under-fives were assessed in terms of anaemia and parasitaemia. Monthly data were collected in a 1-week period by two teams of two people using Personal Digital Assistants. The applicability of a rMIS as a potential complementary M&E tool for National Malaria Control Programmes will be discussed, focusing on the logistics and operational aspects of monthly data collection surveys.

1.1-062

Molecular markers related to *Plasmodium falciparum* resistance in imported isolates from Central Africa in a Spanish hospital

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BACKGROUND Drug resistance is a major problem to control *P. falciparum* infection in endemic countries. During last decade, African countries have changed first line treatment to artemisinin combinations therapies (ACTs); Sulfadoxine-Pyrimethamine (SP) is recommended for Intermittent Preventive Therapy (IPT). In isolates imported from Central Africa we analysed the SNPs related to resistance in the period of transition from SP to ACTs.

MATERIALS AND METHODS Samples were collected from patients with *P. falciparum* infection. A first group was taken between June 2002 and June 2006 (n = 113); a second one between November 2008 and August 2010 (n = 46). We analyzed by nested PCR-RFLP the SNPs 51, 59, 108, 164, in pfdhfr gene; 436, 437, 540, 581, in pfdhps gene; 86, 1246, in pfmdr1 gene and 76, in pfcrt gene.

RESULTS The pfdhfr N51I, C59R and S108N were over to 90% in the two groups; all samples had the I164. In the pfdhps, A437G and A581G, increased up to 80% and 10.9% (P = 0.04) respectively. The K540G decreases (25–15.2%) and the S436A/F disappears at the end (P = 0.004). The K76I-pfcrt stayed over 95% in the two groups. Prevalence of N86Y-pfmdr1 remains throughout the study around 50%; D1246Y pfmdr1 decreased (32.1% to 2.2%, P < 0.001).

CONCLUSIONS Pharmacological pressure should affect the resistance strains prevalence. As for SP, the disappearance of S436A/F and the decrease in K540G suggest that these mutations are not fixed in this area. However the appearance of A581G, not described before in this region, would compromise IPT's effectiveness. On the other hand, studies carried out after ACTs introduction show there was a selection of strains carrying the SNPs N86, D1246 in pfmdr1 and pfcrt-K76. In our work, the prevalence of pfmdr1-D1246 is increasing maybe as a result of selective pressure by ACTs. Continued surveillance is essential.

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1.1-063

Antenatal infection with helminths and malaria increases susceptibility to malaria in Kenyan children

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Co-infections with chronic helminth infection and malaria are common in developing countries. This interaction is more pronounced in pregnant women and may influence susceptibility to malaria in childhood. To examine the impact of helminths [schistosomiasis, lymphatic filariasis (LF) and/or hookworm] on malaria susceptibility we undertook a prospective cohort study of 705 newborns in a malaria endemic region of Kenya in which children were examined every 6 months from birth to 3 years of age for *Plasmodium falciparum* infection and the presence of malaria antigen-specific T cell responses. Overall 26% of the pregnant women were co-infected with helminths and malaria, 16% with malaria and 34% with helminths alone. Off spring with schistosomiasis infected mothers had 1.46 times greater risk of malaria (95%CI = 1.08, 2.4; P = 0.014) infection during follow-up than offspring of mothers without. Children with LF infected mothers had 1.36 times greater risk of malaria infection during follow-up than children of mothers without LF (95%CI = 0.75, 2.47; P = 0.0311). Risk of malaria infection increased in children of mothers with schisto and malaria together and also in children of mothers with LF and malaria. Cord blood mononuclear cells (CBMC) of newborns of women co-infected with LF and malaria had almost no malaria antigen-driven IFN-ã/IL-2 or IL-5/IL-13 (1% and 5% respectively) compared to malaria-specific responses in CBMC from women infected with malaria alone (24% and 25%, P = 0.04). This impaired T cell response persisted into childhood. A greater proportion of CBMC had malaria-antigen-driven IL-10 from women with LF and malaria as compared to women with malaria alone (P = 0.05). Thus, helminths and malaria co-infections during pregnancy enhance risk for malaria infection in their offspring, possibly through a mechanism of immune suppression to protective malaria blood stage antigens. Treatment of helminths in pregnant woman may reduce the risk of malaria for their children.

1.1-064

Artemisinin-based combination treatments for African pregnant women with malaria

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INTRODUCTION Although malaria is the most important human parasitic disease, few studies with antimalarial drugs have been carried out in pregnant women. Pregnant women are at high-risk requiring effective antimalarials but they are systematically excluded from clinical trials for fear of teratogenicity and embryotoxicity. This has complicated evidence-based implications for the prevention and treatment of malaria during pregnancy.

METHODS AND MATERIALS A multicentre (Burkina Faso, Ghana, Malawi and Zambia), non-inferiority trial on the safety and efficacy of four artemisinin-based combinations for the treatment of *P. falciparum* malaria in pregnant women has recently started within the framework of the Malaria in Pregnancy Consortium and the financial support of both the European and Developing

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Countries Clinical Trials Partnership (EDCTP) and the Gates Foundation. Pregnant women in the second or third trimester of gestation and with a confirmed malaria infection are randomised to amodiaquine-artesunate, artemether–lumefantrine, dihydroartemisinin–piperaquine or mefloquine–artesunate. They will be followed up weekly until day 63 post-treatment and then monthly until 4–6 weeks and 1 year post-delivery. The primary end points are treatment failure (PCR adjusted) at day 28 and the safety profiles including significant changes in relevant laboratory values. Explanatory variables for failure, i.e. drug levels and *in vitro* resistance of local malaria parasites are also collected. A total of 870 patients will be recruited to each treatment based on 290 patients in each treatment group in each country (i.e. a total centre sample size of 870 patients), adding up to a total study sample size of 3480 patients.

CONCLUSION A three-arm trial using a 'balanced incomplete block design' allows the treatments to be distributed in a way to allow a head-to-head comparison and the establishment of relative value of the treatment according to a series of outcomes. More than 800 patients have been recruited so far.

1.1-065

Malaria, anemia and malnutrition among pigmeis children in South Cameroon

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INTRODUCTIONS Malaria is a major health problem in Cameroon.

METHODS AND MATERIALS Five hundred and forty-six children in Baka were studied. The study was designed as a cross-sectional observational study. Sample size was calculated according to cluster sampling randomizing the comprehensive list of villages. In every village a census was conducted, every house was mapped GPS coordinates were taken for village, water source, latrine and schools. Children from 6 months to 12 years old were included. When children were not present in the house a second visit was paid to the village later on. Children were tested for malaria using RDT and PCR. Anemia was estimated using Hemocue for hemoglobin measure, conjuntival pallor and capilar refill time. Splenomegaly and temperature were determined as well. To estimate malnutrition weight, height, abdominal distension, edemas, and MUAC were analyzed using the ANTHRO and ANTHRO PLUS WHO softwares. KAP surveys were conducted to establish knowledge about malaria, nutrition and hygiene. The use of bed mosquito nets was determined. Socio-economic and household indicator were collected. Consent for participation was based on oral explanation using local translator and readers and finger print collected in forms.

RESULTS Results show that prevalence of malaria among pigmei children is 79.48%, CI95% (76.10; 82.87). 97.7% presented exclusive *Plasmodium falciparum*. Anemia and malaria was significantly related OR 2.07; CI95% (1.32; 3.25), P = 0.001. No school reported to have malaria education materials or ongoing malaria control activities

CONCLUSION The results show high prevalence of infection among pigmeis children in South Cameroon. They provide a baseline for the evaluation of the National Program to fight Malaria that will be distributing 8.6 million bed nets nation wide and the provision of malaria free treatment for children under five, both activities starting on 2011.

1.1-066

Protective anti-malarial IgG responses are affected by *Schistosoma haematobium* infection

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We conducted a 1-year follow-up study of 305 Senegalese children and identified those resistant or susceptible to malaria . We then compared IgG responses to merozoite surface protein (MSP)-1, MSP-2 3D7, MSP-2 FC27, MSP-3, glutamate-rich protein (GLURP) and apical membrane antigen (AMA)-1 at the end of the follow-up in groups of individuals with defined clinical and parasitological histories of infection with *P. falciparum*. Children resistant to clinical malaria and high-density parasitemia had significantly higher IgG1 responses to GLURP and IgG3 responses to MSP2 than their susceptible counterparts. Among those resistant to malaria, high anti-MSP1 IgG1 levels were associated with protection against high parasitemia. In the same area, urinary schistosomiasis is frequent and we therefore assessed the possible influence of *Schistosoma haematobium* infection on these protective anti-malarial IgG responses. After adjustment for confounders, we found that the levels of IgG1 directed to MSP1 and GLURP were significantly lower in helminth carriers. Our data thus reveal a modulation of *P. falciparum*-specific immune responses in the presence of a trematode helminth infection, potentially increasing infected individuals' risk of plasmodial infection or disease. The data argue for focused efforts to gain more insights into the impact of helminth-malaria co-infections on the immune system, sincecontrol through whatever means, but in particular via vaccines, will depend on a thorough understanding of co-infected individuals' possibly altered states.

1.1-067

A meta-analysis of artemisinin-based combination therapies (ACTs) reveals changes in therapeutic efficacy

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Artemisinin-based combination therapies (ACTs) are recommended by WHO for treatment of uncomplicated *P. falciparum* malaria. In clinical trials, differences in efficacy are recorded due to resistance of the parasites. In the previous meta-analysis published, artemether + lumefantrine (AL) was ranked first based on the efficacy parameter cure rate at day 28, PCR corrected. Pubmed was searched to retrieve all trials published before March 2010 containing the key words 'artesunate', 'artemether' or 'dihydroartemisinin'. Trials were acceptable for analysis if they pertained to a randomized controlled clinical trial and evaluated the clinical efficacy of one or more of the following therapies: artemether + lumefantrine (AL), artesunate + amodiaquine (AS+AQ), artesunate + sulfadoxine (AS+SP), artesunate + mefloquine (AS+MQ), artesunate + sulfamethoxypyrazine-pyrimethamine (AS + SMP) or dihydroartemisinin + piperaquine (DHA + PQ) for treatment of uncomplicated *P. falciparum* malaria. Ninety-two randomized clinical trials were included in a multi-treatment Bayesian random effects meta-analysis, calculat-

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ing the posterior median for the odds ratio (OR) of cure rates in the corresponding treatments over the baseline treatment AL. In addition, a direct comparison was performed to grade the different treatments without taking into account the heterogeneity of the studies. Bayesian random effects meta-analysis did not detect any statistically significant difference between the ACTs. The cure rate of AS + SMP (posterior median of OR 1.2; 95% CI, 0.42, 6.5) was found to be equally effective as AL in the treatment of malaria. While the direct comparison should be interpreted cautiously, AS + SMP was found to be significantly better than AL. Therapeutic efficacy of ACTs changes over time. The current analysis reveals changes in the relative drug efficacy value and proposes artesunate + sulfamethoxypyrazine-pyrimethamine (AS + SMP) as alternative treatment. Apart from efficacy, other factors are important in the overall evaluation of ACTs namely, the ease of administration, cost price, side effects and pharmaceutical stability.

1.1-068

Economic costs of malaria in children in three Sub-Saharan countries: Ghana, Tanzania and Kenya

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INTRODUCTION This study was performed to estimate the costs of malaria in three Sub-Saharan African countries (Ghana, Tanzania and Kenya) in children under 5 years (U5) for 2009.

METHODS Direct and indirect household costs were obtained from a previous study in which exit surveys from approximately 300 children's carers were undertaken. Costs of co-morbidities and complications were estimated from interviews with clinicians, health workers and managers of the malaria control programme. National expenses for malaria prevention, treatment and productivity losses due to death were taken from literature and integrated by information collected on site. Different models were developed to estimate cost per episode, in different age ranges, according to severity (non-complicated, severe, severe with severe anaemia, cerebral and cerebral with neurological sequelae) in two scenarios: with and without treatment seeking behaviour. The cost per episode was multiplied by the total of episodes. Monte Carlo simulations were performed within the constructed ranges.

RESULTS Treatment costs (US$) per malaria episode ranged for non-complicated malaria to cerebral malaria with neurological sequelae from $6.75 to $214.54 in Ghana, $5.20 to $137.74 in Tanzania and $11.24 to $287.81 in Kenya. Productivity losses (US$ in thousands) due to death of children aged 0–1 and 1–4 years were $11.8 and $13.8 in Ghana, $6.8 and $8.0 in Tanzania and $7.5 and $8.8 in Kenya. The national cost (US$) of malaria prevention are $28 million, $103 million and $42 million in Ghana, Tanzania and Kenya respectively. The total annual costs of malaria in U5 children in 2009 were estimated $83 million, $377 million and $468 million nationwide in Ghana, Tanzania and Kenya respectively.

CONCLUSION Costs associated with malaria for health systems and households are substantial in these three countries. This study provides important information to policy makers, and may guide future interventions in these endemic countries.

1.1-069

Probing the binding of *Plasmodium falciparum*-infected red blood cells to polysaccharides by force spectroscopy

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INTRODUCTION Red blood cells (RBCs) infected with the mature stages of the malaria parasite, *Plasmodium falciparum*, bind to the endothelial cells of capillaries and post-capillary venules of tissues such as the brain, heart, lung, placenta and small intestine in a phenomenon called sequestration, which is thought to play a major role in the fatal outcome of severe malaria. The sequestration of *Plasmodium*-infected RBCs (pRBCs) is suggested to be mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a parasite-derived polypeptide expressed at the surface of the pRBC. It has been described that PfEMP1, besides binding to different host cell proteins, adheres to polysaccharides such as heparin, heparan sulfate and chondroitin 4-sulfate (CSA). The measurement and analysis of the binding forces between pRBCs and different polysaccharides is the main aim of this research.

METHODS AND MATERIALS The atomic force microscope (AFM) combines high sensitivity (of the order of picoNewtons) at force detection and subnanometric location resolution. These unique properties of AFM are exploited in the force spectroscopy technique, which has been used in this work to measure the binding force between pRBCs deposited on a poly-L-lysine-coated glass slide and heparin molecules immobilized on the tip of the force sensor called cantilever. The experiments were accomplished in PBS.

RESULT Adhesion between heparin and pRBCs has been detected and quantified. An average binding force of about 40 pN was obtained from the analysis of the force histograms. Control experiments revealed non-significant binding between heparin and non-infected RBCs.

CONCLUSION The results confirm previous evidence of specific adhesion of heparin to pRBCs and not to RBCs. This physical analysis is being extended to other glycosaminoglycans like CSA in an attempt to unravel the molecular basis of sequestration of pRBCs via adhesion to polysaccharides. This research was supported by grants BIO2008-01184, CSD2006-00012, and 2009SGR-760.

1.1-070

Intermittent preventive treatment with sulfadoxine-pyrimethamine does not modify levels of antibodies against variant surface antigens, growth inhibitory antibodies, or cytokine responses to *Plasmodium falciparum* in Mozambican children

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BACKGROUND Previous studies have shown that intermittent preventive treatment in infants with sulfadoxine-pyrimethamine

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(IPTi-SP) is a safe and effective malaria control tool that does not affect the acquisition of IgG or IgM to whole parasite or to specific *Plasmodium falciparum* erythrocyte antigens MSP-119, AMA-1, EBA-175. This study aimed to evaluate whether this intervention had an effect on the acquisition of IgG against *P. falciparum* variant surface antigens (VSA) and growth-inhibitory antibodies, as well as on cytokine responses, in children up to 2 years of age.

METHODS IgG to VSA expressed by MOZ2, R29 and E8B parasite isolates were measured in plasma samples collected at 5, 9, 12 and 24 months of age by flow cytometry. Growth-inhibitory antibodies in dialyzed plasmas using GFP-D10 parasites were measured by flow cytometry at 12 and 24 months. Multiple cytokines and chemokines were quantified in plasma by luminex, and antigen-specific cytokine production in whole blood was determined by intracellular cytokine staining and flow cytometry, at ages 5, 9, 12 and 24 months. In addition, factors affecting the magnitude of these responses were assessed as well as the association between antibody or cytokine levels and protection against malaria.

FINDINGS IPTi-SP did not significantly modify the levels of IgG against VSA nor the growth-inhibitory capacity of antibodies up to 2 years of age. Age but not previous of malaria influenced the magnitude of these responses. In addition, anti-VSA IgG levels were 7% higher in children with current *P. falciparum* infection and were associated with neighborhood of residence. Children aged 24 months had significantly less parasite growth than those aged 12 months. None of the responses were associated with subsequent incidence of malaria. Furthermore, IPTi-SP did not significantly affect the proportion of CD3+ lymphocytes producing IFN-γ IL-4 or IL-10 at any of the time points. Overall, plasma cytokine or chemokine concentrations did not differ between treatment groups. Th1 and pro-inflammatory responses were higher than Th2 and anti-inflammatory responses, respectively, and IFN-γ IL-4 ratios were higher for placebo than for SP recipients. Levels of cytokines and chemokines varied according to age, declining from 5 to 9 months. Plasma concentrations of IL-10, IL-12 and IL-13 were associated with current infection or prior malaria episodes. Higher frequencies of IFN- and IL-10 producing CD3+ cells and elevated concentrations of IL-10, IFN-γ, MCP-1 and IL-13 in plasma were individually associated with increased incidence of malaria, at different time points. When all cytokines and chemokines were analysed together, only higher IL-17 at 12 months was associated with lower incidence of malaria up to 24 months.

CONCLUSIONS IPTi-SP does not negatively affect the development of functional antibody or cytokine responses thought to be major contributors to the acquisition of immunity to malaria in early infancy.

1.1-071

Temperature and humidity conditions of malaria rapid diagnostic tests during storage, transportation and distribution in Burkina Faso

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Malaria rapid diagnostic tests (RDTs) are recommended to diagnose malaria at peripheral level. They are more and more used in endemic countries. However the performance of the test depends on its quality at the moment it is getting used. The RDTs' quality also depends on the physical conditions they are kept in. We studied the temperature and humidity environmental conditions of RDTs during their storage, transportation and distribution in Burkina Faso. Temperatures and humidity were recorded using electronic monitors (Logtag®) stored with the RDTs in storage rooms, and during transportation. The devices were programmed to record every 120 min during long-term storage and every 60 min during in-country transport. Whenever the RDTs were moved, the date and information on the details of transport (place, time, date and type of transport) were recorded. Monitors in storage facilities were placed for 6 and 12 months, respectively. Stored data were downloaded from the monitors, and daily and monthly temperature averages were calculated using Microsoft Excel. Devices were placed in the storage room of the central pharmaceutical products store in Ouagadougou; regional stores (3); health districts (3); rural health facilities (3); and during transportation from central to regional (3); and from regional to district (3) level. The study was conducted from August 2009 to September 2010. The preliminary results cover the six first months. The means and ranges of temperature at the central regional and at some districts level were adequate, but higher and larger at health facilities level. Notable variation was showed during transport. The relative humidity at the different places showed a marked seasonal variation. The mean temperature was also different between the climatic zones. Climate, transport conditions and season should be taken into account in RDTs storage and transportation, particularly at peripheral level.

1.1-072

Development of antibodies and aptamers for nanovector-mediated antimalarial drug delivery

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INTRODUCTION Antimalarial drugs targeting Plasmodium falciparum-infected red blood cells (pRBCs) often exhibit low specificity that requires relatively high administration doses, but on the other hand, non-specific toxic molecules demand low doses thus increasing the risk of delivering sub-lethal amounts and favouring the emergence of resistant strains. We designed an immunoliposomal nanovector for the fast (90 min) and specific antimalarial drug delivery to pRBCs in vitro, using as a targeting molecule the commercial antibody BM1234, whose antigen is unknown. We aim at the identification of the BM1234 antigen for the development of new monoclonal antibodies apt to be optimized through biomolecular engineering. DNA aptamers are a second type of pRBC-specific targeting molecule being developed in our group.

METHODS AND MATERIALS The BM1234 antigen has been partially purified by differential precipitation in the presence of detergents followed by 2D-SDS PAGE and mass spectrometry (MS) sequencing, and its subcellular localization has been investigated by transmission electron microscopy (TEM) pRBC-specific aptamers will be identified through the screening of microarrays containing randomly-synthesized oligonucleotide sequences. Fluorescently labelled liposomal nanovectors have been assayed in mice for their toxicity and blood residence time.

RESULTS We have identified the BM1234 antigen in the pRBC cytoskeletal/plasma membrane fraction as, likely, a knob protein exported by P. falciparum. Polyethyleneglycol-coated liposomes injected intravenously do not show toxicity up to very high concentrations, and they remain in the mouse bloodstream with no significant clearance for 90 min.

CONCLUSIONS Intravenously administered targeted liposomes are an efficient tool for the pRBC-specific delivery of antimalarial drugs. Improvement of our current immunoliposomal prototype shall be

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achieved through the generation of our own hybridoma cell line providing antibodies apt to be developed, together with future DNA aptamers, into smaller, economically affordable, and nonimmunogenic pRBC targeting agents. This research was supported by grants BIO2008-01184, CSD2006-00012, and 2009SGR-760.

1.1-073

The N-terminal region of VAR2CSA induces cross-reactive antibodies that inhibit adhesion of several Plasmodium falciparum isolates to chondroitin-sulfate A

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BACKGROUND Binding to chondroitin sulfate A by VAR2CSA, a parasite protein expressed on infected erythrocytes, allows placental sequestration of P. falciparum-infected erythrocytes. This leads to severe consequences such as maternal anaemia, stillbirth and intrauterine growth retardation. The latter has been clearly associated to increased morbidity and mortality of the infants. Acquired anti-VAR2CSA antibodies have been associated with improved pregnancy outcomes, suggesting a vaccine could prevent the syndrome. However, identifying functionally important regions in the large VAR2CSA protein has been difficult.

METHODS Using genetic immunization, we raised polyclonal antisera against overlapping segments of VAR2CSA in mouse and rabbit. Surface reactivity and adhesion-inhibition capacity of the induced antisera and specific antibodies purified from plasma of malaria-exposed pregnant women were assessed on laboratory-adapted parasite lines and field isolates expressing VAR2CSA. Competition ELISA was used to analyse functional resemblance between antibodies induced in animals and those naturally acquired by immune multigravidae.

RESULTS Antibodies targeting the NTS-DBL2X fragment efficiently blocked parasite adhesion to chondroitin sulfate A, similarly to antibodies raised against the entire VAR2CSA extracellular domain. Interestingly, naturally acquired antibodies and those induced by vaccination against NTS-DBL2X target overlapping strain-transcendent anti-adhesion epitopes. A minimal area of the NTS-DBL2X that concentrates anti-adhesion epitopes was identified.

CONCLUSION This study highlights achievement of an important step towards development of a protective vaccine against placental malaria.

1.1-074

Zanzibar: towards malaria elimination?

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The Zanzibar Malaria Control Program (ZMCP) has implemented comprehensive, well integrated combined malaria control interventions, free of charge and with high coverage starting 2003. The main components are long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) against the vectors, rapid diagnostic tests (RDT) and artemisinin-based combination therapies (ACT) in all public health facilities for malaria case management. The ZMCP initiative has become a unique case study for potential malaria elimination from a malaria endemic area in sub-Saharan Africa. We have studied the respective uptake and overall impact of these interventions more closely in two districts of Zanzibar, North A and Micheweni up to 2010. The impact is assessed with regard to parameters such as incidence of confirmed malaria cases, child mortality as well as community parasite prevalence but also with regard to potential resistance to drugs and insecticides used. Triangulation of data from community based cross-sectional surveys, health facility records and vital statistics provide evidence of sustainable malaria control in Zanzibar to a level equivalent with malaria pre-elimination.

1.1-075

Do classic and variant P. ovale differ in their epidemiology or clinical features?

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INTRODUCTION It has been recently proposed that Plasmodium ovale comprises two nonrecombining species that are cocirculating in Africa and Asia. Scarce information is currently available on epidemiologic and clinical features of both types.

METHODS AND MATERIAL Twelve cases of imported P. ovale infection diagnosed in four hospitals in Madrid were analyzed. The diagnosis was confirmed by seminested multiplex PCR (polymerase chain reaction). Locus amplification and sequencing analysis of the ssrRNA gen were carried out to distinguish between classic and variant P. ovale form. Epidemiological, clinical, microbiological, analytical and therapeutic data were collected from the medical reports and studied retrospectively. Statistical analyses with non parametric tests were performed.

RESULTS Genetic analyses were able to identified eight classic P. ovale and four variant P. ovale. All infections were acquired in West Africa and both types were found in Nigeria and Equatorial Guinea. No significant differences were found in the following items: sex, age, country of origin, type of patient, purpose and duration of travel, time between date of arrival and onset of illness or diagnoses, diagnostic method, parasitemia, chemoprophylasis, clinical features, rate of admission and days of hospitalization, treatment, G6PDH deficit, and analytical results, including hemoglobin, leucocytes, platelets, lactate dehydrogenase, albumin, creatinine, aspartate aminotransferase and alanine aminotransferase. A trend toward deeper thrombocytopenia in variant P. ovale was seen.

CONCLUSIONS No epidemiological, clinical, microbiological, analytical and therapeutic differences were found between both types of P. ovale imported from West Africa. A larger number of imported cases from more countries and Asia would be required to gather more data about possible differences between them.

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1.1-076

Evaluation of the duration of protection conferred by malaria prevention tools

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INTRODUCTION To date, the only way to evaluate the efficacy of a malaria prevention tool is through clinical trials in population exposed to malaria . The main objective of these trials is to evaluate the effect of the intervention in the risk of different outcomes related to malaria disease such as clinical malaria, severe malaria, hospital admissions or anaemia among others. The profile of the efficacy over the time was used to evaluate if the effect of the intervention is constant over time, or if on the contrary, it wanes. However, a change in the profile over the time does not answer the question of how long protection will last.

MATERIALS AND METHODS IPTi is the administration in infants of an antimalarial treatment at established time points. In the context of the evaluation of IPTi, we analysed the profile of the protective efficacy after the dose at 9 months in the trials carried out in Ifakara, Tanzania and in the Manhiça, Mozambique.

RESULTS AND CONCLUSIONS We present the results of different statistical models that incorporate a change-point in the protective efficacy. This type of models allows answering the question of how long protection will last. This method can be used with other prevention tools such a malaria vaccines.

1.1-077

Innate immune responses related to the timing of infection with Plasmodium falciparum during pregnancy

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BACKGROUND Pregnancy associated malaria (PAM) is a major public health problem and an important cause of maternal and infant morbidity and mortality. Newborns born to mothers with PAM exhibit a higher risk of developing malaria in the first year of life, and PAM-mediated modifications to neonatal cellular immune responses are thought to contribute to this increased risk. However, relatively little is known about the role of fetal/neonatal innate immune responses in this context.

METHODS We evaluated the frequencies and activation levels of antigen presenting cells (APC) and natural killer (NK) cells in cord blood of Beninois newborns from the area of Come, southwestern Benin, West Africa, where we are conducting a longitudinal prospective study of 1000 mothers. Pregnant women were enrolled 24 weeks of pregnancy and followed at each antenatal visit until delivery. Cellular immunological assessments were performed on cord blood samples from a subgroup of 225 newborns from women with or without active Plasmodium falciparum infection as detected by microscopy of peripheral blood and placental samples. Immunophenotyping of dendritic cells (DC), monocytes and NK cells and the level of activation (HLA-DR, CD86 expression) were evaluated using flow cytometry on DC and monocytes. Analysis of the results is ongoing.

RESULTS We will compare frequencies of cells and their level of activation according to the timing and pathological outcome of P. falciparum infection in pregnant women. We hypothesize that variations in the timing of P. falciparum infection during pregnancy could variably impair neonatal innate immune responses.

KEYWORDS Plasmodium falciparum, cord blood, monocytes, dendritic cells, NK cells

1.1-078

Imported cases of malaria admitted to a Universitary Hospital of Madrid, Spain, 1991–2011

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BRIEF INTRODUCTION Global travel and migration of people from endemic areas for malaria is increasing. Then, imported cases of malaria constitute an important public health problem in many countries, including Spain.

METHODS AND MATERIALS We reviewed retrospectively cases of malaria to identify the clinical features of those imported cases diagnosed at a Universitary Hospital of Madrid, Spain, between January 1991 and May 2011. Diagnosis was based on thick and thin blood smears and immunochromatography assay (Binax-NOW® Malaria).

RESULTS We identified 55 imported cases of malaria at our institution. Mean diagnosis age was 37.24-years-old (14.37), 63.6% were males. *Plasmodium falciparum* was responsible for most cases, 81.8% (37.8%, parasitemia >1%), followed by *P. vivax* 12.7% (100%, parasitemia 1%), *P. ovale* 1.8%, *P. falciparum–P. vivax* 1.8% and *P. falciparum-P. vivax* 1.8%. From total, 38.2% was spanish residents returning travelers and 30.9% were residents returning migrants (VFR); 89.1% came from Africa (29.1% of total came from Senegal; 87.8% *P. falciparum*), 5.5% from LAC (100% *P. vivax*) and 5.5% from Asia (66.7% *P. falciparum*). Almost all patients presented with fever (96.4%), 85.5% with chills, 58.2% with malaise, and 25.5% with myalgia, among others symptoms. Mean haemoglobin levels on admission were 13.12 g/dl (26.9% <12 g/dl; 50% in females, 12.5% in males, $P < 0.01$); platelets: 100,462 cells/mm^3 (79.2% had platelets below 150,000). Patients were hospitalized by a mean of 6.5 days (max.38 days) being successfully treated, without casualties, in 76.4% with quinine-doxycycline.

CONCLUSIONS Imported cases of malaria in the studied population represent one large series of imported case for a one-single institution in a non-endemic country. These findings illustrate the importance of educating non-immune populations about malaria risk and prevention strategies; and from a public health perspective, the need to further develop malaria prevention strategies at a national level and at the European Union.

1.1-079

Genetic diversity of *Plasmodium falciparum* isolated from Yemen based on the genes of merozoite surface proteins (MSP) 1 and 2

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BACKGROUND Malaria is a major health problem causing substantial morbidity and mortality in Yemen, with *Plasmodium falciparum* being the predominant species. Genetic diversity indicates the intensity of transmission providing a baseline data for

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antimalarial drug efficacy trails. This study represents the genetic diversity of *P. falciparum* based MSP1 and MSP2 genes in Yemen.

METHODS Blood samples were collected from 511 patients with fever. The samples were screened for malaria parasites using Giemsa-stained thick and thin blood films. A total 74 samples had *P. falciparum*, for which their MSP1 and MSP2 genes were studied using nested PCR.

RESULTS All the three families (K1, MAD20 and RO33) of MSP1 and the two families (FC27 and 3D7) of MSP2 were detected in this study. 3D7 allelic family was the most frequent (68%), followed by K1 (45%), RO33 (42%), FC27 (42%) and MAD20 (22%). The four allelic families, (MAD20, RO33, FC27 and 3D7), were significantly more prevalent in the foothills/coastland areas as compared to highland areas of Yemen. The K1 allele type was most frequent in the highland ($P < 0.01$). The multiplicity of the infection (MOI) was higher in the foothills/coastland areas than highland for both MSP1 and MSP2. MSP2 had higher number of alleles than MSP1 (20 vs. 11). The highest number of alleles of MSP1 and MSP2 was observed in the foothills/coastland and the rural areas.

CONCLUSIONS Significant differences in complexity and the distribution of the family alleles of MSP1 and MSP2 genes between foothills/coastland and highland areas were observed, reflecting the intensity of malaria transmission between areas. This observation should be taken into consideration in implementing any antimalarial drug efficacy trails in Yemen.

1.1-081

A comparitive study of quinine v/s artesunate in severe malaria patients in Northwestern Rajasthan, India

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INTRODUCTION AND OBJECTIVES *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) are responsible for most of the global burden of malaria. With changing spectrum of clinical presentation in malaria, newer treatment regimens are evolving. So we plan to study comparative efficacy of Quinine V/S artesunate in treatment of malaria.

MATERIAL AND METHODS The study was conducted among 100 adult patients of severe malaria (Pf, Pv, Pf + Pv). The diagnosis of malaria was confirmed by demonstrating asexual form of parasites in peripheral blood smear and positive rapid antigen test. Amongst study group, 65 patients were treated with artesunate (2.4 mg/kg over 10 min followed by 2.4 mg/kg every 24 h) and 35 patients with Quinine (20 mg/kg loading dose for 4 h in 500 ml D 10% and then 10 mg/kg every 8 h). In both groups results were compared at PCT, FCT, CRT and side effects and hospitalization duration observed.

RESULTS Mean parasite clearance with artesunate was 1.62 days, while mean PCT with quinine was 3.46 days, mean FCT with artesunate was 2.17 days while with quinine it was 3.5 days, CRT with artesunate was 1.33 day while with quinine it was 2.67 days. Hypoglycaemia, QTc prolongation, nausea, vomiting, tinnitus and vertigo were frequently seen side effects of quinine while no such side effects were seen with artesunate. We also found that administration of artesunate was easier than that of quinine. Of 35 patients who were treated with quinine, one died, none of the 65 patients treated with artesunate died.

CONCLUSION Artesunate is preferable to treat severe malaria patients over quinine.

KEYWORDS artesunate, quinine, severe malaria, parasite clearance time, fever clearance time, coma resolution time

1.1-082

Expression of variant surface antigens in Indonesian isolates from severe and uncomplicated *Plasmodium falciparum* malaria patients

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INTRODUCTION Erythrocyte Binding Ligand (EBL) and PfEMP1 are two different protein families of *Plasmodium falciparum* consisting of Duffy-binding like (DBL) domains; they are believed to be involved in erythrocyte invasion and cytoadhesion of infected erythrocytes during malaria infection.

METHODS Blood samples from 22 severe and uncomplicated falciparum malaria cases from Indonesia were collected for parasite culture and molecular investigations. Genomic DNA and RNA were analysed to gain knowledge of different expression patterns. Initially, amplification was performed with *f*ÑAF and *f*ÑBR primers, the amplicons were cloned and subsequently sequenced. UNIEBP primers were additionally used and allowed amplification of further DBL domains and the design of internal var D gene primers. Studies on sequence similarity and modeling of a phylogenetic tree were carried out using the algorithm in DNASTAR MegAlign version 5.7 based upon a ClustalW alignment. Differences between groups were analysed using Fisher's exact tests.

RESULTS Seventy-one non-identical sequences out of 104 DBL1*f*Ñ genomic DNA sequences were identified. There was no evidence for a difference between DBL1*f*Ñ sequences between severe and uncomplicated cases ($P = 0.18$). PCR with UNIEBP primer resulted in multiple bands ranging from 250 bp to 1 kb in all samples. They matched with var genes coding for DBL*f*× and PfEMP1 associated with PAM. Amplification of RNA generated bands for samples from severe malaria patients only. Sequencing showed matching with the eba-175 genes, the DBL1*f*Ñ domain and the DBL*f*× domain isolated from Malawian woman with PAM. Reverse-transcription PCR of var D primers resulted in a single band of 237 bp for samples from severe malaria cases only.

CONCLUSION The DBL1*f*Ñ domains of PfEMP1 showed shared common sequences from severe and uncomplicated malaria isolates. The expression of the var D gene was associated with clinically severe malaria in Indonesian isolates.

1.1-083

Prospective open label trial with artemether–lumefantrine 3 years after its broad introduction in Jimma zone, Ethiopia: first evidence for delayed parasite clearance rates

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BACKGROUND The six-dose regimen of artemether–lumefantrine (AL) for uncomplicated falciparum malaria is the approved first line treatment in more than 40 malaria-endemic countries in sub-Saharan Africa. AL was introduced in Jimma zone, Ethiopia, 2006 and since then provided free of charge at public health centres. The objective of this study was to assess the effectivity of AL in patients with uncomplicated malaria 2–3 years after its broad introduction.

METHODS A prospective open label, single arm study was conducted in four areas in Jimma zone with moderate transmission. Patients with uncomplicated falciparum mono-infection were consecutively enrolled. Diagnosis was confirmed by microscopy. Follow-up visits were at day 2, 3, 7, 28 and 42 or any other day if symptoms occurred. Primary outcome measure was PCR-corrected

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parasitological cure rate by day 42. The outcome variables were stratified by gender, age, nutritional status and by each other; logistic or linear regression models were used.

RESULTS Between the rainy seasons in 2008 and in 2009, 348 patients of at least 1 year of age were enrolled and followed-up for 42 days. Thirty-four patients were lost to follow-up. No early treatment failure occurred but parasite clearance rates were prolonged in six patients. Twenty-eight (8.9%) of 314 developed a new infection during follow-up. PCR-corrected cure rate was 94.7% at day 42. There was evidence for an association of prolonged parasite clearance rates and occurrence of recrudescences (OR 10.1, 95% CI 1.7–59.7, P = 0.01). The prevalence of the chloroquine resistance-related mutation pfcrt 76T was similar to data before introduction of AL. However, a selection for the pfmdr 86N genotype was detected.

CONCLUSION Al is still very effective in Jimma, Ethiopia. However, the delayed parasite clearance is alarming. Mutation patterns seem to change under the different drug pressure but do not favour re-introduction of chloroquine in this region.

1.1-084

Adherence of *Plasmodium vivax*-infected reticulocytes to the human spleen

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Plasmodium vivax is the most widely distributed human malaria parasite and responsible each year for 100–300 million clinical cases including severe disease and death. *P. vivax* invades predominantly, if not exclusively, reticulocytes and it is amply accepted that infected-reticulocytes do not cytoadhere in the deep capillaries of inner organs having an obligate passage through the spleen. The question remains as to how *P. vivax* is able to escape spleen clearance to establish chronic infections. Contrary to the current view on the lack of sequestration in *P. vivax*, it has been recently shown that *P. vivax*-infected reticulocytes adhere to endothelial receptors of lung and placental tissues. Moreover, recent data supports a model of how reticulocyte-prone nonlethal malaria parasites escape spleen-clearance through adhesion of infected reticulocytes to a spleen blood barrier of fibroblastic origin. In here, we addressed adhesion of *P. vivax*-infected reticulocytes to the human spleen. Significantly, results from static adhesion assays using tissue cryosections indicated high adhesion of infected reticulocytes to the red pulp of the spleen. Moreover, as variant surface proteins mediate cytoadhesion in *P. falciparum*, we also performed inhibition assays using sera raised in guinea pig against peptides representing conserved motifs of the variant Vir proteins of *P. vivax*. The data showed a trend in inhibition revealing a putative role of these proteins in spleen adhesion. This study represents the first report on adherence of human malaria parasites to the spleen and suggests that parasite-host interaction in this organ is different from what is currently believed and might actually play a role in chronic infection.

1.1-085

Iron status predicts malaria risk in Malawian preschool children

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INTRODUCTION In Sub-Saharan Africa, between one- and two-thirds of children is iron deficient, the majority being <5 years old. Oral iron supplementation is considered a cost effective strategy to reduce iron deficiency (ID) and anaemia. However, concerns about the interaction between iron status, iron supplementation and (malaria) infection make iron supplementation controversial, though supporting evidence remains inconclusive. A dearth of experimental data and the potential for misclassification of iron status and unmeasured confounding in observational studies likely contribute to this debate.

METHODS AND MATERIALS We prospectively assessed the relationship between baseline iron status and malaria risk in a cohort of 727 Malawian preschool children during 5 months following iron supplementation and presumptive treatment for malaria. Directed acyclic graph theory was used to select a minimal sufficient adjustment set of confounders to be included in multivariable Cox regression. Sensitivity to possible misclassification of iron status due to concurrent inflammation was assessed by including alternative definitions of ID in the regression model.

RESULTS AND CONCLUSIONS The overall incidence of malaria parasitaemia and clinical malaria was respectively 3.3 and 0.9 per person year. ID at baseline (serum ferritin ≤30 μg/l) was associated with a decreased incidence of malaria parasitaemia and clinical malaria; adjusted Hazard Ratio's (95% CI) 0.58 (0.40–0.83) and 0.53 (0.34–0.85), respectively. Consistent results were found using alternative definitions for ID and serum ferritin as a continuous measure (data not shown). Our analyses show that ID independently predicts a decreased incidence of malaria parasitaemia and clinical malaria. These results support a cautious approach towards iron supplementation and indicate its combination with prevention of malaria in preschool children in malaria endemic areas.

1.1-086

Plasmodium falciparum strain carrying the mutant allele a581g related to clinical and analytical profile

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BACKGROUND The spread of resistant *P. falciparum* causes an increase in morbidity and mortality of the infection. Nevertheless, in some studies SNPs linked to resistance have been associated to a less severe disease, because a loss of parasite fitness. We analysed the presence of mutant allele A581G, related with resistance to sulfadoxine, with clinical and analytical outcomes.

MATERIALS AND METHODS Samples were collected before treatment began, in 198 patients who came from African continent with *P. falciparum* infection. The following data were recorded: time elapsed between the arrival from endemic area to the malaria diagnosis (days); parasitaemia (%); symptoms (from asymptomatic infection to severe disease); haemoglobin (g/dl) and platelets (×1000/ìl); hepatic profile: AST (UI/l), ALT (UI/l) and billirubin (mg/dl). We studied the codon 581 of pfdhps gene by nested PCR-

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RFLP. Means and Mann–Whitney test were used to compare wild/mutant allele infection.

RESULTS The mutant allele was found in nine patients (4.5%). Compared with the wild type, it was related to a short time between arrival and diagnosis (5.5 vs. 16, P = 0.02), a lower level of Hg (11.9 vs. 12.6), minor recount of platelets (94 vs. 153, P = 0.04), the highest count of AST (73.13 vs. 37.24), ALT (68.56 vs. 38.15) and bilirubin (4.0 vs. 1.4, P = 0.007) and more severe symptoms (P = 0.03), in spite of lower parasitaemia (0.5 vs. 1.0).

CONCLUSIONS The mutant allele A581G was linked with severe analytical and clinical parameters, to some of them not significantly, probably because of the small number of mutants in our sample. Further research is needed to determine the importance of this trend in malaria control programmes, because some patients with severe malaria could be a reservoir of resistant parasites and could be transmitters to the rest of the population, causing more cases of severe malaria.

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1.1-087

Imported malaria at Hvidovre hospital, Denmark from 1994 to 2010

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INTRODUCTION The worldwide incidence of malaria is decreasing, and in Denmark we have observed a 75% decrease in number of malaria cases. In this study, we describe patients with malaria from Department of Infectious Diseases (DID) from 1994 to 2010.

METHOD We included records of all 300 patients who were diagnosed with malaria at DID. Age, gender, country of origin, destination, purpose of travel, treatment, complications and blood biochemistry were registered.

RESULTS AND CONCLUSIONS Sixty-eight per cent were diagnosed with a *Plasmodium falciparum* infection, 24% with vivax, 4% with ovale, 2% with malariae and 2% with a double infection. We observed a decrease from a mean of 20 cases/year in the first 4 years to a mean of 12 cases/year in the last 4 years. Mean age was 34 (57% male), 72% were imported from Africa, 22% from Asia, and 6% from the Americas or unknown areas. Forty-nine per cent were visiting friends and relatives, 24% were tourists, 13% were work-related travels, and 12% were residents in malarious areas. In the first 8½ years observed, 46% of the patients were ethnic Danish, 33% were African, however in the last 8½ years observed, 32% patients were Danish, and 58% were African. Asians with imported malaria decreased from 4/year in the first period, to fewer than 1/year in the second period. 15% of the cases were defined as complicated malaria, based on parasitaemia >4%, cerebral affection or pregnancy. No deaths were recorded. Forty-nine cases of doctor's delay were observed. Only 13 were registered in the second period, suggesting that travelers and doctors have become more aware of the risk of malaria.

CONCLUSION The world malaria situation is reflected in a decrease in imported cases. Doctors and patients are aware of the risk of malaria; however unnecessary delays in diagnosis were experienced.

1.1-088

Maternal and child health in Northern Angola: malaria, schistosomiasis, geohelminths, anemia and malnutrition in a post war setting

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INTRODUCTION Parasitic diseases are an important cause of morbidity and mortality worldwide. In Angola, although malaria, schistosomiasis and soil transmitted helminth (STH) infections are endemic diseases; there is no detailed updated prevalence data available. The aim of this study was to determine the presence of these infections among pre-school children (<6 years old), school-aged (6–15 year old) children and their mothers in rural and peri-urban areas in Northern Angola (Dande Municipality, Bengo Province). Furthermore, prevalence levels of anaemia and malnutrition were also assessed.

METHODS We conducted a community-based random sampling survey, which included 36 of the 69 hamlets within the CISA Project Demographic Surveillance System (DSS) study area. In total, 972 households were included, representing 960 mothers and their 2379 children (≤ 15 year olds).

RESULTS Malnutrition and anaemia were elevated and should be considered severe public health problems, with a total of 21.4% of children being underweight, a prevalence of chronic malnutrition of 32.2% and anaemia reaching 56.9% among under fives. Malaria prevalence in children was close to 18%, and varied heavily according to geographical location, with some hamlets reaching levels above 50%. Similarly, prevalence levels of urinary schistosomiasis depended heavily on location, reaching an overall prevalence of 16.6% in school-aged children. Finally, STH infections were common, with a prevalence of 31.6% in school-aged children.

DISCUSSION Information gathered during this study will augment previous work by government initiatives and will provide concrete prevalence levels and causal factors for these infections, anaemia and malnutrition on a much smaller geographical scale. The results reported here linking malnutrition with STH infections and anaemia, and the latter with urinary schistosomiasis and malaria, provide further evidence that for the successful achievement of all Millennium Development Goals, sub-Saharan African countries, in particular Angola, should integrate management of neglected tropical diseases and malaria.

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1.1-089

Clinical and laboratory safety of artesunate–amodiaquine and comparator treatments for uncomplicated falciparum malaria – an individual patient data analysis of randomised controlled trials

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BACKGROUND Artesunate plus amodiaquine (AS&AQ) is the second most commonly used artemisinin-combination therapy (ACT) for uncomplicated malaria and has been studied in various clinical trials. Compiling safety summaries is important due to concerns particularly over AQ.

METHODS An analysis of individual patient data (IPD) from randomized comparative trials (RCTs) conducted in Africa with 28-day follow-up. Outcomes analysed were all types of adverse events (AEs) and other safety-related haematology and biochemistry data. Descriptive paired analysis and multivariate logistic regression with random intercept on individuals were used to asses the risks of AEs. RCTs compared AS&AQ to AL (artemether–lumefantrine), DP (dihydroartemisinin–piperaquine), AS + SP (artesunate + sulphadoxine/pyrimethamine), AQ + SP (amodiaquine + SP), CQ + SP (chloroquine + SP), AQ or AS alone.

RESULTS Median age was 3.4 years (range 0.5–65); 72% were <5 years old. AEs and haematology were measured in >7700 patients treated with AS&AQ or a comparator. Heterogeneity was high, AEs incidence rates and haematological parameters varied widely between studies. In Rwanda, the incidence of vomiting after AS + AQ was 11.5% and not different from DP (P = 0.565), but 69% lower than with AQ + SP (19.5%, P = 0.013). In patients on AS&AQ the incidence of neurological AE (headache) was <1%, gastro-intestinal (diarrhoea 10%, anorexia <10%, abdominal pain <5%, nausea 1%), dermatological (pruritus or itching or rash 3%), and other AEs (weakness <10%, muscle pain 1%, joint pain and pallor/jaundice 1%). The risk of AEs on AS&AQ was similar to that with other treatments. Most of the patients experienced only one AE. The risks of leucopaenia, anaemia, thrombocytopaenia decreased significantly during follow-up, while the risks of neutropaenia increased. All deaths occurring during follow-up were reportedly unrelated to treatment.

CONCLUSION AS&AQ was generally well tolerated when used for treating paediatric uncomplicated falciparum malaria. AEs were generally mild and incidence low. IPD analyses are useful to describe and compare safety in large numbers of patients.

1.1-090

Determinants of access to acts and malaria diagnosis: results from a household survey in three regions of Tanzania

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While a general consensus over the choice of artemisinin based combination therapy (ACT) as the most effective malaria therapy has developed, a solid evidence-base for choosing the best ACT deployment strategies to gain optimal impact on malaria morbidity and mortality does not exist. Countries are now beginning to adopt policies to enhance ACT deployment that fall more or less into two basic groups: (i) those making ACTs more readily and speedily accessible to patients, or (ii) those targeting ACTs to patients shown to have malaria parasitemia. To design strategies to address and balance these goals, a detailed understanding of current treatment seeking patterns and their determinants is required. We therefore conducted large scale household surveys in three regions in Tanzania with varying transmission levels. Five thousand four hundred and twenty-nine households and 20,973 people were interviewed in Mbeya, Mtwara and Mwanza Regions between June and September 2010. All members of each household who were present and reported fever in the previous 2 weeks were asked about treatment sought, drugs obtained and the cost of this treatment. Additional data collected covered socio-economic status, net ownership and usage, and knowledge of malaria. Fingerprick blood samples were taken to test for malaria parasitaemia and for anaemia in children under 5 years. We will present results on the following two key outcomes: percent of people with fever who got an ACT (within 24 or 48 h), and percent of people with fever who got a finger-prick or heel stick test. We will explore the determinants of these outcomes, considering the influence of age, socio-economic status, location, knowledge and treatment source. Finally, we will identify policy implications for strategies to improve ACT access and targeting, focusing on the current role out in Tanzania out of rapid diagnostic tests to public health facilities, and subsidised ACT under the Affordable Medicines Facility-malaria.

1.1-091

Changing malaria patterns – a 15-year study in a rural district of Southern Senegal (1996–2010)

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BACKGROUND With effective interventions, malaria is generally receding though cause-effect relationships are difficult to establish. Collections of long-term data are needed to document and ascribe changes. The district of Oussouye (Casamance, Senegal, 39,000 inhabitants, four dispensaries, one hospital) implemented incrementally from 2000 the change in malaria treatment policy from monotherapy for clinically-suspected to ACT (artesunate–amodiaquine) for parasitologically-confirmed malaria. Mlomp started staggered implementation from 2000, Djembereng from 2002, the others from 2006 (artesunate–amodiaquine)-2007 (rapid test, RDT).

METHODS Information on consultations, treatments, parasitological tests (smear or RDT) and age was extracted from clinic registries (five healthcare centres in villages with 62% of the district's population) for 1996–2010.

RESULTS Consultations for fever totalled 395,036 and antimalarial treatments 140,039. From 1996 to 2010, annual rates decreased from 7.5 to 3.9 (fevers) and 2.2 to 0.1/capita (treatments); mean age of confirmed malaria increased from 13.5 ± 15.3 years to 21.1 ± 15.3 years but remained stable (19.8 ± 21.7 years to 23.5 ± 73.2 years) for non-malaria fevers. Overall *P. falciparum*

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was tested for 26% of cases and confirmed in 39% (13,969/36,859); 3954 (45%) of treatments were artesunate + amodiaquine. From 1996 to 2010 the projected prevalence of malaria decreased from 35% in 1996, 69% in 2001 and decreased gradually to reach 3% in 2010. In Mlomp (implementing from 2000) the malaria-attributable fraction of fevers decreased from 44% before 2001 to 20% after 2007 (vs. 38% in therest of district), and projected prevalence of malaria from 39% to 1.5% (3% in the rest of district). In the different facilities, changes followed the implementation of new case-management policy. Bednets are distributed but inconsistently used; rainfalls were stable.

CONCLUSIONS Changes (decreased fever and malaria burden and treatments, altered age patterns) in this district are well-documented and support national trends. Temporal coincidence with changes in case-management practice was apparent.

1.1-092

The influence of wealth index on the knowledge and health behaviors related to malaria in Gaza province, Mozambique

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Malaria is the most important parasitical tropical disease in the world, being responsible for most of the clinical cases in sub-Saharan Africa. In Mozambique, malaria is the primary cause of morbidity and mortality. The Chokwé region, in Gaza province, is the most affected by malaria. The access to health care, in low income countries, still has a lot of challenges to overcome, in order to fallow the social equity parameters desired. The poorer population usually is the most affected by malaria and with less access to health care and means of prevention of the disease. The Tisuna Musototo project to control malaria, in Gaza region, has the objective to enhance the access to means of prevention and treatment of malaria, therefore improving knowledge and empowering the community in order to prevent and manage the disease. The main objective of the study is to analyze the influence of Wealth Index (WI) in knowledge and health behaviors related to malaria in these region, using the data collected by a questionnaire (Survey on knowledge, practices about malaria and health care coverage), applied to 887 women, under the project Tisuna Muzototo in Gaza Province-Mozambique. Through the application of parametric tests (Student's t test and ANOVA) and nonparametric (Mann–Whitney–Wilcoxon and Kruskal–Wallis) to a significance level of 5%, we concluded that women/households had significant higher WI median when: they reported higher levels of malaria knowledge; used effective malaria prevention methods; owned mosquito bed nets; had bed nets in good conditions and had permanent treated bed nets. There was no significant relationship between WI and malaria prevention behavior in women during pregnancy and management of child febrile illness.

1.1-094

The ABO blood group system and *Plasmodium falciparum* infection in three ethnic groups living in stable and seasonal malaria transmission area of Burkina Faso

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BACKGROUND Several genetic factors including red blood cell polymorphisms influence the severity of disease due to infection with *P. falciparum* . However , there is a paucity of information concerning the role of host genetic factors in asymptomatic malaria. This study aimed to investigate the prevalence of the ABO blood group and to explore the relationship between ABO blood type and the prevalence of asymptomatic *P. falciparum* infection in three ethnic groups.

METHODS The study was carried out in rural villages. We performed a cross sectional surveys in children and adults from the three ethnic groups. Blood groups were determined genetically using two polymorphisms (rs8176719 and rs8176746) typed using the Sequenom mass-array platform.

RESULTS A total of 548 subjects; Mossi 163 (29.7%), Fulani 209 (38.2%), and Rimaibe 176 (32.1%) were included in this study. The prevalence of blood groups were respectively A: 25.5% (140/548), B: 26.6%(146/548), AB: 7.3%(40/548) and O: 40.5%(222/548). Blood group O was not only the commonest blood type overall, but was higher in the Fulani 110 (52.6%) than Mossi 48 (29.4%) and Rimaibe 64 (36.4%). Subjects from the Fulani were associated with a reduced risk of infection from *P. falciparum* and lower parasite densities than sympatric populations. The subjects with Non O blood (i.e. A, B or AB) were less susceptible to malaria infection. There was an association between ethnicity (CI = 0.63–0.98, $P = 0.039$) and malaria infection during the high transmission as well as an association between the Non O blood group and malaria infections according to all ethnicity ($P = 0.001$).This was also true when ethnic groups were considered separately (Mossi/Fulani $P = 0.02$, Mossi/Rimaibe $P = 0.03$).

CONCLUSION The Fulani are not only less susceptible to *P. falciparum* malaria infection but when infected have lower parasite densities.

Individuals with Non O blood are at lower risk from infection than other groups.

A correlation between ethnicity and blood group for the risk of malaria infection.

1.1-095

Detection of chloroquine resistance molecular markers (Pfcrt and Pfmdr1) in Pahang, Malaysia

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BACKGROUND Malaria is still a public health problem in Malaysia with *Plasmodium falciparum* being the most virulent species, and Chloroquine (CQ) is still the first line drug in the treatment policy of uncomplicated malaria. However, there is a scarcity of information about the emergence of CQ-resistant *P. falciparum* in Malaysia. This study aims to investigate the presence of point mutations at positions 76, 271, 326, 356 and 371 of the *P. falciparum* chloroquine-resistance transporter gene (Pfcrt) and positions 86 and 1246 of *P. falciparum* multi-drug resistance-1 gene (Pfmdr1).

MATERIALS AND METHODS Thin and thick blood films as well as filter paper blood spots were collected from 728 participants living in malaria endemic areas in Pahang. Moreover, 94 archived malaria positive stained blood films were collected from malaria control units and hospitals in Pahang. Pfcrt and Pfmdr1 point mutations were detected using RFLP-PCR.

RESULTS Among Pfcrt gene positions, threonine substitution for lysin in position 76 (mutant T76) and isolucine for arginine in position 371 (mutant I371) were the highest among Pfcrt gene mutations, 51% and 76% respectively. The gene positions 86 and 1246 of Pfmdr1 were mostly of wild type (sensitive). A significant association between the presence of T76 (mutant) and parasitaemia was reported ($P < 0.001$).

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CONCLUSION It is well documented that the pfcrt 76T point mutation is always associated with CQ-resistant *P. falciparum*. Therefore, the high frequency of pfcrt 76T reported in this study could be considered as a useful marker and warning for the spread of CQ resistance in Peninsular Malaysia. The high prevalence of wild type of both Pfmdr1 86 and 1246 suggests that *P. falciparum* is most probably susceptible for other antimalarial drugs. A further study to examine the implemented malaria drug policy using PCR-corrected antimalarial drug efficacy trail in large sample set nationally, is recommended.

1.1-096

Application of ELISA technique as an alternative to IFI in the serological diagnostic of malaria

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In the Parasitology Service of the ISCIII, routine serological diagnostic of malaria is carried out by Indirect Immunofluorescence (IFI) using as antigen *Plasmodium falciparum* obtained from *in vitro* cultures on human red cells and coated on slides. Low sensitivity for other species of *Plasmodium*, laboriousness and the need of an expert microscopist for the right interpretation of results are the main drawbacks of this technique. The main goal of the present work is to validate an ELISA assay that will detect antibodies against all the *Plasmodium* species, and allow automation of the process. We tested different commercial ELISA kits using a set of sera from patients previously diagnosed by IFI and PCR. The kit that provided best results was selected and further tested with a larger number of sera. Results showed a 90% concordance between IFI and ELISA. The obtained results indicate: (i) higher sensitivity of ELISA in sera from patients infected with other *Plasmodium* species other than *P. falciparum*. These results were subsequently confirmed by PCR; and (ii) those samples reactive by IFI and doubtful or negative by ELISA could be explained by an unspecific reaction or in cases where an active malaria episode caused by *P. falciparum* is present. The studies ELISA kit is a good alternative to IFI for the serological diagnostic since it shows higher sensitivity when detecting antibodies against different species of *Plasmodium*, is simpler and easily automated.

1.1-097

Falciparum malaria in young children of rural Burkina Faso: comparison of survey data 1999–2009

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BACKGROUND Roll Back Malaria (RBM) interventions such as insecticide-treated mosquito nets (ITN) and artemisinin-based combination therapies (ACT) have become implemented with different velocities in the endemic countries of sub-Saharan Africa (SSA) in recent years. There is conflicting evidence on how much can be achieved under real life conditions with the current interventions in the highly endemic savannah areas of SSA.

METHODS The study took place in a rural area of north-western Burkina Faso which was defined as holoendemic in 1999. Clinical and parasitological data were compared in two age-matched cohorts of young children from eight villages. Surveys took place in June and December of the year 1999 and 2009 respectively.

RESULTS Prevalence of mosquito net protection increased from 22% in 1999 to 73% in 2009, with the majority of nets being ITNs in 2009. In 2009, *P. falciparum* prevalence and density as well as falciparum malaria prevalence were significantly lower compared to 1999. Parasite prevalence remained, however, slightly above 50%.

CONCLUSIONS The decreasing malaria burden is likely attributed to the significant increase of ITN protection of young children in the study area. Further surveys will demonstrate how much can be achieved with universal access to the existing RBM tools in the highly malaria endemic savannah areas of West Africa.

1.1-098

Management of severe illness due to malaria in the provincial hospital of Tete-Mozambique

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INTRODUCTION In Tete, Mozambique, programmatic management of severely ill patients diagnosed with malaria has not been recently documented.

METHODS From January to April 2010 (malaria season), patients presenting with malaria suspicion at the emergency ward of the Provincial Hospital of Tete were assessed for clinical or laboratory signs of severity based on the WHO recommendations. Malaria was diagnosed by blood microscopy in children <5 years and by rapid diagnostic tests in older children/adults, according to Mozambican guidelines. Initial treatment of severe malaria consisted of intravenous quinine, completed by oral artemether/lumefantrine.

RESULTS Severe illness was diagnosed in 416 patients: 365 with at least one clinical sign of severity (prostration: 279, 76%; seizure: 68, 19%; respiratory distress: 66, 18%; coma: 31, 8%; shock: 15, 4%), and 51 with a hemoglobin level below 5 g/dl. Male/female ratio was 1.01 and 215 (52%) patients were children ≤5 years. Malaria was diagnosed in 219/416 (53%) severely ill patients, and frequency was similar in both age groups. Sixty-two patients (28%) had been referred from peripheral health facilities with malaria diagnosis, but only 17 (27%) had been given pre-referral antimalarial treatment. Parasite density was ≥5% of the red blood cells in 21/110 (19%) children ≤5 years and in 8/109 (7%) older children/adults (P = 0.01). Of 200 malaria patients with complete follow-up data, 124 (62%) were admitted (including almost all children ≤5 years), 24 (12%) were given transfusion and 7 (3.5%) died.

CONCLUSIONS Malaria accounted for about 50% of severe illness in children and adults in this reference setting with seasonal malaria and high HIV prevalence. Pre-referral administration of antimalarials in suspected cases should be strongly reinforced. The low admission rate of older children/adults diagnosed with severe malaria questions the accuracy of the clinical assessment (e.g. prostration). In-hospital malaria mortality was low.

1.1-099

Effect of malaria test results on treatment practices in the provincial hospital of Tete, Mozambique

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INTRODUCTION In Mozambique, up to end 2010, malaria diagnosis had to rely on blood microscopy in children ≤5 years and could be based on histidine-rich protein 2 rapid diagnostic tests (HRP-2 RDT) in older children and adults. We aimed at evaluating the effect of malaria test results on treatment practices in the reference hospital of Tete, Mozambique.

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METHODS In April 2010, during the malaria season, we enrolled prospectively all patients presenting with malaria suspicion at the emergency ward of the Provincial Hospital of Tete. Treatments prescribed by health providers were registered and compared with malaria test results.

RESULTS During this one-month period, 1705 patients with suspected malaria were evaluated. Male/female ratio was 0.93; 751 (44%) patients were children ≤5 years; 127 (7.5%) were referred from peripheral health facilities. Malaria was confirmed by microscopy in 99/671 (15%) children ≤5 years with complete registry data (89%) and by RDT in 198/873 (23%) older children/adults with complete information (92%). Of the 297 malaria cases, 115 (39%) were admitted. All confirmed malaria cases were given antimalarial treatment; in addition, 35/99 (35%) children ≤5 years and 46/198 (23%) older/children adults diagnosed with malaria received antibiotics. Of the 572 non-malaria cases in children ≤5 years, 27 (7%) received antimalarials and 375 (66%) antibiotics. In older children/adults, 42 (6%) and 437 (65%) of the 675 non-malaria cases were treated with antimalarials or antibiotics respectively.

CONCLUSIONS In this reference setting with seasonal malaria and high HIV prevalence, malaria was frequently diagnosed also in older children and adults. Unlike similar studies, antimalarial prescriptions were uncommon both in patients with negative blood microscopy and those with negative RDT. The frequent antibiotic prescription in non-malaria as well as in malaria cases requires further investigations, even in a reference setting with more severe morbidities.

1.1-100

Cellular-mediated immune factors involved in placental malaria infection and its impact on pregnancy outcomes

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INTRODUCTION Malaria in pregnancy has an important impact on the mother and the fetus. Parasites selectively accumulate in the placenta and are associated with localized inflammation. Factors and pathogenic mechanisms contributing to placental malaria and to maternal and fetal outcomes are unclear. Our aim was to evaluate the relationship between cellular populations and immuno-endocrine mediators and adverse delivery outcomes.

METHODS Leukocytes from peripheral, placental and cord blood of Mozambican women (50 active infections, 72 past infections, 50 without infections in the placenta) and 17 unexposed controls from Barcelona were collected at delivery and phenotyped by flow cytometry using surface markers CD45, CD3, CD4, CD8, CD20, CD14, CD16, CD161, CD56, CD57, CD94. Hormone levels (chorionic gonadotropin, prolactin, 17â-estradiol, progesterone and cortisol) were assessed in peripheral and placental plasma. Cytokines and chemokines (IFN-γ, TNF, IL-1â, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12) were quantified in peripheral, placental and cord plasma by bead-based suspension array technology.

RESULTS Cell subpopulation frequencies differed among periphery, placenta and cord blood, and cytokine/chemokine concentrations were elevated in placental plasma compared to peripheral and cord plasma. Total number and frequencies of some cell populations differed according to infection status, although the effect of placental infection depended on the compartment. Active placental malaria was associated with higher levels of IL-1â in placental plasma, whereas no differences were found in periphery or in TNF concentrations. Also, higher IL-10 levels were found in both compartments and elevated IL-8 was found in the periphery. Similar concentrations of the other TH1 and TH2 cytokines tested were detected among the different infection groups.

CONCLUSIONS Preliminary data show that placental infection induces a pro-inflammatory cytokine response and alters cellular immune populations, not only in the placenta and maternal peripheral blood, but also in the cord blood. Further analysis is ongoing to identify what immune-endocrine factors contribute to maternal and fetal outcomes.

1.1-101

Clinical vs. microscopic diagnosis of malaria at Divina Providencia hospital in Luanda, Angola, 2007–2010

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BACKGROUND According to WHO guidelines malaria treatment should be guided by laboratory diagnosis in order to avoid unnecessary drug treatment and to assist the clinician in the differential diagnosis between malaria and non malarial febrile illnesses (NMFI).

OBJECTIVES To assess the proportion of clinically diagnosed and treated malaria cases confirmed/not confirmed by laboratory among all patients attended at the Hospital Divina Providência, stratified by age groups.

METHODS Data were collected during the period 2007–2010 at Divina Providência Hospital in Luanda, Angola. Each patient was submitted to a thick and thin film, with Giemsa stain. Microscopy was constantly submitted to quality control by expert reading. Rapid Diagnostic tests (RDTs) were regularly supplied in 2007 only.

RESULTS During the study period, 18,084 malaria cases were clinically diagnosed and treated in children under 5, 8882 in children 5–14 years old, 14 950 in patients over 14 years and 919 in pregnant women. The confirmed cases were 1959/34,258 slide examined (5.7%), 1359/14,992 (9.0%), 1250/39,327 (3.2%) and 226/23,128 (1.0%) in the four groups, respectively (in total, 4724/111,705 or 4.3%). In 2007, 347 of 3954 RDTs performed were positive (8.8%), vs. 925 of 17,715 (5.2%) slides examined the same year. Overall, only 4794 out of 42,835 (11.2%) total cases diagnosed and treated as malaria were confirmed by microscopy.

CONCLUSIONS Malaria accounts for <5% of all fevers in the study area. Nevertheless, over 40% of initially suspected cases got a final diagnosis of malaria and were treated accordingly. As a consequence, almost 90% of all cases treated were unconfirmed malaria cases. Patients were thus less likely to receive the proper medical treatment for their condition, moreover drug misuse has also obvious cost implications besides the health consequences.

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1.1-102

Evaluation of asymptomatic carriers of *Plasmodium* sp in an endemic area covered by Atlantic forest, in the state of Espirito Santo, Brazil

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Bromeliad-malaria has been reported in the State of Espirito Santo and it is characterized by low incidence of cases . These epidemiological differences in comparison to the Amazon malaria suggest the presence of simians as reservoirs or asymptomatic inhabitants carrying *Plasmodium* sp. This is a prospective study done in a residual malaria region of Santa Tereza municipality, State of Espirito Santo. The goal is to verify, from the occurrence of a first case of malaria, the persistence and frequency of spontaneous healing of asymptomatic carriers over 2 years. The samples were collected 2 km around the house where the malaria case occurred in 2010. The presence of malaria parasites were determined by thin/thick slides and PCR assay. PCR protocol was that designed by Snounou. Sampling was done every 3 months, after signature of informed consent forms. Ninety-two residents of a rural area have been followed for *Plasmodium vivax* DNA. So far, four collections were made. Among all individuals the presence of *P. vivax* was evident in 16.3% (15/92) of the samples. The percentage of positivity for the first and second collection was respectively, 6.8% (6/88) and 9.0% (7/78). No sample of the first collection maintained its positivity in the second. In the second collection, two individuals were absent while the other four became negative. In the third collection, 2.5% (2/80) were positive, one of them since the second collection. There was no positive sample in the fourth collection. Although some individuals had palpable spleens in the first, second and third collections, none were PCR positive. These findings show a persistent subclinical transmission of malaria in this Atlantic Forest region of Brazil. It seems that the asymptomatic carrier is responsible for the maintenance of the endemic status of malaria. Supported by FAPESP (10/50707-5) and FAPES (edital PPSUS).

1.1-103

Natural infection and feeding preference of anophelines captured in an area of autochthonous malaria occurrence, covered by Atlantic forest, Brazil

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Autochthonous cases of malaria have been notified in Atlantic Forest areas in Grande São Paulo. Therefore a project in the location of Parelheiros, south zone of Sao Paulo city, has been conducted since May 2009. The objective of this study is to detect natural *Plasmodium* infection in anophelines captured in two different environments (anthropic-area of cases occurrence and wild – area of conserved forest) and to analyze feeding preferences. Win *et al.* (2002), and Chang *et al.* (2008) protocols were employed in order to detect natural infection and feeding preference, respectively. Anophelines were captured in wild (4878) and anthropic (267) environments. Most of them were identified as *Anopheles* (Ker.) *cruzii*. PCR reactions for the detection of natural infection were performed in a pool of up to 10 mosquitoes. *Plasmodium vivax* infection was detected in one pool and in only one *An.* (K.) *cruzii* specimen captured in the wild environment with Shannon and CDC traps, respectively. *P. vivax* was also detected in one pool collected with a Shannon trap in the anthropic environment. Ninety-one engorged anopheline females (89 *An. cruzii* and 2 *An. strodei*) were captured in wild (16) and anthropic (75) environments with Shannon traps, except three females that were collected in the wild environment with a CDC trap (1) and in the peridomicile of the anthropic area with an aspirator (2). So far, 86 out of 91 engorged females were tested for feeding preference and 81 *An. cruzii* were positive for human blood. *Anopheles cruzii* has been incriminated as the main vector of vivax malaria in Atlantic Forest areas. Our results indicate the presence of infected anophelines in both anthropic and wild environments and corroborate their anthropofilic behavior. Supported by FAPESP 2008/52016-0.

1.1-104

Malaria outside the Amazon region: natural *Plasmodium* infection in anophelines captured near an indigenous village in the vale do rio Branco, Itanhaém, SP, Brazil

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A few cases of *P. vivax* malaria in which anophelines of subgenus Kerteszia were incriminated as vectors have been reported outside the Amazon region, in the Atlantic Forest. This study was carried out near an indigenous Guarani village in the Curucutu reserve, an environmental protection area in the municipality of Itanhaém in the state of São Paulo, Brazil, on 30 November 2009, 18 February 2010, 29 April 2010 and 26 May 2010. Mosquitoes were captured along the route to the Guarani village where the edge of the Branco river floodplain meets the forests on the mountain slopes. Adult forms were collected with CO_2-baited CDC traps and Shannon traps from twilight to 10:00 PM *Anopheles cruzii* predominated in both traps. The other species captured in the CDC traps were *Anopheles pseudomaculipes/maculipes*, *Anopheles fluminensis* and *Anopheles mediopunctatus/forattinii/costai*. Besides the latter three species, *Anopheles apicimacula/intermedius* and *Anopheles strodei* were also found in the Shannon traps. A total of 506 anophelines were assayed by PCR to detect natural infection by *Plasmodium* species. In the CDC traps, *Anopheles fluminensis* and *Anopheles pseudomaculipes/maculipes* were positive for *Plasmodium malariae*, while in the Shannon traps *Anopheles pseudomaculipes/maculipes* was positive for *Plasmodium vivax* and *Plasmodium malariae* and *Anopheles cruzii* was positive for *Plasmodium malariae*, resulting in a minimum infection rate of 0.24%. Our findings suggest that *Anopheles cruzii* may be incriminated in the transmission of malaria between monkeys and humans, as this species was found to be infected by *P. malariae*. They also highlight the need for an understanding of the role of anophelines from outside subgenus Kerteszia in the transmission of malaria in the Atlantic forest, as these were found to be naturally infected by *P. vivax* and *P. malariae*. Supported by SABESP.

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1.1-105

Finding of low parasitemia plasmodium infection by nested PCR in the suburb of Karachi, Pakistan

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INTRODUCTION Plasmodium causes about 500 000 infections in Pakistan every year. The objective of this study was the detection of low parasitemia cases and mixed infections of *P. falciparum* and *P. vivax* among people of suburban Karachi.

METHODS This study was conducted from July to August 2008 in Mawach goth, Memon goth and Pir sirhindi goth of Karachi. A total of 248 adult patients above 12 years of age, visiting different hospital clinics and medical camps with symptoms of fever chill abdominal pain and headache were examined by peripheral blood smear for the diagnose of different species of malarial parasite (MP). Microscopic examination was done by preparing thin and thick Giemsa stained slides whereas nested polymerase chain reaction was applied for the molecular examination with species specific primers.

RESULTS One hundred and ninety-two *Plasmodium* infections were detected by nested PCR of which 88, 43 and 61 samples were due to *P. falciparum*, *P. vivax* and mixed infection respectively. Microscopic examination detected only 72 cases of which 41 cases were due to *P. vivax,* 26 to *P. falciparum* and five were mixed infections.

CONCLUSIONS Nested polymerase chain reaction is the best method for the detection of low parasitemia *Plasmodium* and accurate results.

1.1-106

Malaria in blood donors from Punjab, India in the Valencia transfusion center

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BACKGROUND According to the National Institut of Spanish Statistics (INE) in January 2008 in the Valencian community the immigrant population increased to 16.7%. Large-scale migration can bring with it emerging and reemerging diseases. Malaria is one reemerging infection disease and is important to set prevention plans for blood donors. In our Center we studied the seroprevalence of *Plasmodium* sp in foreign blood donors or people who lived or travelled to endemic areas, with the objective to know the prevalence of this disease and to evaluate the risk of transfusional transmission.

METHODS In January 2009, blood samples were taken from 96 people at the seat of the Sikh Community, from Punjab, North-East India. Antigens were detected by the Optimal-IT Individual Test for Rapid Malaria Diagnosis (DiaMed) and with ELISA Malaria antigen test (DiaMed). Antibodies were detected by ELISA Malaria EIA, Newmarket Laboratories Ltd (Bio-Rad) and the confirmatory test used was Spot IFI (Bio-Merieux 72751).

RESULTS Of the 96 tests, 75 (78%) were positive for *Plasmodium vivax*. We observed a positive antigen test and two PCR positives. Positive donors were from Orissa, Bihar, Gujarat, Lushiana. Thick smear results were all negative.

CONCLUSIONS With the very high percentage of positive tests, we believe it is necessary to screen donors from malaria endemic areas or who have travelled to them, regardless of prior examination and the guidelines of the Royal Decree of 2005, which establishes 3 years of quarantine for donors who had travelled to endemic areas.

1.1-107

Real-time PCR, nested PCR and immunoassay in blood samples processed in pool, as a platform for molecular and serological diagnosis of malaria on large-scale

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INTRODUCTION Malaria diagnosis is based on microscopy as reference, but more sensitive and faster techniques are required for processing large number of samples in clinical and epidemiological studies or donor screening.

METHODS AND MATERIALS We selected 49 blood samples positive for *Plasmodium* by thick blood smear and 48 negative samples from individuals with no previous malaria. Samples were analyzed in individual and pooled experiments using real-time PCR and nested PCR based on sequences of SSU rRNA genes and immunochromatographic test for detecting antibodies. Pools were assembled using a two-stage algorithm for the processing of 10 samples containing one to three positive ones plus nine to seven negative samples. Pools with only negative samples were used as negative control. DNA was extracted with QIAamp DNA Blood Mini Kit (Qiagen®; Valencia, CA, USA). Real-time PCR using genus-specific primers M60 and M61 plus M62 probe was modified in order to obtain a protocol with lower cost and higher sensitivity. Nested PCR using genus specific (rPLU5 and rPLU6) and species-specific (rFAL1/rFAL2, rMAL1/rMAL2, rOVA1/rOVA2, rVIV1/rVIV2) primers was used for species identification. Antibody detection was performed with SD Bioline Malaria Pf/Pv test (Standard Diagnostics, Inc, Suwon, Korea).

RESULTS In individual tests, the sensitivity of real-time PCR, nested PCR and immunochromatographic test was 93.88%, 93.88% and 69.56%, respectively, and in pooled samples was 86.67%, 86.67% and 73.33%, respectively. In all tests the specificity was 100.0%.

CONCLUSIONS Molecular methods showed good results in accuracy and validity parameters, such as sensitivity and specificity in individual and pooled samples, unlike the immunochromatographic test. Considering the advantages of real-time PCR, this method should be indicated as first choice for large-scale diagnosis, followed by nested PCR for species differentiation. Serological test could be adopted only as complementary method.

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1.1-108

A comparative study of malaria parasitemia in urban and rural areas of South East Nigeria

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Malaria is a serious public health problem in Nigeria. Rural and urban areas of the country have reported presence of parasitemia. However with increased development of rural areas and urban migration, habitat changes have occurred that may favour malaria transmission. This study was undertaken to determine the levels of malaria parasitaemia in Aba ana Umuahia towns in comparison with Umuchieze and Uturu, rural communities in Abia State, South-east Nigeria. A total of 1120 individuals (620 from the rural areas and 500 from the urban towns) were examined for malaria parasites using thick and thin blood films. Of these 857(76.51%) were infected. Infection rates in Umuchieze and Uturu were 72.58% and 74.19% respectively while Aba and Umuahia had 86.4% and 74.4% respectively. Although Aba had a higher rate of

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infection than the others, the difference was not statistically significant (P > 0.05). The infection rates recorded by males and females in both areas did not differ statistically (P > 0.05) though more females were infected. Members of the age cohorts 11–20 and 21–30 recorded the highest rate of infection (82.61%). Farmers and students were most infected in the rural areas while traders were more infected in the urban areas. *Plasmodium falciparum* was the most dominant species recorded. Malaria is still very prevalent in both areas and urgent public health interventions are needed to educate the populace especially given the low LLIN compliance earlier observed in some areas of the country.

1.1-109

Mapping communities in networks for malaria transmission and control in Kenya

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With malaria eradication back on the global agenda and subsequent elimination targets for various low endemic countries, control strategies require a strong quantitative evidence base. The failure of previous elimination efforts has shown that human population movements are important for infection exchange between different transmission areas. For countries such as Kenya, which have overall low transmission but a few high transmission hotspots, population movements from high to low transmission zones within country borders may threaten imported infections and challenge control programs. Here, a unique and extensive mobile phone records dataset was analyzed with network analysis tools, a countrywide *Plasmodium falciparum* transmission map and previously developed transmission models to assess communities within Kenya linked by infection flows. The likely principal sources of imported infections that threaten onward transmission or have clinical significance were mapped at a settlement level. Clusters of settlements were identified and compared to approximate 'natural' malaria-relevant migration boundaries, splitting the country into regions that share malaria-relevant movement characteristics. With elimination as the ultimate goal for Kenya, this provides a quantitative platform for strategic control planning, by targeting control resources at defined spatial and temporal scales.

1.1-110

Chloroquine resistance in Haiti: lessons learned from imported cases

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INTRODUCTION On 12 January 2010, an earthquake, followed by a flood, placed many displaced residents and emergency responders at substantial risk for malaria in Haiti. In the following weeks, US military personal engaged in the relief operations were hospitalised with *P. falciparum* (P.f) malaria resistant to chloroquine(CQ), the first-line treatment for uncomplicated malaria on the island. We investigate if malarial drug resistance profiles (genotypic and phenotypic) of P.f strains detected in imported malaria cases from Haiti could have raised an early warning of chloroquine-resistance prior to this catastrophe.

MATERIALS AND METHODS Data collected in 1988–2010 from malaria surveillance centres in France and Toronto were studied. *In vitro* response of reference and clinical isolates to CQ and the pfcrt 76T molecular marker for CQ-susceptibility were studied in patients with recent travel history in Haiti. In total, 40 P.f isolates were obtained from clinical cases imported from Haiti.

RESULTS Among 3 Canadian clinical isolates, all were pfCRT K76 (wild-type genotype) but after *in vitro* adaptation of two, mutant pfCRT 76T was found. The 50% inhibitory concentrations(IC50) were high for both, 506 and 708 nM. The ratio IC50 isolate/Pf3D7 (CQ susceptible clone) was respectively 19.5 and 27.2. Among 37 French clinical isolates, all were pfCRT K76 and 29 were analysed *ex vivo* with a mean IC50 for CQ of 27 nM [95% confidence interval (CI), 13.5–43.4] and a mean of 3D7 ratio of 1.05 (95%CI, 0.58–1.74). Three and 27 patients in Canada and France, respectively, were infected during and after the earthquake in 2010.

CONCLUSION We did not detect early sign of resistance of CQ but mixed population of parasites in two Canadian samples. It is likely that resistant parasites circulate within a majority of susceptible isolates and the earthquake and flood created the necessary epidemiological conditions, which have contributed to evidence the resistance.

1.1-111

Malaria cases in a hospital in the south of Madrid

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OBJECTIVE Description of 100 cases of malaria diagnosed in the Hospital of Fuenlabrada.

MATERIAL AND METHODS Retrospective analysis of all cases of malaria diagnosed from 2004 to 2010. We analyze demographic, clinical and epidemiological variables.

RESULTS The mean age was 30 years, and 75% of patients were younger than 37 years. 61% were male. Ninety-eight per cent were from sub-Saharan Africa (45% of Nigeria and 44% of Guinea Equatorial). Sixty per cent spent <1 month in risk area, and 77% up to 3 months. 80% of cases were symptomatic <1 week before clinical diagnosis, and 97% <1 month. Two cases had been in Spain for more than 1 year before diagnosis.

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Plasmodium type: 86% *P. falciparum*. Percentage of parasitism: 65% had <1%, and 11% >5%. Prophylaxis: 87% did not take any. Clinical symptoms: 51% had headache, 49% gastrointestinal and 14% respiratory symptoms. Fifty-two per cent had anemia, 70% thrombocytopenia, 97% had no leukocytosis, and elevated transaminases were present in 25 of 87 cases. Other information: three ICU admissions, four pregnant women, and five were HIV positive. Nobody died. Forty-one per cent of patients did not re-review after discharge. Conclusions

In our area of health, malaria must always be included in the differential diagnosis of patients with stay in the past 6 months in a risk area if accompanied by fever, thrombocytopenia, headache or digestive disease, and absence of leukocytosis. *Plasmodium falciparum* is the most common species, and Equatorial Guinea and Nigeria are the most common countries of origin. Most patients did not take adequate prophylaxis, and many disappeared from the health system without further review upon completion of the treatment. Four per cent were pregnant and 5% had HIV infection.

1.1-112

Malaria incidence and prevalence among children living in a peri-urban area on the coast of Benin, West Africa: a longitudinal study

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Clinical malaria incidence was determined over 18 months in a cohort of 553 children living in a peri-urban area near Cotonou. Three cross-sectional surveys were also carried out. Malaria incidence showed a marked seasonal distribution with two peaks, the first corresponding to the long rainy season and the second to the overflowing of Lake Nokoue. The overall *Plasmodium falciparum* incidence rate was estimated at 84/1000 person-months, its prevalence at over 40% in the two first surveys and 68.9% in the third. Multivariate analysis showed that girls and people living in closed houses had a lower risk of clinical malaria. Bed net use was associated with a lower risk of malaria infection. Conversely, children of families owing a pirogue were at higher risk of clinical malaria. Considering the high pyrethroids resistance, indoor residual spraying with either a carbamate or an organophospate insecticide may have a major impact on the malaria burden.

1.1-113

Effectiveness of malaria control strategies among pregnant women and children less than 5 years in a rural area of Burkina Faso: a result from nouna health and demographic surveillance site (NHDSS) survey

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INTRODUCTION Malaria remains the worldwide most important parasitic disease and is a particularly severe problem in sub-Saharan African. The 2000 Abuja summit put emphasis on promoting effective preventative methods and case management for vulnerable groups. The objective of this paper was to assess the availability of insecticide treated bed nets (ITNs) and malaria treatment among children and pregnant women.

MATERIALS AND METHODS Cross sectional studies were undertaken in samples of population derived from the Nouna dataset. The first was constituted by a sample of 2850 households and the second with a sample of 409 children. Comparison was done using Abuja indicators as target goals

RESULTS Overall 89% of households possessed at least two bed nets of which 47.96% were insecticide treated. 24.5% of children had slept under ITNs the previous night, as had 28.4% of pregnant women. 49.7% of children presented a fever and 32.5% tested positive for malaria, of whom 13.7% were treated adequately with ACT. 22.3% of pregnant women received chemoprophylaxis for malaria during their pregnancy. Malaria was the first cause of death (32.7%) and children under 5 years were most affected (71%).

CONCLUSION Many effort remains for strengthening the access to ITNs and preventive intermittent treatment for vulnerable group so that to achieve the Abuja targets and MDGs goals.

KEYWORDS malaria, morbidity, mortality, ITNs, Nouna, Burkina Faso

1.1-114

Long-term malaria prevention with insecticide-treated mosquito nets (ITN) in African children: RCT on the effects on morbidity and mortality

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INTRODUCTION Insecticide-treated mosquito nets (ITNs) have been recognized as effective tools against malaria and are now used as a key component of the global Roll Back Malaria initiative. However, concerns remain regarding possible higher mortality in children protected during early infancy because of interference with immunity development. Furthermore, the long-term effects on malaria prevalence and morbidity are not well described.

METHODS Between 2000 and 2002, a birth cohort was enrolled in 41 villages of a malaria holoendemic area in north-western Burkina Faso. All neonates (n = 3387) were individually randomised to either ITN protection from birth (group A) or ITN protection from 6 months of age (group B). Primary outcome was all-cause mortality; secondary outcome was morbidity in a subsample of six sentinel study villages. A comprehensive census was conducted in 2010 in all study villages.

RESULTS Median follow-up time was 8.3 years. The proportion of children having migrated out or been lost to follow up was 13.1% (443/3387). Among the remaining children 16.4% (484/2944) had died, mostly at home and under the age of five. There were no differences in mortality between study groups (248 deaths in group A, 236 deaths in group B, P = 0.57). Females had a slightly higher survival than males but the difference was non-significant (P = 0.15). The survey conducted briefly after the rainy season in 2009 showed that long-term compliance with ITN protection was good (around 90%) but that more than 80% of study children carried asexual malaria parasites and up to 20% had clinical malaria.

CONCLUSIONS ITN protection in early infancy was not a risk factor for mortality at older ages. However, malaria prevalence was high despite high rates of ITN use. Effective malaria control remains a challenge in high-transmission areas.

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1.1-115

The impact of retail-sector delivery of artemether–lumefantrine on malaria treatment of children under five in Kenya: cluster randomized controlled trial

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BACKGROUND It has been proposed that artemisinin-based combination therapy (ACT) be subsidised in the private sector in order to improve affordability and access. This study in western Kenya aimed to evaluate the impact of providing subsidized artemether–lumefantrine (AL) through retail providers on the coverage of prompt, effective antimalarial treatment for febrile children aged 3–59 months.

METHODS AND FINDINGS We used a cluster-randomized, controlled design with nine control and nine intervention sublocations, equally distributed across three districts in western Kenya. Cross-sectional household surveys were conducted before and after the delivery of the intervention. The intervention comprised provision of subsidized packs of paediatric ACT to retail outlets, training of retail outlet staff, and community awareness activities. The primary outcome was defined as the proportion of children aged 3–59 months reporting fever in the past 2 weeks who started treatment with AL on the same day or following day of fever onset. Data were collected using structured questionnaires and analyzed based on cluster-level summaries, comparing control to intervention arms, while adjusting for other covariates. Data were collected on 2749 children in the target age group at baseline and 2662 at follow-up. Twenty-nine per cent of children experienced fever within 2 weeks before the interview. At follow-up, the percentage of children receiving AL on the day of fever or the following day had risen by 14.6% points in the control arm [from 5.3% (standard deviation (SD): 3.2%) to 19.9% (SD: 10.0%)] and 40.2% points in the intervention arm [from 4.7% (SD: 3.4%) to 44.9% (SD: 11.7%)]. The percentage of children receiving AL was significantly greater in the intervention arm at follow-up, with a difference between the arms of 25.0% points [95% confidence interval (CI): 14.1%, 35.9%; unadjusted P = 0.0002, adjusted P = 0.0001]. No significant differences were observed between arms in the proportion of caregivers who sought treatment for their child's fever by source, or in the child's adherence to AL.

CONCLUSIONS Subsidizing ACT in the retail sector can significantly increase ACT coverage for reported fevers in rural areas. Further research is needed on the impact and cost-effectiveness of such subsidy programmes at a national scale.

1.1-116

Over and under-use of acts at public health facilities in three regions of Tanzania

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INTRODUCTION Artemisinin-based combination therapy (ACT) is the first line drug in most malaria-endemic countries, but there are concerns that quality of care remains poor. While patients needing ACT may not receive it, there is considerable over-treatment due to the lack of accurate diagnosis and inappropriate management. To assess current treatment practices, we conducted health facility surveys before scale up of rapid diagnostic tests (RDTs) in Tanzania.

METHODS We enrolled 1779 patients at 145 randomly selected health facilities in Mwanza, Mbeya, and Mtwara Regions between May and October 2010. Patients with fever in the previous 48 h were enrolled on arrival and interviewed following their consultation. Data were collected on patient characteristics, previous treatment, and care. Fingerprick blood samples were taken by study staff to test for malaria parasitemia.

RESULTS Overall, 66.6% of patients attended a facility with any ACT in stock and 28.6% a facility with all weight-specific doses of ACT available. Only 9.8% of patients received a diagnostic test at the health facility; 82% of those tested received a blood smear and 18% an RDT. Of those tested, 54.8% were reported to have a positive test. ACTs were obtained by 58.5% of patients with a positive test, 11.4% of patients with a negative test, and 36.0% of patients who did not receive a diagnostic test during their consultation. Study RDTs conducted in all enrolled patients found that 24.5% of all patients had a positive RDT. ACTs had been obtained by 44.3% of patients with a positive RDT and 33.7% of patients with a negative RDT.

CONCLUSION Over-diagnosis of malaria remains common, with ACTs frequently prescribed to parasite-negative patients; it is anticipated that national scale up of RDTs should address this issue to some degree. However, under-treatment also remains a key problem, reflecting both ACT stockouts and inappropriate health worker practices.

1.1-117

ABO blood group and the risk of placental malaria in Sub-Saharan Africa

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INTRODUCTION ABO blood group O is more prevalent in malaria-endemic than in malaria free countries. The selective pressure presumably either acts via a blood group-dependent differential risk of malaria infection or via a different survival probability when infected. To evaluate the potential role that the differential risk of infection plays, we assessed the association between ABO blood group and placental malaria.

METHODS AND MATERIALS We compiled data from a study in Lambaréné, Gabon, and from three previously published reports of studies in The Gambia, Malawi and Sudan. We cross-tabulated ABO blood groups with placental malaria stratified by parity and calculated corresponding Odds Ratios (ORs) for the outcome, placental parasitaemia, comparing blood group O vs. non-O mothers. For three studies from areas with perennial hyper/holoendemic transmission we carried out a random effects meta-analysis.

RESULTS AND CONCLUSION The OR for placental malaria comparing mother with group O and non-O for primiparae was 0.3 (95% CI 0.05–1.8) in Gabon 3.0 (95% CI 1.2–7.3) in The Gambia and 2.2 (95% CI 1.1–4.3) in Malawi. For multiparae it was 0.7 (95% CI 0.3–1.8) in Gabon, 0.8 (95% CI 0.3–1.7) in the Gambia 0.6 (95% CI 0.4–1.0) in Malawi and 0.4 (95% CI 0.1–1.8) in Sudan. The random effects meta-analysis of the three studies from areas with

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perennial hyper/holoendemic transmission revealed an OR for placental malaria in blood group O primiparae of 1.70 (95% CI 0.67–4.33) and for multiparae of 0.65 (95% CI 0.44–0.96). The blood group O survival advantage with respect to the threat malaria poses is probably rather due to a milder course of the disease than due to a lower risk of infection, although the data from multiparous women presented here suggest that a lower risk of infection may also play a role.

1.1-118

A randomized clinical trial of artemisinin vs. non-artemisinin-based combination therapy of uncomplicated malaria in Mali

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P. falciparum resistance to artemisinin has been reported in South-East Asia. The potential spread of this resistance is real and makes a search for alternative non-artemisinin-based malaria therapy urgent. We tested the hypothesis that sulphadoxine-pyrimethamine plus artesunate (SP + AS) is as efficacious as sulphadoxine-pyrimethamine plus amodiaquine (SP + AQ) in the treatment of uncomplicated *Plasmodium falciparum* malaria. From August to December 2004 and July to December 2005, we conducted a randomized single-blind trial of SP + AS and SP + AQ in two localities in Mali. Parasite genotyping by polymerase chain reaction (PCR) was used to distinguish new from recrudescent *P. falciparum* infections. We recruited a total of 610 children aged 6–59 months, with uncomplicated *P. falciparum* malaria and followed them for 28 days to assess treatment efficacy. Baseline characteristics were similar in both treatment groups. The analysis revealed no early therapeutic failures (ETF) in both arms; late clinical failures (LCF) were 1.7% for SP + AS ($n = 5$) vs. 0% SP + AQ ($n = 0$) and late parasitological failures (LPF) were 3.4% SP + AS ($n = 10$) vs. 1.4% SP + AQ ($n = 4$; $P > 0.05$). We observed a rate adequate clinical and parasitological response (ACPR) of 94.9% and 98.6% for SP + AS and SP + AQ respectively ($P = 0.98$). Based on msp2 analysis, the rate of re-infection was respectively 4.1% and 1.4% for SP + AS and SP + AQ. After molecular correction, we obtained an ACPR of 99% for SP + AS, and 100% for SP + AQ ($P = 0.98$). Sulphadoxine-pyrimethamine plus amodiaquine therapy is as efficacious as sulphadoxine-pyrimethamine plus artesunate in the treatment of uncomplicated *P. falciparum* malaria in Mali.

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Malaria increase in mauritania and its expansion to non-endemic regions

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BACKGROUD In Mauritania, the current situation of malaria is not clear because of the limited number of health facilities and the lack of experienced laboratory technicians to make accurate laboratory diagnosis. This study aimed at the determination of malaria prevalence during transmission seasons of 2009 and 2010 among febrile outpatients attending health facilities in Nouakchott and Hohd el Gharbi region.

MATERIAL AND METHODS Blood samples were collected from all patients with fever and other malaria-like symptoms who presented at health facilities. Rapid diagnostic test, thin and thick films were performed. An epidemiological record was completed for each patient included in the study.

RESULTS In Nouakchott, of the 192 and 306 included febrile patients in 2009 and 2010 respectively, 56 (29.2%) and 197 (64.4%) were malaria-positive. In Hodh Elgarbi region, malaria prevalence rates were 35.83% (105/293) and 73.78% (273/370) in 2009 and 2010, respectively. A significant increase of positive thick film among febrile patients was observed in 2010 compared to 2009 in both areas ($P < 0.0001$). *Plasmodium vivax* is the predominant species in Nouakchott with a prevalence rate reaching 45% (137/306) at the Health Center of Teyarett district. Inversely, in Hodh Elgharbi *Plasmodium falciparum* remains the main species encountered. The mean prevalence was 34.1% (100/293) and 72.7% (269/370) in 2009 and 2010, respectively. The cumulative prevalence of *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* was 1.7% in 2009 and 1.08% in 2010.

CONCLUSIONS Results suggest a significant increasing of malaria prevalence in the two regions and the involvement of *P. vivax* and *P. falciparum* as the main causative agents. Further study should be carried out to confirm the expansion of malaria in the northern non-endemic regions of the country.

1.1-120

Exploration of hollow liposomes as a new nanovector against malaria

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INTRODUCTION *Plasmodium falciparum* developing inside red blood cells (RBC) needs molecular building blocks to satisfy its growth requirements, including lipids for biomembrane synthesis. Different studies have suggested that two mechanisms are used by the parasite to acquire them: de novo synthesis and the uptake of existing lipids from the RBC plasma membrane. We therefore hypothesized that if lipids toxic for Plasmodium were inserted in the parasitized RBC (pRBC) through a targeted liposome nanovector, they could be incorporated by the parasite and reduce its viability.

METHODS AND MATERIALS The lipid film hydration method was used to prepare immunoliposomes with different lipid formulations that were functionalized with specific targeting antibodies against pRBCs. The toxicity for *P. falciparum* has been measured in *in vitro* cultures through growth inhibition assays where the final parasitemia was estimated by flow cytometry analysis and by direct microscopical visualization. The different formulations were also assayed for their unspecific toxicity and haemolytic capacity. The subcellular localization in pRBCs of liposomes containing a fluorescently labelled phospholipid has been studied by fluorescent confocal microscopy.

RESULTS Certain lipids are particularly toxic for *P. falciparum* when incorporated into a parasite culture in the form of targeted liposomes, without exhibiting significant unspecific toxicity or hemolysis. The preliminary studies on the subcellular localization of liposome-borne lipids indicate that they do not only stay in the pRBC plasma membrane, but instead they also penetrate inside the parasitized cell.

CONCLUSIONS The delivery of toxic lipids in the form of targeted immunoliposomes can be a valid approach for the design new therapeutic strategies against malaria. The relative low immunogenicity of lipids and the possibility of modifying them with (toxic)

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chemical groups open interesting perspectives regarding the applications of targeted nanovectors to the treatment of this devastating disease. This research was supported by grants BIO2008-01184, CSD2006-00012, and 2009SGR-760.

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Impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin

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Urban agricultural practices are expanding in several cities of the Republic of Benin. This study aims to assess the impact of such practices on transmission of the malaria parasite in major cities of Benin. A cross sectional entomological study was carried out from January to December 2009 in two vegetable farming sites in southern Benin (Houeyiho and Acron) and one in the northern area (Azèrèkè). The study was based on sampling of mosquitoes by Human Landing Catches (HLC) in households close to the vegetable farms and in others located far from the farms. During the year of study, 71,678 female mosquitoes were caught by HLC of which 25% (17,920/71678) were Anopheles species. In the areas surveyed, the main malaria parasite, *Plasmodium falciparum* was transmitted in the south by *Anopheles gambiae* s.s. Transmission was high during the two rainy seasons (April–July and October–November) but declined in the two dry seasons (December–March and August–September). In the north, transmission occurred from June to October during the rainy season and was driven by two members of the *An. gambiae* complex: *Anopheles gambiae* s.s. (98%) and *Anopheles arabiensis* (2%). At Houeyiho, Acron and Azèrèkè, the Entomological Inoculation Rates (EIRs) and the Human Biting Rates (HBRs) were significantly higher during the dry season in Households Close to Vegetable Farms (HCVF) than in those located far from the vegetable areas (HFVF; $P < 0.05$.). However, there were no significant differences in HBRs or EIRs between HCFV and HFVF during the rainy seasons at these sites ($P > 0.05$). The knock-down resistance (kdr) mutation was the main resistance mechanism detected at high frequency (0.86–0.91) in *An. gambiae* s.l. at all sites. The ace-1R mutation was also found but at a very low frequency (<0.1). These findings show that communities close to vegetable farms are exposed to malaria throughout the year, whereas the risk in those living far from such agricultural practices is limited and only critical during the rainy seasons. Measures must be taken by African governments to create awareness among farmers and ultimately decentralize farming activities from urban to rural areas where human-vector contact is limited.

KEYWORDS Anopheles gambiae; vegetable farming; malaria; transmission

1.1-122

In vitro drug resistance monitoring of *Plasmodium falciparum* wild isolates from the Peruvian Amazon

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Plasmodium falciparum, the most pathogenic specie of malaria, has developed resistance against all antimalarial drugs around the world. In South America resistance to many classical drugs have been reported included the last line of defense i.e. Artemisinin. Since 2002, the Peruvian Ministry of Health adopted artemisinin-mefloquine combined therapy (ACT). The *in vitro* assay of drug efficacy in local parasite isolates can give an early warning of rising drug resistance *in vivo*. The main objective of this research is to monitor the susceptibility to Chloroquine (CQ), Quinine (QN), Mefloquine (MQ) and Artemisinin (AS) of *P. falciparum* wild isolates from the Peruvian Amazon by the microscopic method. Forty isolates of *P. falciparum* from patients infected with uncomplicated malaria from diverse areas of the Peruvian Amazon were collected during the years 2008–2009. All the samples were assayed by SnM-PCR to confirm the presence of the parasite and specie. Fifteen *P. falciparum* isolates were completely adapted to culture and 10 were exposed to different concentrations of drugs; CQ (940–29.3 nM), QN (924–14.4 nM), MQ (197–6.17 nM) and AS (52.7–0.82 nM). Positive controls; FCR3 (CQ resistant) and Dd2 (MQ resistant) were used to guaranty the assay performance. Growth curves were obtained and the concentration of drug required to inhibit growth by 50% (IC50) was determined graphically by plotting concentration vs. percentage inhibition. All samples were CQ and QN sensitive, the mean IC50 obtained with CQ and QN were 62.8 and 118.9 nM, respectively. For MQ and AS, the IC50 was 48.8 and 18.6 nM, respectively. The number of resistant isolates for MQ and AS were (2/10) and (1/10), respectively. This report shows that the susceptibility to CQ and QN is lower than in 2000 and raises alerts for MQ and AS resistance. These changes can be interpreted in light of the drug pressure exerted on the parasites in the Peruvian Amazon.

KEYWORDS *Plasmodium falciparum*, antimalarials, drug resistance, Peruvian Amazon

1.1-123

Efficacy of fixed-dose combination artesunate–amodiaquine vs. arthemether–lumefantrine for uncomplicated *Plasmodium falciparum* malaria in children under five: a randomised non inferiority trial in democratic Republic of Congo

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Until now, only a limited number of studies have been published in Central Africa measuring the efficacy of artemisinin combination therapies (ACTs) since their introduction. The Democratic Republic of Congo (DRC), one of the largest countries in the region, adopted artesunate and amodiaquine (ASAQ) as first line antimalarial treatment in 2005. We conducted a randomised open-label non-inferiority trial, enrolling children aged 6–59 months with uncomplicated *P. falciparum* malaria in Pweto district, Katanga province. Patients were randomly allocated into one of the two regimens, fixed-dose formulation ASAQ or artemether–lumefantrine (AL). We analyzed the risk of recurrent parasitemia by day 42 adjusted by PCR genotyping, expressed as estimates of failure from survival analysis and as simple proportions (per protocol analysis). Of 1993 children who were referred to the study clinic between April 2008 and March 2009, we enrolled 301 children: 156 with ASAQ and 145 with AL. The proportion of patients with parasitemia was low in both groups at D2 and D3: 6.0% (9/150) in the ASAQ arm and 4.9% (7/143) in the AL arm; and 0.6% (1/150) and 0.7% (1/143) respectively. After PCR correction, cure rates were 98.3% (95% CI, 94.1–99.8) in the ASAQ group and 99.1% (95% CI, 94.9–99.9) in the AL group (difference −0.7%, one sided 95% CI −3.1). Kaplan–Meier PCR-adjusted cure rates were similar: ASAQ, 98.4% (95% CI, 93.8–99.6) vs. AL, 99.2% (95% CI, 94.3–99.9). Both treatment

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regimens were well tolerated. The results show that ASAQ was not inferior to AL and that both ACTs were highly effective as first-line malaria treatment in this area. The logistical constraints of a remote site and the slow recruitment of confirmed cases were among the main challenges and increased substantially the cost of the study. The recommended therapeutic efficacy surveys throughout the territory at repeated intervals are difficult to achieve considering the logistical challenges and the limited technical capacity in a country like DRC.

1.1-124

Impact of malaria in pregnancy on gestational age, birth weight and infant growth: a cohort study in Uganda

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INTRODUCTION With up to 25% of the pregnant women in malaria-endemic areas in sub-Saharan Africa infected with malaria, malaria in pregnancy (MiP) is an important problem. MIP affects birth outcomes through placental infection and results in reduced birth weight. However, other factors as maternal anemia and fever may also be involved and their role as well as the exact effects of the timing, frequency and intensity of MIP need to be clarified. Besides, whereas the consequences of MIP on birth outcomes have been extensively studied, little is known on subsequent impact on infant growth.

METHODS AND MATERIAL Between October 2006 and May 2009, 1218 pregnant women were enrolled in a prospective cohort. After an initial assessment, they were weekly screened for malaria. At delivery, blood smears were obtained from the mother, the placenta, cord and from the newborn. Infants were followed monthly during their first year.

RESULTS Overall 402 malaria infections were observed resulting in an incidence rate of 1.99/100 women-weeks (1.81–2.20) that decreased linearly over time ($P < 0.01$). MIP was positively associated with higher age, HIV infection, lower education level and or rural residence area, whereas placental infection was increased in mothers with more frequent or with infection occurring late during the pregnancy. Weight and height at birth were positively associated with gestational age and parity and negatively associated with a rural residence area. Children of mothers exposed to MIP had lower weight at birth [−0.13 (−0.29; −0.04)] but similar relative change in their weight.

CONCLUSIONS In this study, a systematic screening of malaria, with active follow-up and effective treatment resulted in a steep decline of the malaria risk during pregnancy. However, placental infections and impaired weight at birth in children from infected mothers were observed. There was no evidence for subsequent catch up of this initial impairment.

1.1-125

Relationship between larval density fluctuations of *Anopheles albimanus* and malaria cases in Petit Goave, Haiti

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INTRODUCTION Malaria is considered a major public health problem in Haiti, mainly in areas of flat land and rice crops.

OBJECTIVE To know the relationship between larval density fluctuations of the mainly vector *Anopheles albimanus* and the number of malaria cases in Petit Goave.

METHODS The work was carried out in the town of Petit Goave, where larval surveys were conducted in rice fields the principal breeding sites in the locality from May 2010 to January 2011. Data from suspected malaria cases and diagnosed by thick smear were obtained from the Notre Dame Hospital laboratory located in this commune.

RESULTS The larval density values of *Anopheles albimanus* remained high throughout the studied period, the higher occurred during August, September and January. The total of suspected cases of malaria was 1910 and of these 272 were confirmed by thick smear for a 15% real positive rate with a notable increase in positivity in the number of suspected cases from September to January. In July, the hospital did not collect statistical information on malaria cases.

CONCLUSIONS The number of malaria cases confirmed by blood smear grew in the last quarter of the year after the rainy period favored the presence of the vector in high densities. Parasitological diagnosis of the disease should be promoted to gain more reliable data on the prevalece of malaria in this community.

1.1-126

Refractoriness of apoptotic red blood cells to *Plasmodium falciparum* infection: a putative host defense mechanism limiting parasitaemia

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Recently we have described that apoptosis of red blood cells (RBC) in malaria is not restricted to parasitized cells, occurring also in non-parasitized RBC (nRBC). An addition to pathogenic proprieties, apoptosis also participates in the innate defense through restriction of intracellular pathogens propagation. We investigated the capacity of *P. falciparum* parasites to infect apoptotic RBC. Schizont parasitized RBC concentrated by magnetic separation were cultured with apoptotic RBC obtained by ionomycin treatment and, then, parasite growth was evaluated in Giemsa-stained thin blood smears. While parasites infected and developed normally in control non-apoptotic RBC, cultures performed with apoptotic RBC had a marked decrease in parasitaemia. A great number of free merozoites were observed in apoptotic RBC cultures, indicating that these cells were not susceptible to invasion. We conclude that although RBC apoptosis could be involved in malaria pathogenesis, it could also acting protectively by controlling parasite propagation.

1.1-127

Declining incidence of imported malaria in a reference hospital in Madrid 1999–2010

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INTRODUCTION To evaluate the trends of imported malaria in a reference hospital in Madrid with a high proportion of immigration.

METHODS We collected all cases of malaria diagnosed 1999 through 2010 in our hospital, based on microbiological register. Clinical and epidemiological data were registered. The hospital is a reference center, covers a defined population (250,000 inhabitants) and all microbiological studies are carried in our laboratory. Interior Ministry Statistics were used to estimate immigrant population in Madrid by year.

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RESULTS A total of 136 cases were included. Female 49%, mean age 28.3 (15.3) years. On average, 99% were acquired in sub-Saharian Africa and 88.6% by *Plasmodium falciparum*. All cases lived in Spain and were visiting friends and relatives. Only 12% of patients used appropriated chemoprophylaxis. The incidence of imported malaria evolved from 63, 67, 73, 37, 39 cases per million of inhabitants from 99 through 2003 and declined progressively to 15, 27, 17, 18, 8, 10 and 7 cases for million of inhabitants from 2004 through 2010. Number of registered immigrants increased during the period of the study.

CONCLUSION Importation of malaria in Madrid is declining in spite of an increase of the immigrant population from endemic countries.

1.1-128

Urban malaria scheme: success story of urban malaria control in Surat City, India

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BACKGROUND An understanding of the malaria prevalence based upon recognized ecotypes facilitates the understanding of malaria risks and also helps to identify the tools that are required to meet the local situation, which is dynamic rather than static. Well planned integrated vector management (IVM) can contribute significantly to the reduction of malaria burden, parasite reservoir and sustained reduction in vectorial capacity.

METHODS In Surat, malaria is a major public health problem. This study is a retrospective analysis of the disease burden, species prevalence, restraints and impact assessment of IVM in urban areas of Surat City. Since, 1988 Surat Municipal Corporation (SMC) has established its own urban malaria scheme to combat malaria. SMC is divided in 400 segments, each section has one Primary Health Worker (PHW) for approximately 10,000 population. Fortnightly house to house visits take place for active surveillance, along with intradomestic surveys of mosquitogenic conditions and follow up for previous positive cases. Passive surveillance activities are carried out by 38 Urban Health Centres (UHC) in conjunction with two major hospital working.

FINDINGS With its climate, fast urbanization, vast population growth (1.4–4.5 million) and massive construction projects conditions in Surat City are conducive to the spread of disease. However, between 1989 and 2008, the incidence of malaria dropped dramatically by 81.16% (53,838/10,141) and the last 5 year median (2004–2008), showed (3247/2071) a 36.22% reduction in malaria incidence. The number of *Plasmodium falciparum* cases dropped by (1994/1015) 49.09%. In 2008 regular weekly intradomestic and peridomestic surveillance was carried out. Two lakh fifty-four thousand nine hundred and seventy-nine vulnerable house holds were visited and 557,929 wet container inspected, of which 2932 were breeding spots positive for larvae: 1003 (34.21%) held *Anopheles*, 954 (32.54%) *Aedes*, 587 (20.02%) *Culex* and 388 (13.23%) contained a mix. Along with this activities on source reduction, administrative expenditures were recovered from defaulters who created mosquitogenic conditions on their premises. Between 1989 and 2008 the mean highest *Anopheles stephensi* per man hour density was 1.56 (PMD), observed in July; between 2004 and 2008 it had fallen to 0.74 PMD.

CONCLUSIONS Against this apparently gloomy background, the most pressing challenge is to strengthen management by building up a cadre of technical and human resources, a policy framework and operational capacity of the health system, to exploit the power of IVM to prevent disease transmission, to extend population coverage and achieve a greater reduction in malarial transmission.

KEYWORDS prevalence, transmission, vector, malaria and factors

1.1-129

Optimization of a high-throughput assay to assess antibody-mediated inhibition of *P. falciparum* binding to CSA

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INTRODUCTION Accumulation of *P. falciparum* infected erythrocytes (IEs) in the placenta is thought to contribute to poor delivery outcomes. This phenomenon is mediated by specific interaction between chondroitin sulphate A (CSA) and PfEMP1 proteins expressed on the surface of IEs. The ability of human plasmas to block cytoadhesion may be used as a surrogate of protection against adverse effects of malaria in pregnancy. The optimization of an antibody-mediated inhibition high-throughput assay will be very useful for immunoepidemiological studies.

METHODS AND MATERIALS Decorin, a proteoglycan with one chondroitin or dermatan sulfate glycosaminoglycan chain, was coupled to APC-like fluorescent beads and incubated with ethidium bromide (EtBr) labelled-IEs. Adhesion of IEs to the decorin-coupled beads was analyzed by flow cytometry (FCM) and fluorescence microscopy (FM).

RESULTS FM preliminary Results showed the presence of IE-bead pairs. Furthermore, a double positive (EtBr+ APC+) population was detected by FCM. Experiments to confirm the ability of plasmas from malaria-exposed individuals to inhibit the adhesion of IEs to decorin-coated beads are in process.

CONCLUSION Our results suggest that a high-throughput functional assay based on flow cytometry may be used to quantify antibodies able to block adhesion of IEs to CSA.

1.1-130

Malaria prevalence, morbidity, and drug resistance in Nouakchott, Mauritania

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INTRODUCTION Malaria is becoming a serious public health problem in Mauritania, but limited epidemiological data exist, especially in Nouakchott, the capital city of Mauritania. The objectives were to assess the prevalence and morbidity of clinical malaria among children born and residing in Nouakchott, analyse the current drug resistance status, and determine the *Anopheles* sp. involved in transmission.

MATERIALS AND METHODS Febrile children consulting at three health facilities of Nouakchott in 2009–2010 were enrolled. Laboratory diagnosis was based on microscopic examination of Giemsa-stained blood films and rapid diagnostic test, confirmed by nested PCR. For each *P. vivax* isolate, molecular markers (pvdhfr, pvdhps, and pvmdr1) were sequenced, and the number of pvmdr1 gene copy was determined. Mosquito species were identified by morphological criteria and molecular methods.

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RESULTS Of 301 febrile children, 105 (35%) were malaria-positive by PCR and 87 (29%) by microscopy. *Plasmodium vivax* comprised 97% and *Plasmodium falciparum* 3% of positive cases. Fifty-four of 105 (51%) malaria cases, all with *P. vivax*, had never travelled outside Nouakchott. Individuals belonging to the Moors ethnic group represented 97% of *P. vivax* cases. The majority of the isolates were characterized to be of wild-type pvdhfr haplotype, while the remaining 10 isolates carried double mutations. All isolates had the wild-type pvdhps haplotype. For pvmdr1, 73% were wild-type, and 27% carried the mutant haplotype. The majority (97%) had a single pvmdr1 copy; three isolates carried 2 pvmdr1 gene copies. Anopheles arabiensis was the only anopheles species found in Nouakchott, and 1.6% of the collected mosquitos were infected with *P. vivax*.

CONCLUSIONS The present study demonstrates that malaria is endemic in Nouakchott and *P. vivax* is the principal causative agent. The prevalence of antifolate-resistant *P. vivax* is low, but further *in vitro* and/or *in vivo* studies are necessary to confirm these findings.

1.1-131

A survey on malaria and its vectors in Semnan county, north of Iran

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INTRODUCTION Determination of the age of vector mosquitoes is of particular importance in epidemiological studies of diseases transmitted by them, such as viral and parasitic diseases. The objective of this study was to determine the daily age of *Anopheles stephensi* based on changes in pteridine concentration in female mosquito cuticles by liquid chromatography (HPLC).

METHODS AND MATERIALS Females of *Anopheles stephensi* were raised in an insectary (28°C, 70% relative humidity). At 1, 5, 10, 15, 25, 30 days post-emergence they were divided into groups of 10 mosquitoes each. The mosquitoes in each age group were further divided into three subgroups of 10 each for chromatographic (HPLC, emotion = 355 nm and excitation = 465 nm) pteridine extraction. The chromatograms obtained were compared with respective standards to determine the types of pteridines.

RESULTS AND CONCLUSION Four types of pteridines were detected in the cuticle of *Anopheles stephensi*, including isoxanthopteridine, pteridine-6-carboxylic acid, biopteridine, and xanthopteridine. They were all present in all the cuticle of the mosquitoes; however, no biopteridine in the head or xanthopteridine in the thorax were found. Generally, as the age of the mosquitoes increased, pteridine concentrations kept declining, such that after 30 days the total concentration reached 10% of the original. The findings indicate that there is a negative correlation between the concentration of pteridines in the cuticle and daily age of female mosquitoes. The method described can be used as a standard method to determine the daily age of *Anopheles*, as well as of other mosquito species, since it is fast and precise and needs small samples. Its major limitation is non-availability of HPLC in many parts of the country, although it is possible to freeze dead mosquitoes and transfer them to centers where HPLC is available.

1.1-132

Malaria and malnutrition: risk factors of anemia in young children hospitalized in rural hospital in Kivu Province, DR Congo

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BACKGROUND Anemia remains a public health problem in developing countries primarily in children, associated with increased morbidity and mortality. It can affect long term physical and intellectual development. The aim of this study was to assess association between: (i) anemia and malnutrition, (ii) anemia and malaria in children hospitalized in rural hospital in Kivu.

METHODS Data of children aged 6–59 months at admission at Lwiro hospital from November 2005 to October 2008 were retrospectively analyzed. Anemia was defined as hematocrit <33%, severe anemia hematocrit <21%, malaria as positive blood smear for *Plasmodium*, malnutrition as Z-score weight for height or weight for age or height for age less than −2 or bilateral pitting edema. Statistical analysis included Chi-square and odds ratio.

RESULTS At admission 46.2% (N = 2015) of children were anemic including 9.6% with severe anemia. Of the well nourished children 52% had malaria, whereas 30% of the malnourished [OR = 2.5(2–3), P < 0.0001] did. Anemia was more common in children with malaria (70.5%) than others (36.3%) [OR = 4.2(3.4–5.2), P < 0.0001]. In stratified analysis, malnutrition was also significantly associated to anemia in patients without malaria [OR = 1.8(1.2–2.7), P < 0.01].

CONCLUSION Malaria and malnutrition remain important anemia contributing factors in children in Kivu. A joint fight against malaria and malnutrition would lead to better management of anemia.

1.1-133

Early evidences of chloroquine (CQ) sensitivity to *Plasmodium falciparum*in in Gezira state, Central Sudan 2007

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BACKGROUND Confirmed cases of CQ resistance in Sudan were first reported in 1978. In 2004 Sudan change its malaria treatment policy from CQ to ACTs due to highly evidences of chloroquine resistance.

OBJECTIVE To study the level of CQ resistance of *Plasmodium falciparum* parasite in Central Sudan after 3 years withdrawal from malaria treatment policy, using *in vitro* test and molecular markers.

MATERIAL AND METHODS This study was carried out at three health centers in Wad Medani in November to December 2007, a standard WHO *in vitro* micro test was performed on 70 *P. falciparum* isolates and 100 blood spots of infected patients with falciparum malaria were tested for molecular markers.

RESULTS 25/45 (55.6%) were *in vitro* sensitive to CQ, 17/45 (38.8%) showed low resistance and 3/45 (6.6%) were markedly *in vitro* resistant to CQ. The geometric mean cut-off concentration of schizont maturation was 1492.5 nM. DNA was extracted from

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100 samples, and investigated for the prevalence of the targeted mutation, *Plasmodium falciparum* CQ resistance transporter (Pfcrt) and *Plasmodium falciparum* multi-drug resistance gene 1 (Pfmdr1)' Screening for Pfcrt by real time –PCR revealed that 63/100(63%) carried mutant allele K76T, 37/100(37%) having the wild type. Screening for the mutation of Pfmdr 1 revealed that 46/100 (46%) carried mutant allele Y86N, 49/100(49%) had the wild type and 5/100 (5%) had mixed alleles Pfmdr 1 (wild and mutant). The frequency of the mutations among those sensitive to CQ by *in vitro* micro-test was 55.6%, 14/45 have no mutant genes and 13/14 (92%) were *in vitro* CQ sensitive and 1/14 (8%) was *in vitro* CQ resistant.

Table 1. Mean values of schizonts maturation inhibition (SMI%) among 45 *P. falciparum* isolates.

SMI%	Drug concentration (pmol)
34.92	1
72.20	2
94.74	4
99.35	8
99.96	16
99.99	32
99.99	64

DISCUSSION AND CONCLUSION The low prevalence of an *in vitro* CQ resistance revealed by this study and high prevalence showed by the previous studies conducted in the study area indicate that the *in vitro* CQ resistance level in Central Sudan decreased significantly from 69% in 1999–2000 to 6.6% in 2008. This low frequency of CQ resistance in our study can possibly be attributed to the fact that CQ was withdrawn from Sudan's malaria treatment policy for 3 years, which may have resulted in the recovery of genetic mutations and return of CQ efficacy.

1.1-134

P. falciparum quantitative PCR in a mozambican laboratory to monitor changes in malaria transmission

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BACKGROUND Light microscopy remains the standard method for diagnosing malaria in Africa. Although PCR-based molecular techniques have advantages for epidemiological studies (higher sensibility, objectivity, high-throughput applicability, possibility of quantification), these are still in discussion because of the difficulties in their implementation and sustainability in laboratories from rural areas of malaria-endemic countries. Transfer of PCR-based methodologies are needed to monitor changes in malaria transmission in countries were malaria is endemic.

METHODS A real time PCR (QRT) to detect *P. falciparum* infection was developed in CRESIB and transferred to the laboratory in the Centro de Investigação em Saude de Manhiça (CISM). This PCR is based in the amplification of 18S ribosomal RNA gene using Taqman probe, and the standard curve is prepared with known numbers of ring-stage *P. falciparum* 3D7 parasites from culture. The implementation of QRT was done in the context of a cross-sectional study carried in February 2011 with the objective of monitoring the burden of malaria and impact of different malaria control tools in Manhiça (Mozambique) between 2010 and 2011.

RESULTS QRT was applied in different studies to adults, pregnant women and infants from Manhiça, showing high sensibility and reproducibility. The QRT revealed a high prevalence of sub-microscopic infections. QRT was implemented in CISM in January 2011 and is at present successfully performed by the Mozambican laboratory technicians. Preliminary data shows a *P. falciparum* prevalence of 11% in 2010 suggesting a reduction in the malaria transmission compared to previous years.

CONCLUSIONS A QRT method for the detection of *P. falciparum* has been successfully standardized and transferred to a research centre located in a rural area of Mozambique. The technique is currently being used to determine changes in the intensity of malaria transmission.

1.1-135

Pharmacokinetic drug-drug interaction analysis of pyramax® and ritonavir in healthy volunteers

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Pyramax® is a pyronaridine/artesunate fixed dose combination (PA) tablet developed for the treatment of uncomplicated malaria in adult and pediatric patients. Ritonavir is a known inhibitor and substrate of CYP3A4, CYP2D6, and P glycoprotein (Pgp), as well as an inducer of CYP3A4, CYP1A2 and the UDP glucuronosyltransferases (UGTs). Since CYP3A4, CYP2D6, and CYP1A2 have been shown to be responsible for pyronaridine metabolism and UGTs are known to be responsible for DHA metabolism, there is a potential of a drug interaction between ritonavir and Pyramax®. In a multiple dose, parallel group study, 34 healthy adults randomized (1:1) to Arm A (PA + ritonavir) or Arm B (PA only). Subjects in both arms received PA orally once daily for 3 days. The daily dose of Pyramax® was three or four tablets (60 mg artesunate (AS)/180 mg pyronaridine tetraphosphate (PP) per tablet), depending on subject weight. Subjects in Arm A were administered one 100 mg dose of ritonavir orally on day 1 followed by 100 mg twice daily for 16 subsequent days; PA was administered once daily on days 8–10. Noncompartmental pharmacokinetic parameters for PP, AS, and its active metabolite dihydroartemisinin (DHA) were obtained following the last dose of PA. For ritonavir, parameters were obtained from the first and tenth days of ritonavir dosing. Ritonavir did not produce appreciable changes in PP pharmacokinetics; however, coadministration resulted in an increase of 27% in artesunate exposure and a decrease of 38% in DHA exposure. The decrease in DHA exposure was likely due to ritonavir induction of DHA metabolism by UGTs. Ritonavir exposure increased 3.2-fold more than expected as evaluated by the ratio between the AUC0-12hr on Day 10 (during coadministration with PA) and the AUC0-inf on Day 1 (no PA); this increase likely resulted from PP inhibition of P-gp mediated ritonavir efflux.

1.1-136

Antigen persistence of rapid diagnostic tests and its implications for the diagnosis of malaria in pregnancy. An evaluation in Nanoro, Burkina Faso

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INTRODUCTION Diagnosis of malaria during pregnancy is often complicated by the absence of parasites in peripheral blood, due to sequestration in the placenta. There is a need for different manners

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of diagnosis, such as antigen or DNA detection. HRPII Rapid diagnostic tests (RDTs) are less expensive, more stable and have a lower detection threshold than pLDH tests, they detect only *P. falciparum*, however, and the antigen persists in the blood after parasite death. The aim of this study was to evaluate RDTs for antigen persistence in pregnant women after malaria treatment. In addition, persistence of new antigen targets was investigated, to determine their potential use in malaria diagnosis.

METHODS AND MATERIALS Thirty-five pregnant women with malaria were followed 28 days after treatment. At days 0, 1, 2, 3, 7, 14 and 28, a blood sample was collected. Two RDTs were used: SD Bioline Malaria Antigen P.f (HRPII) and (ADVANTAGE MAL CARD, Pf and PAN (pLDH). Thick and thin smears were prepared and blood is spotted on filter paper for RT-PCR. The persistence of the antigens Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) and Heme Detoxification Protein (HDP) in the samples will be tested in lateral flow or ELISA format.

RESULTS Preliminary Results show that on average, microscopy was negative in 1.2 days, the pLDH RDT in 0.9 days, and the HRPII test in 7.5 days. RT-PCR and extensive analysis are in progress, but so far, the average time to become negative for PCR is 2 days.

CONCLUSIONS HRPII RDTs stay positive much longer after treatment than other tests, due to persistence of antigen. When using this test in the diagnosis of malaria in pregnant women, this issue should be considered, especially if the patient has recently received IPTp. There is a need for RDTs with a better detection threshold than pLDH RDTs, but fewer problems with antigen persistence.

1.1-137

Evaluation of the accuracy of novel antigen detecting tests compared to RDT, microscopy, PCR and histology for the diagnosis of placental malaria in Nanoro, Burkina Faso

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INTRODUCTION During pregnancy, infection of the placenta with *Plasmodium falciparum* is related to poor birth outcome and it adversely affects maternal health. Diagnosis is often complicated by the absence of parasites in peripheral blood, due to sequestration of parasites in the placenta. Rapid diagnostic tests (RDTs) are considered an alternative but are not systematically researched for their potential to detect placental malaria. Therefore different commercial RDTs and newly developed prototype antigen detecting tests were tested for their diagnostic accuracy under field conditions.

METHODS AND MATERIALS Two commercial available tests based on pLDH and HRP-II were compared to ELISA and RDT format tests based on monoclonal antibodies against new target antigens at the district hospital of Nanoro, Burkina Faso. Four hundred and fifty pregnant women are screened for malaria by RDT, SD Bioline Malaria Antigen P.f. (HRPII), ADVANTAGE MAL CARD, Pf and PAN (pLDH) and the new tests. Blood smears are prepared for microscopy and blood is spotted on filter paper for RT-PCR. Additionally, of approximately 150 of these women at delivery, peripheral and placental blood is collected for all tests and a histological slide is prepared.

RESULTS AND CONCLUSIONS From November 2010 till May 2011, 382 women were recruited; of which 121 are SD bioline positive. Patient collection is still ongoing. Preliminary results show a difference outcome between the two RDTs. If the HRPII test is considered as reference test, sensitivity of the pLDH test is 51% (95% CI 40–61%) and specificity 97% (95% CI 92–99%). Microscopy, PCR and prototype testing are in progress.

1.1-138

Imported malaria in a regional hospital in Catalonia: our experience 1995–2010

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INTRODUCTION Malaria continues to plague the developing world and is particularly prominent in areas with limited resources . We have had an important resurgence in cases in our non-endemic area due to a major increase in travel to and emigration from countries where malaria is endemic.

MATERIAL AND METHODS We carried out a retrospective study from a historic cohort of patients diagnosed of malaria between January1995 and December2010. The diagnosis was confirmed by our laboratory database and by the attending physician. We collected clinical, demographic and epidemiological information, reasons for the visit to the endemic area, details of place and time of stay, time of consultation, clinical data, previous prophylaxis and treatment taken.

RESULTS One hundred and eight cases of malaria were reported: 97 were immigrants, 83% from sub-Saharan Africa, 4.6% from Latin America; and 11 were travellers. The study population was young (mean age 26 years) sub-Saharan males travelling to West Africa to visit friends and relatives without antimalarial prophylaxis and pre-travel advice. Fifteen patients were children (all of them immigrant 3–13 years old). The average number of cases/year was 5, and increasing in the last 5 years (minimum in 1998 (one case); with the maximum in 2006 (16 cases) according to migration patterns in our area. Autumn (43%) was the season where most of the cases were diagnosed. Test available for diagnosis were thin blood smears in all patients, rapid antigen test for children and travellers and PCR for identification of malaria species in two cases. Malaria was caused by *Plasmodium falciparum* in 93 cases (86%), *P. vivax* in 10 cases, *P. malariae* in one case and *P. ovale* in one case. Parasitemia was low in 41% of patients, with hyperparasitemia >5% in eight patients. Fever, chills, abdominal pain, headache and myalgias were the clinical presentation, vomiting and diarrhoea in children. All cases came from emergency unit and 48% needed hospitalization. One patient required mechanical ventilation for cerebral malaria with good clinical outcome. Oral and intravenous quinine and doxicycline were the treatment for falciparum malaria; and chloroquine + primaquine for non-falciparum. Those who received primaquine had a negative G6PD test. No resistant case was detected.

CONCLUSIONS Malaria in our area is common in immigrants from sub-Saharan Africa, who are VFR, increasingly in last 5 years. *Plasmodium falciparum* malaria with low parasitaemia is the most common presentation.

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1.1-139

Open-label *in vivo* drug study to evaluate the safety and efficacy of artesunate plus amodiaquine combination in pregnant women with uncomplicated *P. falciparum* malaria in Senegal

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BACKGROUND Malaria in pregnancy remains a major cause of morbidity and mortality in Africa. Artesunate + Amodiaquine (ASAQ) is currently recommended by the Ministry of Health of Senegal as first-line treatment of uncomplicated malaria. WHO has recommended now the ACTs in pregnant women but not yet implemented in West Africa. This study aimed to assess the efficacy, tolerability, and safety of AS + AQ in the second and third trimesters of pregnancy.

METHODS AND MATERIALS An open label trial with one treatment arm was conducted in the Guediawaye health center. Pregnant women in their second and third trimester with confirmed malaria were assessed for enrollment; recruited patients were treated with ASAQ for 3 days. 28 pregnant women were included in the study and followed up until 15 days after delivery. Biological safety was assessed at D0, D7, D28 and D 42 with haematological and biochemistry parameters. Infants were assessed at birth and followed up 9 months.

RESULTS ASAQ was very effective; the parasite geometric means were 9.91 ± 1.24 at day 0, 8.76 ± 1.56 at 8 h after the first intake. At day 1 the mean parasitaemia was 5.23 with ±1.61, at 12 h after the second dose it was 3.43 ± 0.41. At day 2 only one woman presented a positive blood smear and from day 3 to day 42 no subject was found with malaria parasite. No infant presented either a positive blood smear at birth and during follow up or clinical abnormalities No serious adverse events related to the drug were noted. ASAQ was found safe and well tolerated.

CONCLUSION This study confirms the safety, efficacy and tolerability of ASAQ in Senegalese pregnant women with uncomplicated *P. falciparum* malaria in their second and third trimester. However further studies are needed to monitor its long term safety in pregnant women and their bornchilds.

1.1-140

Effectiveness of malaria rapid diagnostic tests in fever patients attending primary health care facilities in the context of low malaria transmission in Zanzibar

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INTRODUCTION Zanzibar has recently undergone a rapid transition from high to low malaria transmission . In the new epidemiological context use of effective tools for confirmatory malaria case detection is critical. Zanzibar introduced *Plasmodium falciparum* specific Rapid diagnostic Tests (RDTs) based on histidine rich protein 2 in 2005. The coverage of RDT now includes almost all public health facilities. This study aimed to evaluate the effectiveness of RDT among health workers in the new epidemiological context in Zanzibar against standard blood slide (BS) microscopy and sensitive PCR for malaria case detection in fever patients.

METHODS The study was conducted during May–August 2010 at 12 public health facilities, six each in North A and Micheweni districts. Prior to study start all health workers were trained in standard malaria treatment guidelines including performance and interpretation of RDT. Three thousand eight hundred and ninety-three patients were enrolled with fever or history of fever during the preceding 24 h. All patients were tested with RDT. Finger prick blood samples were collected for microscopy and PCR from all RDT positive cases as well as from 20% randomly selected RDT negative patients. Consequently, the BS and PCR results retrieved from the RDT negative sub-sample were multiplied with a factor of five for sensitivity and specificity calculations.

RESULTS RDT positivity rate was 122/3893 (3.1%), of whom 34/122 (27.9%) were <5 years of age. Sensitivity and specificity of RDT vs. first BS reading was 105/125 (84.0%) and 3745/3762 (99.6%), respectively. Sensitivity and specificity of RDT vs. PCR was 113/143 (79.0%) and 3735/3744 (99.8%), respectively.

CONCLUSION Effectiveness of RDT among health workers in Zanzibar against first BS reading and PCR was relatively low. This highlights the need for improved quality control of RDT use in primary health facilities, but also the potential need for more sensitive point-of-care malaria diagnostic tools in this new epidemiological context.

1.1-141

Epidemiological effectiveness of insecticide treated nets in the presence of pyrethroid resistance

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INTRODUCTION Insecticide treated nets (ITNs) have become the primary intervention against malaria vectors, partially because the insecticide, typically a pyrethroid, renders them effective even if holed. However, experimental hut studies have shown that holed ITNs killed a much lower proportion of host seeking mosquitoes in areas with high pyrethroid resistance in the vector population compared to areas with susceptible vector populations. The epidemiological implications of this were examined by modeling.

METHODS AND MATERIALS An established computer model was extended to allow detailed modeling of ITN effects. Over a range of transmission settings, new ITNs were mass distributed. ITNs decayed with time both through hole formation and loss of insecticide. The capacity of mosquitoes to withstand ITN effects, depending on the physical and chemical status of the net, was parameterized using experimental hut data.

RESULTS AND CONCLUSIONS ITNs protect users by reducing the number of bites, and reduce malaria transmission by reducing the mosquito survival per gonotrophic cycle, both by reducing the availability of the hosts, increasing the length of the host seeking process and associated mortality, and by direct killing as a result of contact with the insecticide during host seeking. For intact ITNs, protection against resistant and susceptible mosquitoes is similar. Also, the host seeking process is similarly extended, and the pre-prandial killing is similar. With post-prandial killing, negligible with intact nets, there is a difference. With holed ITNs, personal protection, but particularly transmission reduction, is thwarted with resistant mosquitoes. Whereas holed ITNs deter susceptible mosquitoes almost as well as intact ITNs, they deter fewer resistant mosquitoes. Holed ITNs show a large difference in both pre-prandial and post-prandial killing depending on the susceptibility of the vector mosquitoes.

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1.1-142

Molecular genotypes and clearance of different *P. falciparum* sub populations in children treated with ACT

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Plasmodium falciparum has throughout history proven its capacity to develop resistance to virtually all previously deployed antimalarial drugs. The global strategy for malaria control/elimination relies on sustained high efficacy of artemisinin-based combination therapies (ACT). Worryingly, delayed parasite clearance, a characteristic of artemisinin resistance, has been observed in SE Asia. We have analysed parasite clearance in an exploratory study on 50 children with uncomplicated malaria treated with ACT in 2006 in mainland Tanzania. We tried to relate clearance of individual parasite sub-populations of different genotypic markers of antimalarial resistance. The children were treated with artemether–lumefantrine (AL) standard doses according to body weight (www.Clinical.Trials.Gov, I.d. NCT00336375). Blood samples were collected on slides and filter paper at time of diagnosis (−2 h) and 0, 2, 4, 8, 16, 24, 36, 48, 60 and 72 h after initiation of treatment. Artemether, dihydroartemisinin and lumefantrine blood concentrations were determined and RNA and DNA were extracted for each time point. All samples were genotyped for pfmdr1 N86Y, pfcrt K76T SNPs and investigated for pfmdr1 gene copy number variation. Our preliminary results from the genotyping of pfmdr1 N86Y and pfcrt K76T revealed an unexpectedly high dynamic/fluctuation over time; no copy number variation within the pfmdr1 was found. Associations between parasite clearance and specific molecular and clinical characteristics will be further investigated and an update presented.

1.1-143

Design of antimalarial drugs with immunoprotective activity: the Borrelidin model

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Borrelidin is a natural polyketide that selectively inhibits threonyl-tRNA synthetase activity and caspase-3 and caspase-8 activation. Borrelidin has a wide range of biological activities including antibacterial, antimitotic, antimicrobial, antiviral, anti-angiogenic and antitumoral. We tested the antimalarial effect of Borrelidin through *in vitro* and *in vivo* assays. *In vitro* studies have revealed an excellent antimalarial activity with an IC50 value in pM range in the drug-resistant *Plasmodium falciparum* Dd2 strain. *In vivo* 4-days suppressive test with lethal *Plasmodium yoelii* 17XL (PyL) in BALB/c mice showed a strong parasitostatic action of borrelidin on parasite growth. Parasitostatic effect was reverted on treatment withdrawal and then, parasitemia values increased in the following days up to 50–60% before total clearance. Borrelidin-treated mice developed a sterilizing immune response against subsequent re-infections with the same parasite challenge at 1 and 8 months after first infection. Sera analysis of specific antibodies against PyL in the cured mouse showed a significant increase in number and intensity of immunoreactive bands suggesting that the immuno-protected mice trigger a strong and long lived humoral response associated to the presence of infected erythrocytes after primary infection. These results revealed an efficient antimalarial action of borrelidin coupled to immunostimulation and points out its potential to immunize individuals against malaria after a single infection.

1.1-144

Immunoproteomic profiling of the humoral response to *Plasmodium falciparum* in imported malaria

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Antibodies (Abs) are central to malaria immunity, which is only partially acquired after years of exposure to *Plasmodium falciparum* (Pf). Despite the enormous worldwide burden of malaria, the targets of protective Abs and the basis of their inefficient acquisition are still unknown. Addressing these knowledge gaps could accelerate malaria vaccine development. We conducted an immunoproteomic study based on human Abs reactivity against squizont Pf proteins expressed during intraerytrocytic cycle. With this aim, a wild Pf strain isolated from a Guinea Equatorial patient was *in vitro* cultured in human red blood cells at high parasitemias and synchronized at squizont stage (36–48 h). Total protein extract was obtained and separated by 2D-electrophoresis. Immunoblotting was carried out using human sera collection also obtained from Equatoguinean patients and antigenic proteins were identified by mass spectrometry (MS). Our data provided new information about undescribed malaria antigens which can be potential vaccine targets against to the most lethal strain.

1.1-145

Cell biological characterization of the malaria vaccine candidate trophozoite exported protein 1

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BACKGROUND In a genome-wide screen for alpha-helical coiled coil motifs aiming at structurally defined malaria vaccine candidates we identified Trophozoite exported protein 1 (Tex1) (PFF0165c) (1). In extensive preclinical evaluation of Tex1 two long synthetic peptides were identified as promising novel malaria vaccine candidates: an alpha-helical coiled coil region and an intrinsically unstructured domain of Tex1 were recognized with high prevalence by sera from adults from endemic regions throughout the world. Human antibodies against both regions were effective in parasite killing in ADCI *in vitro* assay (2). Clinical evaluation of

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the Tex1 unstructured domain (phase 1 trial) funded by the European Vaccine Initiative (EVI) is in progress.

METHODS Antibodies generated against the intrinsically unstructured N-terminal region of Tex1 and against a coiled coil domain were used to investigate solubility, expression profile and cytological localization by IFA and co-localization studies. PCR products were sequenced to investigate the genetic diversity of Tex1 coiled coil and unstructured domains.

RESULTS The Tex1 coiled coil domain was completely conserved in culture strains and malaria positive field samples. The unstructured region showed very limited polymorphism. The PEXEL-negative Tex1 contains a predicted signal sequence at the N-terminus, is exported into the erythrocyte cytoplasm and localizes to Maurer's clefts in early trophozoite stage, whereas in schizont stage Tex1 associates to the red blood cell membrane. Change in location is accompanied by a change in solubility: from a soluble state within the parasite to a membrane-associated state after export to Maurer's clefts.

CONCLUSION Tex1 was recognized by antibodies naturally generated by infection as well as antibodies elicited against synthetic peptides. Sequence conservation of both selected domains together with demonstration of their antigenicity and immunogenicity validate our strategy for rapid antigen identification by genome-wide bioinformatics, high through-put peptide synthesis followed by stringent experimental selection.

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1.1-146

Factors influencing bed net use in children under five and household bed net ownership on Bioko island, Equatorial Guinea

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BACKGROUND As part of the strategies the Bioko Island Malaria Control Project (BIMCP) has implemented in recent years, more than 80,000 insecticide treated nets (ITN) were delivered to the population in 2007. The aim of this study is to assess the determinants of bed net usage (and bed net ownership) in children under 5.

METHODS Data were selected from households who had at least one child under 5. Outcome variables were: sleeping under a bed net/ITN the night prior to the survey and household ownership of at least one bed net. The explanatory variables were household characteristics and caregiver's knowledge of malaria. To analyse the effect of socio economic status (SES), an asset based score was created using principal component analysis.

RESULTS A total of 3210 households with 5151 children under five participated in the study. Higher bed net usage was associated with being sick at some point in the last 14 days prior to the survey, urban area, more years of education of head of households, household ownership of at least one ITN (rather than an untreated net) and the year in which the survey took place. There was a decline of around 32% in the proportion of households that owned at least one bed net in 2009 compared to 2008. Knowing how malaria is prevented and transmitted, having the house sprayed in the previous 12 months, having fewer children under five in the household and having more children sick at some point in the previous 14 days were associated with higher household bed net ownership.

CONCLUSIONS The big fall in bed net usage from 2008 to 2009 is attributable to the striking decline in ownership. Although ownership is similar in rural and urban areas, rural households are less likely to protect their children with bed nets.

1.1-147

Decreased levels of brain-derived neurotrophic factor across the neurological phenotype of cerebral malaria

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Cerebral malaria (CM) is the most serious complication of *Plasmodium falciparum* infection. Although potentially reversible, long-standing neurocognitive sequelae are often observed, particularly in African children. These sequelae seem to be multifactorial although their causes remain unknown. As neuronal damage is a key process in CM, neurotrophic factors involved in maintaining the integrity of the central nervous system (CNS), neuronal survival and synaptic plasticity could act as potential therapeutic agents. In this context, brain-derived neurotrophic factor (BDNF) expression and brain distribution have been analyzed during the progression of experimental cerebral malaria in mice. A diminished expression of mRNA-BDNF was observed in several brain regions, as the severity of symptoms progressed. Moreover, immunohistochemical assays revealed changes in BDNF distribution pattern, suggesting altered axonal transport. This data points out an important role of this mediator to confer protection during CM. Since BDNF administration has been shown to have protective effects preventing cell death and reducing the neurological signs of several CNS diseases, we suggest that BDNF could be a novel potential target as coadjuvant to antimalarial therapy in CM.

1.1-148

Decreasing efficacy of artesunate + mefloquine on the Thai-Cambodia border part II. Treatment option and the way forward

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With the widespread emergence of multidrug resistant falciparum malaria especially in Thailand, where is the most drug-resistant in the world, new drugs are urgently needed. However, it may take over 5 years to discover one new antimalarial drug. New antimalarial drugs that have been investigated at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, in Phase II and III clinical trials, including several artemisinin combination therapies (such as a pentamidine derivative, the semi-synthetic artemisone and a synthetic trioxolane, a fixed combination of artemisinin and piperaquine, a fixed combination of pyronaridine and artesunate, artemefone and mefloquine, artelolane and piperaquine), and also non-artemisinin combination therapy. Early recognition of malaria complications and their adequate treatment also play an important role as these complications (hypoglycemia, pulmonary edema, acute renal

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failure, metabolic acidosis, and convulsion) often increase the mortality rate. The aim of treating severe malaria is to save the patient's life. Prompt administration of an adequate and effective antimalarial drug is needed once the diagnosis is made. Other symptomatic and supportive treatments include the careful monitoring of fluid input and urine output, the provision of good nursing care and the avoidance of harmful adjuvant therapy. In spite of these efforts, mortality from severe malaria is still high. Moreover, the ineffective to primaquine (15 mg/kg/day for 14 days with relapse rate of 20%) is now also recognized in Thailand and high dose primaquine (30 mg/kg/day for 14 days) is recommended. Finally, new antimalarial agents active against vivax malaria were studied, including tafenoquine, bulaquine and primaquine high doses. Our Results suggest new approaches to overcoming multidrug resistant malaria.

1.1-149

Plasmodium vivax in Papua New Guinea: molecular epidemiology in a population with high endemicity of two malaria species

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Both *Plasmodium vivax* and *P. falciparum* occur at high prevalence of around 50% in the lowlands of Papua New Guinea (PNG), and mixed infections are detected in one third of all positive children. While *P. vivax* incidence in this population decreases after the first year of life, *P. falciparum* incidence peaks at 3 years of age. We collected samples over 16 months from 264 children aged 1–4.5 in PNG, genotyped individual clones of *P. vivax* and *P. falciparum* and assessed basic epidemiological parameters such as diversity and multiplicity of infection (MOI). Both *P. vivax* markers msp1F3 and MS16 showed high diversity (virtual heterozygosity 0.88 and 0.98). Seventy-five percent of all *P. vivax* positive children carried more than one genotype and mean MOI was 2.7, nearly twice as high as for *P. falciparum*. In contrast to the incidence rate, a slight increase of *P. vivax* multiplicity and prevalence was observed with age, while *P. vivax* MOI and prevalence showed little seasonal variation. Comparison of genotyping data from samples collected from the same patient 24 h apart revealed that a single sample usually is sufficient to determine positivity, but in case of complex infections carrying many clones, some of these likely remain undetected. Little difference was observed in detectability of *P. falciparum* vs. *P. vivax* clones. High MOI likely is a result of frequent relapses. High diversity indicates exchange of different parasite populations. These factors highlight the specific challenges to be encountered in the course of malaria elimination in the Pacific.

1.1-150

Therapeutic efficacy of Coartem® for the treatment of uncomplicated malaria in Maputo, Mozambique

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INTRODUCTION In Mozambique, malaria is a major public health problem, accounting for about 48% of outpatient visits, 63% of pediatric admissions and 26.7% mortality. Since 2009, uncomplicated malaria is being treated with Coartem® as first line and ASAQ (aretesunate + amodiaquine) as second line. In this country there are few data on therapeutic efficacy and the prevalence of molecular markers associated with resistance to components of the ACTs. The objective of the present work was to assess the efficacy of Coartem® in Maputo, Mozambique.

METHODS AND MATERIALS Patients aged over 1 year with symptoms suggestive of malaria illness (elevated axillary temperature 37.5°C or a history of fever within the previous 48 h, attending two health facilities in Maputo area were screened for *P. falciparum* mono-infection using thick blood film. Patients were recruited at Centro de Saúde de Boane (rural area) and 1° de Maio (city). Recruiting occurred between March 2009 and March 2011. Clinical outcome was assessed on day 7 and 14. The prevalence of the pfmdr1 SNPs, N86Y and F184Y associated to drug response were determined by PCR-RFLP.

RESULTS Two hundred and eleven patients were included in the study, 109 females and 102 males. Population was divided in three age groups <5 years of age (n = 74), 5 and <15 (n = 86) and ≥15 (n = 51). Most of the patients (60%) went to the health centre after 1 day of developing symptoms, but the time ranged from 1 to 5 days. Most of the patients (71%) referred to having treated the last malaria episode with Coartem® notably 19.4% treated the last malaria, with Fansidar plus artesunate. The prevalence of pfmdr1 SNPs was 22.6% and 60% for 86Y and 184Y respectively. There were no therapeutic failures with Coartem® in the 14 days of follow-up.

1.1-153

The roles of haemoglobin S, haemoglobin C and alpha-thalassaemia in the development of acquired humoral immunity against malaria

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INTRODUCTION Epidemiological studies provided evidence that haemoglobin S (HbS), haemoglobin C (HbC), and alpha-thalassaemia (-thal) protect against malaria phenotypes; however the mechanisms by which this protective effect is mediated are still elusive. While it has been suggested that HbS and HbC may influence the development of acquired immunity, to date no longitudinal study has analysed the influence of HbC or thal on the immuno-reactivity against *P. falciparum* malaria in infancy.

METHODS We investigated a subset of children carrying HbS, HbC, thal and normal HbA, from a longitudinal study in Ghana. Data on episodes of parasitaemia and uncomplicated malaria were collected by active and passive follow-up between age 3 and 24 months. Serum levels and prevalence of total IgG against *P. falciparum* antigens (AMA-1, MSP-119, MSP-2) were measured by ELISA at 9, 15 and 21 months in 490 children (252 HbAA, 76 HbAS, 70 HbAC and 92 thal heterozygotes). Multiple regression analysis and population-averaged models, allowing for correlation between different measurements, were used to analyse the influence of the different genotypes on antibody responses.

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RESULTS In total 1658 episodes of parasitemia (500/μl) were observed, corresponding to a rate of 1.83 per PYAR (95% CI: 1.75–1.92). Antibody titres and seroprevalence against all antigens increased rapidly with age after waning of maternal antibodies. Seroprevalence increased from 8% at age 9 months to 24% at age 21 months for AMA-1, from 20% to 39% for MSP2 and from 44% to 53% for MSP-119. The increase of titres was positively correlated to previous parasite exposure and a modulation mediated by Hb-genotype was observed.

CONCLUSION Identifying the mechanisms by which HbS, HbC and thal influence the development of acquired immunity against malaria might be a useful tool in understanding the crucial steps necessary for inducing protection.

1.1-154

Modelling malaria transmission by *Anopheles funestus* in southern Mozambique (Manhiça district)

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INTRODUCTION Environmental and social factors such as land cover, availability of breeding sites, population density, type of households, control interventions, etc., determine the distribution of malaria vectors influencing malaria transmission. By geoprocessing a combination of field data and remote sensing information, we developed high-resolution models to estimate malaria transmission in Manhiça district.

METHODS AND MATERIALS Field data collected in a longitudinal entomological study conducted during 2006–2007 in two areas from Manhiça, was used to calculate the average human biting rate (HBR) and the entomological inoculation rate (EIR) for *A. funestus* s.l., the main malaria vector in the region. Models based on regression analysis was developed using social (population density) and environmental (proximity of household to rivers, terrain slope, land cover) variables. Normalized difference vegetation index (NDVI) obtained from Landsat 7 ETM + data and distribution of control strategies [indoor residual spraying (IRS) and bednets] were also considered in the modelling process.

RESULTS In 2006, the density of children per km2, terrain slope, shrubland presence and distance to rivers, showed a negative correlation with *A. funestus* HBR, while a positive correlation was observed with the proximity of households to irrigated fields. The regression model created with these variables accurately explained 60% of the *A. funestus* HBR variance. The annual *P. falciparum* EIR estimated with this model was 41.2 (SD: 82.1) infective bites per person. In 2007, *A. funestus* HBR decreased a 92% accordingly to the predicted model, and the reduction was mainly associated with a higher IRS rate.

CONCLUSION We have established regression models to explain the variability of malaria transmission at a local scale through environmental and social factors. Reliable information on vector density and malaria transmission is essential to understand spatial variations of malaria incidence and to monitor intervention programs.

1.1-155

Evaluation of the effect of red cell genetic factors on Pfmdr1 and chloroquine resistance

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INTRODUCTION Chloroquine which used to be the first drug of choice for malaria has been rendered impotent due to resistance. We investigated the effect of red cell genetic factors, sickle cell genetic trait, ABO blood group and glucose-6-phosphate (G6PD) deficiency on chloroquine resistance and its association with Pfmdr1 mutation

METHOD One hundred and twenty patients with acute uncomplicated falciparum malaria were recruited. They were administered 25 mg/kg body weight chloroquine over 3 days and followed up for 14 days for clinical and parasitological responses. Thick and thin blood smears were made for malaria parasite screening. Filter paper samples were collected for DNA analyses. One millitre of blood was collected for determination of blood group, G6PD status, haemoglobin (Hb) genotype and G6PD status. Pfmdr1 gene was amplified by PCR to screen for Y86 mutation. MSP1 and MSP2 polymorphisms were used to differentiate between recrudescence and re-infection.

RESULT Only 53% of the patients were cured with chloroquine. Pfmdr1 Y86 was more common among patients with clinical failure than among those cured. However this was only significant among under 5 years old children. No significant association was found between Pfmdr1 alleles and ABO blood groups or G6PD status. Individuals with Hb AA were 2.3 times more prone to chloroquine resistance relative to Hb AS.

CONCLUSION Association between Pfmdr1 and chloroquine resistance was stronger among <5 year old children. G6PD deficiency and blood group had no effect on chloroquine resistance. Hb AS individuals are less prone to chloroquine resistance.

1.1-156

Effect of artesunate on disposition of orally administered amodiaquine in patients with uncomplicated malaria

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INTRODUCTION The emergence of drug resistance in *P. falciparum* has necessitated that falciparum malaria be treated with Artemisinin – based Combination Therapy (ACT). Amodiaquine (AQ) is one of the drugs used in combination with artesunate for malaria treatment. We assessed the pharmacokinetics of AQ and the effect of artesunate on its disposition in patients with malaria.

METHOD A liquid chromatographic method was developed for analysis of AQ and its metabolite, desethylamodiaquine (AQm). Twelve patients positive for malaria parasite were randomized to receive either AQ or AQ plus artesunate (AS). The doses were AQ 600 mg once daily and fixed AQ/AS daily for 3 days. Blood samples were collected before and at 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 144 and 336 h after drug intake. Plasma was separated and used to assay for AQ. The analytical method was highly sensitive and specific.

RESULTS Calibration curves were linear ($r^2 > 0.99$) in the range of 100–1000 ng/ml for AQ and AQm. The intra-assay coefficients of variation were 1.87–4.94% for AQ and 0.49–5.34% for AQm. While inter-assay coefficients of variation was 1.67–6.37% for AQ and 2.49–6.89% for AQm. The mean values of Peak Plasma Concentration, Cmax (22.7 ± 0.01 vs. 20.43 ± 0.12 ng/ml) and Area Under the Plasma Concentration-time Curve, AUC

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(59.63 ± 0.05 vs. 57.52 ± 0.24 ngh/ml) of AQ were significantly ($P < 0.05$) higher in AQ alone compared with AQ/AS group. Terminal half life, t1/2 was longer (2.83 ± 0.02 vs. 2.69 ± 0.01 h) and oral clearance, Cl/f (9729.54 ± 0.61 vs. 9857.24 ± 42.5 ml/h) was significantly lower in AQ/AS when compared to AQ group ($P < 0.05$). The mean values of peak time of plasma concentration (Tmax) of AQ in the two groups were the same at 2 h. There were however no statistically significant differences in the values of Tmax, Cmax, t1/2, Cl/f and AUC of AQm in both treatment groups ($P > 0.05$).

CONCLUSION Artesunate significantly affected the disposition of the parent drug, amodiaquine but not the metabolite, desethylamodiaquine, when orally administered in combination to patients with malaria.

1.1-157

Asymptomatic infections in blood donors harboring plasmodium detected by molecular and serological tools

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INTRODUCTION Transfusion transmitted (TTM) malaria represents a challenge to disease control, mainly due to occurrence of asymptomatic infections. In the state of Sao Paulo, Brazil, malaria is not endemic, but sporadic autochthonous asymptomatic cases are detected. Plasmodium can survive in stored red cells between 2 and 6°C for up to 3 weeks, with an estimated inoculum of 1–10 parasites per unit.

METHODS AND MATERIALS In 2007, 17 candidates for blood donation were screened based on epidemiological data and one of them reported having knowledge of malaria transmission in their living area. Thick blood smear, PCR, ELISA, IFA and SD Bioline Malaria Pf/Pv immunochromatographic test were requested. Nested PCR was performed with primers genus and species-specific based on SSU rRNA genes of *P. falciparum*, *P. vivax* and *P. malariae*. ELISA, with *P. vivax* MSP119 and *P. falciparum* Zwittergent® extracted antigens. IFA was assayed with total antigens of the three species.

RESULTS Thick blood smear showed rare parasites in two candidates. PCR was positive for *P. malariae* in one sample. ELISA detected 13 reagent samples for *P. vivax* and two for *P. falciparum* antigens. IFA was reactive in eight samples for *P. vivax* and in three for *P. malariae*. SD Bioline detected antibodies against *P. vivax* in 12 samples.

CONCLUSIONS The real risk of TTM presented here suggests the need of accurate knowledge about levels of malaria endemicity when screening blood donors and the possibility of occurrence of asymptomatic infections. All candidates were considered ineligible for donation. Tests with specific markers were efficient in the detection of asymptomatic infection and are safe alternatives for screening of donors. Financial: Superintendência de Controle de Endemias; LIM 48 HC-FMUSP; LIM 49 HC-FMUSP.

1.1-158

Regulatory T cells are not implied on exacerbation of *Plasmodium falciparum* parasites and IgG reponse plays an importan role during malaria infection in individuals living in the Peruvian Amazon

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Several studies have demonstrated that regulatory T cells (Tregs) play a critical role balancing protective immune responses and mediate pathology during malaria infection. These cells suppress cellular immune responses through direct contact with immune effector cells by producing regulatory cytokines as TGF-ß and IL-10, suggesting that Tregs may contribute to the onset of *P. falciparum* infection. It is expected that immunologic memory develops with exposure and age; within this context, IgG response would be present before and with a more rapid increase upon infection associated with lower frequency of fever, as well as, a lower parasite density in both adults and children. The objective of the study was to evaluate Tregs percentage and IgG response as a differential response during *P. falciparum* infection in symptomatic and asymptomatic individuals living in a hypoendemic malaria region. CD4+CD25+CD127loFoxp3+Tregs were identified by flow cytometry and reported as percentage of total CD4+ T cells in three groups: symptomatic (S), asymptomatic (AS) and control (C) individuals. The IgG antibody levels were determined in all blood samples, collected on filter paper, by ELISA assay using recombinant GLURP (R2) antigen. This study showed that S, AS and C groups presented similar Tregs percentage (3.89%, 3.47% and 3.51% respectively) in peripheral blood. Furthermore, there was no positive correlation between parasitemia and Tregs percentage (P-value = 0.47). On the other hand, we observed positive IgG response in S and AS groups (1.19 and 0.85 respectively) with no significant difference between them. All groups (S, AS, and C) presented similar Tregs percentage and there was no positive correlation with parasitemia levels, suggesting that Tregs may are not implicated in the control and/or exacerbation of parasite multiplication. Additionally, positive IgG response in both S and AS groups could imply a very important part of protection and parasite clearance.

1.1-159

Metabolism and disposition of novel liver stage-active acridone antimalarial drugs

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The acridone class of antimalarials shows promise as both a treatment for blood stage malaria and causal prophylaxis. We undertook a series of *in vitro* and *in vivo* studies to characterize the metabolism and disposition related properties of several analogs of a novel acridone chemotype with demonstrated *in vitro* potency against at least three strains of *Plasmodium falciparum*. Incubations with human and mouse liver microsomes showed the compounds were metabolically stable with half-lives in the range of 60 min. Permeability assays using MDR1-MDCK cells indicated the compounds ranked as medium to moderately permeable, while the related efflux ratio suggested a potential Pgp interaction. There were no apparent metabolism-based drug interactions when using expressed CYP450 isoenzymes and selective markers of enzymatic activity. Metabolite identification

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and the relative contribution of the principal CYP450s to the metabolic profile of the lead candidate are under evaluation using hepatocytes and subcellular fractions followed by mass spectrometry analysis. Our *in vivo* imaging system model using mice infected with transgenic bioluminescent parasites to directly assess the effect of drug candidates on liver stage development showed parasite clearance after a three dose regimen with the current lead compound. Efficacy using our Plasmodium cynomolgi/rhesus monkey model and rodent pharmacokinetic studies are in progress. This *in vitro* and *in vivo* research characterizes key metabolism and disposition related properties of a novel acridone antimalarial drug scaffold with demonstrated blood and liver stage activity and contributes to the down-selection process of lead candidates for further development.

1.1-160

Cerebral malaria, a not uncommon presentation in travellers. Experience in a general hospital in the last year

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Brief Introduction Cerebral malaria is the most frequent manifestation of severe malaria, with a mortality approaching 20% despite a correct treatment, requiring experienced staff. Of all the malarial parasites that infect humans, *Plasmodium falciparum* is most commonly associated with neurological complications, which manifest as agitation, psychosis, seizures, impaired consciousness and coma. A substantial proportion of individuals with this condition develop neurocognitive sequelae.

Methods and Materials Review of the cases of cerebral malaria in San Cecilio's Hospital, Granada (Spain) in 2010.

Results Two cases of cerebral malaria were admitted in our center in that period. The principal factors for developing severe disease were ethnicity (Caucasian), age (>50 years, 58 and 62), time since start of symptoms to diagnosis (5 and 4 days, the first episode of malaria and degree of parasitaemia (20 and 25% respectively). Both cases were first episodes of malaria and occurred after a trip to sub-Saharan Africa: Burkina Faso and Equatorial Guinea. Treatment was intravenous quinine in both cases, accompanying intravenous doxicicline. Patient 1 received oral quinine, because intravenous drug was not available at our center. He survived but presented neurological sequelae (paresthesias). Patient 2 died 12 h after admission to the ICU.

Conclusions Cerebral malaria in non-endemic countries is an important cause of mortality. Of particular importance is the need for intravenous therapy in a hospital where the diagnosis is not very common, like ours; survival may depend on it.

1.1-161

Proof of concept of malaria elimination in Mesoamerica: planning for an ambitious goal

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Introduction The Salud Mesoamérica 2015 Initiative (SM2015) is an innovative, regional, public-private partnership between the Bill and Melinda Gates Foundation, the Carlos Slim Health Institute (ICSS), the Government of Spain, the Inter-American Development Bank (IDB) and the Mesoamerican countries. The objective of the malaria component of SM2015 is to provide evidence on whether it is possible to interrupt malaria transmission in two demonstration areas of Mesoamerica.

Methods/Interventions Two demonstration areas were selected: (i) the border area between Honduras and Nicaragua as an example of a high transmission area in the region, (ii) Costa Rica – a country where the epidemiological conditions are already established to advance towards elimination in the short term. The main interventions proposed for the border Honduras-Nicaragua are: (i) Provision of long lasting insecticide-treated bednets to the whole population (1 bednet per person) to achieve universal coverage in the area, from the start of the project. (ii) Start up a diagnostic system (using rapid diagnostic tests) throughout the entire area – universal coverage- and full treatment of all confirmed malaria cases through healthcare posts and staff volunteers organized into immediate response brigades. (iii) Strengthening the system of monitoring, and case surveillance ('surveillance as an intervention'), creating immediate response mechanisms.

The main interventions proposed for Costa Rica are: (i) Adequate diagnosis followed by timely and strictly supervised treatment for 100% of malaria cases. (ii) Strengthening and integration of response capabilities of local and regional malaria diagnostic laboratories in the country as a primary element of the surveillance system. (iii) Strengthening the system of monitoring, and case surveillance ('surveillance as an intervention').

Conclusion This proof of concept project will be crucial to understand whether it is possible to eliminate malaria across Mesoamerica with the current available tools.

1.1-162

Low birth weight and malaria in pregnancy in Brazilian Amazon

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Background *Plasmodium vivax* infection is more common in the Brazilian Amazon than *P. falciparum* infection, responsible for more than 80% of registered cases. *P. falciparum* malaria is recognized as an important determinant of low birth weight (LBW), but this association with *P. vivax* malaria in pregnancy is not well described. The association between LBW and infant mortality is known. This study aimed to estimate the incidence of LBW in newborns of mothers with malaria during pregnancy in a malaria endemic area of Brazil.

Methods and Materials A case-series study at the Tropical Medicine Foundation was carried out in Manaus, Brazil. Five hundred and eight pregnant women with malaria were followed since the diagnosis until delivery of weekly and monthly follow-up. At every visit, blood samples were collected for parasitaemia determination by microscopy and analyzed according to Brazilian standard procedures. Birth weights were secondary data available on the Live births Data System of the Brazilian health system.

Results Between 2005 and 2008, a total of 757 malaria episodes were found in the 508 pregnant women recruited. The frequency by specie was 82.4% (624/757) *P. vivax*, 16.2% (123/757) *P. falciparum*, and 1.3% (10/757) mixed infections. 17.7% (90/508) of recruits were younger than 18 years. The total incidence of LBW was 8.7% (44/508). The distribution of maternal infections was 68.2% (30/44) *P. vivax*, 28.5% (13/44) *P. falciparum*, and 2.3% (1/44) mixed infections. Mother when separated by episodes, whe find the following frequency of LBW: 8.3% (32/387) women infected with *P. vivax* only, 11.6% (10/86) with *P.*

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falciparum only, 16.7% (1/6) mixed infection, and 8% (2/25) *P. vivax* plus *P. falciparum* episodes at same pregnancy.

CONCLUSIONS The LBW incidence in malaria infected women was higher (or similar) than in the Manaus general population (8.2%). When mother were separated by episodes, the LBW incidence in falciparum infected women and mixed infected women was higher than in women infected with *P. vivax* only and *P. vivax* plus *P. falciparum* episodes at same pregnancy. It is necessary evaluate others factors that may cause LBW as maternal anemia, gestational age and neonatal diseases.

1.1-163

Assessing information, education and behavior change intervention in a malaria control program implemented in Ouahigouya district, Burkina Faso

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The impact of information, education and communication (IEC) and behavior change communication (BCC) intervention towards malaria was evaluated in Ouahigouya district, Burkina Faso. IEC/BCC intervention consisted of educating the community on management and prevention of malaria. In October 2007, prior to intervention, a questionnaire was used to obtain information on knowledge of and practices towards malaria of 146 adults living in the rural community of Tangaye. Prevalence of asymptomatic parasitemia in children of <5 years, proportion of the households using mosquito nets and proportion of pregnant women using intermittent preventive treatment were also evaluated. The same questionnaire was administered to 128 adults of the same villages and intervention indicators were measured 2-years post-intervention. A comparison of pre- and post-intervention data indicated significant changes in (i) parasite prevalence in children of <5 years (from 89% to 64%), (ii) coverage of households with mosquito nets (from 19% to 62%), (iii) proportion of pregnant women using intermittent preventive treatment (from 71% to 96%), (iv) proportion of persons treating malaria with traditional medicines (from 21% to 6%), (v) proportion of persons seeking antimalarial medications from a healthcare facility for malaria case management (from 43% to 64%). Cases of severe malaria registered in Tangaye fell by 60% during the same period. These preliminary data demonstrate that implementation of IEC/BCC in rural communities and provision with commodities at affordable cost is a central strategy to reduce malaria.

1.1-164

Impact of treating *Plasmodium falciparum* asymptomatic carriers on the dynamic of malaria transmission

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INTRODUCTION *Plasmodium falciparum* malaria is associated with approximately one million deaths every year, mainly in children aged <5 years. In areas that have already implemented strategies to reduce malaria transmission [e.g. distribution of insecticide-treated bed nets (ITNs), widespread adoption of artemisinin-based combination therapy (ACT)], additional complementary interventions are required to further accelerate reduction in disease burden. Asymptomatic *P. falciparum* carriers serve as parasite reservoirs for malaria transmission; and community screening and treatment of these asymptomatic carriers with ACT may reduce the pool of infectious gametocytes and influence malaria transmission in that area.

MATERIALS AND METHODS Description of malaria epidemiology is often focused on clinical parameters such as prevalence of parasitemia. However, entomological parameters such as vector species and density, proportion of infected mosquitoes, and entomological inoculation rate (EIR) are essential for understanding the malaria epidemiology in a specific area and planning control measures. The EIR is a standard measure of transmission intensity, obtained by multiplying human-biting rate with proportion of sporozoite-positive mosquitoes. This longitudinal survey will assess EIR in five villages in Burkina Faso where mass screening and treatment of asymptomatic carriers is being implemented, and in five control villages. To measure the impact of the intervention, entomological parameters will be assessed before and after ACT treatment. Mosquitoes will be collected twice a month using indoor spray catch method, a standard method for collecting indoor resting adult mosquitoes.

CONCLUSIONS Data collected will be used to infer the human-biting rates (the number of biting mosquitoes per human per night). Moquitoes collected will be processed by ELISA assay to estimate sporozoite index and EIR in each site. The population will be provided with long-lasting ITNs, and susceptibility of malaria vectors to insecticide will also be determined.

1.1-165

Prevalence of pigment containing leukocytes during uncomplicated malaria in malnourished children: cases of the general hospital of Kindu, D.R.Congo

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INTRODUCTION Malaria diagnosis based on parasitological evidence is difficult in a context of self-medication and poverty.

MATERIALS AND METHODS Parasitemia and pigment-containing leukocytes were screened in a prospective case–control study of 250 malnourished children (cases) and 750 children with a good general condition (controls), by thick and thin blood smears.

RESULTS 52.8% males. Mean age: 29.3 ± 18.1 months old (2.4 ± 1.5 years old). Malaria prevalence in cases: 47.6% (CI 95%, 41.1%–53.8%) vs. 46% (CI 95%, 42.6%–49.9%) in controls. Prevalence of pigment-containing leukocytes: 11.2% (CI 95%, 7.6%–15.8%) in cases vs. 6% (CI 95%, 4.5%–8%) in controls. Comparing pigment-containing neutrophils, cases and controls, the difference was statistically significant, $\chi^2 = 7.49$, $P < 0.05$, OR = 0.50 (CI 95%, 0.30–0.80) whereas it was not for pigment-containing monocytes $\chi^2 = 0.6$ $P > 0.05$, OR = 1.1 (CI 95%, 0.8–1.5). Pigment-containing leukocytes sensitivity was 12.5%, specificity 97.2%, Positive Predictive Value 79.4%, Negative Predictive Value 56% and global value 14.7%. 5.8% (58/995) of concordance between positive thick blood smears and pigment-containing neutrophils vs. 34.6% (344/995) pigment-containing monocytes and positive thick blood smears. Discordance between negative thick blood smears and pigment-containing neutrophils was 1.5% (15/995) vs. negative thick blood smears and pigment-containing monocytes was 36.8% (366/995).

CONCLUSIONS The sensitivity of pigment-containing leukocytes was weak in uncomplicated malaria. In cases of negative thick blood smears the screening of pigment-containing monocytes would contribute better to malaria diagnosis than pigment-containing neutrophils.

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1.1-166

Malaria in malnourished children case of the nutritional center of the general hospital of Kindu, Maniema, D.R.Congo

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INTRODUCTION During this post-conflict period, malaria and malnutrition are predominant in children of Kindu and its rural areas.

METHODS AND MATERIALS Parasitemia was determined in a prospective case–control study, 250 malnourished children (cases) and 750 children with good general condition (controls). Thick and thin blood smears were done and stained with Giemsa. Study period: March to July 2010. Study settings: age, sex, brachial perimeter, weight and height. For statistical analysis: mean, standard deviation, chi square, CI 95% and Odds Ratio.

RESULTS 52.8% males vs. 47.2% females. Mean age: 29.3 ± 18.1 months old (2.4 ± 1.5 years old). We had: 54.1% (CI 95%, 47.6–60.5%) of marasmus, 45.5% (CI 95%, 39.1–52%) of kwashiorkor and 0.4% (CI 95%, 0–2.3%) of marasmus-kwashiorkor. Overall malaria prevalence was 46.5% (CI 95%, 43.4–49.9%), the prevalence in cases was 47.6% (CI 95%, 41.1–53.8%) vs. 46% (CI 95%, 42.6–49.9%) in controls: $\chi^2 = 0.95$ (CI 95%, 0.71–1.27). Malaria prevalence was 26.2% in marasmus, 20.5% in kwashiorkor and 0.41% in marasm-kwashiorkor ($\chi^2 = 1.34$, $P > 0.05$). The overall mean parasitic load was of 282 ± 266. The mean parasitic load was 241 ± 222 in cases vs. 405 ± 340 in controls ($P > 0.05$). In cases, the parasitic load was 418 ± 340 for marasmus vs. 395 ± 346 for kwashiorkor cases ($P > 0.05$). *Plasmodium falciparum* was the only specie found.

CONCLUSION Malaria affected all malnourished children without significant difference.

1.1-167

Severe *Plasmodium vivax* malaria in splenectomized patient

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BRIEF INTRODUCTION Malaria is still one of the most common infectious diseases in tropical and subtropical countries with enormous public health impacts. In Iran, two species of malaria are present; *P. vivax* and *P. falciparum* have been present mainly in three provinces of southeast. The role of the spleen in the elimination of intra-erythrocytic parasites has been reported in several animal models. Splenectomy, the surgical removal of the spleen, is increasingly practiced in malaria-endemic tropical countries. The procedure leaves patients more susceptible to serious bacterial and parasitic infections, including malaria. Here, we report for the first time in Iran a severe case of *P. vivax* malaria in a patient who had undergone splenectomy due to hemolytic anemia.

METHODS The patient who was admitted to the hospital with severe anemia after undergoing splenectomy. Clinical signs subsided intermittently but later a more complicated clinical profile emerged characterized by severe fever. There was no evidence of bacterial or viral infections; serological tests for visceral leishnamiasis were nagative. In spite of antibiotic therapy for two consecutive weeks, clinical signs did not improve. Microscopy revealed malaria parasites in patient's peripheral blood smears. Molecular test (PCR assay) and DNA sequencing were performed on blood smears for final confirmation of the causative agent. Eventually the patient received antimalarial drugs several days after diagnosis, when developing serious clinical symptoms. The patient did not respond to anti-malarial chemotherapy and died after a coma.

RESULTS Microscopic and molecular tests confirmed the presence of *P. vivax* in blood smear of the patient, which the showed *P. vivax* as the causative species.

CONCLUSION Clinicians should consider the possibility of malaria in patients with fever who are previously splectomized and unresponsive to antibiotic therapy especially in malaria endemic areas.

1.1-168

Pharmacokinetics of artemether and dihydroartemisinin for two commercially available artemether intramuscular injections in a black adult population affected with uncomplicated malaria

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Artemether is on the list of essential medicines of the WHO since 1995. A study on the pharmacokinetic behaviour of artemether, injected intramuscular from different oils could be of interest. Since artemether is metabolised by a P450 enzyme to dihydroartemisinin (DHA), the behaviour of this active metabolite is also recorded. The current study determines the plasma levels of artemether and dihydroartemisin after administration of an artemether injection in a single dose of 4 mg/kg in African patients with uncomplicated malaria. The study was conducted in Ivory Coast. Artemether was administrated as 4 mg/kg at time 0, in a single injection in the outer upper quadrant of the quadriceps muscle. Patients were randomized to receiving one of the two study drugs: Artesiane® i.m. injection (Dafra Pharma) contains 80 mg/ml artemether in miglyol, Paluther® i.m. injection (Sanofi-Aventis) contains 80 mg/ml artemether in arachis oil. A total of 23 patients were included in the study and randomized, 13 patients received Artesiane® and 10 received Paluther®. Age and sex-distribution were similar between the two groups. The parasite count in the group receiving Paluther® was higher (two sample *t*-test = 0.05). The individual plasma levels of artemether obtained with the two different formulations varied considerably. The plasma levels of dihydroartemisinin stayed very low throughout the study. The tmax for both formulations was around 8–12 h, but due to the limited amount of sampling points this could not be precisely determined. The average Cmax for artemether and dihydroartemisinin was 59.8 and 7.1 ng/ml respectively for Artesiane® and 47.8 and 3.3 ng/ml respectively for Paluther® ($P > 0.05$). In all patients, fever and parasite clearance occurred within 48 h. The longer period of elevated plasma levels of artemether after intramuscular artemether can explain the finding that the recrudescence and reinfection after a full artemether i.m. treatment course over 5 days is less likely than with oral artesunate since plasma concentration above the EC50 will be maintained for at least 7 days. Acute administration of Intramuscular artemether prepares ideally for a follow up with ACT medication when the initial complications are over.

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1.1-169

Treatment of Korean vivax malaria patients with the fixed-dose combination of pyronaridine: artesunate

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A randomised, double-blind, double-dummy, comparative clinical study was conducted in patients in Korea diagnosed with acute, uncomplicated *Plasmodium* (P.) *vivax* malaria to compare the efficacy and safety of the fixed dose formulation of pyronaridine:artesunate (PA) *vs.* chloroquine. In total, 30 patients were recruited from two sites in Korea and were randomised in a 1:1 ratio to receive either oral PA (180:60 mg tablets) plus chloroquine-placebo or oral chloroquine (155 mg tablets) plus PA-placebo, once-a-day for three consecutive days (Days 0, 1, and 2). Patients aged between 3 and 60 years and with a body weight between 20 and 90 kg, were recruited who had acute uncomplicated *P. vivax* mono-infection confirmed with fever and positive microscopy of *P. vivax* with parasite density = 250/mcL of blood (including at least 50% of asexual parasites) and a rapid test negative for *P. falciparum*. Patients were followed for safety up to 42 days. The primary efficacy endpoint of cure was at Day 14. Except for patients who were deficient in G-6-PD, patients completing the study up to Day 28 received a 14-day course of primaquine (15 mg/day) starting on Day 28. Secondary efficacy endpoints were the proportion of patients cured at Days 28 and 42, parasite clearance time, fever clearance time and the proportion of patients aparasitemic on Days 1, 2 and 3. Safety and tolerability was assessed through monitoring of clinical safety laboratory evaluations for haematology, biochemistry and urinalysis as well as 12-lead ECG. This trial is consistent with the results of a multicenter study that was conducted in South East Asia and India by demonstrating a high level efficacy with a similar safety profile of PA for the treatment of blood stage *P. vivax* malaria in Korea. The results of this study and those of the multicenter study will be pooled and presented.

1.1-170

Using RDTS as a malaria diagnostic tool in wad Medani different hospitals – Central Sudan

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Malaria is overestimated if the diagnosis is based solely on clinical signs. Therefore, laboratory confirmation is essential. Microscopy is the gold standard method and reliable technique when performed correctly and unreliable when poorly performed. But rapid diagnostic tests (RDTs) offer a good alternative with the advantage that it is an easy and rapid method, and may assist in diagnosis and improving the practices prescription. From 2007 to 2008, in Wad Medani Central Sudan, 931 patients with symptoms of malaria attended the different specialized hospitals and were enrolled in this study, RDT and blood smears methods were performed to diagnose malaria, and nested PCR were done as a confirmative tool. The results obtained by this study revealed that, 131/931 (14.1%) and 63/931 (6.7%) were positive when performed by microscopy and RDT respectively. Also the finding showed that 68 of 131 positive by microscopy were negative by RDT. But the nested PCR confirmed that, 25/931 (13.4%) were positive while six were false positive by microscopy and 62 were false negative by RDT, indicating that there is a significant difference between the rates of malaria cases diagnosed by microscopy and RDT ($P = 0.001$) This study concluded that the implementation of RDT as a diagnostic tool could not be an alternative method to diagnose malaria. But the microscopy is still the gold standard need to be improved.

1.1-171

Evidence scientific to stretch of the early diagnosis of HIV in the Guinean population

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Proyecto financiado por la AECID y la RICET

INTRODUCTION To be realised voluntarily the early diagnosis of HIV is at present one of main recommendations in the field of the prevention and treatment of HIV. Nevertheless as soon as it is had specific recommendations to its implementation. Great part of it must to the specificity on the different contexts where it can be implanted and to the little scientific evidence on its beginning. So that an investigation was realised in Equatorial Guinea in order to generate knowledge to designed the implementation of this strategy.

METHOD Cross-sectional study with qualitative design of investigation. Study environment: Bata, Equatorial Guinea. Analysis unit: barriers and facilitators for the stretch of the early diagnosis. Intentional sample: homogeneous of sub-group. Techniques for collect information: semistructured interview (30) and groups of discussion (8). Analysis method: Grounded Theory. Triangulation of the data: internal by two observers.

RESULTS The existence of cultural, economic, social, institutional and political aspects can interfere, as a barriers or facilitators, in the acceptance of this measurement of prevention of HIV in the general population. In this study it has been identified like barriers:

The social value that the health acquires in the Guinean society.
The religious vision on the health an its protection.
The existence of gender roles about the care of the health.
The social representation of HIV.
The lack of cover in the health service, and the other.

AS STRENGTHS HAS BEEN IDENTIFIED:

The feminine roles of the familiar and individual care health.
Great minded respect prevention of disease in masculine population with a high educative level.
Great cover of the services of diagnosis in Bata, among other.

CONCLUSIONS The individual and specificity of identified about the barriers and facilitadotes in Equatorial Guinea show the relevance to realise previous studies in the contexts where it is going to be implementation to stretch of the early diagnosis.

1.2 HIV/AIDS, TB and Sexually Transmitted Infections

1.2-001

Prevalence and risk factors of infection with hepatitis C in a population of patients with HIV-AIDS in Tijuana, B. C., Mexico

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OBJECTIVES To determine the prevalence of infection by hepatitis C virus (HCV) and associated risk factors in a population of patients positive for HIV-AIDS Specialist Services General Hospital (ISESALUD) from Tijuana, B.C., México.

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MATERIAL AND METHODS One hundred and thirty-fore samples (33.5%) underwent serological survey and test-Ortho HCV 3.0 ELISA, HCV RNA quantitation and genotyping by Real Time PCR.

RESULTS The prevalence of HCV seropositive persons was 10.0% (CI 95% 5.0–15.0), confirmed HCV RNA in 71%, of which 100% corresponded to genotype Ib. Risk factors for HIV-HCV co-infection were: intravenous drug use (RMP = 10.0, CI 95% 2.7–41.4), with increased risk in women (RMP = 13.3, CI 95% 0.8–214.6), history of tattoos (RMP = 3.28, CI 95% 1.06–10.17), with increased risk in men (RMP = 3.7, CI 95% 1.0–13.6) and the unemployed (RMP = 4.8, CI 95% 0.6–40.4).

CONCLUSION It is urgent to expand the monitoring of HCV infection especially in high-risk populations; of the 71% of seropositive persons with active infection, 94% had at least one risk factor for progression to liver disease and 100% matched genotype Ib, These persons with co-infection HIV-VHC are candidates to receive antiviral therapy to prevent severe liver complications and improve quality of life.

KEYWORDS hepatitis C, genotypes, co-infection, HIV-AIDS

1.2-002

Multiple partners and risk of HIV infection among vulnerables youth attending free targeted HIV counseling and testing services including in Benin, West Africa

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OBJECTIVES To measure the association between multiple partnership and HIV infection among young people in Benin.

BACKGROUND In Benin the HIV epidemic has become generalized, with an HIV prevalence of 1.2% in the general population (Benin DHS III, 2006). Among youth aged 15–24 years, the prevalence is 0.7%. In collaboration with two health facilities and a mobile clinic, Counseling and Testing (CT) services was implemented in two departments of Benin (Atacora and Donga) trough a project funded by the Kreditanstalt für Wiederaufbau (KfW) Germany. These interventions targeted vulnerable youth (15–24) and included STI prevention, screening and management, using clinical screening algorithms. In 28,868 clients requesting HIV counselling and testing were registered from 2008 to 2010 trough a database.

METHODS The socio-demographic, behavioral and biological data of each young voluntary tested were recorded on a monitoring form well structured. Descriptive analysis and logistic regression was performed on the database with SPSS 17. The dependent variable is: Result of HIV test (positive or negative). The independents variables were: having multiple partners the 3 months and socio-demographic characteristics.

RESULTS The quarter (26.4%) of the young people had multiple partnerships the last 3 months. HIV prevalence was 0.7% (0.6–0.8%). Having multiple partners is significantly associate to IST infection (OR = 1.8; P = 0.003). Education level (OR = 0.7; P = 0.007) and sex (OR = 0.2; P = 0.000) were the significant socio-demographic characteristic associated with STI.

CONCLUSIONS Data findings indicate that 'multiple partners' is a big risk factor for HIV among vulnerable young attending counseling and testing services in Atacora/Donga in Benin. Finding suggests such intervention (counseling) focused on reducing number of partner, improved to outreach activities. It may reduce HIV incidence among vulnerable youth.

1.2-003

Spectrum of opportunistic infectious diseases among hospitalized patients with HIV/AIDS in Boo-Ali Hospital, Zahedan-Southeastern Iran

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BACKGROUND/AIM Infection with HIV virus reduces the immune system ability to fight against infections. Certain bacteria, viruses, fungi, and other organisms, which do not usually cause infections in healthy people, can cause protean infections in people with a weakened immune system. Here in, we aimed to study the spectrum of opportunistic infectious diseases in a series of hospitalized HIV patients in our hospital.

METHODS In this cross-sectional, descriptive study, we studied all files of patients with HIV/AIDS who admitted to Boo-Ali hospital in Zahedan (a city in the Southeastern Iran) from November 2000 to October 2010.

RESULTS We evaluated 65 patients (52 male, 13 female, age range: 7–55 years) with HIV/AIDS infection. Eighteen patients (27%) had tuberculosis, 14 (21%) bacterial pneumonia, two (3%) *Pneumocystis carinii* pneumonia, 10 (15%) Gastroenteritis, 10 (15%) Hepatitis, two (3%) Toxoplasmosis, four (6%) malignancy, two (3%) Cellulitis, one (1%) Herpes Zoster, two (3%) *Candida esophagitis* infection. Of 18 patients with tuberculosis infection 14 (77%) had pulmonary tuberculosis.

CONCLUSION Our study showed that the most common infection in patients with HIV/AIDS in this region was tuberculosis and pulmonary TB was more prevalent than other forms of TB. We suggest all patients with HIV should evaluate for TB infection, especially in this area which TB is endemic.

1.2-004

Cotrimoxazole, effective molecule for the treatment of the cerebral toxoplasmosis in the event of AIDS

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INTRODUCTION Cerebral toxoplasmosis (CT) is the principal cerebral infection during AIDS. The traditional curative treatment is based on association pyremethamine and sulfamethoxazole, molecules not always available in certain countries.

OBJECTIVE To evaluate the effectiveness and the tolerance of cotrimoxazole (CTX) in the treatment of CT among patients infected by HIV.

MATERIALS AND METHODS Study retrospective 2005–2009. Twenty-five HIV+ patients with CT were treated by CTX in infectious diseases department in Oran hospital (because of its availability) TMP 10 mg/kg/J and SMX 50 mg/kg/J during 6 weeks. Cerebral scans were taken at admission and after 2 weeks of TRT.

RESULTS Male prevalence (Sex Ratio = 2:1); median age: 36 years. CT inaugural and revealing of the AIDS: 32% and first opportunist infection: 16% of the cases. Clinical symptoms were headache, fever in 36%, neurological deficits in 32%, convulsion in 12%, impaired consciousness in 20%. 100% of the patients had IgG antibodies without IgM and 8% with IgM. Total lymphocytes $<800/mm^3$ I, 80% of the cases. The cerebral scanner was in favors of the toxoplasmosis diagnosis among all patients. The diagnosis is based according to the clinical and radiological criteria and the immunosuppressant person. Treatment was based on CTX considering its availability, its cost and the experiment of our area, associated with the corticosteroids. The outcome was favorable in 80%. The tolerance was good in all of the cases. Relapses were noted in 20%.

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CONCLUSION Cotrimoxazole was a curative, well-tolerated treatment (parenteral and *per os*) of cerebral toxoplasmosis at the PVVIH. Its lower cost recommends it especially in poor countries.

1.2-005

A case of co-existent *M. avium* and *M. tuberculosis* lymphadenitis

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INTRODUCTION Cervical lymphadenitis (CL) is the most common head and neck manifestation of mycobacterial infections and should be considered in the differential diagnosis of a cervical mass, since the incidence has increased. CL due to non tuberculous mycobacterial (NTM) is primarily a disease of the childhood. Mycobacterium avium complex (MAC) is an unusual cause of cervical lymphadenitis in immunocompetent adults.

METHODS AND MATERIALS An 85-year-old female, with previous carcinoma of the vulva (2005), presented gradually enlarging supraclavicular and laterocervical multiple lymphoadenopathies in 2 months time and weight loss. Neck and chest CT confirmed the known multiple adenopathies (largest diameter of 2 cm) and a small calcified nodule in the upper apical lobe bilaterally. Both a Mantoux skin test and a Quantiferon TB Gold test were positive. An excision of a lymphonode was undertaken, standard bacterial and fungal cultures, direct microscopic examination for AFB and PCR testing for *M. tuberculosis* were negative. Histopathologic examination revealed caseating necrosis and granulomatous inflammation, therefore antituberculous chemotherapy (isoniazid, rifampicin, ethambutol and pyrazinamide) was started, confirmed after the culture became positive for a totisensitive *M. tuberculosis*. Later, in the same specimen, MAC was isolated too and, due to the disease's extension, medical therapy was preferred to surgery and pyrazinamide was replaced with clarithromycin in order to have a therapeutic effect on both pathogens.

RESULTS Since the neck CT after 6 months of therapy revealed that the lymphadenopathy had reduced in size, isoniazid was suspended while the treatment for MAC, more difficult to eradicate, continued for other 5 months, with further improvement of neck CT at 12 months of therapy.

CONCLUSIONS Mycobacterial CL is caused either by tuberculous or NTM in elderly people too. This is in our knowledge the first case of co-infection. Their diagnosis and distinction are necessary because the treatment is different.

1.2-006

Screening of HTLV I infection in a subsaharian immigrant population

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BACKGROUND In the last 10 years human T-cell leukemia virus (HTLV) infection has emerged as a worldwide health problem. The numbers of HTLV-I infections in USA and Europe is increasing in relation to migration phenomena.

OBJECTIVE We describe the results of a screening program of HTLV-I conducted in subsaharian immigrant population attended at the Tropical Medicine Unit of Hospital Universitario Central de Asturias during the year 2009.

STUDY DESIGN We determined anti-HTLV-I antibodies in all subsaharian immigrant patients that were followed-up at the Tropical Medicine Unit of Hospital Central of Asturias during 2009. The serologic screening tests used for HTLV I were a chemiluminiscent immunoassay. The positive results were confirmed with Western-Blot analysis and polymerase chain reaction.

RESULTS Seven-three subsaharian immigrants were screened (63% male, mean age: 28.8 years, range 15–59). The countries of origin were: Equatorial Guinea (51%), Senegal (28%), Nigeria (16%), Kenya and Mali (2% respectively). The average time of permanence in Spain was 335 days. All patients were asymptomatic. No haematologic abnormalities were observed in any patients. Positive antibodies were found in a patient from Mali (2%). Western Blot and polymerase chain reaction were positive for HTLV-I. The patient had a CD4+ lymphocyte count of 1003 cells/mm^3. There was no evidence of neurological or hematological disease. The patient present a indeterminate pattern in the Western-Blott for HIV.

CONCLUSIONS The presence of infection by HTLV-1 in latent phase could be a problem under-diagnosed in immigrants from endemic area. The presence of HTLV-I-II infection must be suspected in patients with indeterminate reaction to HIV. However, although the current sample is small, it appears that the prevalence of infection is still low in our environment.

1.2-007

Standardization of in-house real time PCR for the identification of *Mycobacterium tuberculosis* in sputum

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BACKGROUND Tuberculosis (TB) is a chronic infectious disease that remains a major health problem worldwide. Smear microscopy is the easiest, fastest and cheapest technique for laboratory-confirmed diagnosis of TB, but has a low sensitivity. Cultivation followed by phenotypic identification is still the 'gold standard', but has the disadvantage of taking a long time. So there still is a need for a rapid, simple and cost-effective diagnostic tool for detection of *Mycobacterium tuberculosis*. The aim of this study was to standardize a real time PCR directly from sputum through non-commercial kits to detect *M. tuberculosis* complex.

METHODS We collected sputum samples from consecutive patients with suspicion of TB. Decontamination was done through the hypertonic saline and sodium hydroxide method. The pellet was resuspended in TE buffer and heat-inactivated (20 min at 95°C). After centrifugation the supernatant containing DNA was precipitated with ethanol and stored. The target for the PCR was the small mobile genetic element IS6110, which is unique to the *M. tuberculosis* complex, and the human erv-3 sequence was used as internal control to monitor for inhibition. Lowenstein–Jensen (LJ) culture from the same decontaminated sample was used as 'gold standard'.

RESULTS We analyzed 70 sputum samples. With LJ culture 94% (66/70) of samples were positive and 6% (4/70) remained negative. The qPCR analysis confirmed 97% (64/66) of the culture-positive samples, and predicted 100% (4/4) of the negative samples. The qPCR showed a specificity of 100% and a sensitivity of 97% as compared to LJ culture.

CONCLUSION This assay is simple, rapid, inexpensive, and reliable. It is suitable for use in clinical laboratories as well as research facilities.

KEYWORDS real time PCR, *Mycobacterium tuberculosis*, element IS6110

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1.2-008

'Granulomatous inflammation' in an HIV positive patient: not just tuberculosis

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INTRODUCTION Infections with NTM usually affect immunocompromised patients. *M. xenopi*, a mild pathogen, rarely causes spondylodiscitis, mostly post local surgery (exogenous). Seven are the known cases of not postoperative spondylodiscitis (endogenous) from *M. xenopi*. The etiologic diagnosis should be pursued as *M. xenopi* has a profile of drug treatment that results in difficulties with the risk of complications for the patient.

CASE REPORT A 40-year old patient, known to be HIV+ since 1993 (stage C3), in HAART, in 2001 had been diagnosed (imaging and blood cultures) with *S. epidermidis* related L1-L2 spondylodiscitis. In 2006, for recurrence of symptoms, M. Pott's L1-L2 was diagnosed histologically, on bone biopsy (granulomatous inflammation), without microbiological confirmation. The empirical anti-tuberculosis therapy, poorly tolerated, was very difficult, with apparent improvement. In August 2009, a lumbosacral MRI control (patient asymptomatic, CD4 880/iL, suppressed viremia) detected recurrent disease, with a multilocular abscess to the muscle-ileum right psoas, confirmed on PET F18-FDG (SUV max 30). From the purulent material drained from the muscle *M. xenopi* was isolated. Then, in October 2009, the patient received moxifloxacin, ethambutol, and clarithromycin. Lumbosacral MRI at 1 month of treatment was unchanged, while the F18-FDG PET had significantly improved (SUV max 10). At the later radiological control (3, 6 and 12 months of therapy), the picture progressively improved. To date, the patient is on therapy.

DISCUSSION It is the third report of spondylodiscitis endogenous *M. xenopi* and the first of ileus psoas muscle abscess in patient HIV+ who had never undergone spinal surgery. Nonspecific clinical and histological appearance similar to *M. tuberculosis*, make the risk of mis-diagnosis high. In HIV+ patient with spondylodiscitis histologically attributable to mycobacteriosis, should be pursued a microbiological date to exclude NTM, even rare, like the *M. xenopi*, in order to start adequate treatment, as the prognosis is unfavorable if not appropriately treated.

1.2-009

Attitudes and risk perception of HIV among attendees at the center for voluntary anonymous counseling and testing in monastir (Tunisia)

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INTRODUCTION The frequency of HIV infection is still low in Tunisia. However, risk factors are growing constantly. The implementation of voluntary counseling and testing centers is an essential service to enhance prevention strategies. The objective of this study was to document knowledge, attitudes and practices of HIV among the attendees at the Center for Voluntary Anonymous Counseling and Testing in Monastir (Tunisia).

METHODS A cross-sectional study was conducted between 1 January 2008 and 31 December 2010 and including all attendees at the Center for Voluntary Anonymous Counseling and Testing in Monastir. Sociodemographic characteristics, knowledge and sexual behavior data were collected using a structured questionnaire.

RESULTS We included 218 participants. Means age was 27.3 ± 7.4 with a female predominance (sex ratio = 0.96). Results showed that 65% knew unprotected sex as the main risk factor and 89% reported having had sexual intercourse during their lifetime. Of rgese only 31% always used condoms and 10% engaged in homosexual sex. A logistic regression model showed that unprotected sex occurred in males, adolescents and adults <30 and those having little knowledge of HIV/AIDS.

CONCLUSION The results of this study indicated that much greater efforts are needed to improve HIV/AIDS knowledge, to promote safer sex particularly among those engaging in risky behaviors.

1.2-010

Predictors of antiretroviral treatment associated tuberculosis in Ethiopia: a nested case–control study

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BACKGROUND Little is known about the predictors of antiretroviral treatment (ART) associated tuberculosis (TB) in developing nations. The objective of this study was to determine predictors of ART associated TB in adults with HIV infection at Jimma University Hospital, Ethiopia.

METHODS A nested case–control study was conducted in October, 2009. The study population consisted of adults with HIV infection (>14 years) who developed active TB in the first 6 months of ART initiation and controls that did not develop active TB. Data were collected using a structured and pre-tested questionnaire. Cox proportions hazards analysis was done to determine predictors of ART associated TB.

RESULTS A total of 357 patients (119 cases and 238 controls) participated in the study. After 6 months of follow up, cumulative incidence of ART associated TB was 5.2% (123/2355). Forty (33.6%) cases were lost to follow up after they developed ART associated TB and 11 (9.2%) died. Fifty-one (21.4%) controls interrupted ART and 11 (4.6%) died. A CD4 lymphocyte count increase >0.5/l/day (AHR = 19.80, 95% CI: 9.52: 41.12, P < 0.001), a base-line CD4 lymphocyte count <200 cells/l (AHR = 9.59, 95% CI: 2.36: 39.04, P = 0.002), WHO clinical stage 3 or 4 (AHR = 3.04, 95% CI: 1.62: 5.69, P < 0.001), night sweats during ART initiation (AHR = 1.53, 95% CI: 1.06: 2.21, P < 0.001) and high ART adherence (AHR = 1.30, 95% CI: 1.13: 1.50, P < 0.001) were independent predictors of ART associated TB.

CONCLUSIONS HIV infected adults with a low CD4 lymphocyte count, night sweats for more than 2 weeks, or in WHO clinical stage 3 or 4 at ART initiation should be cautiously followed for the development of ART associated TB. Good ART adherence and a good immunological response during ART were associated with ART associated TB probably because of an immune reconstitution inflammatory syndrome unmasking the TB.

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1.2-011

'It's not all about AIDS': perceptions of illness, health seeking behaviors and barriers to health care among hijra (transgender people) in urban Dhaka

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The aim of this study was to understand emic notions of illness among the hijra (transgender) community, health-seeking behavior patterns, and barriers to health care services in Dhaka, Bangladesh. A qualitative exploratory study was conducted with 31 hijra and 37 health service providers. Focus group discussions and informal discussions were conducted during the initial stages to gather perceptions of illness, and then followed up with in-depth interviews. Participatory methods such as city mapping, and body mapping were also used to gather information about illnesses faced and health services available. Outcomes of the study showed that hijra face a number of health concerns, which are not limited to the mainstream-philosophy that labels hijra as deviant and sexualized beings. Illnesses of highest concern in the hijra community included, sexually transmitted infections, anal injuries, skin diseases, physical injuries, birth control side effects, and psychosocial and psychosexual illnesses. Furthermore, many of these illnesses could not be cared for in the current health care system in Bangladesh. Social and structural violence were seen throughout every stage of this study; in particular societal perceptions of hijra, donor and research priorities, and government policies have all in some way limited hijra agency in the health care system and left them vulnerable to acquiring particular diseases. Thus, the impact of larger social structures on health must not be overlooked, as they are the source of deep-rooted causes of illness and barriers to healthcare in the hijra community.

1.2-012

Molecular assessment on the prevalence of cervical infections with *Trichomonas vaginalis* and *Mycoplasma genitalium* among symptomatic women in Shiraz, Iran

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Cervicitis is an inflammatory condition of the cervix. It is common with rates as high as 30–45% in some sexually transmitted infection (STI) clinic populations and is generally considered to be associated with sexually transmissible pathogens. Both *M. genitalium* and *T. vaginalis* are common causes of nongonococcal cervicitis or nonspecific cervicitis but their prevalence and their pathogenesis in some developing countries such as Iran are still limited reported. A cross sectional study of 503 Iranian women aged between 17 and 56 years (January 2010 to January 2011) having Pap smears were investigated for presence of cervicitis at the family planning clinics affiliated to Shiraz University of Medical Sciences, and department of Parasitology and Mycology, Medical School of Shiraz, Iran. Of 503 cases, 238 women (range, 17–56, and the mean age of 36 years) with cervicitis were recruited. The microscopic diagnosis of cervicitis was determined by using Papanicolaou test (the number of polymorphonuclear leucocytes per hpf; e1000, oil immersion) and different cut off thresholds usually >10 PMNL/hpf. PCR amplification for detection of *T. vaginalis* and *M. genitalium* infections were performed on cervical smears. The prevalence of *M. genitalium*, 25.63% (61/238), was higher and statistically significant than *T. vaginalis*, 19.32% (46/238), among symptomatic patients diagnosed with cervico-vaginal discharge. *T. vaginalis* and *M. genitalium* were frequently observed with discharge and dysuria. Co-infection with both pathogens was observed in 11 cases (4.62%). Our result shows that an infection caused by *T. vaginalis* and/or *M. genitalium* is associated with higher incidence rate and screening for their occurrence in women with cervicitis is essential.

1.2-013

Purulent cervicitis and *Trichomonas vaginalis* in Shiraz, south of Iran

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In some countries, *Trichomonas vaginalis* is one of the commonest pathogens associated with cervical inflammation and vaginitis. Its reported contribution to the etiology of cervicitis and vaginitis is highly variable; reflecting local prevalence and it is considered to be frequently under-diagnosed due to the relatively low sensitivity of wet-mount microscopy. This survey was conducted to determine prevalence of cervicitis and vaginitis caused by *Trichomonas vaginalis*. Pap smears from 503 women attending several family planning clinics affiliated to Shiraz University of Medical Sciences, south of Iran were evaluated in the Parasitology and Mycology laboratory of Shiraz University of Medical Sciences by a pathologist between January 2010 and January 2011. Of 503 cases, 238 subjects had inflamed cervix diagnosed by Pap smear. Another cervico-vaginal smear was prepared from each woman. DNA was extracted from 238 cervico-vaginal smears. *T. vaginalis*-specific primers were used to amplify a 300 bp fragment of the *T. vaginalis* genome. Of 238 cases with inflammation in their cervix, 46 women with *T. vaginalis* infection were identified by PCR results, resulting in an estimated prevalence of 19.32%. The mean age of the women was 36 years (age range, 17–56 years) and the highest infection rate was seen in the age group of 21–40 years. Positive cases were treated suitable drug, which recommend a package of care, including metronidazole, husband notification, and condom distribution. The results of this survey show that T. vaginalis, known to be associated with cervicitis is a major problem in our Iranian women population with cervicitis. Also, education, compulsory examinations, treatment and follow up on this group of people is very important and further studies are strongly recommended in Shiraz considering the high prevalence of trichomoniasis.

1.2-014

Prevalence of specific cervicitis among women with inflammatory cervix in Shiraz, south of Iran

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In some sexually transmitted infection (STI) clinic populations, *Chlamydia trachomatis* and *Neisseria gonorrhea*, the most sexually transmitted pathogens are account for less than half of specific cervicitis cases varying widely in the literature from 11% to 50% depending on population sampled, cervicitis definition and detection methods. This study was aimed to determine the incidence of cervical infections caused by Chlamydia trachomatis and *Neisseria gonorrhoeae* in women attending several family planning clinics affiliated to Shiraz University of Medical Sciences, Iran. The most common reasons for clinic visits were signs and/or

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symptoms of cervicitis. Of 503 women having Pap smears over the 1 year period between January 2010 and January 2011, 238 cases with inflammatory condition of cervix diagnosed by Pap smear, clinical manifestations of cervicitis, were enrolled. From 238 recruited women, cervical smears were collected and stained with Gram-staining to detect neutrophils and Gram negative intracellular diplococci (*N. gonorrhea*). DNA amplification for identification of *C. trachomatis* and *N. gonorrhea* were done on Gram-stained cervical slides. Of the 238 women with cervicitis, nine (3.78%) were positive for *C. trachomatis*. No microscopic detection or PCR amplification was obtained for *N. gonorrhea*. The age range of the women was 17–56 years and the highest percentage was related to the 20–25 years age group. Women with diagnosed Chlamydiosis were treated. Although based on studies performed in western and European countries, *C. trachomatis* and *N. gonorrhea* account for a noticeable percentage of cervicitis cases. The presence of inflammatory cells in cervical smears is not necessarily due to these pathogens and other noninfectious or other infectious agents such as *Trichomonas vaginalis* and/or *M. genitalium* may be responsible for cervicitis in our country. So, given the diversity of causes, treatment should not be prescribed without an accurate diagnosis.

1.2-015

High rates of *Trichomoas vaginalis* among HIV-infected patients in fars province, southern Iran

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Several studies have shown that Trichomoas vaginalis infection is associated with increased of HIV infectivity and transmission. A study of *Trichomonas vaginalis* in 354 HIV-infected adult patients (273 males, 81 females), based on the detection of motile trophozoites by spun urine wet smear and urine-based PCR technique, was carried out from June to December 2010. This cross-sectional study was conducted in a clinic of behavioral disorders affiliated to Shiraz University of Medical sciences, and Department of Parasitology and Mycology, Medicine School of Shiraz, southern Iran. From each patient without any noticeable genito-urinary symptoms, 20 ml first voided urine specimens were collected and centrifuged. The deposits were put on the glass slides and examined microscopically for by observation of the jerky motility. PCR testing was performed on pellets using specific-primers. In a total of 354 (273 males, and 81 females) specimens, 135 (38.1%) cases 38.09% (104 males), 38.27% (31 females) tested positive for *T. vaginalis* by both microscopy and PCR methods. Of these, 12 (3.4%) cases were positive in both spun urine wet smears and PCR of urinary sediments, 123 (34.74%) positive in the PCR amplification but negative in the wet smear. None of PCR-negative samples were positive by wet mount microscopy. The age range of the women was 19–69 years and the highest percentage was in the 31–40 years age group among both males and females. There was no significant difference in the occurrence of *T. vaginalis* between the sexes ($P > 0.05$). Our survey suggests that trichomoniasis is a common but less well known sexually transmitted infection affecting HIV positive men and women, and the eradication of this infection is possible with mass public health education and the administration of specific therapeutic agents to patients with trichomoniasis and full treatment of trichomoniasis can help reduce the risk of HIV transmission.

1.2-016

High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey

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BACKGROUND Southern African countries bear an inordinate burden of the HIV/AIDS pandemic. Monitoring the epidemiology dynamics is critical to identify populations at greatest risk for the infection and to guide control strategies.

METHODS A cross-sectional community-based study to determine age and sex-specific HIV prevalence among individuals aged 18–47 years was carried out in Manhiça, southern Mozambique. Participants were randomly selected from the demographic surveillance system in place in the area and voluntary HIV counselling and testing was offered at the household level.

RESULTS A total of 841 individuals were invited to participate. Of them 722 were recruited (50.8% women). The overall HIV prevalence in the community was 40.30% (95% CI: 36.09–44.55). By age: 23.61% (95% CI 18.13–29.08) in individuals aged 18–27 years, 42.62% (95% CI 36.39–48.85) in those aged 28–37 years and 45.31% (95% CI 39.05–51.56) in the 38–47 year-old group. HIV prevalence was higher among women than men in all age groups. The overall HIV estimate for women in the community (43.07%; 95% CI 37.66–48.49) was 1.4-fold times higher than the average of those attending the antenatal clinic (29.35%; 95% CI: 26.70–32.00).

CONCLUSIONS The high HIV prevalence found in this region of Mozambique suggests a mature stable phase of the epidemic. The lower rates in the ANC as compared to those of the community alert that ANC evaluations may underestimate community HIV prevalence. Resources to monitor the HIV dynamics are needed to guide targeted control strategies in countries suffering the greatest toll of the epidemic.

1.2-017

Safe sex practice and health care seeking: sex-workers struggle for survival from HIV in Bangladesh

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BACKGROUND Sex workers and their clients in Bangladesh are playing an important role as vectors for STI/HIV transmission among the general population. This qualitative study on brothel based Female Sex Workers (FSWs) explored factors that influence safe sex practice and health care of FSWs within an integrated HIV intervention.

METHOD Focus group discussions, in-depth interviews and key informant interviews took place in four brothels. Young and older FSWs, Madams (employers of young FSWs), program managers and providers were the subjects.

RESULT Condom use was not consistent by types of FSWs – bonded and free young FSWs, elderly FSWs and their regular (Babu) and irregular clients. Bonded FSWs reported being maltreated by 'Madam' for refusing to have sex without condom. For 'Babus', FSWs needed to struggle more to convince on condom use, many FSWs also did not offer condom to 'Babus' because they were known as regular clients. The clinical services that included regular screening coupled with prevention messages did influence FSW's increased condom use and reduced STI symptoms. FSW's preference for the NGO clinic over a referral center was due to respectful attitude and services offered in locations that were closer to their brothels. However, limited clinical services were criticized,

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expanding services for maternal and child health from this outlet was desired. The key informants expressed concern that withdrawal of NGO services might limit FSWs' care seeking pattern.

CONCLUSION Future HIV prevention programs should provide more effort on 'Madams' and 'Babus' for promoting mutual support towards the FSWs to prevent HIV epidemic in Bangladesh. Continuing NGO services required long term donor's funding and such investments are critical. One direction would be to integrate the targeted interventions to reduce STI/HIV transmission through commercial sex in a national reproductive health effort of Bangladesh.

1.2-018

Implementation of diagnostic counselling and testing for HIV in tuberculosis patients: service user experience in a rural Tanzanian tuberculosis clinic

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OBJECTIVES To preliminarily assess the effectiveness of a policy change towards diagnostic counselling and testing, from voluntary HIV testing, in a rural Tanzanian tuberculosis (TB) clinic. In addition, to determine the knowledge of HIV and the experience of HIV testing and treatment in patients attending the clinic.

DESIGN Population-based cross sectional survey using questionnaires, carried out during August 2006.

SETTING Teule District Hospital Muheza, Tanzania. TB outpatient's clinic. Participants: 78 adult patients (≥18 years) attending for routine TB treatment. Main outcome measures: Rates of HIV testing at the clinic. Previous testing for HIV and current status. Level of knowledge of HIV and TB co-infection. Current medical treatment for HIV infections.

RESULTS A 98% (n = 53) uptake rate was found in patients offered an HIV test at the clinic; however only 68% (n = 78) of the group reported that they were given this opportunity. Co-infection rate was 53% (n = 64). No formal documentation of testing status and those who opted-out was performed. Testing for HIV within the clinic was having a direct positive impact on the number of patients aware of their HIV status and thus receiving medical treatment. Apprehension about sero-status, lack of knowledge of treatment, and fear of stigma were preventing people from being tested for HIV.

CONCLUSIONS The TB clinic successfully identifies patients who are co-infected with TB and HIV, enabling them to receive treatment. Further improvement is necessary to achieve the WHO aims of 100% testing. Service users were knowledgeable and receptive to testing. DCT coupled with integration of services at this hospital has a high chance of success and is likely to provide a model for others to follow in the future.

1.2-019

Validation of interferon gamma release assays (IGRA) on frozen peripheral blood mononuclear cells (PBMC)

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INTRODUCTION The aim of this study was to evaluate the effect of freezing PBMC on IGRA outcome. If valid, this would extend the possibilities for retrospective cohort studies.

METHODS Twenty-three volunteers (of whom three had no history of past, active or latent tuberculosis) were recruited for blood collection in Quantiferon (QFT) and heparin tubes. QFT and T-spot TB test (T-spot) were performed on fresh specimens according to manufacturer's guidelines. PBMC were isolated from heparin blood and stored in liquid nitrogen (vapour phase). After a minimum of 60 days PBMC were thawed, washed and resuspended before resubjecting them to QFT and T-spot. An analysis, by an automated plate reader, will be provided for discrepancies between two independent spot counts.

RESULTS Incubation of the QFT with fresh and frozen PBMC revealed 2/23 (9%) and 0/23 (0%) positive results, respectively. Nineteen (83%) samples showed a reproducible qualitative T-spot result (15 twice positive, four twice negative). Freezing PBMC with a mean of 132 (85–246) days resulted in an increasing number of aspecific spots (mean: 0.6^3 3.2) and a decrease of specific spots (mean: 29^3 19). Three of six initial negative samples became equivocal after freezing; one initially positive sample turned invalid. Duration of freezing (85–246 days) did not influence the effects.

CONCLUSION Qualitative results of T-spot on frozen PBMC are highly reproducible when compared to results of fresh specimens. We observed little loss of sensitivity but some loss of specificity because of spontaneous interferon gamma release.

1.2-020

Towards a sustained reduction of tuberculosis case fatality in La Habana, Cuba

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INTRODUCTION Tuberculosis (TB) mortality is an important impact indicator of TB programme performance. We assessed the level and potential determinants of TB case fatality in La Habana, to effect a further decline.

METHODS We conducted a retrospective study. Outcomes for the TB cases of cohorts 2006–2009, as notified by the TB program, were identified. Deaths from TB (DFT) were also extracted from the National Vital Statistics (VS) records. We reviewed clinical records, autopsy reports and conducted interviews with close relatives of dead TB patients. Data on the date of death, underlying causes, age, sex, type of TB, place of diagnosis, technique of diagnosis and HIV infection status were obtained. We performed bivariate and multivariate analyses.

RESULTS All TB patients registered at the TB program who died were included in the vs. records. Amongst 753 TB cases there were 95 (12.6%) TB related deaths. Fifty-eight of them (61%) were DFT and 44 of these were HIV (−) (76%). The crude TB case fatality rate (CFR) was 7.7%. Of the 58 DFT, 16 (28%) were diagnosed by autopsy and 29 (50%) died during the treatment, 19 (66%) within 60 days of diagnosis. Associated risk factors for DFT were HIV infection (RR = 1.8), age ≥65 years (RR = 7.2), being diagnosed in hospital in HIV (−) (RR = 13) and by clinical diagnosis in HIV (+) (RR = 3.2). Age (RR 1.06; 95% CI 1.04–1.08), HIV (+) (RR 3.3; 95% CI 2–10) and being diagnosis in primary hospital (RR 10; 95% CI 2.5–16.7) were statistically significant in multivariate analysis.

CONCLUSION The estimated TB CFR is low compared with figures reported worldwide. It could possibly still be improved if the

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diagnosis could be made earlier, particularly in elderly and HIV (+) patients.

KEYWORDS tuberculosis, mortality, case fatality, surveillance, Cuba

1.2-021

Access to care and quality of services for plwha in Benin: what options are viable and sustainable?

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Since 2002, Benin has adopted a global care approach for people living with HIV/AIDS (PLWHA). Healthcare services are delivered both by public and private as well as associative health centers. This communication analyzes the demand profile of three healthcare centers located in Cotonou, one public (CTA/CNHU) and two private (NGO RACINES and NGO Arc en ciel), in relation to delivery costs, quality of care and approach to medical care. The methodological approach is based on the comparison of data collected from the healthcare structures (03), patients (32) and prescribers (12) through questionnaires, guided interviews and participant observations. For cost analysis, a specific algorithm was designed that takes account of free ARVs, main activities and available statistics. The three studied centers reported strong demand. All active files exceed 1200 patients. However, that demand reflects different realities. At CTA/CNHU, the average cost of care is the highest against an average quality of service. But its care approach is non global. However, its attractiveness is linked to the nutritional support provided and to social networks. 'Arc-en-ciel' also offers partial support but virtually no direct cost and therefore increases access to car to poor PLWHA, with an average quality of service. At the NGO Racine, support is indeed comprehensive and extends to the family of PLWHAs at low cost. However, that centre denies entry of new people into its active file to ensure the quality and completeness of care to the initial registered patients. The patient flow towards the various centers is indicative of the social profile of patients and the degree of discrimination in the system. Universal access to quality services and care for PLWHA is primarily a question of approach, organization and policy option whose sustainability must be rethought.

KEYWORDS HIV/AIDS, universal access, cost, quality, Benin

1.2-022

Evaluation of an improved giemsa technique for staining *trichomonas* species

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BACKGROUND *Trichomonas vaginalis* is a cosmopolitan parasite, found in the reproductive tracts of both men and women all over the world. Wet mount remains the most frequently clinical test of infection though it has a sensitivity of only 50–60%. Due to the restrictions and problems in the diagnosis, particulary in the staining of *Trichomonas vaginalis* we sought to evaluate a useful procedure for staining clinical or cultured isolates of this flagellate.

METHODS Fresh clinical isolates of *T. vaginalis* from symptomatic women and from isolates were grown in TYI-S-33 Diamond's modified axenic medium. We compared the use of modified Giemsa, ordinary Giemsa and Gram stains on either axeniced or fresh sample of *T. vaginalis* and *T. galinarum*. Smears from each sediment of all isolates fixed by methanol and stained by both methods in various dye dilutions, temperatures and times.

RESULTS We determined that modified Giemsa stain, evaluated in this study, can readily distinguish the organelles such as nucleus as well as the cytoplasm and also the Vacuoles of the Trophozoite is clearly visible in the stained slides. All smears stained with 1/40 dilution and hot fixing revealed a better quality of staining than when stained with either ordinary Giemsa without hot fixing or with Gram stain.

CONCLUTION This study offers an appropriate method for staining *Trichomona* spp. The only limitation the procedure is that it takes a longer time of about 1 h for staining.

KEYWORDS *Trichomonas*, Giemsa, staining

1.2-023

Molecular detection of coccidiosis and microsporidiosis among HIV/AIDS patients with chronic diarrhea, Shiraz, Iran

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Most of the HIV-patients die of infections other than HIV. Diarrhea is one of the most common clinical manifestations in theses patients. The opportunistic protozoa causing HIV/AIDS-associated diarrhea are enteric *Coccidia* and *Microsporidia* spp. Among microsporidia infecting humans, *Enterocytozoon bieneusi* is the most common identified agent of chronic diarrhea. This study was done to determine the prevalence of these parasites in this particular group with diarrhea, CD4+ T-cell count and related risk factors. Stool specimens from 356 HIV-positive (83 females, and 273 males) patients 10–69 year old with diarrhea and/or history of diarrhea alternating with constipation were examined to screen oocysts of *Cryptosporidium, Isospora, Cyclospora*, and spores of *E. bieneusi*. Unpreserved fecal samples were collected and microscopically examined for cysts, ova, and larvae of non-opportunistic parasites using physiologic saline solution-ethyl acetate concentration technique. A modified acid fast-trichrome staining was used to observe oocysts and E. bieneusi spores. All fecal pellets were also investigated by known genus-specific primers in nested-PCR protocols. All PCR-positive samples were sequenced to confirm obtained results. Blood samples of patients were also analyzed for CD4 counts by flow cytometry. In this particular group, the most common opportunistic and non-opportunistic pathogens were *Cryptosporidium* spp., *E. bieneusi*, *Giardia lamblia*, and *Blastocystis homonis*; 34, 8, 23, and 14 cases respectively. *Isospora belli* and a *Cyclospora*-like parasite were found in two and one patient respectively. The only helminths Enterobius vermicularis (three cases), and *Hymenolepis nana* (one case) were observed. CD4 count <200 cells/μl was significantly associated with the presence of opportunistic parasites and diarrhea ($P < 0.05$). Opportunistic intestinal parasites should be suspected in any HIV/AIDS patient with chronic diarrhea. Also the importance of non-opportunistic enteric parasites among these subjects should not be neglected in Iran.

KEYWORDS coccidiosis, HIV/AIDS Patients, *Enterocytozoon bieneusi*

1.2-024

Literature review and qualitative study of HIV positive women's experiences of the prevention of mother to child transmission (PMTCT) of HIV program in West Java, Indonesia

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With nearly 230 million cases, Indonesia is facing one of the most rapidly growing HIV epidemic in South East Asia. The concen-

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trated epidemic used to be driven by people who engage in high risk behaviours: injected drugs users and those who practise unsafe sex with multiple partners, now begins to spreading to their lower risk sexual partners. A recent projection model shows that new infections from heterosexual transmission are predicted to rise and affect a significant proportion of low risk women. The latest official Ministry of Health publication announced that more women from this intimate/steady partner of keynotes population were reported as being infected with HIV. In West Java province solely, over 314,000 individuals are estimated being susceptible contracted the virus from their couple. Numbers of children infected by their HIV positive mothers at birth or through breastfeeding have been predicted to double within 6 years. Hence, primary prevention of HIV in women is the most sensible and effective means to prevent burden of paediatrics HIV in the future. Although approximately 10,000 mothers and infants are estimated to need HIV testing prophylaxis, <1% of them are covered by such services in Indonesia. Recently the Indonesian National AIDS Commission adopted PMTCT as part of the prevention program. No study in Indonesia has been published so far to explore experience among HIV positive mothers in PMTCT program. A better understanding to this topic will inform public health professional and clinical practitioners, how to improve the PMTCT program and therefore benefit both HIV positive pregnant women and their babies.

1.2-026

Position paper on the prevention and management of sexually transmitted infections (STIS) in immigrant populations in Italy

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INTRODUCTION Epidemiological and socio-behavioral risk factors make immigrants from low income countries a vulnerable population for STIs. In addition, access to healthcare services may be particularly limited for immigrants.

AIM To draft a consensus document on prevention and clinical management of STIs in migrants, to improve healthcare management of such populations in Italy.

METHODS The consensus document was prepared by a multidisciplinary group of experts in the field of dermatology, infectious diseases and migration medicine and it was based on systematic literature review.

RESULTS Data on STIs from the Surveillance System of the National Institute of Health show that immigrants represent 30% of notified cases but incidence or prevalence data cannot be generated. A few sporadic surveys identify three high risk groups for STIs among migrants: male transgender engaged in prostitution, female prostitutes and VFRs (visiting friends and relatives) returning to their country of birth.

CONCLUSIONS Integrated out-reach interventions are recommended for active tracing of male transgender and female prostitutes. Pre-travel advice on STI should be delivered to VFRs. Risk reduction interventions that address the socio-cultural characteristics specific of each migrant group and that actively involve the target population are recommended. No difference in the diagnostic and therapeutic algorithms is envisaged for migrants care; however, qualified intercultural mediators play a pivotal role in education and counselling and should be included as part of the healthcare team. Training of healthcare workers and appropriate information about health legislation for undocumented immigrants are needed to reduce barriers to healthcare access.

Project funded by the Italian Ministry of Health. †STIs and migrants study group: P. G. Calzavara Pinton, S. Pecorelli, R. Basché, V. Bergamaschi, P. R. Puzzi, A. C. Carvalho, I. El Hamad, A. Matteelli, M. Orobello, C. Benedetti, C. Fornabaio, O. Armignacco, A. Barelli, A. Beltrame, T. Bini, M. Busso, M. Cusini, S. Delmonte, F. De Seta, A. D'Antuono, M. Giuliani, B. Guerra, F. Lillo, G. Liuzzi, V. Martini, R. Murri, G. Orlando, C. Pajno, A. Rossi, C. Signorelli, MC. Salfa, F. Starace, G. Tempera, F. Vichi, A. Volpi, G. Zuccati.

1.2-027

Modified ziehl neelsen staining and prevalence of coccidian parasites in HIV patients with diarrhea; a series of 12 cases, Bukavu, D.R.Congo

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INTRODUCTION The purpose of this study is to determine the prevalence of coccidian parasites in HIV patients with diarrhea by using the modified ziehl neelsen staining.

MATERIALS AND METHODS We had 12 HIV positive patients presenting with diarrhea, we collected punctually the stools, conditioned them with formalin-ether 10% and kept at 4°C. Thin smears of stool were realized and stained according to the modified ziehl-neelsen method. Coccidian slides from CDC/Atlanta were used as for quality control. For statistical analysis we used Epi-info 3.5.

RESULTS 66.7% females vs. 33.3% males. The CD4 cells mean count was 289 ± 168/µl. Among patients with <200 CD4/µl, 60% (CI 95% 14.7–94.7%) of subjects were infected by more than one opportunistic digestive parasite, no case of polyparasitism was found in patients with >200 CD4/µl ($P < 0.05$). Prevalence of parasites found: cryptosporidium parvum 25% (CI 95% 5.5–57.2%), *Cyclospora cayetanensis* 16.7% (CI 95% 2.1–48.4%), *Mycobacterium tuberculosis* 8.3% (0.2–38.5%). All cases of cryptosporidium and cyclospora were found among patients with <200 CD4/µl, *Mycobacterium tuberculosis* in a patient with >200 CD4/µl ($P < 0.05$).

CONCLUSIONS In these preliminary results, we can note that cryptosporidium parvum is the major cause of diarrhea, followed by *Cyclospora cayetanensis*. Digestive opportunistic parasites are related to low CD4 cells counts in this study as well as cases of polyparasitism. The modified Ziehl–Neelsen staining is an easy-use technique.

1.2-028

Malnutrition among tb patients: is it alarming in Bangladesh

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OBJECTIVES To study the malnutrition situation among TB patients at different time periods of TB treatment, to study the influence of treatment on the anthropometric indicators of nutritional status, and to recommend possible ways to overcome the situation.

METHODS A total of 1068 TB patients and 910 healthy controls (HC) were included in this study. The TB patients were categorized into three groups: before starting treatment (BST), after 2 months of treatment (2MOT), and after completion of treatment (ACT). Height, weight, and mid upper arm circumference (MUAC) were taken at registration, 2 months, and 6 months of TB treatment of same patients. The index of nutritional status like body mass index (BMI) was computed.

RESULTS The percentages of chronic energy deficiency (CED) based on body mass index (BMI < 18.5 kg/m^2) for the different

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treatment periods were 67.1% (BST), 58.9% (2MOT), 49.9% (ACT), and 22.9% (HC) respectively. The CED based on mid upper arm circumference (MUAC < 22.0 cm) was 42.2% (BST), 38.9% (2MOT), 33.5 (ACT) and 8.6% (HC) respectively. Based on the World Health Organization BMI classification, the prevalence of CED (BMI < 18.5 kg/m^2) among tuberculosis patients in the different stages of treatment was from high to very high indicating a critical situation. Qualitative findings revealed that poverty, frequent changes of food prices, dependency on other members of family acted as main barriers in accessing adequate nutritional care. Support from local food providers, social fund, health workers, and family members could play an important role in improving nutritional status of patients rather than government initiative/intervention.

CONCLUSIONS Half of the TB patients were malnourished after completion of treatment. Regular intake of medicines could improve nutritional status of patients. However, the findings can be used to advance the argument for nutritional support urgently for TB patients in Bangladesh.

1.2-029

Acceptance to human immunodeficiency (HIV) test among people with risks of hiv infection: an urgency to prioritize young men

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AIM The study aimed (1) to estimate proportion of people with risks of Human Immunodeficiency Virus (HIV) infection accepted HIV test within Voluntary Conseling and Testing (VCT) program, and (2) to analyse determinant factors to the acceptance to HIV test.

METHODS We used cross-sectional study design. The sample was all people with risk of HIV infections at Surakarta municipality, particularly Injected Drug Users who were involved in activities under a Non Governmental Organization and patients who were referred to a public VCT clinic during 1 June to 30 July 2010 (n = 78 respondents). Data were collected through self-administered questionnaires. Fisher Exact test, t-test and logistic regression were used in data analysis.

RESULTS 80.7% of people with risk of HIV infection accepted the HIV testing. There was a statistical significant difference of acceptance between men and women (P = 0.01). Younger people were likely to refuse HIV test (P = 0.01). Being afraid of HIV test procedures was significantly associated with acceptance to HIV test (P = 0.03).

CONCLUSION Younger men tend to reject HIV test offers. Interpersonal approach is needed to provide knowledge on HIV test procedures to people at risk of HIV infection, particularly young men.

1.2-030

Home-based voluntary HIV counseling and testing yields very high uptake compared to clinic-based: results from a cluster-randomized trial in Zambia

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INTRODUCTION Voluntary HIV counseling and (VCT) is promoted as essential for access to prevention, treatment and care for HIV. Although clinic-based VCT has been scaled up, uptake remains low. Innovative approaches which are locally acceptable are strongly needed. The objective of this study was to compare uptake and acceptability of home-based VCT to standard VCT (ISRCTN53353725).

METHODS AND MATERIAL Thirty-six rural clusters were pair-matched based on baseline data and randomly assigned to the intervention and the control arm. All adults in the intervention clusters were offered VCT in their homes by local lay counsellors trained in the home-based approach and in proper handling of the process of consent. Control clusters had access to standard clinic-based VCT. Six months after the intervention a follow-up survey was conducted. Community mobilization was performed at each stage to inform about the study.

RESULTS In the intervention arm 88% accepted counselling, testing and receipt of test result in their homes, and 20% received counselling only. Sixty-three percent of couples were counselled together. From baseline to follow-up the proportion reporting being tested (anywhere except PMTCT) during the previous year increased in the intervention arm from 18% to 74% among women and from 21% to 71% among men; and from 15% to 41% among women, and from 20% to 34% among men in the control clusters. At baseline the likelihood of being tested increased by level of education, but in the follow-up this inequality was restricted to the control clusters. HIV prevalence among tested was 9.1% in men and 9.5% in women.

CONCLUSION Home-based VCT using lay counsellors had an exceptionally high acceptability and uptake compared to clinic-based VCT, and levelled out social inequalities in access. The high level of couple counselling represents an important opportunity to reduce horizontal and vertical HIV transmission.

1.2-031

Reduction in HIV-related stigma: findings from a cluster-randomized trial in Zambia

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INTRODUCTION HIV-related stigma is a barrier to testing, care, treatment and prevention. This study aimed to investigate changes in HIV-related stigma after a cluster-randomized trial on the uptake of home-based voluntary HIV counseling and testing (VCT) in Monze district in Zambia (ISRCTN53353725).

METHODS AND MATERIAL The trial was conducted in 36 rural clusters, which were pair-matched based on baseline data. Baseline and follow-up surveys (6–12 months before and 6 months after intervention) contained eight Likert-scaled stigma questions. Factor analysis yielded two factors: 'equality' and 'perceived discrimination'. Changes in the two factors and overall stigma were investigated. Linear regression was performed with changes in overall stigma as the dependent variable. Non-self proportions were used as a measure for community stigma. Social mobilisation at various stages was conducted at various stages through different channels.

RESULTS There was a significant decrease in overall stigma (P < 0.001) in both the intervention and control arms. In both arms the mean score for 'perceived discrimination' was higher than for 'equality stigma', but there was a larger decrease in 'equality stigma' (control P = 0.035, intervention P = 0.014). In the intervention arm high baseline stigma and being tested in the previous year were independently associated with a decrease in overall stigma. In the control arm, high baseline stigma and readiness to be tested were associated with decreased stigma, whereas worries about being HIV-infected and high community levels of 'equity stigma' were associated with increased stigma.

CONCLUSION Being tested in the past year was associated with reduced stigma in the intervention arm, but the reduction might

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not be attributed to exposure to home-based VCT alone since there were reductions in both arms. However, substantial social mobilisation might have contributed in both arms. The stigma reduction in both arms might also be indicative of on-going social processes, and increased social desirability bias cannot be excluded.

1.2-032

Knowledge, attitudes and practices on HIV/AIDS, and HIV prevalence in the city of Sucre, Bolivia

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BACKGROUND Describe and analyze knowledge, attitudes and sexual practices (KAP) for HIV/AIDS and estimate HIV prevalence in Sucre's residents, Bolivia.

METHODS Cross sectional study applying KAP population-based survey. Residents between 15 and 49 years old were randomly selected from all districts of Sucre 2008–2009. Blood samples were collected in Whatman filter paper, processed in the National Centre of Microbiology of Spain by ELISA method Genscreen HIV-1/2. Descriptive analyses of socio-demographiccharacteristics and indicators based on UNAIDS as lack of HIV/AIDS knowledge, sexual risk practices and discrimination attitudes against people living with HIV (PLHIV) were performed. Multiple logistic regression was used to study associations between response and exposure variables.

RESULTS Of 1499 residents, 885 (59%) were women. Unknowledge of HIV/AIDS transmission and prevention was observed in 67% of respondents, 69% in women vs. 65% in men. Associated risk factors were: rural residence vs. urban (ref) OR = 7.3 (95% CI 2.6; 21.0) in men and OR = 3.9 (95% CI 1.9; 8.29) in women, speaking native language vs. only spanish (ref OR = 1.6 (95% CI 1.1; 2.3) in men and OR = 1.4 (95% CI 1.0; 1.9) in women. Only in women: low vs. medium/high education level (ref) OR = 7.8 (95% CI 3.1; 19.7) and low vs. high income (ref) OR = 2.0 (95% CI 1.4–2.7). Discrimination attitudes were seen in 85.1% Associated factors: residence (rural) OR = 2.9 (95% CI 1.4; 5.0) low educational and economic level OR = 2.1 (95% CI 1.1; 4.2) and OR = 2.0 (95% CI 1.4; 2.7). Unsafe sex practices were observed in 10%; men 17% and women 5%. Associated factors in urban areas: female vs. male (ref) OR = 0.3 (95% CI 0.2; 0.4), age 25–49 vs. 15–24 (ref) OR = 0.4 (95% CI 0.2; 0.6) and marital status (lived in pair) OR = 2.9 (95% CI 1.4; 6.1). HIV prevalence was 0.0% (95% CI 0.0; 0.4%).

CONCLUSION In spite of low prevalence of HIV infection, there is little knowledge, frequent discriminatory attitudes and sexual risk practices. The identification of these indicators is useful to improve health interventions.

1.2-033

Evaluation of diagnosis and treatment of tuberculosis in a rural setting in Angola

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BACKGROUND Tuberculosis (TBC) is one of the most prevalent infections in Angola and the main cause of hospital admission in adults. The lack of diagnostic and therapeutic tools in Africa makes it difficult its diagnosis, treatment and follow-up. The aim of this study is to evaluate the efficacy of the national treatment protocol for TBC in a rural setting in Angola.

METHODS Observational study of all cases of TBC diagnosed between October 2010 and May 2011 in the Hospital Nossa Senhora da Paz de Cubal, a regional hospital in Angola. Diagnosis of TBC was based on direct exam of clinical samples (Ziehl Nielssen stain). Culture was not done in any case. A clinical diagnosis of TBC was made in patients with a consistent clinical picture despite the absence of microbiological diagnosis. All patients were treated with rifampin, isoniazid, ethambutol and pirazinamide for the first 2 months. Evaluation of the treatment efficacy was analyzed only in patients with positive baciloscopy. We attempted to evaluate the efficacy by the proportion of patients who had a negative exam in sputum at 2 months after having started treatment.

RESULTS Five hundred and nine patients with TBC were diagnosed. 95.1% had pulmonary TBC. A microbiological diagnosis was obtained in 336 (66%) of patients. The remaining 173 (33%) were diagnosed on a clinical basis. Thirty-five (6.9%) patients had HIV co-infection. At 2 months 64.9% of patients with an initial positive bacilloscopy in sputum became negative. In 17.8% a sputum sample could not be obtained and in 17.4% of patients, baciloscopy in sputum remained positive.

CONCLUSION Despite the lack of diagnostic tools the proportion of patients with microbiological diagnosis is relatively high. The number of patients with positive bacilloscopy at 2 months is a matter of concern that necessitates tools to detect resistance to antituberculous drugs.

1.2-034

Western blot method as supplemental tool for syphilis diagnosis and suggesting possible clinical phases of the disease in recently HIV infected patients

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HIV persists as public health issue worldwide. The increasing incidence of the disease is caused by sexual promiscuity, drug abuse, poverty, increased population migration. As HIV disease and syphilis are both sexually transmitted infections, co-infected patients are commonly observed. The aim of this study was to evaluate a well characterized cohort of recently HIV infected patients the incidence of syphilis and according with the patterns of IgG response to define protein bands by Western Blot in view to discuss possible phase of the disease.

METHODS AND MATERIAL We performed FTA-Abs, TPHA and Western blot (WB Tp-IgG) and VDRL in 156 sera samples from a cohort of recently infected HIV-1 patients. WB Tp-IgG was performed as describe by Lemos et al. (2007) and the other tests were performed according to the manufacture instructions.

RESULTS Using VDRL as screening test 30 sera samples from 156 were reactive. From these 25 were also reactive for TPHA and FTA-Abs. Of five sera samples, two samples were reactive for TPHA, one sample for FTA-Abs and two samples were considered as false positive. Treponemal tests were more reactive than VDRL (WB TP-IgG 37; FTAabs 32; TPHA 36), related to the sensibility and specificity of each assay or to the presence of IgG antibodies, with no clinical value, after the treatment. According to Lemos et al. (2007) criteria five patients had primary infection, five had secondary syphilis; 15 early latent syphilis, 11 patients late latent syphilis and one had tertiary syphilis.

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CONCLUSIONS The combination of different tests for syphilis diagnosis is important in this population to assure discrimination between infected and non infected patients. Serological patterns are important for the treatment and the follow up of patients

1.2-035

Missed opportunities to accessing HIV testing and antiretroviral therapy during routine patient-provider encounters in Sub-Saharan Africa: a systematic review of the evidence

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BRIEF INTRODUCTION The routine offer of HIV testing during patient-provider encounters is becoming a pivotal component of HIV treatment and prevention programs. The aim of this review was to examine the implementation of Provider-initiated Counselling and Testing (PICT) programmes in Sub-Saharan Africa (SSA). We systematically searched the data bases Pubmed, Embase, GlobaL Health, the Cochrane Library and Jstor. Grey literature was explored through the websites of international and non-governmental organizations. Eligibility of studies was based on systematic screening of abstracts and full texts by two researchers.

RESULTS We retained 45 studies of the 5.088 references retrieved. While many SSA counties have issued PICT polices the translation of policy guidance into practice has had mixed results. The challenges encountered encompass a wide range of areas from logistics, to data systems, human resources, management and coordination, reflecting some of the weaknesses of health systems in the SSA region. PICT approaches are potentially effective at identifying large numbers of previously undiagnosed HIV+ individuals but often fall short at linking them to follow-up assessments and antiretroviral treatment. The acceptance of the offer of the test varied widely across sites but the determinants of uptake remained largely unexplored. The behavioural outcome of PICT polices was rarely addressed and the extent to which the approach was particularly well-suited to identify HIV+ cases at earliest stages of infection remained unclear.

CONCLUSIONS Without additional investments, opportunities to identify HIV+ individuals who enter in contact with the health care system, and to allow them to successfully access the further HIV-related care and treatment that they require, will continue to be lost. As programs are underway and data become available, operational research using quantitative and qualitative tools is required to inform the development and adaptation of PICT programmes on an ongoing basis.

1.2-036

Differential characteristics of syphilis and gonococcal infection by migration status in Catalonia

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INTRODUCTION The aim of the study is to describe the characteristics of syphilis and gonococcal infections cases by origin status in Catalonia.

METHODS In Catalonia, nominal mandatory notification of syphilis and gonoccocal infection started in 2007. STI reports are registered at the Centre for Epidemiological Studies on Sexual Transmissible Infections and HIV/AIDS of Catalonia (CEEIS-CAT). This is a retrospective, descriptive study of all new cases of syphilis (infectious and undeterminate) and gonoccocal infection reported to the CEEISCAT between 1st January 2008 and 31st December 2010.

RESULTS A total of 1176 syphilis and 1267 gonoccocal infection were reported. Fourty-two percent of all cases (2443) were born in Spain, 26% were from Latin America-Caribbean, 9.5% from Western Europe and 6.3% from North Africa. 86.6% were men. Foreign origin participants were younger than the Spanish patients, for gonococcal infections were significantly more common among men ($P = 0.05$). Immigrants had a lower educational level ($P = 0.005$). Forty seven (4%) of those diagnosed with gonococcal infection and 25 (2.1%) with syphilis reported having practiced commercial sex in the last year. Of these, 40 of gonococcal cases and 21 of syphilis cases were immigrants. No differences were found in HIV co-infection. Immigrants with syphilis reported more previous STI in the last year ($P = 0.003$), more use of prostitution in the last year ($P \leq 0.001$) and were less likely to have used condoms during the last sexual contact ($P \leq 0.001$) than Spanish patients.

CONCLUSIONS The study of populations at higher risk, such as immigrants, is essential for understanding the epidemiology of STI in countries with high immigrant flow and appropriately targeting public health actions.

1.2-037

Progress toward millennium development goal 6 in global fund-supported HIV/AIDS programs

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INTRODUCTION HIV/AIDS programs co-financed by the Global Fund were providing antiretroviral treatment (ART) to three million people, and had distributed 2.7 billion condoms among other prevention services in low- and middle-income countries by end-2010.

METHODS We evaluated progress of countries with HIV grants toward Millennium Development Goal (MDG) 6. Assessments use UNAIDS estimates of ART coverage (people currently on ART, as proportion of need according to WHO's 2010 eligibility criteria), HIV incidence and HIV deaths for 117 low- and middle-income countries based on the Epidemiology Projection Package/Spectrum modelling.

RESULTS ART coverage increased from 7% (2000) to 35% (2009) among the 20 countries that received the largest cumulative HIV disbursements, from 13% to 36% in 79 other countries with HIV grants and from 47% to 55% in nine low- and middle-income countries without HIV grants. HIV incidence decreased from 139 to 88 per 100,000 person-years in the 20 largest-disbursement countries, and from 77 to 59 in other supported countries. HIV mortality peaked around 2004–2005, with the steepest subsequent decline apparent in the top-20 countries. ART coverage increases and HIV incidence and death reductions were also larger in the 20 countries with highest per-capita HIV support, compared to other countries with or without HIV support.

CONCLUSIONS Global Fund HIV financing is concentrated in those countries with highest need, i.e. with initially the lowest ART coverage and highest HIV incidence and deaths rates. HIV grants are associated with increasing progress towards MDG 6 targets, which may reflect that grants accelerate progress, and/or that good implementation capacity and program progress are pre-conditions for ongoing, performance-based financing. To reach universal

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access to ART by 2015, treatment roll-out must be accelerated, especially in sub-Saharan Africa. Mortality trend estimates will benefit from improved measurement of patient retention and survival on ART, and of population-level mortality.

1.2-038

Retention in care of children and adolescents in an HIV programme in Kampala, Uganda: the impact of a multifaceted approach

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BACKGROUND The rapid scaling up of ART in resource-limited settings brings some relief however; retention in care of children and adolescents remains a major challenge requiring innovation and creativity to deal with. Currently about 64% of the accredited ART sites in Uganda provide HIV paediatric services, but, little is known about the retention in care of children and adolescents in such programmes. Our objective in this paper was to evaluate the impact of Antiretroviral Therapy (ART), home visits and psychosocial support services including quarterly counselling workshops on retention in care of a cohort of HIV-infected children and adolescents in Kampala, Uganda.

METHODS From 2003 to date, HIV-infected infants and children from age 0 to 14 years are enrolled into the 'Tukula Fenna' Project of the Home Care department of Nsambya Hospital to receive standard HIV care. The first child in this cohort was enrolled during the last quarter of 2003, and all children were followed up until 30 September 2010, excluding deaths. Overtime, several interventions were introduced to this cohort: the ART programme was introduced during the first quarter of 2005; psychosocial support workshops were introduced during the first quarter of 2006 and home visiting commenced during the first quarter of 2007. The intention of this analysis was to assess the independent impact of these complementary interventions on the levels of retention in care, which has been defined as 'still in care 1 year after recruitment'. This was achieved by constructing quarterly time-series segmented autoregressive error models with time-dependent disturbances.

RESULTS Overall, 1181 children were included in this analysis; 588 (50.5%) were female, 184 (15.8%) orphans and 538 (46.2%) were on therapy by the date of assessment. The median (IQR) age at enrolment was 65.0 (19.0, 116.5) months and all stayed an average of 4.1 ± 2.1 years in care. Over time, the overall levels of children who were retained in care 1 year after enrolment was 86.7%. Introducing the ART programme was independently associated with a significant 28.6 [95% Confidence Interval (CI): 20.9–36.3] mean percentage increase in the levels of retention in care. Psychosocial support workshops were independently associated with a significant 13.4 (95% CI: 5.1–21.7) mean percentage increase in the levels of retention in care, whereas home visits were independently associated with a significant 16.5 (95% CI: 7.8–25.2) mean percentage increase in the levels of retention in care.

CONCLUSION The multifaceted approach adopted by the project did not only provide some practical solutions to some of the important challenges faced by clients while accessing care, but also generated potent synergistic effects from the complementary interventions which led to an appreciable retention in care 1 year after recruitment.

1.2-039

Seroprevalence of HIV, HBV, HCV, HTLV and *Treponema pallidum* among patients in a rural hospital in southern Ethiopia

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INTRODUCTION Human immunodeficiency virus type 1 (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic virus type 1 (HTLV and syphilis represent major public health problems in sub-Saharan countries. Ethiopia is among the countries where HIV-1 and HBV infections are highly prevalent. However, information on seroprevalence of these infections among rural care attendees is very scarce and the majority of studies have been conducted in patients from urban areas.

OBJECTIVE To determine the seroprevalence of HIV, HBV, HBC, HTLV and syphilis infections among patients attended in at rural hospital in southern Ethiopia.

METHODS A cross-sectional study was conducted among consecutive patients in whom a screening of HIV was performed provided by health assistant attending in outpatient clinic during July 2010.

RESULTS A total of 568 patients were included. The seroprevalence of HIV-1 was 2.5% [95% confidence intervals (CI): 1.4–4.2%] (n = 14). HBsAg seropositivity was 4.6% (95% CI: 3.1–6.7%) (n = 26), all cases were HBeAg negative. Past infection of HBV (Anti HBc seropositivity with HBsAg seronegativity) was detected in eight patients (prevalence: 1.4%; 95 CI: 0.7–2.9%). The seroprevalence of HCV was 0.2% (95% CI: 0.7–2.9%) (n = 1). No co-infection of HIV and HBsAg was found. No cases of HTLV infection and syphilis were found (95% CI: 0–0.7%).

CONCLUSIONS A percentage of patients of whom a provider-Initiated HIV counselling and testing from rural areas harbour HIV, and notably, HBV infections. No cases of HTLV-1 infection and syphilis were found. Continuing efforts to comprehensive screening for patients provider-Initiated HIV counselling and testing are necessary

1.2-040

Trabajadoras sexuales en la frontera sur de méxico: violencia y VIH/SIDA

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INTRODUCCIÓN En la frontera México-Guatemala, la violencia es un problema de grandes dimensiones que afecta a la población local y migrante, entre los cuales figuran las mujeres trabajadoras sexuales (MTS). Ellas enfrentan el estigma y discriminación, viven situaciones de violencia que incrementan su condición de vulnerabilidad al VIH/ITS, y ejercen el trabajo sexual en condiciones desprotección.

OBJETIVO Analizar la condición de vulnerabilidad a la violencia sexual y el VIH/ITS en MTS mexicanas y extranjeras. Se esperaría que ambos grupos presenten un perfil socio-demográfico semejante, pero que la prevalencia de violencia sexual se relacione con el estatus migratorio indocumentado.

MATERIALES Y MÉTODO Entre 2009 y 2010, se realizó una encuesta en una muestra no aleatoria de 425 MTS de las cuales 31.5% son mexicanas y el resto centroamericanas. Se realizó un análisis descriptivo para conocer la distribución de la violencia sexual en ambos grupos.

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RESULTADOS No hay diferencias estadísticamente significativas en las variables socio-demográficas de las MTS. La media de edad es de 28 años, con un promedio de escolaridad de 7 años, 80% son madres solteras (2.2 hijos por mujer). La frecuencia en el uso del condón con cliente nuevo en MTS mexicanas fue de 72% mientras que en las extranjeras fue de 66% ($P < 0.05$). El 24.5% de las MTS reportó haber sufrido violencia psicológica, 21.4% violencia física y 10% violencia sexual, sin diferencias estadísticamente significativas entre mexicanas y extranjeras. El 47% de las extranjeras reportan haber sido obligadas a tener relaciones sexuales como una forma de sobrevivencia o protección.

CONCLUSIÓN Se requiere fortalecer las iniciativas que incorporen la prevención y atención de la violencia sexual y del VIH/SIDA de manera articulada, con sistemas de identificación y referencia adecuados a las especificidades de las trabajadoras sexuales mexicanas y migrantes.

1.2-041

HIV testing status of family members of art clinic attendants in public hospitals of addis Ababa, Ethiopia

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BACKGROUND Family members of ART clinic attendants are at risk of infection for HIV infection. Furthermore, being a family disease, care of HIV/AIDS needs a family centered approach with chronic care model. Family centered care has a potential to improve adherence to ART and quality of life. Prevention of HIV transmission and identification of infected family members is another task of family centered care. To this effect, HIV counseling and testing of undiagnosed family members of ART clinic attendants is a corner stone of family centered care.

OBJECTIVE To assess HIV testing status of family members of ART clinic attendants.

METHOD A cross-sectional facility based study carried out between 24 February and 5 April 2011 using structured, interviewer administered questionnaire among ART clinic attendants of three hospitals in Addis Ababa (Zeditu, Yekatit and Menillik hospitals).

RESULT Of 415 (98.8%) respondents who have spouses, 330 (79.5%) said that their spouse knew his/her HIV status, 85 (20.5%) said their spouse did not. Of 411 reported children <18 years of age, 230 (56%) were reportedly tested for HIV and the rest 185 (44%) were not. Non-disclosure rate to spouse/partner was 12.5%, discordant couple rate was 38.5%.

CONCLUSION AND RECOMMENDATION There are considerable numbers of family members of ART clinic attendants, especially children, who are not tested for HIV. Therefore it is recommended that attention should be given to those segments at risk. Counseling service to clinic attendants needs to be strengthened to access their untested family members.

1.2-042

Outcome of severe AIDS-related kaposi's sarcoma treated with two-weekly bleomycin/vincristine and antiretroviral treatment in the district Hospital of Buhera, Zimbabwe

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INTRODUCTION Literature about outcomes of severe Kaposi's sarcoma (KS) treated with Highly Active Antiretroviral Therapy (HAART) and chemotherapy is scarce, especially for the chemotherapy regimens presently used in resource limited settings. We assessed the toxicity and effectiveness of HAART with Bleomycin-Vincristine, the treatment currently used for severe HIV-related KS in the District Hospital of Buhera, Zimbabwe.

METHODS Between June 2009 and December 2010, we included prospectively all adult HAART-naïve (or on HAART for <3 months) and chemotherapy-naïve HIV patients presenting with severe KS (tumor stage T1) at the District Hospital of Buhera. Six cycles of intravenous Bleomycin 15 mg and Vincristine 2 mg were administered two-weekly and, if not yet given, HAART (NNRTI-based) was initiated within 2–8 weeks. Patients were followed till 6 months post-chemotherapy and treatment outcomes (AIDS Clinical Trials Group criteria) were documented.

RESULTS Thirty-one eligible patients were enrolled, including one KS-immune reconstitution inflammatory syndrome (KS-IRIS). The male:female ratio was 0.55:1, and median age was 34 years (IQR 30–42). Pretreatment median CD4 count was 206 (IQR 135–330). Six (19.4%) patients presented concurrent tuberculosis. At inclusion, 77.4% presented extensive mucocutaneous KS, 67.7% had KS-associated edema and 38.7% had suspected visceral involvement. The overall response rate at month 6 post-chemotherapy was 19.2% (five of 26): one complete and four partial responses. Fourteen (53.8%) patients had an unfavorable outcome: nine KS relapses and five deaths prior to the 6 month follow-up visit. Definitive outcomes could not be determined (yet) for 12 patients: five still in follow-up period and seven lost for follow up (now actively traced). Side effects, mainly peripheral neuropathy ($n = 8$), were minimal and tolerable. One patient developed KS-IRIS during treatment.

CONCLUSIONS The treatment of HIV-related advanced Kaposi's sarcoma with HAART and six cycles of Bleomycin-Vincristine was rather well tolerated but showed poor outcomes with high relapse and mortality rates.

1.2-043

Short-term tolerability of doxorubicin-based chemotherapy in patients with advanced hiv-related Kaposi's sarcoma in the provincial Hospital of Tete, Mozambique

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INTRODUCTION Conventional anthracyclines, mostly in combination with Bleomycin (B) and/or Vincristine (V), belong to the commonly used cytotoxic agents against advanced Kaposi's sarcoma (KS) in resource limited settings. Anthracycline-related toxicity remains however a significant concern. We aimed at evaluating the short-term tolerability of Doxorubicin-based chemotherapy combined with HAART, the recommended treatment for severe HIV-related KS in Mozambique.

METHODS From January to November 2010, we included prospectively all adult HAART-naïve (or on HAART for <3 months) and chemotherapy-naïve HIV patients presenting with severe KS (tumor stage T1) at the Provincial Hospital of Tete. Three-weekly intravenous Doxorubicin (D) 40 mg/m^2 was co-administered with Vincristine 2 mg and, if not yet given, HAART (NNRTI-based) was initiated within 2–8 weeks. Adverse events were evaluated using the Division of AIDS (DAIDS) adverse events grading table (2004).

RESULTS Twenty-seven patients with severe KS were enrolled. The male:female ratio was 1.8:1, and median age was 34 years. At enrolment, median hemoglobin was 11 g/dl (IQR 9.2–12), four (14.8%) had a CD4 < 100 and seven (26%) had concurrent tuberculosis. Extensive KS-associated edema (86%), mucosal involvement (66%), ulceration (34%) and lymph node involve-

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ment (41%) were common. By November 2010, 13 patients had received four to six cycles of DV, 10 two to three cycles and four only one cycle. Early toxicity was as follows: anemia (grade 3), 15%; neutropenia (grade 3), 26%; peripheral neuropathy (grade 2), 33%; alopecia (grade 2), 30%; and nausea and vomiting (grade 2) despite premedication, 26%. Nine (33%) patients reported palmar hyperpigmentation. One patient died in hospital with severe anemia possibly aggravated by chemotherapy.

CONCLUSIONS Treatment of HIV-related advanced Kaposi's sarcoma with HAART and 3-weekly cycles of Doxorubicin-Vincristine was associated with considerable hematologic and non-hematologic toxicity. Clinical and laboratory evolution needs to be strictly monitored, seriously limiting the use of doxorubicin-based regimens in peripheral, less equipped health facilities.

1.2-044

Prevalence and spectrum of ophthalmologic manifestations in HIV patients in Kumasi, Ghana

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BRIEF INTRODUCTION Ocular complications significantly contribute to HIV-associated morbidity in resource-poor countries. We investigated the prevalence and spectrum of ocular symptoms and pathologies among HIV patients in Ghana.

METHODS AND MATERIALS HIV patients attending the HIV outpatient department of a university teaching hospital in Kumasi, Ghana, were recruited for the study and underwent complete eye examination, including visual acuity (Snellen chart, E chart), slit lamp examination of the anterior segment, applanation tonometry, and dilated fundus examination with indirect ophthalmoscopy. Patients were also interviewed on ocular symptoms.

RESULTS A total of 105 patients (27% male, mean age 43 years) were recruited. CD4 cell counts were 200/μl in 30% and 100/μl in 10% of patients. Visual acuity (binocular) was <6/18 in 14%, <6/60 in 11% and <3/60 in 6% of patients. Two patients had no perception of light in one eye. The commonest complaints reported by patients were itchy eye (27%) and visual disturbances (29%). None of the patients had previously presented to the eye clinic. On ophthalmologic examination, 41% of patients had some pathological finding; 16% presented with anterior segment or neurophthalmic findings, and 25% presented with posterior segment signs. Most important anterior segment findings were corneal scarring after herpes keratitis in 6%. Most important posterior segment conditions were toxoplasmic retinochorioiditis (7%), signs of HIV retinal microvasculopathy (7%), dense vitreous opacities (6%), and chorioretinal scars of unknown cause (4%). Only one patient had retinal scarring similar to post-CMV retinopathy.

CONCLUSIONS HIV-related ocular manifestations are common in HIV patients in Kumasi, Ghana. Ocular symptoms are equally common, but patients usually do not report to the eye clinic. If routine ophthalmologic screening is not feasible, HIV patients should be interviewed for ocular symptoms and undergo visual acuity screening. Those with symptoms or reduced vision should be referred for ophthalmological examination to detect treatable pathologies, such as toxoplasmic retinochorioiditis.

1.2-045

No evidence of persistent or chronic hepatitis E virus infection in HIV patients in Ghana

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INTRODUCTION Until 2008, hepatitis E virus (HEV) was associated only with acute infections. Recently, it has been shown that hepatitis E virus (HEV) can also cause chronic infections in immunosuppressed patients. Data on chronic HEV infections in HIV patients is scarce, but prolonged courses of HEV infection in HIV patients, and two cases of chronic HEV infection in HIV patients have been published. No data exist on persistent or chronic HEV infections in HIV patients in Sub Saharan Africa, where HEV is highly prevalent, and most HIV patients present with severe immunosuppression.

METHODS AND MATERIALS Plasma from 830 HIV-infected patients attending the outpatient HIV clinic at the Komfo Anokye Teaching Hospital in Kumasi, Ghana, was collected. Clinical and biochemical data were extracted from medical files. Testing for HEV RNA was conducted by RealStar® HEV RT-PCR Kit 1.0 (Astra Diagnostics, Hamburg, Germany) after nucleic acid preparation using the QIAamp®MinElute® Virus Spin kit (Qiagen, Hilden, Germany).

RESULTS Of the 830 patients, 594 (72%) were female and mean age was 39.8 ± 9.7 years. CD4 counts were available for 702 patients, mean CD4 count was 381 ± 237 cells/μl. CD4 cells were <200/μl in 144 patients and <100/μl in 61 patients. 383 (46%) patients were on antiretroviral therapy at the time of recruitment. Serum transaminases were available for 421 patients, of which 57 patients (14%) had elevated AST levels, and 67 patients (16%) had elevated ALT levels. HEV RNA was not detected in any of the 830 serum samples.

CONCLUSIONS There was no evidence of acute or persistent/chronic HEV infection in the studied HIV patients in Ghana. The study population comprised patients with severe immunosuppression, and elevations of serum transaminases were common. We conclude that hepatitis E virus infection does not play a role in liver pathology in HIV patients in Ghana.

1.2-046

Progressive disseminated histoplasmosis (PDH) in an art naïve migrant with advanced infection

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INTRODUCTION PDH is an AIDS defining condition rarely reported in Europe. Given the unavailability of the urinary specific antigen test in Europe, the diagnosis can be achieved by histology or culture isolation.

CASE REPORT On December 2010 a 35 year-old Ecuadorian man was admitted to the Infectious and Tropical Diseases Unit, Careggi Hospital, Florence, Italy, with a 1 month history of fever, weight loss and cough. Physical examination was unremarkable, except for fever (39°C). Blood tests showed AST 196 UI/ml, ALT 129 UI/ml, LDH 1.681 UI/ml, CRP 108 mg/dl, ferritin >16.000 ng/ml. Chest CT-scan showed a medium lobe pseudonodular infiltrate, bilateral ground glass areas and 3 cm mediastinum adenopathies.

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At abdominal ultrasound presence of splenomegaly was reported. HIV1–2 tested positive (CD4 31/mm^3). Hepatitis markers, Quantiferon, TST, sputum for mycobacteria and blood cultures tested negative. ART was started with empirical first line antitubercular treatment although it was stopped 2 days later for hepatotoxicity and replaced with a regimen including amikacin, ethambutol and rifabutin without improvement. Further investigations included bone marrow and liver biopsy, bronchoscopy, test for serum galactomannan antigen (AGM) and antibodies for H. capsulatum (Hc) and T. cruzi. Fifteen days after admission, the unexpected positivity of AGM (DO 4.49) prompted to start treatment with liposomal amphotericin-B (4 mg/kg/day) following by recovery after within 5 days. Few days later, liver and bone marrow histology resulted positive for Hc beside tested negative for antibodies. AGM became negative after 3 weeks induction therapy and the patient was discharged. Hc was isolated from the blood culture only after the discharge.

CONCLUSIONS This case highlights the need of considering PDH in the differential diagnosis of AIDS and the importance of a rapid aggressive approach. False positive Results of AGM can be useful in Europe to suspect and monitor PDH, as has already been reported.

1.2-047

Baseline study for a lifestyle intervention on HIV children with dyslipidemias in El Salvador

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INTRODUCTION At present time there are no many options in the treatment of metabolic disorders in HIV-infected children and adolescents. Hypolipemiants and changes in antiretroviral treatment (ARV) are limited options for this population that is at increased risk of developing alterations in lipids metabolism caused by the virus itself and the ARV. It is important to determine the safety and effectiveness of lifestyle interventions for this particular problem.

METHODS We are carrying out an intervention study based on lifestyle changes (diet and exercise) in HIV-1-infected children (0–18 years) attended at the Children HIV Reference Center in El Salvador (CENID). The inclusion criteria for the study were: HIV-infected children between 6 and 18 years presenting at least borderline values for hypercholesterolemia and/or hypertriglyceridemia. Fasting triglycerides, cholesterol and glucose tests as well as anthropometric assessments were performed. Dyslipidemias and nutritional status were defined according to international references.

RESULTS Of 280 children surveyed 63.9% (N = 179) fulfilled the inclusion criteria. The mean age of the children to be included in the intervention was 10.5 (6.14, 17.13); 50.8% were female and 42.2% male; 16.2% had hypercholesterolemia, 62.9% hypertriglyceridemia, and 0.6% hyperglycemia; 33.7% were identified as being stunted, 2.8% as being wasted and 7.8% as being overweight or obese. All children except for one were under ART. 33.5% were taking protease inhibitors and 63.3% ITINAN. The way of HIV transmission in 97.2% was vertical transmission.

CONCLUSIONS More than 60% of the population screened fulfilled the inclusion criteria for the intervention study. Among them, 63% presented hypertrygliceridemia but only 8% were identified as being overweight or obese. These Results highlight the importance of metabolic alterations in HIV-infected children and the necessity of developing appropriate interventions for their management. This project was supported by ESTHER, 'Red en Investigación Cooperativa en Enfermedades Tropicales (RICET)' and AECID.

1.2-048

Incidence and risk factors of antiretroviral toxic neuropathy in rural mozambique: correlation with chronic immune activation

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BACKGROUND Most of the patients receieving combind antiretroviral therapy (cART) in sub-Saharan Africa (SSA) are currently receiving stavudine, a well know cause of antiretroviral toxic neuropathy (ATN). We conducted a study to assess the incidence and risk factors for ATN development in a cohort of HIV-infected ART-naïve adults initiating cART in Manhiça, Southern Mozambique. The relation of chronic immune activation with ATN is largely unknown.

METHODS One hundred and thirty-six consecutive ART-naïve HIV-1-infected patients initiating antiretroviral treatment (ART) at the Manhiça district hospital were prospectively followed for development of antiretroviral toxic neuropathy (ATN) over 16 months. Plasma HIV RNA, CD4 and CD8 counts, and T-cell activation markers were assessed at the pre-cART visit and at 4 and 10 months after cART initiation. Survival analysis by Cox regression was performed to assess risk factors for ATN development.

RESULTS All patients received cART, 104 (76.5%) stavudine-based, and 32 (23.5%) zidovudine-based regimens. Thirty-two patients (23.5%) developed ATN, corresponding with an incidence rate of 3.23 cases/100 per month of ART (95% CI 2.29–4.57). The median time to ATN diagnosis was 126 days (IQR 85–194) from ART initiation. After adjustment for all the relevant variables, multivariate analysis identified four independent pre-ART predictors of ATN: (i) BMI < 18.5 (HR 3.35, 95% CI 1.49–7.5, P = 0.003), (ii) stavudine used as initial ART regimen (HR 4.2, 95% CI 1.4–12.4, P = 0.009), (iii) Age > 40 years old (HR 2.44, 95% CI 0.98–6.1, P = 0.055), (iv) percentage of CD38-expressing CD8 T cells (HR 1.61 per 10% increase, 95% CI 1.05–2.5, P = 0.028).

CONCLUSIONS ATN was common in our cohort of ART-naïve HIV-infected patients mainly treated with stavudine-based ART combinations. Low BMI, stavudine use and older age are known risk factors for ATN development. We have identified an additional risk factor, which is the baseline percentage of activated CD38-expressing CD8 T cells. We hypothesize that over-expression of CD38 could lead to increased consumption of nicotinamide adenine dinucleotide (NAD) and to a decrease in overall cellular and mitochondrial stores of NAD. CD38-mediated depletion of NAD would lower the NAD-mediated neuroprotective effect and facilitate the development of ATN in these patients. Earlier initiation of ART in the course of HIV/AIDS disease may result in lower pre-ART levels of CD38-expressing T cells, and thereby in a lower incidence of ATN.

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1.2-049

Knowledge on and risk perception of HIV/AIDS among university students in Bangladesh

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BACKGROUND In Bangladesh, very few studies have been conducted among university students to explore their sexual behavior. Thus, this study was conducted to explore sexual behavior and risky sexual practices for HIV infection among these youths who are often out of parental control.

METHODOLOGY Data were collected from 500 Honours to Masters level students from randomly selected one public and two private universities. A pretested semi-structured questionnaire was used to collect data for the research.

RESULTS Overall knowledge on HIV/AIDS among University students was satisfactory (mean = 16.36 out of 23). But knowledge of prevention, treatment and symptoms was poor. Mass media (television was most frequent) were the predominant source of students' knowledge on HIV/AIDS compared to parents and teachers. The perception of self risk of being infected was comparatively lower than friends' risk of being infected. Relatives as student's knowledge source were a significant predictor in the perception of their self risk while doctors was the significant predictors of the perception that their friend might be infected. Significant predictors of perception of AIDS as the most dangerous disease were radio and knowledge score; however no significant predictor was found in regard to no treatment of HIV/AIDS.

CONCLUSION Therefore, this study may suggest the necessity of involving of parents, teachers along with mass media role to increase knowledge of students about HIV/AID.

1.2-050

An assessment of condom use among HIV inflicted youth to prevent pregnancy and control STIS in urban Zambia

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INTRODUCTION The study was conducted in 2009, in the Copperbelt Province of Zambia, to assess knowledge and attitudes of condom use in young people aged 18–21 years old. Low levels of education, lack of income by the young women, caring for the orphans and older people and seeking treatment were studied. Youth experiences and impact of HIV in the age group were examined.

METHODS Data were obtained from key informant interviews and Focus Group Discussion. Qualitative data included interviews with parents of young men and women in communities where young people resided. Qualitative data was also obtained from health facility staff.

RESULTS Most young people strong associated HIV transmission with traditional beliefs and gender of individuals (85.6%). Girls were more likely to mention lack of finances as a reason for increased HIV infection than boys. Over two thirds of youth often indicated that they provided care for older people and orphans. Nearly 90% of the youth experienced stress due to loss of both parents and burden of caring for the orphans. Consequences for emotional, social and economic well-being were strongly related to lack of employment and high percentages of early pregnancies. There were 76.4% of health care providers who mentioned putting great efforts on caring and seeking treatment from traditional healing system. Negative attitudes to early pregnancies required extensive time for young women (63.2%). Parental and community support were regarded as paramount by key informants (84.7%).

CONCLUSION Providing means and knowledge for youth to correct use of condoms according to gender will help reduce early pregnancies and HIV infection among the Youth.

1.2-050b

Utility of galactomannan antigen in the diagnosis of disseminated histoplasmosis in HIV infected patient. A case report

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INTRODUCTION Disseminated histoplasmosis (DH) in patients with infection of Human Immunodeficiency Virus (HIV) is difficult to diagnose due to the low incidence in our area . More than 25% of DH were misdiagnosed in high prevalence areas of both diseases. In the European Union (EU), there is not a helpful rapid test for DH diagnosis but cross-reactivity with galactomannan antigen (GM) has been demonstrated. GM might be useful in the diagnosis and follow-up of the patients.

MATERIAL AND METHODS We present a HIV infected from Equatorial Guinea with 4-month history of diarrhea and vomiting, and we propose the performance of GM in serum to help in the diagnosis of this infection.A woman 40 year-old native from Equatorial Guinea who resided in Spain for the last 10 years, seeked for medical assistance for 4-month history of diarrhea, vomiting, fever and weight loss. During the admission, HIV serology and different cultures were sent to the Microbiology Department. A broad-spectrum antibiotic treatment was started but the patient had a poor response. A Giemsa stain from broncoalvelolar lavage (BAL) and bone marrow was made. We also performed a GM study in serum.

RESULTS The HIV serology was positive. Other laboratory test findings showed lymphopenia and a lactate dehydrogenase: 2736 U/L. All microbiological cultures performed were negative. The Giemsa stain of BAL and bone marrow showed yeast compatible with Histoplasma capsulatum. The GM result assay was positive with a 12.5 index due to cross-reactivity existence. The patient was diagnosed of DH and after treatment with amphotericin B, she had a good outcome.

CONCLUSIONS In our area, the GM assay in a patient with AIDS who come from endemic areas may be useful for DH diagnosis in absence of other specialized tests. More studies might to be performed to demonstrate the GM usefulness in the DH diagnosis.

1.2-051

Gender based violence and HIV in India: rhetoric *vs.* reality

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An estimated 2.5 million women in India are living with HIV, equivalent to approximately 0.36% of the adult population. The transmission route is predominantly sexual (87.4%) and approximately 75% of the HIV+ women were infected from their husbands or partners. Several Asian research studies indicate that between 15% and 65% of women experience physical and/or sexual violence inintimate partner relationships, placing them at increased risk of HIV infection. The fundamental gender-related

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structural factors that interact to fuel intimate partner transmission in India is gender inequality, unequal balance of power, prevalent sexual norms in Indian society and the Indian culture where sex and sexuality is a subject of taboo. Subjugation, low status of women and their exclusion from decision making, render them highly vulnerable to contracting HIV from their partners. Not only that, the strong patriarchal Indian culture severely limits a woman's ability to negotiate sex in intimate partner relationships. Gender-based violence and HIV both can only be effectively addressed through ensuring the creation of gender-equitable norms within communities and families. There is an urgent need to enhance awareness and gender responsive action by all stakeholders including the donors, local and international organizations, government agencies, enforcement agencies, and decision makers. The Policy makers must address the underlying power structures through educational outreach on sexual and reproductive health that effectively keep women in subjugation. There is a need to integrate protection of women into all HIV prevention programs. There is also a need for structural interventions for vulnerable women and their male sexual partners and research to have the understanding of the dynamics of HIV transmission among intimate partners.

1.2-051b

'He will say I have killed him' the dilemma of HIV disclosure to sexual partners by pregnant women tested for HIV during antenatal care in rural Uganda

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BACKGROUND Routine HIV counselling and testing as part of antenatal care in Uganda is currently being expanded to lower level health facilities as an entry point for women into the PMTCT programme. We explored experiences of pregnant women tested for HIV as part of antenatal care in rural Uganda with regard to disclosure of HIV status to their sexual partners.

METHODS We conducted a qualitative study at Mbale Regional Referral hospital in eastern Uganda between January and May 2010. Data were collected using in-depth interviews with 15 HIV positive and 15 HIV negative pregnant women attending follow up antenatal clinic. Content thematic approach was used for data analysis.

RESULTS All HIV negative women had disclosed their HIV status to their sexual partners. However, most of their sexual partners were reluctant to go for HIV testing on the assumption that they were also HIV negative and the perception that HIV testing as part of ANC was only for women. Most of the HIV positive women had not disclosed their HIV status to their sexual partners for fear of domestic violence, abandonment and accusation bringing HIV infection in the family.

CONCLUSIONS Scale-up of HIV counselling and testing as part of the PMTCT programme should incorporate strategies to support women with disclosure of HIV status and attracting men to test for HIV so as to maximize opportunities for HIV prevention. Community mobilization, partnerships with community organizations and networks for persons living with HIV may help to bridge this gap.

1.2-052

Advocacy for the implementation of interferon gamma release assays (IGRAS) for diagnosis of latent tuberculosis infection (LTBI) in Manitoba

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OBJECTIVE The purpose of this paper is to advocate the usage in Manitoba of Interferon gamma release assays (IGRAs) for Mycobacterium tuberculosis (MTB) and latent tuberculosis infection (LTBI) , in accordance with the 2008 recommendations of the Public Health Agency of Canada (PHAC) guidelines on implementation of IGRA. IGRAs are a significantly more effective diagnostic tool than the lone tuberculin test, in use for the last century, the Tuberculosis Skin Test (TST). The implementation of IGRAs in Manitoba could prevent iatrogenesis, wasteful human and financial resources for unwarranted, injurious treatments, as in adverse effects of chemoprophylaxis and hepatotoxicity due to false-positive TST results. It is the collective responsibility and moral obligation of Public Health, physicians, nurses, and all healthcare professionals to be informed and to deliver the best available patient care. Improved TB diagnostics, drugs, vaccines, education, and healthcare policies aimed at improving the health system are vital, working towards the fair and equitable distribution of health care resources for all Canadians. Public Health Care of Manitoba should implement IGRAs for diagnosing LTBI according to PHAC diagnostic guidelines. Alberta, Ontario, British Columbia, Quebec, and Newfoundland already follow these guidelines as an integral part of a global commitment at all levels to control TB before it overwhelms our already overtaxed Canadian healthcare system. Currently two IGRAs are commercially approved and licensed for use in Canada and other countries: QuantiFERON-TB Gold assay (also available in QuantiFERON-TB Gold In-Tube) is manufactured by Cellestis Ltd, Carnegie, Victoria, Australia. The QFT-GIT assay is an ELISA-based, whole-blood test that uses peptides from three TB antigens (ESAT-6, CFP-10, and TB7.7) in a simple, in-vitro format. The T-SPOT.TB (Oxford Immunotec, Oxford, UK) is an enzyme-linked immunospot (ELISPOT) assay performed on separated peripheral blood mononuclear cells; it uses ESAT-6 and CFP-10 peptides. The result is reported as the number of IFN-? producing T cells (spot forming cells).

1.3 Neglected Diseases

1.3-001

Effect of Tamarindus indica seeds extract on Biomphalaria pfeifferi, the intermediate host of Schistosoma mansoni

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Screening of plant extracts represents a continuous effort to find new bioactive molecules or extracts. In the present study a series of laboratory experiments was conducted to assess the molluscicidal activity of *Tamarindus indica* seeds (locally known as Ara'daeb) against *Biomphalaria pfeifferi*. The sensitivity of the snails to Ara'daeb seeds extracts was directly proportional to the age of the snails. The chronic effect of the extracts on adult snails' can also be observed in the significant decrease in fecundity rates of the snails. The study also shows that the seed of Ara'daeb include high amount of saponin. This may account for the molluscicidal activity which is observed. Attempts were made to apply the experimental findings obtained in laboratory conditions in field situations.

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1.3-002

A preferential IFN biased immune response enables the antimony mediated therapy for a possible cure of experimental VL in BALB/c mice

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Visceral leishmaniasis is a macrophage associated disorder which is linked with a profound decrease in the immunotherapeutic potential of the infected subjects leading to a marked reduction in the CD4 associated Th1 protective immune response. Antimonials and the recently introduced package of new drugs are all directly leishmanicidal towards intracellular *Leishmania donovani* amastigotes. However the use of new regimen of drugs is compounded by problems like cost efficacy and side effects. Moreover the effectiveness of Sb in intact animal is determined by the host cell-mediated immune response. We have previously reported that stimulation of CD2 epitope with antiCD2 antibody leads to a remarkable increase in the Protein kinase C alpha mediated phosphorylation on CD2 co receptor on CD4 T cells, induction of IFN- led Th1 dominated immune response, a substantial increase in the lymphoblast population. This response remained Th1 dominated even in the presence of Th2 predominant conditions signified with rIL4 (Bimal *et al*., 2007). In the present part of the study we tried to evaluate the use of CD2 antibody as an immunotherapeutic agent along with SAG in ensuring treatment of BALB/c mice induced with experimental visceral leishmaniasis. In context to our results the use of CD2 antibody along with SAG led to induction of IL-2 synthesis, expression of CD-25 and subsequent production of IFN and TNF thereby leading to induction of i (NOS) gene and nitric oxide mediated parasite killing, which is further validated by the remarkable decrease in the parasite load of spleen. Triggering with CD2 antibody provides a missing signal leading to signal transduction and T cell proliferation hence its use along with SAG might provide impetus to some drastic innovations in the development of immunetherapy against visceral leishmaniasis.

1.3-003

Amphotericin B and miltefosine combination in the treatment of Indian post-kala-azar dermal leishmaniasis

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INTRODUCTION Post-kala-azar dermal leishmaniasis (PKDL) is an uncommon sequel seen in 5–10% of patients treated for the predominantly anthroponotic form of kala-azar in the Indian subcontinent. It is characterized by a variety of lesions and clinical presentations, recalcitrance to therapy, and forms a reservoir of infection during the inter-epidemic periods. No satisfactory treatment has emerged for PKDL, particularly for the extensive form of the disease. The parenteral forms of therapy (sodium stibogluconate and amphotericin B) and oral miltefosine when used as monotherapy have been found impractical either because of difficulty of administration of a high volume of the drug, side-effects or prolonged in-patient care. Combination of drugs would be a good alternative in selected instances to bring about cure. Our previous experience with miltefosine showed that in those with widespread PKDL the condition did not subside with the recommended regimen and higher doses were not tolerated by the patient.

MATERIAL AND METHODS Two men diagnosed with PKDL presented with extensive skin affection. In one the entire body was erythematous and studded with papulonodules, most prominent over the face and scattered elsewhere. The other patient had widespread nodulation of skin. The diagnosis was made by the demonstration of LD bodies in slit-skin smears, histopathology and molecular tests. Non-liposomal amphotericin B was started i.v. 50 mg/day along with miltefosine capsules 50 mg twice or thrice daily. Patients were monitored for signs of subsidence of disease and toxic side-effects. Parasite load was determined by PCR.

RESULTS Both patients achieved cure after receiving a total of 1 g of amphotericin B and 130 capsules of miltefosine. They have completed 2 years of follow-up without signs of relapse.

CONCLUSION Miltefosine along with amphotericin B when given together is an effective combination in selected cases of PKDL.

1.3-004

Distribution of *Leishmania major* zymodemes in relation to populations of *Phlebotomus papatasi* sand flies

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Phlebotomus papatasi (Scopoli) (Diptera: Psychodidae) is the main vector of *Leishmania major* Yakimoff and Schokhor (Kinetoplastida: Trypanosomatidae), the causative agent of zoonotic cutaneous leishmaniasis in the Old World. Multilocus enzyme electrophoresis (MLEE) was extensively used to type different *L. major* stocks allover the world. Multilocus microsatellite typing (MLMT) has been recently used to investigate *P. papatasi* sand flies at population and subpopulation levels. In this article, the association between geographical distribution of *L. major* zymodemes and the distribution of populations and subpopulations of *L. major* vector; *P. papatasi* are discussed.

1.3-005

Human African trypanosomiasis: a review of non-endemic cases in the past 20 years

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BACKGROUND Human African trypanosomiasis (HAT) is caused by sub-species of the parasitic protozoan *Trypanosoma brucei* and is transmitted by tsetse flies (*Glossina*), both of which are endemic only to sub-Saharan Africa. Several cases have been reported in non-endemic areas, such as North America and Europe due to travellers, ex-patriots or military personnel returning from abroad or due to immigrants from endemic areas.

METHODS Non-endemic cases reported over the past 20 years were reviewed. Cases were selected via searches of PubMed (search terms: 'trypanosoma OR HAT OR African trypanosomiasis OR sleeping sickness NOT Chagas NOT animal NOT reservoir') and ProMED-mail as well as personal communication.

RESULTS A total of 68 cases were reported, 19 cases of gambiense HAT and 49 cases of rhodesiense HAT. Of these, 57 were found through PubMed search and through a bibliographic search of articles. Three cases, all related, were obtained by personal communication (P. Büscher). And eight cases were reported only on ProMED-mail which were not encountered via the PubMed search. Many of these cases were also reported on TropNetEurop (http://www.tropnet.net/special_reports/tryps_ex_serengeti.pdf), a European surveillance network for imported infectious diseases. Patients ranged in age from 19 months to 72 years. Of the 19 cases of gambiense HAT, nine cases were diagnosed in the first stage of the disease, eight were diagnosed in the second and two were not specified, but all were successfully treated. Two rhodesiense cases died. Physicians in non-endemic areas should be aware of the signs

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and symptoms of this disease, as well as methods of diagnosis and treatment especially as travel to HAT endemic areas increases.

Conclusion We recommend extension of the current surveillance systems such as TropNetEurop and maintaining and promoting reference centres of diagnostics and expertise. Important contact information is also included, should physicians require assistance in diagnosing or treating the disease.

1.3-006

Outbreak of acute eosinophilic pneumonia in the Brazilian Amazon: a flag for Löffler's syndrome

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Introduction The original description of Löffler's syndrome in 1932 listed *Ascaris lumbricoides* infection as its most common cause. Outbreaks of this syndrome are rarely reported.

Patients and Methods This is a descriptive study of a series of cases of Löffler's syndrome occurred in Manaus (Brazilian Amazon).

Results Between June and July 2010, 16 cases of eosinophilic pneumonia (EP) were identified in four distinct families and four isolated cases. Nine (56%) were male (19–57 years). All cases presented with fever, cough, dyspnea, pulmonary infiltrates on chest x-rays and hypereosinophilic syndrome. The median peripheral eosinophilia was 33% (13–60%). Eight patients who underwent computed tomographic scan of the chest, all revealed bilateral scattered ground-glass opacities and peripheral consolidation. There was no history of use of medications or similar episodes among the patients and in 14 (88%) there was no history of asthma or atopy. The parasitological exams of stool (three samples) of all patients were negative for helminths at this moment. During the acute episode of EP, all patients were hospitalized and treated with steroids evolving with clinical improvement and normalization of eosinophilia in a 14-day follow-up. Of the 12 patients who completed the 3-month follow-up in the outpatient clinics, all had gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain, between 60 and 90 days, with positive stool for *Ascaris lumbricoides* eggs and/or larvae. In all, at the time of diagnosis of intestinal ascariasis, chest x-rays were normal and peripheral eosinophilia reappeared. All had resolution of gastrointestinal symptoms in use of the standard treatment with mebendazole.

Conclusion In tropical sites, Löffler's syndrome is an obligatory differential diagnosis of EP. The epidemic occurrence is uncommon and may be related to socio-environmental characteristics of the population. Due to the natural history of Löeffler's syndrome, diagnosis based on stool examination is possible only after 60–90 days.

1.3-007

Interferon-gamma release assay (modified quantiferon) as a potential marker of infection for *Leishmania donovani*, a proof of concept study

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In areas endemic for visceral leishmaniasis (VL), a large number of infected individuals mount a protective cellular immune response and remain asymptomatic carriers. We propose an interferon-gamma release assay (IFN-ãRA) as a novel marker for latent *L. donovani* infection. We modified a commercial kit (QuantiFERON) evaluating five different leishmania-specific antigens; H2B, H2B-PSA2, H2B-Lepp12, crude soluble antigen (CSA) and soluble *Leishmania* antigen (SLA) from *L. donovani* with the aim to detect the cell-mediated immune response in VL. We evaluated the assay on venous blood samples of active VL patients (n = 13), cured VL patients (n = 15), non-endemic healthy controls (n = 11) and healthy endemic controls (n = 19). The assay based on SLA had a sensitivity of 80% (95% CI = 54.81–92.95) and specificity of 100% (95% CI = 74.12–100). Our findings suggest that a whole-blood SLA-based QuantiFERON assay can be used to measure the cell-mediated immune response in *L. donovani* infection. The positive IFN-ã response to stimulation with *Leishmania* antigen in patients with active VL was contradictory to the conventional finding of a non-proliferative antigen-specific response of peripheral blood mononuclear cells and needs further research.

1.3-008

How reliable is PCR for diagnosis, staging and follow-up of gambiense sleeping sickness?

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Introduction The polymerase chain reaction (PCR) has been proposed for diagnosis, staging and post-treatment follow-up of sleeping sickness but no appropriate evaluations of its diagnostic accuracy have taken place in clinical practice. We report on the sensitivity and specificity of a PCR for diagnosis, disease staging and detection of treatment failure in 360 *T. b. gambiense* sleeping sickness patients and 129 endemic controls from the Democratic Republic of the Congo.

Methods and Materials The infection status of the patients was monitored during 2 years post-treatment. Reference standard tests were trypanosome detection for diagnosis and trypanosome detection and/or increased white blood cell concentration in the cerebrospinal fluid (CSF) for staging and detection of treatment failure.

Results When performed on blood, the PCR that targets the 18S ribosomal RNA gene, showed a sensitivity of 88.4% and a specificity of 99.2% for diagnosis. For disease staging, PCR on CSF had a sensitivity of 88.4% and a specificity of 82.9%. During follow-up after treatment, PCR on blood had low sensitivity (12.5–50%) to detect treatment failure. In the CSF, PCR positivity weaned slowly. Unexpectedly, the PCR on CSF remained positive until the end of the 2 year follow-up in around 20% of successfully treated patients.

Conclusions For *T. b. gambiense* sleeping sickness diagnosis and staging, this PCR performed as well as the most sensitive parasite detection techniques. PCR is however not appropriate for post-treatment follow-up. Continued PCR positivity in one out of five cured patients points to persistence of living or dead parasites or their DNA after successful treatment and may necessitate the revision of some paradigms about the pathophysiology of sleeping sickness.

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1.3-009

Comparative gene expression analysis throughout the life cycle of *Leishmania braziliensis*: diversity of expression profiles among clinical isolates

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INTRODUCTION *Leishmania* are protozoan parasites responsible for a wide spectrum of clinical forms. This clinical pleomorphism has been related to both host and parasite features. Most of the *Leishmania* genome is reported to be constitutively expressed during the life cycle of the parasite, with a few regulated genes. Inter-species comparative transcriptomics (*L. major*, *L. infantum* and *L. braziliensis*) revealed species-specific expression features, albeit in a low number. This type of research being undertaken among other reasons for a better understanding of the differences in virulence and pathogenicity of the respective species, it is of utmost importance to ensure that the observed differences are species-specific and not simply specific to the strains selected as representative of the species. The relevance of this concern is illustrated by current study.

METHODS AND MATERIALS We selected five clinical isolates of *L. braziliensis* characterized by their diversity of clinical and *in vitro* phenotypes. Real-time quantitative PCR was performed on promastigote and intracellular amastigote life stages to assess gene expression profiles at seven time points covering the whole life cycle. We tested 12 genes encoding proteins with roles in transport, thiol-based redox metabolism, cellular reduction, RNA poly (A) – tail metabolism, cytoskeleton function and ribosomal function.

RESULTS The general trend of expression profiles showed that regulation of gene expression essentially occurs around the stationary phase of promastigotes. However, the genes involved in this phenomenon appeared to vary significantly among the isolates considered. Our results clearly illustrate the unique character of each isolate in terms of gene expression dynamics.

CONCLUSIONS The gene expression pattern of one *Leishmania* strain is not necessarily representative of a given species. Further studies should integrate this important dimension of intra-species diversity and take extreme care when comparing the profiles of different species and extrapolating functional differences between them.

1.3-010

Molecular approaches to the identification of *Bulinus* species in Nigeria and observations on natural infection with schistosomes

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INTRODUCTION *Schistosoma haematobium* infection is endemic in Nigeria. Freshwater pulmonate snails of the genus *Bulinus* act as the intermediate hosts for *S. haematobium*. Knowledge of the geographical distribution of *Bulinus* species present in Nigeria and their frequency of occurrence is important in the design of treatment/control programs.

METHODS AND MATERIALS *Bulinus* snails were collected from 28 localities within eight Nigerian states and were identified using both DNA sequencing of a partial cytochrome oxidase subunit 1 (cox1) fragment and restriction profiles obtained from ribosomal internal transcribed spacer (its) amplicons. The use of Rsa1 restriction endonuclease to cleave the ribosomal its of *Bulinus*, as a method of species identification, was adopted for the majority of samples this being a quicker and cheaper method, better suited to small laboratory environments. PCR amplification of schistosome Dra1 repeat within each of the collected *Bulinus* samples determined the extent and distribution of infected snails within the sample areas.

RESULTS Majority of *Bulinus* samples belonged to the species *Bulinus truncatus* whilst two were *Bulinus globosus*. Amplification of the Dra1 repeat demonstrated that 29.7% of snails were infected with schistosomes, while amplification of schistosome its from a small subset of snail samples suggested that some snails were either penetrated by both *S. haematobium* and *S. bovis* miracidia or hybrid miracidia from the two species.

CONCLUSIONS This study has made a significant contribution to discovering the identity of snail species responsible for the transmission of schistosomiasis in the study areas and has also revealed the dominant intermediate host species in these regions, which is *B. truncatus*. The use of its primers to supplement those of Dra1 in an attempt to identify the infecting schistosome species has raised some interesting questions relating to the possible species status of the parasite and this needs to be addressed in future studies.

1.3-011

Serological evidence of exposure to leptospires in horses bred at stud farms, equestrian centers and at the urban environment of mounted police, Bahia, Brazil

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INTRODUCTION There are few reports using ELISA as a complementary method for microscopic agglutination test (MAT) in serosurveys of equine exposure to leptospires. In addition, exposure to leptospires in horses living in the urban environment, such as those from mounted police, has not been previously studied.

MATERIAL AND METHODS We describe an in house indirect ELISA assay that was used, along with MAT, in a serosurvey of 1200 horses: 1031 from stud farms, 93 from the mounted police troops and 76 from Equestrian Centers from Salvador, a large urban area with 2.9 million inhabitants, where human leptospirosis is associated with rainy seasons, floods and poor sanitation.

RESULTS All horses had no signs of clinical leptospirosis. Using strict criteria for controls (positive controls with MAT titers 200), IgG ELISA showed 73% sensitivity and 90% specificity. There was, however, poor agreement between MAT and ELISA when testing the 1200 field samples. Seroprevalence by MAT was 7% in stud farms, 10.5% in equestrian centers and 17% in Mounted Police horses. The most common infecting serogroup (inferred by MAT highest titers) was Australis (60%) in stud farms, Australis and Icterohaemorrhagiae (25% each) in equestrian centers and Sejroe (44%) in mounted police horses. ELISA positive samples were obtained in 36% of stud farms, 18% in equestrian centers and 35.5% in Mounted Police horses.

CONCLUSIONS These results suggest that the environment and the methods of horses management influence on the exposure to leptospires as well as interfere in which serovars will be the infecting ones in each setting.

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1.3-012

Incidence of *Taenia solium* cysticercosis in southern Ecuador: a longitudinal community-based study

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INTRODUCTION Cysticercosis is recognised as an important but neglected cause of acquired epilepsy in developing countries where *Taenia solium* occurs. Data on the transmission dynamics of the parasite are scarce. Individuals living in endemic areas are likely to be highly exposed to the parasite, but relatively few of them develop active infections. The present study aimed at estimating the incidence of the disease in a South Ecuadorian endemic community.

MATERIALS AND METHODS A longitudinal community-based study was conducted between April 2009 and April 2010 in a community located in the *T. solium* endemic province of Loja. All inhabitants were three times blood sampled with 6 months intervals. Both antigen and antibody serological detection tools were used, reflecting infection with the parasite and exposure to the parasite, respectively.

RESULTS The proportion of antigen positive individuals is significantly lower than the proportion of antibody positives. Active infections are significantly higher in the elderly. Preliminary results show a yearly incidence rate of 0.5% and a yearly exposure rate of about 12%. About 38% of the antibody seropositive individuals were negative after 1 year (seroreversion rate) and 18% of the seronegatives became positive in the same time period (seroconversion rate).

CONCLUSIONS This is the first study allowing an estimation of the incidence of human cysticercosis, and of the rate of exposure to *T. solium*. These estimates allow characterizing the transmission patterns of the parasite inside an endemic population where immunity acquisition and immunosenescence probably play important roles.

1.3-013

Development and application of a loop-mediated isothermal amplification method for a rapid identification and differentiation of *Taenia* species from humans

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Cestode parasites *Taenia solium, T. saginata* and *T. asiatica* cause taeniasis in humans. Although taeniasis is relatively innocuous, cysticercosis caused by *T. solium* larvae is one of the most serious diseases in human in developing countries. The morphological examination of proglottids, scolices and eggs to differentiate *Taenia* species lack sensitivity and specificity. Therefore, a simple, rapid and reliable method to distinguish Taenia species is imperative for control and prevention of these diseases in endemic areas. In this study, we developed and applied a loop-mediated isothermal amplification (LAMP) method for differential detection of Taenia species targeting cathepsin L-like peptidase (clp) and cytochrome c oxidase subunit 1 (cox1) genes. Primer sets based on cox1 genes could differentiate three species, and primer sets based on clp genes could differentiate *T. solium* from *T. saginata* and *T. asiatica*. Comparison of the performance of the LAMP method with that of a multiplex PCR using 43 fecal samples from taeniasis patients revealed that the LAMP method, without false-positives, had a higher sensitivity (88.4%) than the multiplex PCR (37.2%). Furthermore, the LAMP method was applied to a field survey to identify Taenia tapeworms from humans. Of 51 proglottid samples recovered from 35 carriers, nine, one and 41 samples were identified as *T. solium*, *T. asiatica* and *T. saginata*, respectively. Identical results were obtained afterwards in the laboratory, except one sample. These results suggest that the LAMP method being a rapid identification method of parasites will contribute to efficient control of *Taenia* infections in endemic areas.

1.3-014

Radiological findings in immigrants patients with imported urinary schistosomiasis

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BACKGROUND Schistosomiasis is the second most important parasitic disease after malaria, which causes increased morbidity and mortality worldwide. An estimated 200 million people are infected worldwide, 95% in sub-Saharan Africa. *Schistosoma haematobium* is endemic in 53 countries worldwide. The infection is associated with severe complications, such as hydronephrosis, renal failure, terminal and squamous cell carcinoma of bladder. Radiological investigations are crucial in the diagnosis and monitoring of this disease.

METHODS AND MATERIAL We studied 149 immigrant patients who attended at the Tropical Medicine Unit of Hospital de Poniente (El Ejido, Southern Spain) with urinary schistosomiasis diagnosed by methods of direct visualization of the parasite. One hundred and forty-seven were men (98.6%). The mean age was 26.17 years (15–46). Eighty-four percent of patients were in an irregular administrative situation.

RESULTS One hundred and thirty patients had abdominal X-rays, being pathological in 29 cases (22.3%). The most common findings were calcifications bladder to varying degrees. Abdominal and bladder ultrasound was performed in 124 patients with lesions of the disease in 56 cases (45.2%). The most common findings were diffuse thickening of bladder (22 patients), bladder focal thickening (14 patients), bladder nodules (13 patients) and hydronephrosis (two patients). One patient had a large lesion in the bladder and was finally diagnosed with squamous cell carcinoma.

CONCLUSIONS The imaging studies (plain abdominal radiography and ultrasound of abdomen and urinary tract) is essential in patients with known or suspected diagnosis of schistosomiasis, helping both the diagnosis and control monitoring and aftercare of patients. Also allow early diagnosis of potentially serious injuries, and establish therapeutic measures in place to prevent the progression of them.

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1.3-015

Vector control interventions for the visceral leishmaniasis elimination initiative in South Asia, 2005–2010

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The Visceral Leishmaniasis (VL) elimination initiative in the Indian subcontinent was launched in 2005 as a joint effort between the governments in the region (India , Nepal and Bangladesh) and the World Health Organization (WHO). The objective is to reduce the annual VL incidence below 1/10 000 inhabitants by 2015 based on detection and treatment of VL cases and vector control. This paper presents a review of studies published in the period 2005–2010 on the efficacy of different tools to control Phlebotomus argentipes. The review indicates that the current-Indoor Residual Spraying (IRS)- and novel vector control methods -mainly Insecticide Treated Nets (ITN)- have low effectiveness for several reasons. Efforts to improve quality of IRS operations and further research on alternative and integrated vector control methods should be promoted to reach the VL elimination target by 2015.

1.3-016

Leishmaniases in the XXI century – Portugal

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Leishmaniases, caused by *Leishmania infantum*, are endemic zoonosis in the Mediterranean basin. Dogs are considered the major host for these parasites, and the main reservoir for human visceral infection. Parasites are transmitted by phlebotomine sandflies, being *Phlebotomus perniciosus* and *P. ariasi*, the proven vectors in Portugal. Visceral leishmaniasis was a pediatric disease up to the eighties but in the last decades the number of cases in children has decreased with an increase of infection in adults, namely associated with AIDS. This work updates *Leishmania* infection in humans, dogs, cats and vectors between 2000 and 2010 in Portugal. Two hundred and twenty-one new cases of human visceral leishmaniasis (74 immunocompetent adults and children and 147 immunocompromised patients) were diagnosed in the Leishmaniases Laboratory/IHMT. Despite cutaneous leishmaniasis is barely known, in the last 10 years, 21 cases were identified. The Portuguese national leishmaniasis observatory (www.onleish.org) was created in 2008 in order to implement an epidemiological surveillance on canine leishmaniasis. In 2009 a national canine survey was conducted and an overall seroprevalence of 6% was found. *L. infantum* infection in cats has also been evaluated and *Leishmania* DNA was detected in 28/138 screened animals. Phlebotomine surveys have been carried out and vector species were found infected with *L. infantum*. Data reveal that Portugal continues an endemic country for leishmaniases and the prevalence of canine and feline infections is a serious concern for the increase and spreading of leishmaniases. Moreover, the number of human cases has not diminished as expected with HAART in opposition to what was observed in other European countries. The development of national and international epidemiological networks would promote opportunities to advise health authorities about the most effective measures for prevention and control of this zoonosis.

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1.3-017

Leishmania braziliensis: *in vitro* evaluation of D-L-alanine, L-proline and L-glutamine on promastigote's chemotaxic responses

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Leishmania infection begins with its migration towards the host cell, skin macrophages. Chemotaxic responses are fundamental in this initial process. Amino acids are among essential metabolites in all *Leishmania* life stages used as carbon sources. Herein we investigate the chemotaxic effect of three amino acids (D-L-alanine, L-proline and L-glutamine) in *Leishmania* (*L.*) *braziliensis*. For the experiments we used the modified 'oetwo-chamber capillary chemotaxis assay'. Tips of a multichannel micropipette (inner chamber) were filled with amino acids (10–6 M); while the *L. braziliensis* cell suspension (4 × 107 cell/ml) was located in microtitration plates (outer chambers). Apertures of the tips served as connecting junctions between the two chambers. After 30 min of incubation time, the samples were fixed (2% formaldehyde/PBS); the cell density was estimated in a hemocytometer. Our results suggest that L-glutamine (10–6 M) with a polar uncharged side chain induces a significant positive chemotaxic response in *L. braziliensis*. By contrast, D-L-alanine with a hydrophobic side chain and L-proline with a hydrophobic pyrrol ring did not alter the number of cells that migrated to the inner chamber with respect to control. Our data thus indicate that L-glutamine produces a positive chemosensory response *in vitro* in *L. braziliensis* and suggests that this amino acid has a role on chemotaxis that could be receptor mediated. Additionally the results suggest that the hydrophobic property of amino acids may impair chemotaxis. As aminotransferases with L-glutamine and L-cysteine specificities are functional in Trypanosomatidae, and since these enzymes are distinctly expressed during their life cycle and differently distributed in the intracellular compartments, the future goal of our studies would be to evaluate the response of *L. braziliensis* to L-glutamine. This is a fundamental study to analyze taxic responses in tsetse fly hemolymph where glutamine may be present.

1.3-018

Leishmania braziliensis: *in vitro* evaluation of methotrexate based peptides on promastigote's chemotaxic responses

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Chemotherapy is a key factor for control and management of leishmaniasis. However, the effectiveness of current treatments is variable due to factors related to host and parasite, including drug resistance. These features highlight the urgency to identify new therapeutic targets and develop drugs against *Leishmania* parasites. Understanding the host-parasite interaction at the beginning of infection is critical for the development of alternative chemotherapies. Chemotaxic responses are fundamental both during *Leishmania* differentiation to the infective stage and during infection. Drugs such as methotrexate (MTX) inhibit the entry of folate into the cell and are active against *Leishmania*. The conjugation of poly-lysines as carriers for MTX may improve drug-targeting and increase MTX effectiveness against *Leishmania*. This increased targeting may reduce the appearance of resistance and minimize drug adverse effects. Herein we analyzed the activity and chemotaxic properties of compounds designed as poly-lysine-MTX. For this aim we have used a modified two-chamber capillary chemotaxis assay. *L. braziliensis* susceptibility to the conjugates was evaluated *in vitro*. The concentrations of

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EAK(γMtx-GFLGC) and of SAK(αL-Mtx-GFLGC) that produced a 50 % reduction in promastigotes growth were 1.50 and 3.24 μM respectively. Concentrations up to 5 μM of SAK(αD-Mtx-GFLGC) did not decrease the growth of *L. braziliensis* and the IC_{50} of SAK (γ-Mtx-GFLGC) has not yet been determined. Our results demonstrated that SAK(αL-Mtx-GFLGC), at 10^{-8} M elicited a significant negative chemotaxic response, while SAK(γ-Mtx-GFLGC) has a dual effect being positive at concentrations lower than 10^{-9} M and negative at concentrations higher than 10^{-8} M. In conclusion, our results indicate that chemotaxis produced by the poly-lysines depends on specific chemical properties. Indeed, the terminal amino acid significantly influenced the response as the presence of serine instead of glutamine elicited chemotaxis, and in the serine conjugates, α-isomers elicited a strong chemotaxic response while γ(–isomers exhibited a dual response.

1.3-019

Specific pharmacovigilance system in the use of the new therapeutic combination nifurtimox – eflornithine in the treatment of second stage of gambiense human African trypanosomiasis

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INTRODUCTION The new nifurtimox and eflornithine combination treatment (NECT) showed in clinical trials a non-inferior level of safety and efficacy that previous elective treatment of eflornithine monotherapy for the treatment of second stage of gambiense human African trypanosomiasis (HAT). In April 2009, NECT was included in the 16th WHO Essential Medicines List (WHO EML). Following the inclusion in the WHO EML, the NECT has been adopted as first line treatment of advanced stage of gambiense HAT by 10 endemic countries (Cameroon, Central African Republic, Chad, Cote d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Guinea, Sudan and Uganda). These 10 countries currently notify the 95% of cases of gambiense HAT. The WHO Essential Medicines Expert Committee recommended to strength the pharmacovigilance of this new therapy at the moment to use NECT by National Sleeping Sickness Programmes (NSSCP), usually in health facilities located in rural remote areas.

MATERIAL AND METHODS Nifurtimox and eflornithine are donated toWHO through a Public-Private-Partnership by the pharmaceutical companies, Sanofi-Aventis and Bayer. They are supplied free of charge to disease-endemic countries by WHO in form of treatment kits, which includes all additional material required for its use. Therefore WHO took the responsibility to set up a pharmacovigilance system in collaboration with NSSCP and non-governmental organizations.

RESULTS AND CONCLUSIONS One year after the implementation, this pharmacovigilance system adapted to this specific situation is showing to be an effective tool to get useful information about safety and efficacy of a new implemented treatment.

1.3-020

Tumor necrosis factor alpha antagonist drugs and leishmaniasis in Europe

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Leishmaniasis is endemic in Europe and the prevalence of latent infection in the Mediterranean region is high. Reports describing opportunistic leishmaniasis in European patients treated with tumor necrosis factor (TNF) alpha antagonist drugs are rapidly accumulating. For other granulomatous infections, risk of opportunistic disease varies by mode of TNF-alpha antagonism. This study explores whether this may also be the case for leishmaniasis. We ascertained the relative frequency of exposure to different TNF antagonist drugs among published cases of opportunistic leishmaniasis in Europe and compared this to the prescription of these drugs in Europe. We found that risk of opportunistic leishmaniasis is 8-fold higher in patients receiving anti-TNF monoclonal antibodies (infliximab or adalimumab) compared to patients treated with the TNF-receptor construct etanercept. Clinicians may want to be cautious and balance the risks and benefits of different anti-TNF therapies for individuals with transient or ongoing exposure in areas endemic for leishmaniasis.

1.3-021

Successful treatment of cutaneous leishmaniasis due to *Leishmania aethiopica* with liposomal amphothericin B in an immunocompromised traveler returning from Eritrea

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Cutaneous leishmaniasis (CL) due to *Leishmania aethiopica* is rarely encountered outside endemic areas and clinical trials evaluating its pharmacotherapy are missing. Under resource limited conditions antiparasitic treatment of *L. aethiopica* infection relies largely on pentavalent antimonials. Treatment failure, however, is frequent and their systemic application potentially harmful. Evidence for the efficacy of less adverse chemotherapeutics is needed. We describe the treatment of cutaneous leishmaniasis due to *L. aethiopica* using liposomal amphothericin B in an immunocompromised traveler returning from Eritrea. This is the first description of the treatment of CL due to *L. aethiopica* using liposomal amphothericin B (LAmB) and the first report of its treatment outside endemic regions at all.

1.3-022

Epidemiology of caninne leishmaniasis in amudat district in Uganada

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Visceral *Leishmaniasis* is an important public health disease amongst the Pokot pastoralists in Amudat district in Uganda. A cross sectional study was performed in all the villages of Amudat district using the dip Stick method and microscopic examination of lymph node biopsies. A total of 1245 dogs from 124 'Manyatas' were tested sequentially for *Leishmania* antibodies using the direct agglutination test or rK39 anti-gen-based dip sticks and micro-

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scopic examination of lymph node smears. Prevalence of 14.1% was recorded in female dogs whiles 28.1% were recorded in the male dogs population; while prevalence of 12.6% for females and 25.7% for male using the microscopic smear examinations were recorded. *Leishmania* prevalence varied according to area and grazing stategy. Age, sex, geographical location and history of migrations had independent effects on the seroprevalence. This study establishes that canine *leishmaniasis* is endemic in dog populations owned by pastoralist communities in Uganda. The implications of these findings with respect to the epidemiology and control of canine *leishmaniasis* in Karamoja are discussed.

1.3-023

Visceral leishmaniasis: determination of agent by PCR in Yasouj, south of Iran

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BACKGROUND Visceral Leishmaniasis is an endemic disease in some parts of Iran. *Leishmania* infantum is the agent of disease in studied areas. In Yasouj city, new cases of patients had reported recently from some villages. In this study, the agent of visceral leishmaniasis determined by PCR in infected dogs.

METHODS In this study 15 infected dogs selected from five endemic villages. These dogs had sign and symptoms of canine visceral leishmaniasis. All cases tested by DAT for evaluation of leishmanial antibody titers. After necropsy parasitology study conducted by use of impression smear of liver and spleen. Nested PCR examination conducted on smears and tissues of liver and spleen.

RESULTS Fourteen of 15 dogs had antibody titers above of 1:320; one tested negative. *Leishmania* amastigote was seen in 13 smears of liver and spleen (13 cases). The agent of disease in 14 dogs determined as *L. infantum* and in one dog as *L. major* by nested PCR method.

CONCLUSION The most common causative agent is *L. infantum*, as in other regions in Iran. But a new finding is *L. major's* reservoir hosts are gerbils not dogs. This needs further studies.

KEYWORDS PCR, DAT, visceral leishmaniasis, dog, Yasouj

1.3-024

Anti leishmanial effect of *Plantago psyllium* (ovata) and white vinegar on *Leishmania major* lesions in BALB/c mice

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BACKGROUND *Leishmania major* causes rural leishmaniasis in some parts of Iran and For treatment the currently used drug is meglumine antimoniate (Glucantime) by injection on the site of the ulcers. The aim of this study was to evaluate the anti leishmanial effects of topical *Plantago psyllium* (ovata) with vinegar in *L. major*-infected BALB/c mice.

METHODS In this study 30 infected BALB/c mice divided in five groups. All mice had leishmanial ulcers confirmed by microscopic examination. Group 1 (10 mice): treated with the combination of *P. psyllium* (ovata) powder and white vinegar. Group 2 (five mice): treated with glucantime. Group 3 (five mice): treated with white vinegar. Group 4 (five mice): treated with the combination of *P. psyllium* (ovata) powder and water. Group 5 (five mice): without any treatment (control). All groups were treated for 28 days. Lesions were measured weekly and smears prepared on the last day for microscopic examination.

RESULT In group 1, six mice healed and in groups 2 and 3, four mice treated and in group 4, three mice healed and in control group all mice not healed. The results showed a significantly ($P < 0.001$) smaller lesion size in the mice in the treated groups specially in glucantime and vinegar groups compared to mice in the control group. Anti leishmanial effect of vinegar is the same as glucantime and the effect of *P. psyllium* (ovata) is smaller than those.

CONCLUSION The combination of *P. psyllium* (ovata) powder and white vinegar is used to treat leishmanial lesions traditionally in Iran. It seems the most anti leishmanial effect is related to vinegar and supported by Plantago. However, the route of treatment with this combination is very simple and painless in comparison with injection. So by additional study scientists could design effective and more easily used drugs.

KEYWORDS Leishmania major, Plantago psyllium (ovata), Iran, treatment

1.3-025

Seroepidemyology of human Alveolar Echinococcosis in rural population of Moghan plain, Ardebil province of Iran in 2009

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Alveolar Echinococcosis (AE) caused by metacestode of *Echinococcus moltilocularis*, the most lethal helminthiasis of humans, is confined to the northern hemisphere. In Iran, all confirmed AE cases were reported from a limited area, Moghan plain in Ardebil province, in the northwest of the country. For establishment of a control program, we needed to know the prevalence of the disease derived from serological screening in the region. One thousand and one hundred residents from 53 villages including stable and nomad complexes in Moghan plain were selected randomly. After filling in a questionnaire, 5 ml of blood were collected from each person. The sera were examined by using a commercial kit of ELISA (Bordier® Affinity products, Switzerland) at parasitology laboratory in Pasteur institute of Iran. One percent (11/1100) were positive for AE. The most infected age group was 21–30 years old with 46% (5/11) of cases. 73% (8/11) were housewives; 27% (3/11) were male animal keepers. With respect to risk factors, 73% (8/11) were dog owners, 91% (10/11) used to consume wild (self-grown) vegetables and fruit and 9% (1/11) had a history of a contact to red fox skin. None of the positive cases were hunters. Finally 82% (9/11) of cases were living in villages and 18% (2/11) were nomads. In conclusion, the results showed an unexpectedly high prevalence of AE in Moghan which warrants a control program for the disease in this region.

1.3-026

Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with *Opisthorchis viverrini*: a randomised, exploratory, open-label, phase 2 trial

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BACKGROUND Praziquantel is the only drug available for treatment of *Opisthorchis viverrini*, although *in-vivo* studies point to activity of mefloquine, artesunate, and tribendimidine against this liver

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fluke. We aimed to assess the efficacy and safety of these drugs compared with that of praziquantel in patients with *O. viverrini* infection.

Methods We did a randomised open-label trial between February and April 2010, in the Saysetha district, Attapeu Province, Laos. Eligible patients were school children aged 10–15 years who had *O. viverrini* infections. Patients were randomly assigned to one of five different treatment groups by use of a computer-generated randomisation code. We assessed efficacy as cure rate and egg reduction rate in intention-to-treat and per-protocol analyses. The trial was registered with Current Controlled Trials, ISRCTN23425032.

Results One hundred and twenty-five children were randomly assigned: 25 received mefloquine, 24 artesunate, 24 mefloquine-artesunate, 27 tribendimidine, and 25 praziquantel. Nineteen patients were lost to follow-up. In the intention to treat analysis, 14 patients receiving praziquantel were cured compared with none with mefloquine, one with artesunate (odds ratio 0.03, 95% CI 0.004–0.29), one with mefloquine-artesunate (0.03, 0.004–0.29), and 19 with tribendimidine (1.87, 0.60–5.85). Egg reduction rate was 98.4% for praziquantel, 30.2% for mefloquine (egg reduction-rate ratio 1.61, 95% CI 0.21–0.72), 31.5% for artesunate (0.43, 0.23–0.80), 41.3% for mefloquine-artesunate (0.60, 0.31–1.10), and 99.3% for tribendimidine (1.00, 0.44–2.30). Most adverse events were mild or moderate and affected all treatment groups; serious adverse events – vertigo, nausea, vomiting, and anxiety-were reported only by patients taking mefloquine or mefloquine-artesunate.

Interpretation Tribendimidine seems to be at least as efficacious as the drug of choice, praziquantel, for the treatment of *O. viverrini* infections; both drugs were well tolerated. Mefloquine, artesunate, and mefloquine-artesunate did not show an effect. Tribendimidine should be further investigated with large clinical trials.

1.3-027

Identification of genes mediating resistance in *Leishmania* by overexpression/selection followed by comparison with the genome database

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Employing a gene targeting method based on homologous recombination as well as in the fact that drug resistance in *Leishmania* is basically developed by protein overexpression often generated by gene amplification, we isolated more than eight genes/loci related with drug resistance in *Leishmania*. Briefly, we started from a genomic DNA library constructed into the shuttle vector cLHYG transfected into new wild type cells. Cosmids that codified for a protein whose overexpression is able to confer resistance to a determinate compound were selected in the presence of high concentration of the same drug. Using anti-*Leishmania* agents (Pentamidine, Terbinafine, Itraconazole, Tubercidin, Cyclosporin-A, Sinefugin, Miltefosine, WR-6026 and Pyrazofurin), we isolated more than 40 different loci approximately 35–40 kb long, defined by restriction and southern-blot analysis. Functional tests with insert deletions still capable to render cells resistance after transfection were performed. Chromosome localization was determined by nucleotide sequence associated with the *Leishmania* genome database. The final gene/loci identification can be done by gene interruption, amino acid comparisons, molecular analyses and functional tests with related compounds and/or inhibitors. Some of these proteins have recently been characterized in *Leishmania*. The association of these isolated proteins with drug resistance in leishmaniasis and its fundamental role in the pathology and pharmacology is a significant field in leishmaniasis chemotherapy, contributing enormously as a practical tool on this new functional genomic era.

1.3-028

In vitro leishmanicidal activity of imidazole- or pyrazole-based benzo[g]phthalazine derivatives against *L. infantum* and *L. braziliensis*

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The *in vitro* leishmanicidal activity of imidazole-based (1–4) and pyrazole-based (5–6) benzo[g]phthalazine derivatives has been studied against intra- and extracellular parasite forms of *L. infantum* and *L. braziliensis* species. Compounds 1–6 proved to be largely more active and less toxic than glucantime in the two *Leishmania* species considered. Infection rates and amastigote mean numbers data obtained from infected J774.2 macrophage cells showed that the monosubstituted benzo[g]phthalazine derivatives 2, 4, and 6 were clearly more active than their disubstituted analogs 1, 3, and 5 in both *L. infantum* and *L. braziliensis*. The inhibitory effect of the tested compounds on the protective antioxidant enzime Fe-SOD of promastigote forms of the parasites was remarkable, whereas inhibition of human CuZn-SOD was negligible. Ultrastructural alterations observed by electron microscopy in promastigote forms treated with 1–6 confirmed the greater toxicity of the monosubstituted compounds compared to their respective disubstituted partners. Finally, the modifications observed by 1H NMR in the composicion of the catabolites excreted by the parasites after treatment with 1–6 suggested that different action mechanisms could be involved, depending on the structure of the side-chains linked to the benzo[g]phthalazine moiety.

1.3-029

Effect of fasting and host genetics on the anthelmintic efficacy of plant derived cysteine proteinases

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Gastrointestinal nematode infections affect more than two billion people in the tropics causing extensive morbidity and death to human populations. Despite the prevalence of parasitic worms, anthelmintic drug discovery in tropical medicine is neglected by the majority of the pharmaceutical industry. Even those drugs that are used routinely for human treatment were first developed as veterinary medicines. The problem is being worsened by the increase of resistance to the limited number of drugs available for treatment of GI nematode infections. Therefore development of new, alternative treatments is urgently required. Plant cysteine proteinases from papaya latex, pineapple fruit and stem extracts has been demonstrated to be substantially effective against gastrointestinal nematodes of rodents and sheep. The current study investigated the effect of fasting on the efficacy of papaya latex supernatant (PLS), and compared efficacy in a range of inbred mouse strains of contrasting genotype. The results showed that

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fasting before treatment of mice did not significantly improve efficacy and by avoiding fasting the side effects of treatment were minimized. Comparison of efficacy in a range of mouse strains indicated that efficacy varied between mice of different genotype, a factor that will have to be taken into account when developing these agents further for use in human and domestic livestock.

1.3-030

Tropical pulmonary eosinophilia in two immigrants from India

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INTRODUCTION The main cause of eosinophilia in immigrants from tropics is parasitic infection. An absolute eosinophil count above 3000/mm^3 associated with respiratory symptoms in a patient coming from filaria endemic countries should be suggestive of Tropical Pulmonary Eosinophilia (TPE). TPE results from a hypersensitivity reaction to occult filariasis endemic in Southeast Asia, India, and Africa. Two cases of TPE diagnosed in Italy are reported. Case 1: A 29-year-old Indian man presented with a 3-month history of cough, weakness, weight loss (5 kg), eosinophilia. He was HIV negative. Case 2: A 36-year-old Indian man recently diagnosed for HIV infection presented with right-hemiplegia, persistent dry cough, expiratory wheezing, eosinophilia.

METHODS To confirm TPE diagnosis, for both patients we performed total and differential leucocyte counts, serum IgE level, radiograph and TC of chest, day and night blood samples for microfilaria, stools and urine microscopy for eggs, filarial serology. The therapy utilized was diethylcarbamazine 6 mg/kg daily for 21 days. Clinical examination and blood analysis were performed 2 and 3 weeks after beginning therapy and 3 weeks after its interruption.

RESULTS Hypereosinophilia (14,800 and 29,760/mm^3) and elevated serum IgE levels (IgE 103,000 and 10,999 kUI/l) were confirmed in both cases. Peripheral blood specimens tested negative for microfilariae in both patients. Filarial serology tested positive only for case 1, allowing confirming the diagnosis. Chest CT detected interstitial markings in case 1 and bilateral infiltrates in case 2. During follow-up, symptoms and laboratory findings improved for both patients. The response to treatment of case 2 indicates TPE diagnosis.

CONCLUSIONS TPE must be considered in patients who have peripheral blood hypereosinophilia, persistent respiratory symptoms and exposure history to filariasis endemic areas, particularly India. Being a neglected disease, drug is not readily available, in our case WHO support was essential for treatment.

1.3-031

Improving strongyloides spp. diagnosis – a novel specific real-time PCR for rapid and highly sensitive confirmation

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BACKGROUND *Strongyloides* spp. are soil-transmitted helminths which affect about 30,100 million people worldwide, mainly in tropical and subtropical countries. Due to rising migration from and tourism to endemic regions, this neglected infection is tools are often inadequate, means to detect and control the infection are limited. The purpose of this study was the evaluation of a novel real-time PCR for *Strongyloides* spp. to be implemented as a routine diagnostic tool in clinical practice algorithms.

METHOD Stool samples from patients with positive stool microscopy, a positive blood ELISA for *Strongyloides* ssp., eosinophilia or typical clinical symptoms were collected and DNA extracted with a special lysis method and amplified by a LightCycler real-time PCR. Gene sequencing was performed for confirmation.

RESULTS Out of 34 stool samples from patients clinically suspected of strongyloidiasis, nine samples showed positive results by real-time and conventional PCR confirmed by sequencing. Evaluation showed a specificity of 100% and no cross-reactivity with other helminths. Sensitivity of microscopy and serology were only 44% and 67%, respectively.

CONCLUSION The here described real-time PCR proved to be a highly sensitive and specific, inexpensive and rapid tool for the detection of strongyloidiasis and seems superior to microscopy and serology. Infection should be considered also in microscopically and serologically negative patients if typical symptoms or eosinophilia are present.

1.3-032

Epidemiology and monitoring of the effect of treatment of soil-transmitted helminth using multiplex real-time PCR

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INTRODUCTION Recently, real-time PCRs were developed and validated for the detection and quantification of a wide range of parasitic infections, and proved to be highly sensitive and specific. In the present study, a multiplex real-time PCR for the detection of soil-transmitted helminths was used to obtain the epidemiological characteristics and risk-factors of STH infections and monitor the effect of treatment.

MATERIALS AND METHODS A longitudinal double-blind household randomized study was performed, in a semirural area in Nangapanda, Flores Island, Indonesia with repeated treatment with albendazole (400 mg) or placebo at three monthly intervals. Before and 1 year after the intervention, stool samples were examined for STHs using microscopy after formol-ether concentration and using multiplex real-time PCR for the simultaneous detection of *Ancylostoma duodenale, Necator americanus*, *Ascaris lumbricoides*, and *Strongyloides stercoralis*.

RESULTS In the baseline study, microscopy and multiplex real-time PCR was available in approximately 1200 cases. *N. americanus* was found as the dominant species (72%) compared to *A. lumbricoides* (33.5%), *T. trichiura* (25.2% with microscopy only), *A. duodenale* (5%) and *S. stercoralis* (1.9. Quantitative real-time PCR revealed a typical decrease of the *Ascaris* prevalence with age whereas the prevalence and intensity of *N. americanus* increased until approximately 20 years of age and stabilized at later age. Factors that were associated with STH infections were: education level, occupation, house materials, water resource, toilet, waste processing, and main food. A significant decline in prevalence and intensity of *N. americanus* and *A. lumbricoides* infections was found in the albendazol treated cases as compared to the placebo group whereas no effect of treatment was found for *T. trichiura*.

CONCLUSIONS Multiplex real-time PCR can be used for sensitive and specific measurement of prevalence and intensity of infection in the epidemiology of STHs and monitoring the effect of Mass Drug Administration.

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1.3-033

Assessment of immunologic salivary proteins of *Glossina* sp. as an epidemiological tool, comparedto entomological method

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Glossina sp. (Tsetse flies) transmit the protozoan parasite *Trypanosoma* sp, responsible for sleeping sickness in livestock (Nagana disease) and Human (Human African Trypanosomiasis, HAT). In natural *Glossina* sp. populations, *Trypanosoma* sp. prevalence has been estimated to be around 5–10% in endemic regions of African trypanosomiasis. The transmission of the parasite is therefore the result of an intense interaction between the host and the vector. Exposure of populations to the *Glossina* sp. is evaluated by entomologic methods (e.g. capture by traps), but such methods cannot evaluate heterogeneous individual exposure. *Glossina* salivary proteins appear to be an epidemiological tool, useful to assess this interaction between host and vector. Indeed, many study have demonstrated that specific antibodies was directed against *Glossina* sp. salivary protein, but so far this potential tool has never been compared to entomological methods. This will be a transversal study conducted in Kasai/DRC, an endemic area for HAT. During this study we will determine the distribution and density of *Glossina* sp. in different parts of Kinshasa, by using capture by traps. Simultaneously we will make a screening of the related population, looking for antibodies against *Glossina* salivary proteins by assays. The aim of the study is to compare data from entomological method to those from serological screening. The results of this study will bring more information about this host/vector interaction, and will allow us to use the detection of antibodies against *Glossina* salivary proteins as a more efficient epidemiological tool for: identification of populations exposed to a high risk of transmission, individuals who may benefit from monitoring and for optimization of epidemiologic surveillance. This will help to conduct efficient entomological lute in the well identified regions.

1.3-034

Single-dose safety, pharmacokinetics (PK) and pharmacodynamics (PD) of fexinidazole

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BACKGROUND Fexinidazole is a 2-substituted 5-nitroimidazole, which exhibits activity against *Trypanosoma brucei* rhodesiense and *T. b. gambiense*, the causative agents of human African trypanosomiasis (HAT). This first in man study investigated the safety, pharmacokinetics and pharmacodynamics of single doses in healthy sub-Saharan male volunteers.

METHODS Seventy-two subjects were randomized in nine cohorts of eight subjects (six active + two placebo) to receive single ascending doses of 100–3600 mg FEXINIDAZOLE oral suspension. Assessment included clinical and laboratory safety and ECG recordings. Fexinidazole, fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2) were quantified in plasma and urine by LC-MS/MS.

RESULTS All subjects completed the study (mean, bSD age 27, b5.9 years). There were no clinically relevant abnormalities in laboratory parameters, ECGs or vital signs. Adverse events (AEs) were rare and no severe or serious AEs were reported. Fexinidazole and M1 exhibited similar kinetic pattern i.e. rapid absorption and elimination process (median Tmax 3.00 h–4.00 h and geometric mean (Gmean) T1/2 9–15 h for fexinidazole vs. median Tmax 2.00 h–5.00 h and Gmean T1/2 8–15 h, but slower for the second metabolite (M2) (median Tmax 18.00 h–24.00 h and Gmean T1/2 18–25 h). There was no saturation of the metabolism of fexinidazole and metabolites.

CONCLUSIONS Fexinidazole was generally well tolerated up to the maximum orally administered dose 3600 mg OAD. Within the dose range 100–3600 mg, the dose proportionality was not demonstrated for Cmax and AUCs neither for fexinidazole nor M1 nor M2. The elimination route of fexinidazole and metabolites M1 and M2 was almost entirely extra-renal.

1.3-035

Polyparasitism – A systematic review of gastro-intestinal co-infections with soil-transmitted nematodes (STN)

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INTRODUCTION Infections with Soil-Transmitted nematodes (STN: primarily *Ascaris lumbricoides* (Al), *Trichuris trichiura* (Tt), hookworms (Ho); and *Strongyloides stercoralis*) are common in the tropics, particularly in children, but the frequencies and types of co-infections (=polyparasitism) with different species are not well characterized.

METHODS We performed a systematic review of the literature (Pubmed/Medline and Embase for papers published during 2001–2010) to document and quantify the prevalence of co-infections with STNs in children and adolescents living in the tropics. Predefined selection procedures and STROBE-based quality criteria were applied.

RESULTS Of the 675 non-duplicate papers identified, 24 (~26,000 individuals, >24,000 children) met all criteria and were retained. Nine studies were from Asia, 10 from Latin America, five from Africa; 12 in rural, eight urban, four mixed settings. No obvious publication bias was found except for small studies with low co-infection prevalence. One or more STN infections were reported in 0.5–100% of subjects. Co-infection with 2–4 STN was reported in 5685 cases (5001 children) (range 0–72.6% of the subjects enrolled, or 0–99.7% of those infected). The largest subgroup of studies considered triple infections (Al, Tt, Ho) (13 studies enrolling 20,751 subjects, 79.8% of total). The crude aggregated rate for any infection was 53.6%. Triple infections ranged 0–31.1%, crude aggregated rate = 3.9% (95% CIs 3.6–4.2), but the results are heavily influenced by one study in Brazil contributing 64.0% of subjects. No triple infection was reported in five studies (2/4 African, 1/5 Latin American and 2/4 Asian studies).

CONCLUSIONS The majority of papers report marginal prevalences, so data could be extracted only from a fraction of the literature. Polyparasitism (especially double infections) is present in variable proportions (from none to all) of subjects (essentially children) in rural and urban tropical settings. Polyparasitism has consequences for individuals' health and should also be considered when assessing treatments' performance.

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1.3-036

Difficult but easy – the presentation and diagnosis of acute fascioliasis

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Fascioliasis is a food-borne trematode infection with important public health implications in areas of high endemicity and furthermore an emerging problem in travelers and migrants all around the world. This parasitosis presents in two clinically and diagnostically distinct forms: (i) the acute or hepatic stage caused by migrating larvae, (ii) the chronic or biliary form caused by adult trematodes. The diagnosis and management is challenging, especially in areas where fascioliasis occurs only sporadically or as an imported infection. Here we present five cases of acute fascioliasis diagnosed in an urban hospital in Santiago, Chile, and discuss the typical clinical presentation and the diagnosis including its pitfalls. All patients were adults suffering from acute upper abdominal pain accompanied by marked eosinophilia and focal hepatic lesions in CT scans. Some patients also presented with other abdominal and cutaneous symptoms. Diagnosis was confirmed by serological tests. Most patients were inhabitants of urban areas and only some had a history of consumption of raw aquatic or semiaquatic plants. The triad of acute upper abdominal pain, marked eosinophilia, and focal hepatic lesions is highly suspicious for acute fascioliasis. Diagnosis should be confirmed by *Fasciola* serology, whereas coprodiagnostic examinations are useless since the disease takes place during prepatency. Anamnestic data on food consumption and other risk factors might be misleading at least in areas of low endemicity such as Chile.

1.3-037

Chagas disease: new disease in Hospitalet de Llobregat, Barcelona

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CONTEXT Recent trends have spurred migration from Chagas-endemic areas to Europe.

OBJECTIVE To describe characteristics of Chagas' disease patients in our hospital.

MATERIAL AND METHODS Medical history of all patients with Chagas disease was reviewed. We included epidemiological data, clinical findings, electrocardiogram, echocardiography, and barium contrast studies. The proportion of turn-up to scheduled visits was calculated.

RESULTS One hundred and eleven patients were referred to our hospital because of a suspected diagnosis of Chagas disease. Eighty-nine attended and diagnosis was confirmed (80%). 72% were female (16 pregnant). Median age: 37 years (24–61). All but three patients were from Bolivia. Ninety percent arrived in Spain between 2003 and 2007. Eighty-six percent were unaware that they suffered from the disease when they arrived, but 39% reported family cases. Symptoms were present in 45%: 16 had constipation (18%), nine dyspnea 10%), five atypical chest pain (6%), five palpitations (6%) and four lipothymia (4%). Sixty-five percent did not complain of any symptoms. Physical examination revealed nine systolic murmurs (10%), six bradycardia or extrasystole (7%), two heart failure (2%). Electrocardiogram were taken for 65 (73%) and showed pathological signs in 18 (28%). Echocardiograms showed abnormalities in nine of 48. Three of seven barium contrast studies showed pathological findings. Half of scheduled visits were attended.

CONCLUSIONS Chagas disease presents an increasing challenge for clinicians. It is most common in females (72%) and young people (median age 37 years). Almost all patients were from Bolivia and had arrived between 2003 and 2008. Although 39% had a family history of Chagas disease, 86% were unaware of their own illness when they left their country. Sixty-five percent were symptom-free. The most frequent symptoms were constipation and dyspnea. Bradycardia/extrasystolia was the most frequent findings. Two had signs of heart failure, and ECG showed abnormalities in 28%. Transthoracic ecography confirmed structural pathology in nine. Patients found it difficult to attend scheduled visits.

1.3-038

Deworming improves asthma and temporarily deteriorates atopy: longitudinal anthelminthic treatment study in Cuba

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INTRODUCTION Soil-transmitted helminth (STH) infections have been suggested to protect from atopy and atopic diseases, although there is still no consensus on their relationship. However if the relationship is true, anthelminthic treatment would increase the prevalence of atopic disease in STH endemic populations. We investigated the effect of deworming and STH (re) infections on atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis.

METHODS We examined 389 Cuban schoolchildren aged 4–13 in six-monthly intervals for 24 months. STH infections were diagnosed by stool examination. Atopic diseases were diagnosed by International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and atopy by skin prick testing (SPT). STH infections were treated with one single dose of 500 mg mebendazole at every measurement period.

RESULTS After deworming the frequency of asthma significantly decreased ($P < 0.001$). The percentage of SPT positives temporarily increased from 10.2% (95% CI 4.5–15.9%) to 34.0% (95% CI 24.8–43.1%) and subsequently nearly returned to baseline values (14.4%, 95% CI 7.4–21.4%). (Re) infection with *A. lumbricoides* and *T. trichiura* was positively and hookworm negatively associated with having atopic diseases, while for atopy an opposite trend was seen.

CONCLUSION Our results indicate that atopic diseases improve after anthelminthic treatment while atopy increases. As this increase appears only temporarily, anthelminthic treatment does not seem to be a risk factor for the development of atopy, nor for atopic disease. Effects of STH (re) infections on atopy and atopic diseases appear to be species-specific.

1.3-039

Fruit-derived cysteine proteinase as novel anthelmintic for tapeworms?

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Gastrointestinal (GI) helminths pose a significant threat to livestock industry and are a recognized cause of global morbidity in humans. Control relies principally on chemotherapy but in the

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case of nematodes is rapidly losing efficacy through widespread development and spread of resistance to conventional anthelmintics and hence the urgent need for novel classes of anthelmintics. Cysteine proteinases (CPs) from plants such as papaya, pineapple and figs are effective against three murine nematodes *H. bakeri*, *P. muricola* and *T. muris in vitro* and *in vivo*. Preliminary evidence suggests an even broader spectrum of activity with efficacy against the canine hookworm *A. ceylanicum*, juvenile stages of parasitic plant nematodes of the genera Meloidogyne and Globodera and a murine tapeworm *H. microstoma in vitro*. This project focused on tapeworms, with *in vitro* experiments on two different rodent tapeworms *H. diminuta* and *H. microstoma* confirming that cysteine proteinases from papaya latex and pineapple do indeed affect cestodes by causing a significant reduction in motility leading to death of the worms. Observation of damage by scanning electron microscopy revealed tegumental damage. These findings were also verified by pilot *in vivo* studies in which treatment with papaya latex on rodent hosts infected with *H. diminuta* and *H. microstoma* demonstrated significant reductions in worm burden and biomass. However, worm fecundity was not affected in both rodent cestode models studied.

1.3-040

A survey of bancroftian filariasis for microfilariae, disease and drug coverage in four sentinel sites of sikasso in Mali

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A study was conducted to assess the prevalence of filarial infection and that of its complications as well as drug coverage before the community directed treatment (ComDT) in four sentinel sites of the district of Sikasso from December 2004 to January 2005. Social mobilization and training of laboratory workers tokk place before data collection and treatment. Prevalence of circulating filarial antigen (CFA) was estimated by immunochromatography test (ICT): 100 µl blood was collected from each individual by venepuncture. Night blood smears (20 µl blood) collected between 22:00 and 01:00 h were examined to detect microfilariae (Mf). Clinical examination was performed according to WHO criteria to classify filarial disease. By ICT test, 52.5% of blood samples were positive for CFA and the results were significantly different between sites (P = 0.013). The overall microfilariae prevalence was 26.1%, the prevalence was significantly higher in Kola with 37.0% (185/500) than in the other sites ($P < 10^{-6}$). Three chronic manifestations were observed: lymphoedema (6/500), leg elephantiasis (14/500) and hydrocele (5/500). The community directed treatment (ComDT) coverage rate after using a single-dose ivermectin (200 µg/kg) and albendazole (400 mg) was 91.1% (1987/2180). It varied significantly between sites ($P < 10^{-6}$).

KEYWORDS *Bancroftian filariasis*, microfilaraemia, antigenaemia, sentinel sites, Mali

1.3-041

Age-stratified serum cytokine profile (IL-6, IL-10, TNF-ζ) in Kenyan children with early *Schistosoma haematobium* infection

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In a study of children with polyparasitic infections in a *Schistosoma haematobium* (Sh) endemic area, we examined the hypothesis that infection-associated inflammation and disease precedes detection of Sh infection by standard urine filtration. Children 5–18 year old were surveyed in August–October 2009, and tested for *P. falciparum* by ICT card and for Sh both by urine filtration and anti-SWAP detection. IgG4 anti-SWAP positive children ($n = 221$) were compared to anti-SWAP-negative children ($n = 62$) for levels of pro-inflammatory cytokines IL-6, TNF-á, and down-regulatory IL-10. In the á-SWAP positive children, regardless of age, there were higher serum IL-6 levels compared to á-SWAP negative children, with the greatest difference seen at 11–13 year (mean 5912 ng/ml). Increased serum IL-6 correlated with parasitic infection, anemia, and acute and chronic malnutrition. IL-10 levels peaked at 9–11 year in the á-SWAP positive group (mean 430 ng/ml) and were inversely correlated with IL-6 levels. Children in the á-SWAP positive group and infected with hookworms and *P. falciparum* had significantly increased serum levels of IL-10 (P = 0.045 and P = 0.015). Elevation of TNF-ζ in the á-SWAP group was also associated with malaria infection in 7–9 year olds (P = 0.009). Our results show a marked difference in the cytokine profile among á-SWAP positive vs. á-SWAP negative children, with an early inflammatory response in á-SWAP positive young children (5–7 year old), measurable by increased IL-6 and low IL-10, before eggs are detected in urine. Schistosomiasis-malaria co-infection strongly correlated with higher pro-inflammatory cytokines in serum, suggesting an important morbidity-related interaction between these parasite species in children.

1.3-042

Impact of polyparasitic infections on anemia and growth retardation among Kenyan children living in a *Schistosoma haematobium* endemic area

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Objectives were to measure the co-prevalence of endemic parasites – *Schistosoma haematobium*, filariasis, malaria, hookworm, and other geohelminths among residents 5–18 years old in coastal Kenya, and to determine the relationship between co-infection (and parasite load) with the disabling outcomes of anemia and growth retardation. Two thousand and thirty-five children were surveyed in four villages. Both single and multiple parasitic infection were highly prevalent in the area with some significant heterogeneity among communities. *Schistosoma haematobium* was most prevalent in all villages (25–62%) followed by hookworm (11–28%) and malaria (8–24%). Anemia was highly prevalent (45–58%) in all villages, as were both acute and chronic undernutrition, measured as wasting (10–18%) and stunting (25–45%) respectively. Regression modelling indicated marked gender- and age-related differences in the prevalence of these morbidities. Significant predictors for anemia were young age (5–7 years old) combined with malaria in boys, and with high intensity schistosomiasis in girls. Filariasis had a significant association with malnutrition only in girls. There were also striking gender differences in the association between polyparasitic infections and the study outcomes: Hookworm-malaria for boys and schistosomiasis-hookworm and schistosomiasis-malaria for girls were significantly associated with greater odds of anemia. Synergy between co-infection with schistosomiasis-filariasis was associated with both wasting and stunting, but only in girls. Our results highlight the continuing high prevalence of polyparasitism in a high-risk endemic area. They also highlight the potentially synergistic, negative effect of the co-infections on children's health. The marked gender and age differences we observed underscore the need to research related differences in immunological response and in environmental exposure. Such knowledge will help target at-risk populations in future control initiatives, and will further

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our understanding of the complex disease-producing pathways that affect children harbouring multiple parasitic infections.

1.3-043

Associations between atopic markers in asthma and intestinal helminth infections in Cuban schoolchildren

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BACKGROUND Total serum IgE (tIgE), allergen-specific IgE (sIgE), and skin prick test (SPT) are commonly used markers for atopy and atopic disease. The association between these measures and their relationship to clinical symptoms, differ in affluent and non-affluent countries.

OBJECTIVE We investigated the role of intestinal helminth infections in observed variations in atopic markers and asthma, and possible diagnostic and epidemiological consequences.

METHODS A cross-sectional study was conducted in Cuban schoolchildren (n = 1285; 4–14 years). Atopy was determined by SPT, sIgE and tIgE; asthma by ISAAC questionnaire; and intestinal helminth infections by stool examination.

RESULTS Percentages of tIgE, sIgE and SPT positives were 88.9%, 25.5% and 16.5%, respectively. Asthma was found in 20.8%, and helminth infections in 20.9% of the children. All three atopic markers were significantly associated with each other and with asthma. Median tIgE levels were higher in helminth infected than in uninfected children, irrespective of their status of atopy/asthma, and vice versa. Discordant results between SPT and sIgE were observed in 22.6% of the children. Among SPT positives, 41% were sIgE negative. The proportion of SPT negatives among sIgE positives was 74% in helminth infected and 58.4% in uninfected children ($P < 0.05$).

CONCLUSION Helminth infections affected tIgE levels, reconfirming the limited value of tIgE for diagnosis of atopy and asthma in tropical areas. Higher frequencies of sIgE than positive SPTs were observed, especially in helminth infected children. This corresponds with current hypotheses on the role of helminths in atopy. However, the observed proportion of sIgE negatives among children with positive SPT suggests that other mechanisms may also be.

1.3-044

Randomized clinical trial on ivermectin vs. thiabendazole for the treatment of strongyloidiasis

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INTRODUCTION Strongyloidiasis may cause a life-threatening disease in immunosuppressed patients. This can only be prevented by effective cure of chronic infections. Direct parasitologic exams are no prove of cure if negative. We used SEROLOGY (IFAT) along with direct methods for patient inclusion and efficacy assessment.

SUBJECTS AND METHODS Prospective, randomized, open label, phase III trial conducted between 2004 and 2007 at the Centre for Tropical Diseases (Verona, Italy) to compare efficacy and safety of ivermectin (single dose, 200 ìg/kg and thiabendazole (two daily doses of 25 mg/kg for 2 days) for strongyloidiasis. Consenting patients responding to inclusion criteria (positive IFAT for *S. stercoralis*) were randomly assigned to one of the treatment arms. Primary outcome was: negative direct and indirect (IFAT) tests at follow-up (4–6 months after treatment) or subjects with negative direct test and drop of two or more IFAT titers.

RESULTS Considering 198 patients who concluded the follow-up, efficacy was 56.6% for ivermectin and 52.2% for thiabendazole ($P = 0.53$). If the analysis is restricted to 92 patients with IFAT titre 80 or more before treatment (virtually 100% specific), efficacy would be 68.1% for ivermectin and 68.9% for thiabendazole ($P = 0.93$). Considering direct parasitological diagnosis only, efficacy would be 85.7% for ivermectin and 94.6% for thiabendazole ($P = 0.21$). In ivermectin arm, mild to moderate side effects were observed in 24/115 patients (20.9%), vs. 79/108 (73.1%) in thiabendazole arm ($P = 0.00$).

CONCLUSION No significant difference in efficacy was observed, while side effects were far more frequent in thiabendazole arm. Ivermectin is the drug of choice, but efficacy of single dose is sub optimal. Different dose schedules should be assessed by future, larger studies. Trial Registration Eudract n. 2004 004693 87. The full article has been submitted to Plos Neglected Tropical Diseases.

1.3-045

Burden of filarial diseases in Bangladesh

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Bangladesh has one of the largest burdens of lymphatic filariasis (LF) in the world, with an estimated 70 million people at risk of infection, and 10 million suffering from various forms of clinical deformity including grossly swollen limbs and genitals. The national programme to eliminate LF is prioritizing morbidity management in line with global objectives. The highest rates of disease occur in the far north western districts of the country, and in 2003 the Syedpur Filarial Hospital opened to treat and provide morbidity management to the tens of thousands of patients in the region. The Filarial Hospital routinely collects data on patients, and the aim of this study was to examine these data to determine the number, distribution and demographic profile of LF patients utilising this specialised health service. Data from 2003 to 2010 were available for analyses, and information on the number of patients, place of residence, age, sex, clinical manifestations (i.e. lymphodema, hydrocele) and type of surgical procedures were examined using statistical and mapping software. These analyses found a total of 1174 individuals with LF clinical manifestations were admitted as inpatients, 19,162 attended outpatient clinics, and more than 3200 sought treatment at satellite clinics or mobile camps. The annual number of patients increased steadily from 31 to 893 inpatients and 893 to 4422 outpatients between 2003 and 2010. Most LF patients registered as new patients and came from Nilphamari, Dinajpur and Rangpur Districts, and were aged 25–50 years. The majority of inpatients were males, admitted for hydrocelectomy. There were similar numbers of males and females seeking treatment at outpatient and satellite clinics, predominantly for leg lymphodema. This study highlights the physical burden of LF disease on individuals in endemic communities, which can have a major psychosocial and economic impact and affect their quality of life.

1.3-046

Scaling-up of insecticide-treated bed net coverage and the impact on filariasis transmission in Africa

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 and the majority of endemic countries are implementing of the WHO recommended mass drug administration (MDA) to interrupt transmission of the parasite. However,

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many LF endemic countries in Africa have just started, or are yet to implement the GPELF MDA strategy, which does not include vector control. Nevertheless, the dramatic scale up in usage of insecticide treated/long lasting nets (ITNs/LLINs) for malaria in Africa may significantly impact LF transmission because the parasite is transmitted mainly by *Anopheles* mosquitoes. Therefore, this study aimed to examine the magnitude and geographical distribution of ITNs/LLINs in African countries, and the extent to which they have scaled up over time. National data on mosquito nets, ITNs/LLINs were obtained from published literature, national reports, surveys and datasets from public sources such as Demographic Health Surveys, Malaria Indicator Surveys, Multiple Indicator Cluster Surveys, Malaria Report, and Roll Back Malaria websites. The type, number and distribution of ITNs/LLINs were summarised and mapped at sub-national level, and comparison made between urban and rural communities, and with known or potential LF distributions. These analyses found that ITNs/LLINs had increased significantly since 2005, with a 3-fold increase in ITN ownership overall. However, coverage varied dramatically, with some regions reporting >70% ITNs ownership, while others had very low coverage in LF endemic regions, especially among children under 5 years. Sub-national maps showed coverage distributions across countries, and highlighted differences between urban and rural communities over time. Although African countries are behind with initiating MDA, the continued global financial support and rapid scale up of ITNs/LLINs for malaria control, has the potential to help GPELF in reaching its target of global elimination by 2020.

1.3-047

A global comparative evaluation of commercially available rapid diagnostic tests for visceral leishmaniasis

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INTRODUCTION Access to prompt and accurate diagnosis of visceral leishmaniasis (VL) is a major hurdle to disease control. Antibody-detecting rapid diagnostic tests (RDT) create an opportunity to safely and efficiently link diagnosis and treatment. However, there are multiple products to choose from on the market and published reports of variable test performance.

METHODS Five commercially-available VL rapid diagnostic tests containing bound rk39 or rkE16 antigen were evaluated at baseline and after heat incubation (37°C, 45°C × 60 days) using archived human sera from confirmed VL cases (n = 750) and endemic non-VL controls (n = 754) in the Indian subcontinent (ISC), Brazil and East Africa to assess regional sensitivity and specificity with 95% Confidence Intervals. Inter-lot and inter-observer variability was assessed.

RESULTS All test brands performed well against ISC panels [sensitivity range (92.8–100.0%) specificity range (96.0–100.0%)]; however sensitivity was lower (61.4–91.2%; 36.8–87.2%) against Brazil and East African panels, respectively. Specificity was consistently >95% in Brazil and ranged between 90.8% and 98.0% in East Africa. Outside of the ISC, rK39 tests generally performed better than rkE16-based tests. One RDT consistently returned negative and/or invalid results after incubation at 45°C for 60 days; while others remained stable. Agreement between lots and readers was good to excellent (kappa > 0.73–0.99).

CONCLUSIONS Diagnostic accuracy of VL RDTs varies between the major endemic regions. Many tests performed well and showed good heat stability in the ISC; however, reduced sensitivity against Brazilian and East African panels suggests that in these regions, used alone, most RDTs are inadequate for excluding a VL diagnosis. Therefore, use in combination with a more sensitive test is recommended. Performance of some products is adversely affected by high temperatures. Between reader agreement was excellent. More research is needed to assess ease of use in field conditions and to compare test performance using whole blood instead of serum.

1.3-048

Risk factors for post-kala-azar dermal leishmaniasis in Nepal, a retrospective cohort study

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INTRODUCTION Post-kala-azar dermal leishmaniasis (PKDL) is a late complication of visceral leishmaniasis (VL) and is considered a potential reservoir in the transmission of *Leishmania* donovani. We studied the probability and risk factors to develop PKDL after VL treatment in the eastern region of Nepal.

METHODOLOGY Between February and May 2010 trained field workers traced the patients who had received VL treatment between 2000 and 2009 in five endemic districts and screened them for PKDL-like skin lesions. Suspected cases were referred to a tertiary care hospital (BPKIHS) for confirmation by parasitology (slit skin smear (SSS)) and/or histopathology. Demographic, socioeconomic and clinical risk factors were assessed by computing adjusted odds ratios in a logistic regression model.

RESULTS Of 680 past-treated VL patients, 37 (5.4%) with active skin lesions suspect of PKDL were detected. Thirty-three of them presented for dermatological assessment, and 16 (2.4%) were found with probable (n = 2) and confirmed (n = 14) PKDL. All 16 PKDL cases had been treated by sodium stibogluconate (SSG) in the past. Skin lesions developed after a median time interval of 21 months [interquartile range (IQR) = 15–45]. There was a significantly higher PKDL rate (29.4%) in those who received inadequate VL treatment compared to those who were treated with SSG 20 mg/kg daily for more than 20 days (2.0%). In the logistic regression model, ambulatory treatment at government health facilities [odds ratio (OR) = 11.4, 95% CI 1.5–90.0], and inadequate treatment (OR = 30.5, 95% CI 6.2–150.9) were significantly associated with PKDL.

CONCLUSION The occurrence of PKDL after VL treatment in Nepal is low compared to neighboring countries. Supervised and adequate treatment of VL is essential and could reduce the risk of PKDL development. Active surveillance for PKDL is needed.

1.3-049

Are intestinal parasites fuelling the rise in dual burden households in Venezuela?

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INTRODUCTION In developing countries undergoing rapid economic development the prevalence of dual burden (co-existing over-

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weight/obesity and stunting) households is increasing. While intestinal parasites are usually prevalent in these countries, their role in the dual burden phenomenon of overweight/obesity and stunting has so far been neglected. We studied the associations between dual burden households and intestinal parasite infection in a rural community of Venezuela.

METHODS We examined data of 41 households (142 children and 99 adults) in a low income community with measured data on height, weight and parasitic infections. Dual burden households were defined as households with an overweight/obese adult (BMI > 25) together with a stunted child (height for age *z* score < −2.) Intestinal parasite infection was determined by direct and ferric haematoxylin stained smears of two faecal samples.

RESULTS Sixty-five percent of the adults in the community were either overweight (39%) or obese (26%) with almost equally high prevalences of parasitic infection (38% geohelminth and 15% *Giardia lamblia*). In addition, 14% of the children were stunted. In more than one in four households (26%) we found an overweight adult together with a stunted child. Members of such a dual burden household were 2.05 times more likely to have intestinal parasites, a result that was statistically significant [Confidence Interval (CI) = 1.03–4.09] and remained true both for geohelminth infection OR = 1.90, CI = 1.02–3.53 and *G. lamblia*: OR = 2.35, CI = 1.04–5.31.

CONCLUSIONS This cross sectional study shows that parasitic infection is strongly associated with the dual burden of adult overweight/obesity and childhood stunting, pointing to a triple burden of disease in this community in Venezuela. While the relationship between parasitic infection and stunting has been well established, the association of intestinal parasite infection with overweight/obesity in adults needs to be explored further.

1.3-050

Evaluation of cutaneous leishmaniasis in patients referred to pasteur institute of Iran

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Cutaneous leishmaniasis (CL) is a widespread tropical infection which has a high incidence rate in Iran. *Leishmania tropica*, the causative agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, which causes zoonotic cutaneous leishmaniasis (ZCL), are endemic in various parts of Iran with a high incidence rate. The aim of this study was to evaluate the reappraisal of the diagnosis and epidemiology of CL in Iran, by different clinical, parasitological and molecular assays among patients suspected of CL referred to the Department of Parasitology, at the Pasteur Institute of Iran during 2006–2009. Two hundred samples from patients with ulcerative skin lesions were collected, clinical analyses were applied, data questionnaire was completed and samples were examined for CL by using both direct microscopic and culture methods. Moreover, PCR assay was applied for detection of *Leishmania* species in CL isolates resulting from parasitological assay. Of 200 patients, *Leishmania* body was observed in 77 samples (38.5%) by direct smear and 40% by cultivation assay. Most patients (21.3%) had a travel history to Isfahan province, one of the most important endemic areas of CL located in center of Iran. PCR assay by kDNA indicated 32 and 18 of 50 isolates respectively had similar patterns with standard *L. major* and *L. tropica*. In conclusion, clinical manifestations and an appropriate diagnostic assay with a parallel molecular characterization of CL may lead to a screening evaluation of disease, prognosis, treatment and control strategies.

KEYWORDS parasitological analysis, molecular biology, leishmaniasis, cutaneous, Iran, epidemiology

1.3-051

Leishmania infantum infection induces in its later stage severe nuclear degeneration of the host cell

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INTRODUCTION A number of works have reported on the encapsulation in liposomes of drugs against *Leishmania* as a strategy to increase efficacy and decrease unspecific toxicity, but a few knowledge gaps still obscure some of the subcellular processes underlying this effect.

METHODS AND MATERIALS Peritoneal macrophages were obtained after stimulation of female Swiss mice with sodium thioglycolate, seeded in a chamber slide system and incubated for 24 h. The medium was then removed and a late stationary phase promastigote culture of the *L. infantum* strain MCAN/ES/92/BCN503 was added in RPMI medium. The culture was incubated for a further 24 h and then washed to eliminate free promastigotes. After addition of liposomes containing quantum dots (QDs) followed by 1-h incubation, confocal microscopy was used to observe liposomes, lisosomes, nuclei, Leishmania, and phagosomes.

RESULTS QD fluorescence could be observed inside the cells as whole internalized liposomes 30 min after the addition of liposomes to macrophages. Colocalization analysis of QDs and lisosomes revealed that internalized liposomes fuse with lisosomes. In the early stages of infection single promastigotes can be observed inside the phagosome, whereas lisosomes are still scattered throughout the cell. In those macrophages where *Leishmania* has replicated, lisosomes are absent from the cytosol and their specific fluorescence is strongly localized inside the phagosome where the parasite has already divided, but not in those containing only one amastigote. In later stages, when the parasite has multiplied several times, we have observed strong staining of the macrophage nucleus with dansyl-cadaverine, a phagosome marker.

CONCLUSIONS Lisosomes can be used to target liposomal anti-*Leishmania* drugs towards the parasite-containing phagosomes. Only after intraphagosomal replication of *L. infantum*, phagolysosomal fusion proceeds. *Leishmania* infection induces in its later stages severe nuclear degeneration and possibly apoptosis of the host cell.

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1.3-052

A serological and molecular study of *Coxiella burnetii* in human, vertebrates and arthropods in Rio de Janeiro, Brazil: preliminary results

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INTRODUCTION The study was conducted after the occurrence of the first Q fever case confirmed by polymerase chain reaction (PCR), in Itaboraí municipality, Rio de Janeiro State, Brazil. An epidemiological investigation was carried out at two steps: (i) the

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samples of patient's family and pets living at the patient's house were collected, (ii) the inquiry was made in the property to where the goats were transferred; animal handler's family and pets samples were taken to investigate *Coxiella burnetii* infection.

METHODS Blood samples from the family and domestic animals as well as milk, faeces, vaginal, nasal and fecal mucus samples of goats and arthropods were collected. Serum samples collected in the two stages of the study were tested for antibodies anti-C. burnetii phases I and II, using a commercial indirect immunofluorescence assay (IFA) for IgG (PANBIOTM);cut-off titer at 64. DNA extracted from all samples was tested for the presence of C. burnetii using a PCR targeting the heat shock protein (htpAB) gene.

RESULTS AND CONCLUSIONS At step one of the study, the patient's wife and two of 13 dogs showed reactivity to phase II at titer 128 and 64, respectively. At step 2, blood samples from patient's family and animals, except goats, were tested to IFA and not showed reactivity. All blood samples were negative to PCR. A pool of arthropods, collected from a horse, identified as *Amblyomma cajennense*, showed expected amplicon size (687 bp) as also six milk samples from seven adult goats and one anal swab. Only two of the three milk samples were analyzed, and nucleotide sequence of the amplicon generated showed 99% identity to the homologous sequence of the *C. burnetti* transposon gene deposited at Genebank. This study revealed the circulation of *C. burnetii* in Itaboraí municipality and the goats are probably involved in this outbreak.

1.3-053

Help-seeking for pre-ulcer and ulcer conditions of buruli ulcer disease in Ghana

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BACKGROUND Since the mode of Buruli ulcer (BU) transmission is unknown, public health programmes in affected countries encourage early medical treatment to alleviate suffering and avoid disabilities. Studies of affected persons are required to highlight the influence of social, cultural, economic and behavioural factors on help-seeking (HS) and delays in medical treatment. This study examined socio-cultural features of help-seeking for BU-affected persons with pre-ulcers and ulcers in an endemic area of Ghana.

METHODS A sample of 181 respondents, were purposively selected from three BU treatment centres and 67 endemic communities. The Fisher's exact test was used to compare HS variables for pre-ulcers and ulcers. A two-sample test for proportions was used to compare the perceived effectiveness of treatment from different providers. Qualitative phenomenological analysis of narratives clarified the meaning and content of selected quantitative HS variables.

RESULTS For pre-ulcers, herbal dressings were placed on affected body parts to expose necrotic tissues and subsequently used as dressings for ulcers. Analgesics and left-over antibiotics were used to ease pain and reduce inflammation. Respondents were more likely to seek medical care for ulcers (62.4%, P = 0.001) than pre-ulcers (16.6%). Choices for outside-help were influenced by the perceived effectiveness of the treatment, the closeness of the provider to residences and the influence of family and friends. Herbal treatment was easily accessible since most herbalists were itinerant and family members.

CONCLUSION Health education is required to emphasise the risk of self-medication with antibiotics, the importance of medical treatment for pre-ulcers, and to caution against the use of herbs to expose necrotic tissues which could lead to co-infections. Access to medical treatment should be improved to encourage early medical care. Since herbalists contribute to delay in seeking medical care, their role as health educators and advocates for early medical treatment should be explored.

1.3-054

An outbreak investigation of visceral leishmaniasis among urban residents of Dharan town, eastern Nepal

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INTRODUCTION Visceral Leishmaniasis (VL) is a predominantly rural disease, common in the lowlands of Nepal. Since 1997, VL has also been reported from the city of Dharan. We investigated risk factors for VL among residents of Dharan town.

METHODS We conducted an outbreak investigation including a case–control study; cases were all urban residents treated for VL between 2000 and 2008 at BPKIHS. Controls were healthy persons with no previous history of VL and were selected from a census list frequency-matched for age. Cases and controls were interviewed in their homes with a structured questionnaire. A multilevel model with ward (neighborhood) as random effect was used in the data analysis.

RESULTS A total of 158 VL cases and 448 controls were enrolled. The distribution of cases was clustered with 70% of cases vs. 32% of controls resident in three out of 19 wards. Proximity to other VL cases was a strong risk factor (OR 4.8, 95% CI 2.6–8.6). Other associated factors were: 'Blood transfusion' (OR 3.6, 95% CI 1.4–9.1), 'Regular forest visits' (OR 2.9, 95% CI 1.7–5.1), 'Daily wage earner' (OR 2.5, 95% CI 1.4–4.4), 'Earthen floors' (OR 2.2, 95% CI 1.1–4.4). Sleeping on a bed (OR 0.31, 95% CI 0.13–0.78), ownership of cattle (OR 0.11 95% CI 0.01–0.92) and 'Socio-economic status' (OR 0.01, 95% CI 0.001–0.05, for richest vs. poorest quartile) were protective factors against VL.

CONCLUSION The distribution of VL was strongly clustered; proximity to other VL cases was a strong risk factor as were housing conditions. All of this suggests local transmission of VL in Dharan town, but requires further entomological evidence. The association between VL and blood transfusion could be due to reverse causality.

1.3-055

Study of the *Leishmania infantum* metacaspase

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Leishmania are flagellated protozoan parasites of the Trypanosomatidae family. They cause leishmaniasis which are responsible for approximately 60,000 deaths each year. The WHO considers the leishmaniasis as one of six priority diseases of the research program on tropical diseases. The medical importance of theses parasites and the lack of really satisfactory treatment lead to the urgent need of new therapeutics and of the discovery of new therapeutic targets. In this purpose, but also to better understand the biology of this parasite, we are interested in a key process: programmed cell death. Contrary to mammals, *Leishmania* do not possess caspases, key enzymes of mammalian apoptosis. However *Leishmania* express metacaspases, peptidases related to caspases in terms of amino acid sequence, structure and characteristic catalytic

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domains. The exact role of metacaspases remains controversial. In order to better understand the role of these proteins in *Leishmania* biology, we are studying the metacaspase of *L. infantum*. This species is endemic in the south of France, more particularly in the Marseille area and is responsible for the most severe form of the disease. Furthermore, little is known about *L. infantum* apoptosis. To understand the role of this protein, we are studying its cellular localisation after GFP-fusion, as well as its expression during apoptosis induced by stationary phase and serum deprivation. To correlate the various apoptosis phases to the expression of the metacaspase, apoptosis markers like phosphatidylserin exposure, mitochondrial membrane potential modifications and DNA fragmentation are used to establish the apoptosis sequence. This study will allow to confirm or to counter the role of the metacaspase during the programmed cell death of *Leishmania*.

1.3-056

The economic burden of visceral leishmaniasis on households in south-eastern Nepal

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INTRODUCTION Visceral leishmaniasis (VL) is a deadly disease affecting poor people living in precarious conditions. Studies from other countries showed that VL leads to substantial direct and indirect costs. We examined the economic impact of VL on households living in urban and rural areas of south-eastern Nepal.

METHOD We interviewed 283 recent VL patients (115 urban and 168 rural) using a structured questionnaire about health seeking behaviour, direct medical and non-medical costs and coping strategies (both time and financial coping strategies). We assessed the income of the patient and household as well as productivity losses resulting from illness.

RESULTS A median number of two providers were visited prior to treatment. The majority of urban patients (52%) visited an unqualified provider first, while rural patients tended to visit a public provider (51%). The median delay between onset of symptoms and presentation to a qualified health provider was shorter for urban compared to rural patients (14 vs. 24 days). None of the interviewed patients reported expenditure for drugs and diagnosis of VL. The total median cost of a VL episode to the patient's household was similar for urban and rural patients (i.e. US$183). Loss of income represented more than 60% of total costs for both groups. Overall, the median total expenditure (direct and indirect) represented 67% and 57% of annual per capita income of urban and rural patients respectively. Using savings and taking a loan were the most commonly reported coping strategies to cover expenditures.

CONCLUSION Despite drugs and diagnostics being provided free of charge, VL patients and their household still incur substantial costs. In addition strategies to cope with VL health expenditure such as borrowing money at high interest rates may result in households being pushed further into poverty. Ignoring these costs will seriously hamper VL control efforts.

1.3-057

Baseline burden estimate for Brucellosis and Bovine Tuberculosis, planning of an intervention, and demonstration of cost-effectiveness of one-health intervention package

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ICONZ or the 'Integrated Control of Neglected Zoonoses' is a large collaborative project supported by the EU, incorporating seven Case Studies, one of which is being conducted in Nigeria and is of focus here. The Nigeria Case Study is comprised of three phases. The first phase is currently underway and involves collection of data (using cluster sampling methodology) on the 'burden' of the bacterial zoonoses cluster (comprising Brucellosis and Bovine Tuberculosis) on human and animal populations in two contrasting study sites (the Jos Plateau where the pastoral Fulani practise traditional migration, and the Kachia Grazing Reserve where populations are purportedly sedentary but in reality still practise dry season migration). The 'burden' estimate for Brucellosis will be derived from human and cattle seroprevalence (using the Rose Bengal Test), and socioeconomic data on cattle production losses (e.g. fertility), monetary expenditure on human and animal health and non-monetary losses to human health. For Bovine Tuberculosis, occurrence of disease will be estimated using the Comparative Intradermal Skin Test. Phase two will consist of designing and planning an intervention based on the evidence collected during Phase 1. Cost-effectiveness of the intervention will be demonstrated in Phase 3 (in real terms if the intervention is administered or modelled if it is not). This will be accomplished by comparing the costs in terms of burden or losses of Brucellosis or Bovine Tuberculosis against the benefits and costs (modelled or real) of disease control, thereby demonstrating the added value of transdisciplinary, holistic or 'one-health' intervention packages.

1.3-058

Evaluation of selective periodic mebendazole treatment against soil-transmitted helminth infections in Cuban schoolchildren

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BACKGROUND A single dose of 500 mg mebendazole is one of the recommended treatments for soil-transmitted helminth (STH) infections by the WHO. Based on randomized, placebo-controlled trials, mebendazole shows high cure rates for *A. lumbricoides*, but not for *T. trichiura* and hookworm. We evaluated the impact of mebendazole treatment on STH infections in Cuban school-children under field conditions.

METHODS During a 3 year period, we followed up 268 STH-positive Cuban schoolchildren (5–16 years) at 6 months intervals, and assessed the effect of selective periodic treatment with a single dose of 500 mg mebendazole on STH infections. Infections with *A. lumbricoides*, *T. trichiura*, and hookworm were diagnosed by stool examination (two 25 mg Kato-Katz examinations). Common risk factors related to STH were assessed by parental question-naire.

RESULTS A significant reduction in the number of STH infections between 58.7% and 93.9% was obtained after 3 years with the highest reduction for *T. trichiura* and the lowest for hookworm. Between two consecutive follow up periods we found cure rates between 56.3–82.6%, 52.4–76.9% and 44.4–76.7% for *A. lumbricoides*, *T. trichiura* and hookworm, respectively. After

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two treatment rounds, approximately 75% of the children infected were cured, with important differences between helminth species (95.2% for *A. lumbricoides*, 80.5% for *T. trichiura*, and 76.5% for hookworm). At the end of the study, cumulative cure rates were almost 100% for all three STHs. No differences in common risk factors for helminth infections were observed between children who were cured after one treatment and those who were still helminth positive after at least four treatments.

CONCLUSION Our results indicate that periodic selective treatment with a single dose of 500 mg mebendazole is effective in reducing the number of STH infections in Cuban schoolchildren, although not equally for all helminth species, and only after at least two rounds of selective treatment.

1.3-060

Subcutaneous sparganosis in a Japanese immigrant caused by *Spirometra erinaceieuropaei*

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INTRODUCTION Sparganosis is a neglected parasitic disease caused by the plerocercoid stage (sparganum) of *Spirometra* sp., a pseudophyllidean tapeworm. The disease is most often found in East Asia, were humans become infected by eating raw or undercooked meat of fish, amphibians, or reptiles which contain the larval stage. The spargana invade the brain, eye, viscera, and the subcutis, and can cause serious illness. A rare and complicated form, disseminated proliferative sparganosis, is caused by a branching, maldifferentiated plerocercoid provisionally termed *Sparganum proliferum*.

METHODS An abdominal subcutaneous nodule was surgically removed from a 60-year-old female Japanese immigrant, with preceding breastcancer. The removed tissue contained wormlike structures that were embedded in paraffin for further histological examination. Later DNA was extracted from the paraffin embedded tissue and subjected to a cestode-specific mitochondrial 12S rRNA gene- and cytochrome c oxidase subunit 1 (cox1) -PCR for species identification by sequencing of the PCR-product.

RESULTS Morphological and histological analysis of the helminth showed typical aspects of a cestode, i.e. calcareous corpuscles in a spongy stroma surrounded by an aspinous tegument. As a characteristic feature of a pseudophyllidean cestode larva (plerocercoid or sparganum), the anterior region showed an invagination and no proper scolex had been developed. The organism was pseudosegmented without any strobilar structures. Sequence analysis of the 440 and 425 bp amplicons of the 12S rRNA gene- and cox1-PCR showed 100% and 99% identity with *S. erinaceieuropaei* (AB374543.1 and AF096237.2, AF096238.2). Histological sections of the parasite were also used to set up an immunofluorescence test. When incubated with the patient's serum a tegumental signal was detected at concentrations of 1:100.

CONCLUSION *Spirometra erinaceieuropaei* has a cosmopolitan distribution, but most human cases occur in Asia possibly due to local eating habits. PCR analysis unambiguously identified the species responsible for the patient's disease and thus excluded the more pathogenic *S. proliferum*, a larval cestode for which the adult strobilar stage is unknown.

1.3-061

Emergence of *Dirofilaria repens* infections?

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INTRODUCTION *Dirofilaria repens* is a filaria species of dogs and other carnivores which is transmitted by various mosquitoes. It is endemic in many tropical and subtropical countries as well as in the Mediterranean region. Recently single cases of infected dogs have been reported from north of the Alps. *D. repens* can accidentally infect humans usually resulting in a subcutaneous nodule containing a single infertile parasite.

RESULTS AND DISCUSSION We analyzed multiple *D. repens* samples, which were sent to our diagnostic laboratory. The worms had been removed from humans, who acquired the infection in various parts of the world. The patients showed diverse clinical presentations. We performed phylogenetic analyses using mitochondrial sequences including the mitochondrial 12SrRNA-gene. The analyses indicated the existence of regional genetic variants of *D. repens*. The genetic variants did not correlate with the development of certain clinical symptoms. In the recent years we have diagnosed more cases of human *D. repens* infections than ever before. This may just be coincidence, but it suggests an increasing rate of infections. It has been postulated before, that climate warming will lead to a spread of *D. repens* to regions north of the Alps. We therefore analyzed more than 20,000 mosquitoes captured in Southwest Germany in the years 2009 and 2010 for the presence of filariae using PCR. We detected different filariae of animals, such as *Setaria tundra*, but we did not find evidence for the presence of significant numbers of *D. repens*-infected mosquitoes.

CONCLUSION There are different regional genetic subtypes of *D. repens*. *D. repens* may be spreading, but German mosquitoes do not (yet?) pose a significant risk for transmitting *D. repens* infections.

1.3-062

Molecular identification of *Leishmania* parasites in endemic areas of Bolivia

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INTRODUCTION Leishmaniasis is a significant health problem in Bolivia, where the greatest ratio (approximately 20% of all cases) of mucosal leishmaniasis to localized CL cases in Latin America is reported. Although it seems that most cases are caused by L. (V.) braziliensis, data on *Leishmania* species distribution in Bolivia are very fragmented. Currently at least four apparently sympatric *Leishmania* species persist in endemic areas. In this specific setting and in the light if their differential virulence and pathogenicity, species identification is highly relevant for improved prognosis and adequate treatment. This work reports the use of molecular species typing method; Heat Shock protein (HSP) 70 PCR-RFLP to identify strains isolated from patients across most endemic areas from Bolivia.

METHODS AND MATERIALS Two hundred and forty-three *Leishmania* strains were isolated over a 5 year period (2005–2010) from cutaneous and mucocutaneous lesions on human patients across several endemic foci. Species identification was achieved via HSP70 PCR-RFLP in relation to suitable reference strains.

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RESULTS AND CONCLUSIONS Molecular typing identified 89% of strains as *L.* (*Viannia*) *braziliensis*, 5% as *L.* (*V.*) *lainsoni*; 4% *L.* (*Leishmania*) *amazonensis* and 2% cases have also been found to be caused by *L.* (*V.*) *guyanensis*. Sympatric species diversity was found primarily in the center and north of the country. These findings facilitate improved evaluation of clinical and epidemiological risk as well as provide a base for future disease control in Bolivia.

1.3-063

Larynx leishmaniasis: an atypical leishmaniasis presentation

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INTRODUCTION Leishmaniasis, caused by heterogeneous group of protozoan parasites belonging to the genus *Leishmania*, results in a variaty of different clinical syndromes. Specific organisms are often associated with a typical clinical picture. Mucosal leishmaniasis (ML) is an infrequent clinical manifestation of this parasite. ML only occurs in the New World and is mainly associated with *L. braziliensis* infection. Years after resolution of the primary cutaneus lesion, recurrence at a distal mucosal site can follow due to hematogeneus or lymphatic dissemination (espundia fenomenon). Larynx leishmaniasis a infrequent localization of a ML. Is more frequent an erosive disease of mucosal surface of the nose, nasal septum or mouth.

METHODS AND MATERIALS We describe a case of Larynx Leishmaniasis with dysphonia in a patient without recent history of travel to South America. The only risk factor was multiple myeloma and quimiotherapic drugs.

RESULTS AND CONCLUSIONS Leishmaniasis is an atypical disease in Europe, but with the new biological treatments and a more aggressive quimotherapic the disease, even with rare forms of presentation, may become more common.

1.3-064

Impairment of protective immunity against *L. Major* infection in HIV-coinfected patients is associated with worse disease outcome

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INTRODUCTION In the present study, an epidemiological/immunological study on cutaneous leishmaniasis (CL) and Leishmania/HIV co-infection was conducted in Northern Cameroon as both diseases occur in the region.

METHODS AND MATERIALS A total of 32,466 individuals were surveyed; underlying immune mechanisms for susceptibility to *Leishmania* and HIV were characterized.

RESULTS One hundred and forty-six patients with active CL were identified. Serological testing of all CL patients revealed seven (4.8%) HIV positive patients. Clinically, the disease ranged from localized to disseminated CL with 1–20 lesions/patient. A therapeutic regimen of amphotericin B/metronidazole or allopurinol was successfully used. We detected elevated levels of Leishmania-specific IgG in all sera; with significantly lower levels in HIV+ subjects. Multiplex analysis of blood Th1/Th2 cytokines revealed significantly decreased levels IL-6 and IL-8 and higher Th2-associated cytokines IL-4 and IL-5 in samples of HIV+ patients. Analyses of skin biopsies showed fewer epidermal LC, CD1a+ dermal DC, CD68+ macrophages, as well as fewer CD4+ T cells and CD20+ B cells in HIV co-infected individuals. HIV co-infected patients also showed reduced degranulation of skin mast cells in CL lesions. The cytokine levels in skin were altered towards Th2 and decreased numbers of IFN+ cells as well.

CONCLUSIONS We demonstrated *Leishmania*/HIV co-infections in Cameroon in ~1/20 CL patients. Also, our results confirm prior studies demonstrating worsened disease outcome in HIV infected patients, indicating that an increased susceptibility to progressive disease after infection with an otherwise dermatotropic strain (*L. major*) is observed in HIV-infected patients. In the skin, we noted severe alterations in the protective immune response initiated by antigen presenting cells and mediated by IFN-producing T cells. Our findings provide important baseline data for the development of successful control/management programs against CL and HIV co-infection.

1.3-065

Different clinical assessments and immune reactions between HIV+ and HVI– *Opisthorchis felineus* infected persons. Case report

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INTRODUCTION Patient A, M, 44 year, HIV+, and Patient B, F, 38 year, HIV–, attended the Infectious Diseases Outpatients Ward because they were involved in an opisthorchiasis outbreak some months previously.

MATERIALS AND METHODS Both had consumed raw fillets of tenches fished at the Bracciano Lake (Central Italy). At the admission, B showed clinical symptoms with elevated peripheral eosinophilia and a moderate IgE level. A was asymptomatic with a mild peripheral eosinophilia and elevated IgE. Stool samples collected at different days were tested for helminths and protozoa after Ritchie's concentration by microscope and by PCR. Anti-*Opisthorchis felineus* IgG were searched by an in-house ELISA.

RESULTS All faecal samples of A tested positive for *Opisthorchidae* eggs; whereas, all but one faecal sample of B were negative. Eggs were identified as belonging to *O. felineus* by PCR. Anti-*O. felineus* IgG were detected in sera of both patients. Both patients were treated with praziquantel (75 mg/kg). Three months after the infection at the follow up, faecal samples tested negative and anti-*O. felineus* IgG were strongly reduced.

CONCLUSIONS Apart from individual variability observed in immune response to *Opisthorchidae* infection, the case report shows the clinical differences between HIV+ and HIV– people parasitized by *O. felineus* and stresses the need to include this foodborne zoonotic disease in the differential diagnosis of infections of Italian people.

1.3-066

Significant drop of worm burden after treatment of lymphatic filariasis in Kyela district in south west Tanzania

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BACKGROUND Efforts are undertaken by the 'Global Alliance to Eliminate Lymphatic Filariasis' to eradicate this disease, using

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repeated annual mass drug treatment. In July 2009, the campaign started in South Tanzania. Mapping of the prevalence in different regions and evaluation of treatment outcome is needed to assess the success of the program.

Objectives Evaluation of the prevalence of Lymphatic Filariasis (LF) in a randomized population before and after two rounds of mass treatment with Ivermectine (200 μg/kg)/Albendazole (400 mg).

Methods Since 2006, a population based survey (EMINI), including all age groups, is conducted annually in the Mbeya Region, South Tanzania. In one of the study sites clinical examinations from our surveillance suggest a relevant prevalence of the disease. A commercially available ELISA (TropBio® Og4C3 serum ELISA; Townsville, Australia) which detects the circulating filarial antigen (CFA) was used to test 1000 sera collected in 2009 before treatment and in 2011 after two treatment rounds were issued through a Government program.

Results In 2009 we detected CFA in 22.5% of the participants, compared to 19.4% in 2011 (P = 0.0026). In infected participants the level of antigen dropped significantly. Fifty-nine percent of infected participants had an antigen level >32,000 units in 2009, but only 16% in 2011 (P < 0.0001), reflecting a lower worm burden after treatment.

Conclusions Lymphatic filariasis in Kyela region had a high prevalence of 22.5% before the Government program commenced in 2009. Two rounds of mass drug administration led to a substantial reduction in prevalence and a significant lower worm burden in infected participants. The carefully randomized cohort and large number of participants in the EMINI survey allow an estimation of treatment success after the initial two rounds of the treatment program. Treatment success rates are necessary to estimate the years of treatment needed to stop the transmission of LF in that area.

1.3-067

Intrinsic insulin-like growth factor I present in macrophages affects *Leishmania* major growth

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Introduction In *Leishmania* infection specific and non-specific immune factors contribute to its evolution, including growth factors such as insulin like-growth factor I (IGF-I). We have shown that extrinsic IGF-I favors the parasite proliferation and the infection development, with effect on macrophage and parasite arginase activation. However, IGF-I is constitutively present in macrophages what we propose to study here.

Aim To study the role of intrinsic IGF-I in *Leishmania* infection, we evaluated the parasitism in *Leishmania* (Leishmania) major-infected macrophages upon IGF-I silencing with interference RNA (iRNA).

Methods Macrophage cell line RAW 264.7 (5 × 105 cells) was infected with stationary phase *L. major* promastigotes (8 parasites/cell). Cells were transiently transfected before or after the infection with 150 μM IGF-I-iRNA using Lipofectamine 2000 for 6 h. Paremeters were evaluated at 24 and 48 h after transfection. IGF-I expression was quantified by Real Time PCR relative quantification method and by confocal microscopy using anti-IGF-I antibody (1:75) and anti-mouse-IgG Alexa Fluor546 (1:200). The parasitism was evaluated in 100 cells per glass cover slip.

Results Silencing IGF-I with iRNA resulted in 70% inhibition of mRNA expression accompanied by a significant reduction in the immunostaining of IGF-I under confocal microscopy. In parallel, we evaluated the parasitism. When infected cells were treated with iRNA afterward, 105 parasites (median, in control) decreased to 90 parasites per 100 cells at 24 h, and 100 parasites to 54 per 100 cells (P < 0.0001) at 48 h. When iRNA treated cells were infected afterward, the parasitism decreased from 146 parasites to 134 per 100 cells at 24 h, and from 122 to 117 parasites per 100 cells at 48 h.

Conclusion These results emphasize the importance of IGF-I in *Leishmania* infection and suggest its direct role in on the growth of the parasite within macrophages.

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1.3-068

Dihydroartemisinin, a new agent against *Schistosoma japonicum*

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Brief Introduction Praziquantel is virtually the only current drug of choice for treatment of human schistosomiases. However, there is a growing concern that praziquantel resistance or reduced susceptibility may emerge. Screening and development of novel antischistosomal agents, is therefore given high priority. Artemisinin derivatives like artemether and artesunate exhibit effectively antischistosomal activities. However, the antischistosomal efficacy of dihydroartemisinin, the main metabolite of the mother compound artemisinins, as well as of artemether and artesunate, remains unclear.

Materials and Methods Male ICR mice were infected with 39–41 *Schistosoma japonicum* cercariae, and then grouped and administered with dihydroartemisinin or dihydroartemisinin plus praziquantel orally using different treatment protocols. And the reductions in total and female worm burdens were calculated.

Results Single oral doses of dihydroartemisinin (at 300 mg/kg) reduced total worm burdens of 1.07–64.81% and female worm burdens of 11.90–90.48%, and the greatest reductions was seen when treatment was given either 7 or 35 days post-infection. However, no marked dose-response relationship was observed. During the schistosomulum stage (7 day), the combined treatment of dihydroartemisinin and praziquantel, or administration of praziquantel, followed by treatment of dihydroartemisinin, both resulted in lower efficacies of dihydroartemisinin against *S. japonicum*. However, no marked changes of antischistosomal activities were observed when dihydroartemisinin was given first, followed by praziquantel. At adult stage (35 day), a significantly higher antischistosomal efficacy was found for combination therapy with dihydroartemisinin given first, followed by praziquantel, compared to dihydroartemisinin alone, or praziquantel given first followed by dihydroartemisinin. However, no significant difference was observed between the effects of combined treatment of dihydroartemisinin and praziquantel and administration of praziquantel alone.

Conclusions Dihyartemisinin is a novel antischistosomal agent against *S. japonicum*. The *in-vivo* treatment of *S. japonicum* at the schistosomulum stage with a combination of praziquantel and dihydroartemisinin appears no more effective than treatment with dihydroartemisinin alone.

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1.3-069

Risk factors for visceral leishmaniasis in a rural district of Bihar, India, a multilevel analysis

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BACKGROUND Published studies investigating risk factors for visceral leishmaniasis (VL) on the Indian subcontinent have all been cross sectional with a focus on outbreaks or high incidence villages. Contradictory results have emerged, in part explained by inadequate control for confounding by socio-economic factors. Moreover, associations found in high incidence villages are not necessarily generalizable.

METHODS Over a 3-year period we conducted three annual surveys in an area of Bihar, India, comprising of 50 villages. We registered and verified all cases of VL for the year preceding each survey; we collected data on assets owned and on various factors potentially associated with VL, including coverage with residual insecticide spraying (IRS). All households were georeferenced and plotted on a map; data on land cover obtained from a satellite image were added. Data was analyzed using a binomial multilevel model with village as random effect.

RESULTS We enrolled 81,210 individuals and confirmed 207 VL cases. Average annual VL incidence at village level ranged from 0 to 936 per 100,000 (median 41). IRS coverage by household for 2009 was 12%. Poor housing was a risk factor for VL, independent from socio economic status (OR 1.9, P = 0.04 for improvised brick house; OR 2.5, P = 0.004 for thatched house). We found a weak but statistically significant association with ownership of goats (OR 1.5, P = 0.006). Land use is very diverse; we found no significant associations between VL and type of land cover. The Mushahar caste were at highest risk (OR 2.4, P = 0.005).

CONCLUSION This large and comprehensive risk factor study with adequate control for confounding by socio-economic status and clustering at village level corroborates earlier findings about the risk of poor housing. It did however not identify any other risk factors amenable to change; more benefit can be expected from improving the poor IRS coverage observed.

1.3-070

Genotyping of echinococcus granulosus cyst isolates from different geographical areas in north India

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INTRODUCTION Hydatidosis caused by the cestode parasite, *Echinococcus granulosus*, is of immense public health importance. A high degree of intra-specific variation has been detected within *E. granulosus* and a number of well characterized strains are now recognised, which may have implications in epidemiology and control of this infection. Several techniques such as RFLP, RAPD polymerase chain reaction and single stranded conformation polymorphism (SSCP-PCR) have been used for molecular typing of *E. granulosus* cysts in different endemic areas worldwide. However, the reports from sheep isolates from India are scarce and none with the use of human isolates.

OBJECTIVE To assess the genotype (s) of *E. granulosus* cysts collected from infected sheep and patients from three different geographical areas in north India.

METHODS Forty-two hydatid cysts were collected, 39 from freshly slaughtered and infected sheep from slaughter houses at three geographical areas of North India (Chandigarh = 31, Shimla, Himachal Pradesh = 3, Srinagar, Kashmir = 5) and three cysts from patients (Shimla, Himachal Pradesh = 2, Srinagar, Kashmir = 1). Genotyping of cysts was carried out by using the known primers (OPB-1, OPB-4, OPB-7, OPB-10, OPB-15, OPI-1, OPI-15) 1,2. The DNA extracted from protoscolex isolated from the fertile cyst or cyst wall was subjected to RAPD-PCR. The numerical analysis of RAPD results was carried out by UV Photo MW and NTSYS software.

RESULTS Forty-two isolates were grouped in three clusters. Three sheep isolates from Shimla were placed in one cluster, while 10 sheep isolates from Chandigarh, five sheep isolates from Srinagar and three human isolates (one from Srinagar and two from Shimla) were placed in second cluster and rest of 21 sheep isolates from Chandigarh were placed in the third cluster.

CONCLUSION The report indicates the presence of strain variation within *E. granulosus* species in North India.

1.3-071

Twenty-five years fighting against sleeping sickness in equatorial Guinea

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INTRODUCTION Since 1985, the National Sleeping Sickness Control Programme has successfully controlled the four endemic foci in Equatorial Guinea, achieving a significant reduction in the incidence of the disease.

METHODS AND MATERIALS Epidemiological surveillance and control are carried out by surveys with mobile teams (active detection) and passive detection in the health facilities of affected district. From these activities, key epidemiological indicators to monitor the endemic situation and the impact of control measures are obtained.

RESULTS Human African Trypanosomiasis (HAT) cases have not been reported in Luba focus (southern Bioko Island) since 1995. Campo focus (frontier with Cameroon) is the less important one on the mainland, based on its geographical extension and number of cases reported. Mbini (centre of mainland) and Kogo (frontier with Gabon) are the most active foci. In the last 5 years, it has been reported cases in 13% (4/31) and in 60% (15/25) of the localities with high risk of transmission, Kogo and Mbini respectively. In 2009, the number of new cases was only one in Kogo and six in Mbini districts, and 30 cases were being monitored in all foci.

CONCLUSION In accordance with the current epidemiological pattern, the Equatorial Guinea foci can be considered under control and some of them near elimination. However, this epidemiological context poses new challenges to the national control programme, highlighting; (i) integration of surveillance and control activities in primary health care of affected districts, (ii) ensuring community attendance in the control activities (iii) be guaranteed the technical and financial resources for supporting the activities. Given the proximity of a feasible elimination of the sleeping sickness after 25 years of a successful control strategy in Equatorial Guinea, a renewed effort should be made by authorities and partnerships to achieve the international goals.

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1.3-072

IL-27 and IL-21 are associated with T cell IL-10 responses in human visceral leishmaniasis

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INTRODUCTION IL-27, a member of the IL-6/IL-12 cytokine family, has been shown to contain inflammatory responses by inhibiting Th17 lineage development, and by promoting the differentiation and expansion of IL-10 producing T cells, in part by up-regulating their production of autocrine IL-21. In this study, we investigated whether IL-27 and IL-21 are associated with human VL.

MATERIALS AND METHODS Parasitologically confirmed VL patients were enrolled with prior consent. The splenic aspirates taken for diagnostic purpose were collected at pre and post treatment level for mRNA expression. Control spleen was obtained from healthy organ donors. Paired plasma samples were used to detect cytokines by ELISA. Heparinized blood was collected from VL subject before start of treatment and from endemic controls for whole blood culture assay.

RESULTS IL-27 was significantly elevated in the plasma of patients compared to endemic controls (EC). At pre-treatment, cells in the VL spleen showed significantly elevated mRNA levels of both the sub units IL-27p28, EBI-3 and also IL-21 compared to paired post-treatment samples. Further, CD14+ cells were the main source of IL-27 mRNA while CD3+ T cells were the main source of IL-21 and IL-10 mRNA. By stimulating monocytes derived macrophages from normal donors under conditions appropriate to those present in the VL spleen, IFN-ã and IL-1â were shown capable of inducing both IL-27 subunits. Finally, by using whole blood assay to detect antigen-specific cytokine secretion, we could demonstrate that the majority of VL patients, and none of the ECs, produced both IFN-ã and IL-10, and that the IL-10 response could be augmented by treatment with rhIL-21.

CONCLUSION The pro- inflammatory cytokines acting on macrophages in the VL spleen have the potential to up regulate production of IL-27, which in turn can induces IL-21 to instructs T cells to produce IL-10 as a mechanism of feedback control.

1.3-073

Study of toll like receptors 2, 4 and 9 in human visceral leishmaniasis

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INTRODUCTION Toll like receptors (TLRs) are type1 trans-membrane proteins capable of sensing organisms upon detection of pathogen associated molecular patters (PAMPs) and can induce the synthesis of inflammatory cytokines. In contrast to the ample evidences for the recognition of Bacterial PAMPs, very little work has been done on the role of TLRs in the host response with eukaryotic *Leishmania* parasite. Studies from animal models have been demonstrated that TLR 2, TLR4 and TLR9 contribute to the immune response against *Leishmania* infection. However, there is no data available with role of TLRs in human Visceral Leishmaniaisis (VL). In this study, we tried to investigate the expression of TLR 2, 4 and 9 genes in splenic aspirate and peripheral blood mononuclear cells (PBMC) of VL patients.

MATERIALS AND METHODS Parasitologically confirmed VL subjects were enrolled with prior consent. Splenic aspirate taken for diagnostic purpose and PBMC were collected at pre and post treatment stage for *ex-vivo* mRNA expression. Splenic cells were sequentially enriched for CD3+, CD14+ and CD19+ to compare the expression profile of these TLRs in these cell populations.

RESULTS Quantitative measurement of splenic mRNA at pretreatment showed significantly elevated levels of TLR 2 ($P < 0.001$) and TLR 4 ($P < 0.01$) compared to 3–4 weeks paired post-treatment samples but no significant difference in the expression pattern of TLR 9 ($P = 0.346$). Further, splenic CD14+ cells have higher expression of TLR 2 and 4 compared to CD3+ and Cd19+ cells. No difference was observed in expression of these TLRs in the PBMC of VL Subjects at Pre and Post treatment stage.

CONCLUSION These preliminary results suggest the site specific expression of TLR 2&4. Higher expression of these TLRs in splenic CD14+, major parasitized cell population, may play a possible role in the immune regulation and pathogenesis of disease.

1.3-074

Analysis polymorphism of nucleotide binding oligomerization domain 2 (NOD2) gene locus exon 4 802 on leprosy patients in Makassar

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BACKGROUND Several studies have suggested that changes in the NOD2 gene prevent the protein from recognizing bacteria and influence susceptibility to infection disease. We analyze polymorphism at NOD2 gene of locus exon 4 802 between the leprotic patients of PB, MB types and normal people, and finding out the relationship between polymorphism of NOD2 gene and antibody titre on the leprotic patients of PB and MB types.

METHOD This research involved 40 leprotic patients samples (20 PB type and 20 MB type) and 20 normal people. Polymorphism detection was then carried out with PCR-RFLP technique and lateral flow test for detection antibody titre

RESULT The result of the research revealed that of 40 samples, two samples (10%) of leprotic patients showed the mutation of NOD2 gene while normal people did not experience the mutation. Fisher's Exact Test between the result of PCR-RFLP and the antibody titre resulted in $P = 0.487$.

CONCLUSION There are no significant differences between polymorphism NOD2 gene in the leprotic patient of PB, MB types and normal people. There is no significant relationship between polymorphism NOD2 gene and antibody titre.

KEYWORDS leprotic, polymorphism, NOD2 gene, antibody titre, PCR-RFLP

1.3-075

Causes of eosinophilia in immigrants treated at the tropical medicine unit of hospital de poniente de almería (UMTHP), Spain

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INTRODUCTION Eosinophilia is a common finding in people from tropical and subtropical regions, both immigrants and travelers. Parasitic diseases, especially helminthiasis, are one of the main diagnoses in this group of patients.

OBJECTIVES Description of the diagnoses made in immigrants referred to the UMTHP because of eosinophilia from January 2008 to April 2011 after the implementation of the UMTHP eosinophilia study protocol for inmigrants.

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METHODS All immigrants referred to the UMTHP because of eosinophilia (>500 eosinophils/μl) were studied with the same protocol which consist of: clinical and epidemiological history, exhaustive physical examination and complementary examinations which are applied in three consecutive phases if diagnosis has not been reached. (i) First phase: Blood test (including CBC, hepatorenal profile and iron metabolism), Mantoux test, plain abdominal and chest X-ray, serum serologies for Strongyloides, filariasis and schistosomiasis (for patients from endemic areas) and sampling of: urine (parasites search, up to three samples on alternate days if the patient comes from or has been in endemic areas for *S. haematobium*), stools (search for parasites, three samples on alternate days by stool concentration technique-Ritchie method-), skin (looking for microfilariae with the skin snip test in buttocks and shoulders, if any suggestive symptoms or the patient comes from or has traveled to endemic areas of filariasis), (ii) Second phase: search for parasites in urine and stool following the method above, stool culture for *Strongyloides*, and serologies for anisakiasis, toxocariasis, hydatidosis, fascioliasis and trichinosis, (iii) Third stage: search for parasites in urine and stool following the methodology described, Mazzotti test (if onchocerciasis is suspected), abdominal and bladder ultrasound, duodenal aspirate – biopsy, cystoscopy with bladder biopsy (if hematuria is present or abnormal bladder ultrasound), rectal biopsy (if the patient comes from or has traveled to endemic areas of schistosomiasis) and abdomino-pelvic CT (if ultrasound is not conclusive). Furthermore, demographic variables are recorded: age, sex, country of origin, length of residence in Spain and administrative status.

RESULTS During the study period, 118 patients were referred to the UMTHP because of eosinophilia (>500 eosinophils/μl), three did not complete the study due to moving away. The characteristics of the 115 patients who completed the study were: 103 men (89.6%) and 12 women; mean age of 29.97 years + 8.56 (range 10–58 years); 73 patients (63.5%) were in an irregular administrative situation; the average time of residence in Spain was 39.93 + 43.45 months (range 2–204 months); the countries of origin were (96.52% came from Africa): 29 Senegal, Guinea Bissau 27, Mali 25, Gambia 10, Guinea Conakry 6, four Equatorial Guinea, Ghana 3, three Mauritania, Morocco 2, Colombia 2, Nigeria 1, Ecuador 1, Lithuania 1 and Ivory Coast. The mean value of eosinophils in blood was 1151.95 + 753.50/μleosinophils; 25 patients (21.73%) had moderate or severe eosinophilia (>1500 eosinophils/μl). An etiological diagnosis was made in 93 patients (80.86%), with 27.95% (26 patients) with more than one cause. Etiologic findings were as follows: six patients with non-infectious causes (two atopic dermatitis, allergic rhinoconjunctivitis 2, two bronchial asthma) and 87 (93.54% of patients in whom the diagnosis was achieved) with an infectious cause for eosinophilia (three anisakiasis, one trichinosis, 1 toxocariasis, one Trichuristrichiura, one Hymenolepis nana, one Loa loa, 18 Mansonellaperstans,), 43 strongyloidiasis, 20 hookworm, 14 *Schistosoma haematobium*, four *S. mansoni,* seven *Schistosoma* spp., and two *Ascaris lumbricoides*).

CONCLUSIONS Infectious causes are the most common cause of eosinophilia in immigrants from Africa studied at the UMTHP. By using the eosinophilia study protocol of the Tropical Medicine Unit of 'Hospital de Poniente de Almería', an etiologic diagnosis is reached in a very high percentage of cases, achieving optimal use of resources.

1.3-076

Liposomal amphotericin B for visceral leishmaniasis in HIV-coinfected patients: 2-year treatment outcomes in Bihar, India

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BACKGROUND Reports on treatment outcomes of visceral leishmaniasis (VL) -HIV coinfection in India are lacking. None studied the efficacy of liposomal amphotericin-B in VL-HIV coinfection. We report the 2-year treatment outcomes of coinfected patients treated with liposomal amphotericin-B followed by antiretroviral treatment (cART) in Bihar, India.

METHODS In 2007, a VL treatment program was launched, with liposomal amphotericin-B as first-line treatment, routine HIV testing and cART (D4T/3TC/NVP) after VL treatment. All patients with newly diagnosed VL-HIV coinfection and initiating treatment with liposomal amphotericin-B (20–25 mg/kg over 4–15 days) between July 2007 and September 2010 were included. Kaplan-Meier estimates of cumulative incidence of death/treatment failure were calculated.

RESULTS Fifty-five patients (83.6% male, median age 35 years; 62% migrant labourers; median follow-up 1 year) were included. Mean CD4 cell count at VL diagnosis was 85 cells/μl. Twenty-seven patients (49.1%) experienced a VL relapse. Overall, tolerance of liposomal amphotericin-B was excellent, while none experienced treatment interruption. In total, eight cases of relapse were documented, six patients died. None had initial treatment failure. Survival by 1 and 2 years after VL treatment was estimated at 85.5%. A probability of VL relapse of 0.0%, 8.1% and 26.5% was found by 0.5, 1 and 2 years after VL treatment respectively; relapse rates were similar for primary and relapse VL. CD4 count <200 cells/μl at 6 months after cART initiation was predictive of subsequent relapse. The mean CD4 cell count at 6 and 24 months after cART initiation was 187 and 261 cells/μl respectively. Retention in HIV-care was 83.6%.

CONCLUSIONS This is the first study reporting long-term treatment outcomes of VL-HIV coinfection in India. Whereas excellent initial treatment response and good long-term survival was obtained, long-term VL-relapse remained frequent. Our findings highlight the need of increased availability of liposomal amphotericin-B and high accessibility of HIV care in resource-constrained settings.

1.3-077

Characterization and vaccination of two novel *Schistosoma japonicum* genes screened from a cercaria cDNA library

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Two novel genes, SJCWL05 and SJCWL06, were harvested from screening of *Schistosoma japonicum* (Sj) cercaria cDNA library by using pig sera vaccinated (VPS) with Sj immature egg ws-vaccine (SjiEw). Prokaryotic recombinant plasmids pGEX-4T-1/SJCWL05 and pGEX-4T-1/SJCWL06 were constructed to analyze their immunogenicity, which was confirmed by SDS-PAGE and Western blotting. Two eukaryotic recombinant plasmids pcDNA3/SJCWL05 and pcDNA3/SJCWL06 were constructed and their ability to protect mice against challenge of Sj was evaluated. All mice vaccinated with pcDNA3/SJCWL05 or pcDNA3/SJCWL06 developed ELISA specific anti-SjSIEA (Sj soluble immature egg

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antigens) antibody. Immunoprotection experiments showed that worms and liver eggs reduced 34.64% and 39.14% in the pcDNA3/SJCWL05 group and those reduced 27.17% and 27.95% in the pcDNA3/SJCWL06 group, respectively. The reduction rates of intestine and uterine eggs in female worms of both groups reached 39.45% and 38.5% as well as 30.02% and 28.7%, respectively. Results of our study suggest that novel genes SJCWL05 and SJCWL06 are potential vaccine candidates against schistosomiasis japo.

1.3-078

In vivo efficacy and tolerability of oleylphosphocholine (OLPC) in laboratory models of visceral leishmaniasis

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The alkylphospholipid oleylphosphocholine (OlPC) can be considered as a potential new therapeutic option for visceral leishmaniasis (VL). Activity of OlPC on intracellular amastigotes of *Leishmania* donovani and *L. infantum* show IC50 values in the low micro molar range, similar to miltefosine (MIL) and of higher potency compared to SbV and paromomycin. These promising *in vitro* data prompted us to confirm the efficacy of OlPC in a hamster model of VL, in which two formulations (aqueous and liposomes) were compared. Hamsters were infected with $2 \times 10E7$ *L. infantum* amastigotes on day 0. At day 21 post-infection, animals were orally treated with the test formulations at 20 and 40 mg/kg for five consecutive days. Evaluation parameters for efficacy were reduction of amastigote burdens in the target organs liver, spleen and bone-marrow 10 days after the last treatment, compared to the burdens in the organs of vehicle-treated infected controls. Both OlPC formulations were well tolerated and equipotent, and had markedly higher efficacy compared to MIL: at 20 mg/kg/day, parasite reduction in liver, spleen and bone marrow of MIL/PBS-treated animals was 0%, 61% and 39% compared to 91%, 99% and 98% in OlPC/PBS-treated animals. In a follow-up experiment, total doses of 25, 50 and 100 mg/kg were compared when administered as single or as multiple doses. Comparable parasite suppression levels were obtained when the doses were given either as single or as multiple administrations, suggesting that the overall systemic availability (AUC) is more important than peak plasma concentration (Cmax) for therapeutic effect. Interestingly, single dosing as high as 100 mg/kg was well tolerated and no toxicity or intolerance was observed. These data suggest that OlPC may become a promising candidate to improve and simplify current case management of VL. Additional studies are ongoing to assess the 'drug candidate' potential of OlPC.

1.3-079

Diagnosis of Mediterranean visceral leishmaniasis by detection of *Leishmania* antibodies and *Leishmania* DNA in oral fluid samples collected by ORACOL device

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INTRODUCTION Current visceral leishmaniasis (VL) diagnostic methods require invasive sampling procedures such as visceral aspiration and/or blood drawing. The development of diagnostic tests using oral fluid, which is easier to collect, would be more simple and practical for VL diagnosis especially under field conditions.

MATERIALS AND METHODS Oral fluids from 37 VL cases and 40 healthy controls were collected by ORACOL devices. Blood samples and oral fluid specimens from both groups were analyzed by rK39 Enzyme Linked Immuno-Sorbent assay and quantitative real-time PCR.

RESULTS Antibodies detection in the oral fluid had a sensitivity of 100% and a specificity of 97.5%. Antibody levels measured in sera and oral fluid showed a significant positive correlation (=0.655 and P = 0.01). *Leishmania* DNA detection in oral fluid had a sensitivity of 94.6% and a specificity of 90%. The median parasitic load estimated in blood was 133 parasites/ml (IR: 10–1048) whereas that accessed in oral fluid specimens was 3 parasites/ml (IR: 0.41–92). However, there was no significant linear relationship between parasitic loads assessed in both biological samples (=0.31, P = 0.06).

CONCLUSIONS VL diagnosis based on specific antibody detection and *Leishmania* DNA identification in oral fluid sample was equivalent to blood in accuracy, and therefore promising for clinical use.

1.3-080

Genetic structure of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in the Republic of Guinea Bissau

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INTRODUCTION *Glossina palpalis gambiensis* is the most common tsetse fly in Guinea-Bissau and a major vector of Human African Trypanosomiasis in West Africa (HAT) . Despite its widespread distribution, no vector-mediated HAT transmission has been reported in the country since the late 1970s. Wild populations of the palpalis group display different levels of intraspecific variation that may influence vectorial capacity. Therefore, accurate knowledge on species identity and population structure is essential to predict the possible reestablishment and spread of HAT transmission in Guinea-Bissau.

METHODS Genetic variation was analyzed in *Glossina palpalis gambiensis* samples from four districts of Guinea Bissau, using 11 microsatellite loci. Three of the districts are in the mainland and one represents the insular part of the country.

RESULTS Very low levels of genetic differentiation were observed among populations (FST = 0.06). This result is in agreement with a model-based clustering analysis that revealed the presence of a single population clustering grouping all samples regardless of geographic origin. These results suggest very little population substructure in *G. palpalis gambiensis* from this region.

CONCLUSIONS Genetic evidence suggests considerable gene flow among *G. palpalis gambiensis* populations within mainland and between islands and mainland Guinea-Bissau. In the case of focal reestablishment of HAT transmission, the possibility of parasite dissemination through tsetse fly active dispersal should be taken into account when planning vector control actions in Guinea Bissau.

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1.3-081

Investigation on distribution of intermediate host of *Echinococcus multilocularis* in Shiqu county of Sichuan province, China

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Shiqu County in Sichuan Province is highly endemic for mixed alveolar hydatid diseases. *Rodentia* and *Lagomorpha* are the local dominant mouse species with a biggest amount of *Ochotona curzoniae*, which is the important intermediate host of *Echinococcus multilocularis*. This research is to find out the distribution and its characteristics of intermediate hosts of *Echinococcus multilocularis* in Shiqu County with GIS/RS technology and provide basis for the epidemiological features of *Echinococcus multilocularis* in the area.

METHODS To investigate the density of caves of *Rodentia* and *Lagomorpha* mice at 97 sites of pastures in winter and summer with the grid sampling method and to estimate their distribution in different landscapes with RS£Remote Sensing Technology.

RESULTS Based on the densities of *Ochotona curzoniae, Microtus fuscus* and their cave densities in survey points, regression equations were fitted respectively (*Ochotona curzoniae*, $P < 0.0001$, $R^2 = 0.8705$; *Microtus fuscus*, $P < 0.0001$, $R^2 = 0.9736$). Their cave density in summer pasture was higher than that in winter pasture ($F = 36.65$£, $P < 0.0001$). The cave densities of *Ochotona curzoniae* and *Microtus fuscus* in wasteland and semi-wasteland were higher than in grassland ($F = 7.73$£, $P < 0.001$).

CONCLUSION The regression relationship between the densities of *Ochotona curzoniae*, *Microtus fuscus* and their cave densities in survey points existed, which meant that the cave densities could reflect the animal densities and the cave density in summer pasture was higher than in winter pasture. The main distribution areas of the intermediate hosts were in wasteland and semi-wasteland.

1.3-082

Soil transmitted helminths (STH), anemia and malnutrition mong pigmei children in South Cameroon

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Soil transmitted helminths are a public health problem in Cameroon. Traditionally nomadic the Baka pygmies are now undergoing a process of sedentarization hat may be determining new patterns of disease transmission for them. Five hundred and five Baka children were studied in a cross-sectional observational study. Sample size was calculated according to cluster sampling randomizing the comprehensive list of villages. In every village a census was conducted, houses were mapped; GPS coordinates were taken for villages, water sources, latrines and schools in the given area. Children from 6 months to 12 years old were included. When children were not present in the house a second visit was paid. Children stools were tested using Ritchie modified technique. Eggs were identified for *Ascaris lumbricoides*, *Trichiuris trichura* and Hookworm. Anemia was estimated using Hemocue for hemoglobin measure, conjuntival pallor and capillary refill time. Malnutrition weight, height, abdominal distension, edemas, and MUAC were analyzed using the ANTHRO and ANTHRO PLUS WHO softwares. KAP surveys were conducted for nutrition and hygiene. Socio-economic and indicator were collected. Prevalence of STH among pigmei children was 77.62% CI 5% (73.8–81.25). 34.05% of the children harboured more than one species (CI 5% 29.92–38.19) and 4.75% harboured all three CI 5% (2.89–6.60). Prevalence of *Ascaris lumbricoides* was 41.98%, of *Trichiuris trichura* 63.96% and of hookworm 10.49%. The prevalence of anemia was 59.8% (severe anemia <7 gr/l Hb: 4.97%, moderate 7–9 gr/l Hb: 31.21%, light 9–10 gr/l Hb: 23, 66%), malnutrition (height for age) and anemia were inversely related with STH: anemic or malnourish children were less likely to have intestinal parasites. Results were mapped using GeoMedia Professional software. Pigmei children in south Cameroon are highly infected by intestinal parasites. Deworming, water, sanitation and hygiene education are needed in this region.

1.3-083

Stamping out sleeping sickness in Uganda: evidence of *T. brucei* s.l circulating in cattle reservoir within three principal livestock markets

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Since colonial times, African trypanosomiasis has been an important health and economic drawback, accounting for up to 50% of livestock deaths in sub Saharan Africa. The disease also has a zoonotic side as two of these parasites, namely *T. brucei rhodesiense* (acute) and *T. b. gambiense* (chronic), can cause death in the human population. Currently the two species are spatially discrete however; they are at their closest in Uganda, the only country where both the chronic and acute human infective forms occur within a single nation. With already huge strain upon the existing Ugandan health infrastructures, co-existence of these parasites cannot be afforded. To compound this problem, the acute disease has spread in the last decade from the endemic south of the country as a result of cattle movement; thus drawing closer to localities endemic for the chronic disease in the north. This encroachment prompted the initiation of the Stamp Out Sleeping sickness (SOS) campaign. This public-private partnership has been shown to work in controlling the northward spread of the acute disease by treating cattle within newly effected, and at risk, districts in the centre of the country. However with weak national legal requirements to treat livestock from affected areas prior to sale at markets, disease introductions by way of infected animals remain an ongoing problem. Here we show evidence *T. brucei* s.1 circulating in the cattle reservoir within three principal livestock markets of the SOS intervention region.

1.3-084

Causa: the development and validation of a risk score for household infestation by *Triatoma infestans*, a bolivian vector of chagas disease

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INTRODUCTION Chagas disease, primarily spread by infected vectors such as *Triatoma Infestans* that reside in households, persists as an important public health problem throughout Latin America. Preventative insecticide campaigns often rely on anecdotal evidence and there is a need for an accurate classification score to correctly identify 'at risk' houses.

METHODS AND MATERIALS Data were collected from 337 households on 11 pre-specified risk factors through the use of a standardised questionnaire and survey. Multivariate logistic regression analysis was used to identify risk factors for infestation. From this, a risk

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score was developed and validated on a separate cohort of 165 houses.

Results Five significant risk factors were identified; cracks in the walls of houses; adobe walls; junk in the *peridomiciliary area*; no insecticide spraying (< 2 years) and freely ranging animals. A risk score was generated and then calculated for each house. Three risk categories were defined: low risk, medium risk and high risk. In the development cohort the infestation rates for these three groups were 2%, 18% and 69% respectively. The corresponding infestation rates in the validation cohort were 7%, 30% and 75%. Sensitivity and specificity for this test were 81% and 84% respectively when classification as high risk was defined as a positive test result. The positive predictive and negative predictive values were 71% and 90% respectively for the score.

Conclusion A risk score was developed to classify houses by risk of infestation by *T. Infestans*. This could be used to inform decision making in under-funded multilateral prevention initiatives.

1.3-085

Epidemiology of intestinal helminths, schistosomiasis andectoparasites in schoolchildren in Burkina Faso

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As part of a large nutritional and health survey in schoolchildren, the prevalence, intensity and spatial distribution of intestinal parasites; schistosomiasis; and skin parasitic infestations were evaluated in Burkina Faso primary schools in 2006. A national wide cross-sectional survey was conducted on a regional basis of randomly selected provinces, and schools. A random sample of 270 children was selected in each school. Children were clinically examined for external parasitic infestations. Finger-prick blood samples were taken for haemoglobin level measurement with a HemoCue photometer; and faecal and urine samples were provided to diagnose intestinal helminths infections and urinary schistosomiasis. Faeces and urine were analysed using the Kato-Katz and filtration method, respectively. A total of 3480 children (44.5% females and 55.5% males) were examined in 130 public primary schools of 13 provinces. The children were aged 8–12 (median 10 year). Mean weight was 28.4 (+10.1) kg, mean haemoglobin level 11.8 gr/dl (+1.2) and 40.6% were anaemic (Hb < 11). Intestinal helminths infection prevalence was 10.4% (range 0–21.9% by province), and equally distributed between girls and boys (P = 0.41). Species prevalence were the following: Hookworms: 3.7%; Ascaris lumbricoides: 2.0%; Hymenolepis nana: 4.5%; Trichuris trichiura 0.1%; Taenia spp 0.1% and Enterobius vermicularis 0.1%. Infection with Schistosoma haematobium was 8.1% and S. mansoni 0.85%. Mean infection intensity of Hookworms, Ascaris lumbricoides, Hymenolepis nana, and Schistosoma mansoni was 79, 74, 784 and 609 eggs per gram of faeces, respectively. Body lice, tinea and scabies prevalence were 8.5%, 7.5%, and 0.1%, respectively. The spatial distribution and prevalence of parasitosis varied between provinces. However in four provinces all the helmints species were prevalent at the highest rates. H. nana was the most commonly found parasite (10/11 provinces, 0.4–10.5%). Helminths infections and ectoparasites are common in schoolchildren in Burkina Faso and constitute a public health problem.

1.3-086

Stamping out sleeping sickness in Uganda: evaluation of 31 months' interventions targeted at the cattle reservoir of disease

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Since colonial times, African trypanosomiasis has been an important health and economic drawback, accounting for up to 50% of livestock deaths in sub Saharan Africa. The disease also has a zoonotic side as two of these parasites, namely T. brucei rhodesiense (acute) and T. b. gambiense (chronic), can cause death in the human population. Currently the two species are spatially discrete however; they are at their closest in Uganda, the only country where both the chronic and acute human infective forms occur within a single nation. With already huge strain upon the existing Ugandan health infrastructures, co-existence of these parasites cannot be afforded. To compound this problem, the acute disease has spread in the last decade from the endemic south of the country as a result of cattle movement; thus drawing closer to localities endemic for the chronic disease in the north. This encroachment prompted the initiation of the Stamp Out Sleeping sickness (SOS) campaign. This public-private partnership was designed to control the northward spread of the acute disease by treating cattle within newly effected, and at risk, districts in the centre of the country. Here we present an assessment of the effectiveness of the SOS intervention 31 months following initiation. In summary, our analysis shows that the disease is not penetrating new ground but remains associated with distance from the cattle markets of the SOS intervention region.

1.3-087

Use of Ln-PCR and DNA sequencing as a tool for detection and identification of new world Leishmania species in different hosts

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Leishmaniases in the New World have a very complex epidemiology due to the participation of various species of Leishmania, sand fly vectors and mammalian hosts. Wild, synanthropic and domestic mammals have been found infected by different Leishmania species. The identification of Leishmania species infecting reservoirs is crucial to set up efficient control measures. Leishmania nested PCR (Ln-PCR) targets the SSUrRNA gene is highly specific and sensitive. Ln-PCR was used for Leishmania detection in biological samples from 162 dogs and 97 small mammals (rodents and marsupials) from an endemic area for cutaneous and visceral leishmaniasis in Brazil. DNA sequencing of the PCR products was performed to identify the Leishmania species. Detection of Leishmania DNA was positive in 62/162 (38.3%) dogs and 63/96 (65%) small mammals in at least one tissue. Among the positive dogs, 53% were infected by L. infantum, 42% by L. braziliensis and two dogs (3.25%) were infected by L. amazonensis. The majority of small mammals were infected by L. braziliensis complex. Three rodents collected in the peridomicile had a mixed infection (L. braziliensis and L. infantum). Ln-PCR and DNA sequencing showed up as an efficient tool in the detection and identification of New World Leishmania species in different hosts. Additionally, the results obtained reveal the complexity of Leishmania transmission cycles in the study area.

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1.3-088

Study of genetic variability of Leishmania (Viannia) Braziliensis vianna, 1911 from different regions of Brazil

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Leishmania (Viannia) braziliensis is the most prevalent species causing human cutaneous leishmaniasis in the Americas. Genetic polymorphism occurs in Leishmania populations and it is possibly linked to adaptative process to different vectors/reservoirs which are associated with specific environments. In this study, genomic DNA polymorphisms were analyzed using different genetic markers, comparing the intra-specific variation of 80 L. braziliensis samples from different Brazilian geographical regions and biomes. Among the stocks analyzed by MLEE, 68 (87.3%) presented a profile identical to the L. (V.) braziliensis reference strain MHOM/BR/75/M2903 (IOC/Z27). Eight (10.1%) of the samples showed different electromorphs for the IDHNADP enzyme and two (2.6%) showed different patterns for the G6PDH, MDH and IDHNADP. These samples were designated as L. braziliensis variants. The consensus tree was generated by parsimony with the concatenated data of three markers: RAPD-PCR using the primer M1340F, and SSR-PCR using the primers CARY and K7. This analysis revealed two clusters: (i) one composed of samples from the Amazon biome/Northern region and the Atlantic Forest biome/Northeastern region; (ii) the other including samples from the 'Cerrado' biome/Midwestern region, the 'Cerrado' and Atlantic Forest biomes/Southeastern region, and the Atlantic Forest biome/Southern region. These results agree with the analysis performed by the method of genetic distance (UPGM). The average index of similarity between samples was approximately 70%. The results suggest an association between genetic variability and geographical origin.

1.3-089

Leishmania infantum recombinant Hsp 83 in the serodiagnosis of canine visceral leishmaniasis

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INTRODUCTION Transmission control measures for visceral leishmaniasis include elimination of infected dogs, important reservoir. However the diagnosis of infected dogs is difficult due to the absence of efficient and standardized sensitive and specific test. In this study we compared ELISA using recombinant Leishmania infantum Hsp 83 antigen with other tests in samples from dogs retained in two Centers of Zoonosis (CZ), and in samples obtained in a serological survey in dogs in their domiciles.

METHODS AND MATERIAL Serum samples from Sao Paulo State, Brazil: 239 from CZ-1, 77 from CZ-2, and 128 from serological survey. In dogs from CZs, parasitological exams were available. Serological assays: a) ELISA using purified recombinant L. infantum Hsp 83 (E-Hsp83), b) ELISA using L. major-like whole antigen (E-Lm) (Bio-Manguinhos), indirect immunofluorescence using L. major-like antigen(IFI) (Bio-Manguinhos), and the immunochromatographic test using rK39 (Diamed).

RESULTS In CZ-1 the positivity was 37.7% (E-Hsp83), 51.1% (E-Lm), 45.6% (rK39), 38.5% (IFI), and 33.1% (parasitological). In CZ-2 the positivity was 79.2% (E-Hsp83), 89.6% (E-Lm), 88.3% (rK39), 41.0% (IFI), and 84.4% (parasitological). In the serological survey samples the positivity was 29.9% (E-Hsp83), 15.6% (E-Lm), 7.8% (rK39), and 10.9% (IFI).

DISCUSSION AND CONCLUSIONS In CZ-1 and CZ-2 the results of the tests were closer to the detection level of parasitological exams, except with IFI that showed lower detection level. In the serological survey samples performance of the assays were divergent may be due to lower parasite burden pushing the sensitivity of the assays. In the latter samples the detection level was very low with rK39 rapid test suggesting that this test would not be appropriate for the serological surveys. Further we consider ELISA using recombinant L. infantum Hsp 83 a promising assay for use in serological survey of dogs in VL endemic areas.

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1.3-090

Transmission of cutaneous leishmaniasis during an outbreak investigation in bandiagara, Mali

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INTRODUCTION Cutaneous leishmaniasis (CL) caused by L. major has been reported in Mali, but disease epidemiology is still poorly described . Given the risk of leishmaniasis epidemic and following recent occurrence of superficial skin lesions in the touristic area of the Dogon country, we conducted an outbreak investigation in Bandiagara.

METHODS AND MATERIALS Investigation was centered on declared suspected cases of CL. The multidisciplinary field survey team performed clinical examinations, thin smears and punch biopsy of the skin lesions, venous blood draw. Thin smears were Giemsa stained and examined by microscopy. Punch biopsy tissues were used for culture and PCR. Venous blood was used for Western Blot and IFI assays. Entomological survey used CDC miniature light and sticky papers to capture sand flies. Alive and dead sand flies were dissected for identification of Leishmania. Engorged females were analyzed by PCR for ITS2 Leishmania rDNA gene detection.

RESULTS Fifty patients with skin lesions were included. All patients had negative microscopy. Eighty six per cent of the 14 patients in which biopsy PCR was performed were positive for L. major. Western Blot and IFI were positive respectively in 92% and 17% out of 12 sera assayed. Out of 1324 sand flies analyzed, four species of Phlebotomus and 16 of Sergentomia were identified; S. (Spelaeomyia) darlingi and P. duboscqi were more frequent respectively 505 (38%) and 177 (13.4%). DNA of L. major was identified by PCR in P. duboscqi and S. darlingi females. This implies a role of S. (Spelaeomyia) darlingi in the transmission of CL for the first time in Mali.

CONCLUSION Bandiagara area is prone to outbreak of CL. S. (Spelaeomyia) darlingi may play a role in CL transmission. Further investigation is required to clarify the epidemiology of CL in Mali.

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1.3-091

Dynamic of spleen alterations associated with clinical manifestation in canine visceral leishmaniasis

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INTRODUCTION The spleen as a secondary lymphoid organ, initiates a response to intravascular foreign antigens. Depending on the response to infection, a wide range of changes occurs, contributing to the establishment of the general spectrum of visceral leishmaniasis events. We evaluated spleen alterations of symptomatic and asymptomatic dogs, comparing to the clinical manifestation.

MATERIAL AND METHODS Dogs with visceral leishmaniasis were grouped according to number of clinical signs and spleens were evaluated to pathological alterations.

RESULTS Initially we observed depletion of periarteriolar lymphatic sheath cells in spleen from dogs presenting three or more symptoms. Depletion of follicle was observed only in the group presenting one symptom and follicle hyperplasia was higher in the group showing more than five clinical signs. In addition, we show increasing number of granuloma in the group exhibiting five signs and eosinophil granulations in plasma cells were greater in the group with five clinical manifestations compared to those with one, two, three and four and asymptomatic dogs.

DISCUSSION Follicle depletion in the group showing only one clinical sign probably resulted from the initial interaction of the parasite with the marginal zone of the lymphoid follicle and follicle hyperplasia and follicles with little activity were seen in hamster in late infection in agreement with the results of this study. The data concerning neutrophil infiltration in symptomatic dogs could suggest that the parasite component has a role in neutrophil action, similar to the protection against apoptosis provided by macrophages. These structural changes occurring in the spleen in the course of VL can help to identify cellular and molecular components to understanding of the immunopathological changes that occur with progression of canine visceral leishmaniasis.

CONCLUSION Our results show that the spleen undergoes profound immunopathologic changes that can influence the outcome of infection.

1.3-092

Improving protection against visceral leishmaniasis by heterologous prime-boost vaccination based on DNA and insect-derived recombinant proteins

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BACKGROUND Leishmaniasis, a neglected disease affecting 12 million people, lacks an effective vaccine. We assayed three vaccination strategies against visceral leishmaniasis (VL), the most severe form of the disease, using the KMPII, TRYP, LACK, and PAPLE22 vaccine candidate antigens in the hamster model.

METHODS Four groups of hamsters were immunized using the following approaches: (i) raw baculovirus-infected Trichoplusiani larvae extracts expressing the four recombinant proteins (Prot); (ii) naked pVAX1 plasmids carrying the four genes individually (DNA); (iii) a prime-boost strategy involving DNA vaccination followed by Prot inoculation (DNA-Prot); or (iv) a prime-boost strategy using empty pVAX1 plasmid and raw wild-type baculovirus-infected T. ni larvae extract (Control). Hamsters were challenged with 1×107 L. infantum promastigotes and maintained for 20 weeks.

RESULTS While Prot vaccine was not protective, DNA vaccination achieved protection in spleen (90%, $P = 0.015$). Only DNA-Prot vaccination induced significant NO production by macrophages, accompanied by a significant parasitological protection in spleen (86%, $P = 0.030$) and blood (99%, $P < 0.001$).

CONCLUSIONS Prime-boost strategy with DNA plus raw Trichoplusiani larvae extracts containing recombinant KMPII, TRYP, LACK, and PAPLE22 proteins could be an immunogenic and effective vaccine against VL, better than DNA or protein alone with the same antigens.

1.3-093

Identifying causal agents of Indian Kala-Azar: a pilot study

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INTRODUCTION Historically Kala-azar (KA) in India is caused by *Leishmania donovani*. The has been challenged by several authors claiming *L. tropica* and *Leptomonas* sp. may be associated with the disease. In the present study, we characterized recent nine clinical isolates of KA and PKDL patients with the help of Random Amplified Polymorphic DNA (RAPD) analysis, PCR-amplification of the Internal Transcribed Spacers (ITS) of the rRNA operon and ITS1-RFLP, followed by sequencing and aligning of the ITS1 amplicons.

METHODS AND MATERIALS Nine clinical isolates from VL and PKDL patients were collected in the year 2006–2010, of which three were from Bangladesh. RAPD PCR analysis was done using random primers. The amplification of ITS regions of the rRNA operon was carried out. The amplified ITS and ITS1 regions were digested with restriction enzymes. The ITS1 amplicons were sequenced and aligned for the homology analysis.

RESULTS Amplification of genomic DNA of all clinical isolates along with WHO reference stains, DD8 (*L. donovani*) and K27 (*L. tropica*) showed that T5 was matching exactly with K27 while banding patterns present of rest clinical isolates including Bangladeshi KA isolates, were all very similar to DD8. Amplification and digestion of ITS1 amplicon with the restriction enzyme HaeIII revealed the band patterns corresponding to DD8 for all eight isolates examined while T5, was again very similar to K27. Sequencing and homology alignment supported the above observations.

CONCLUSION This pilot study revealed that *L. donovani* is not the only agent causing Indian KA giving credence to the single report claiming *L. tropica* is also responsible for the disease. The observations thus emphasized the need for rigorous systematic typing of the clinical isolates of Indian KA as proper drug regimen

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against the disease demands appropriate identification of the parasites.

1.3-094

Intestinal parasitic infection among schoolchildren in Fogo Island, Cape Verde

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INTRODUCTION Intestinal parasitic infections are a public health problem in developing countries where adequate water and sanitation are lacking. Those infections are often asymptomatic and may contribute to the delay of the children's growing up, weight deficit and learning difficulties. Studies on intestinal parasitic infections are scarce in Cape Verde. This study aims to determine (i) the prevalence of intestinal parasites among school children in Fogo Island; (ii) their nutritional status; (iii) risk factors for infection.

METHODS AND MATERIALS A stratified sample was obtained from all schools of Fogo Island during September 2009. Stool samples were examined using direct observation and Ritchie's concentration techniques. Anthropometric variables (age, height, weight) were obtained from each child of the sample. Data on housing conditions, water, sanitation and personal hygiene habits were obtained by questionnaire.

RESULTS Two hundred children participated in the study. The age range was 6–16 years; 41.7% were male. The infection rate was 51.8%, a third of them with more than one species of parasites. Specific rates: *Entamoeba coli* 58.4%, *E. histolytica/dispar* 49.5%, *Giardia intestinalis* 21.8%, *E. vermiculares* 7%, *A. lumbricoides*, *H. nana* and *I. buetschlii* each 1%. Fifty percent of the children presented acute malnutrition, 14.8% chronic malnutrition, 28.4% general malnutrition. Statistical associations and comparisons reveled that infected children lived in homes with fewer rooms (P = 0.004), did not have complete bathroom in their houses (P = 0.004), did not always wash their hands after defecation (P = 0.005), did not drink chlorinated water at school (P = 0.03).

CONCLUSIONS Intestinal parasitic infections are a public health problem in this population. The identification of non pathogenic species reveals contamination of food and water. Half of the children have a kind of malnutrition. Housing conditions, water and sanitation, and personal hygiene habits must be improved.

1.3-095

Antibiotic treatment outcomes of Buruli ulcer in Akwapem South and Suhum-Kraboa-Coaltar districts of Eastern region, Ghana

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BACKGROUND Buruli ulcer is a neglected chronic, indolent , necrotizing disease of the skin caused by *Mycobacterium ulcerans*. It is characterized by a painless nodule, papule, plaque or oedema evolving into a painless ulcer with undermined edges leading to functional disability if not diagnosed and treated early. Surgery has previous been used as the main mode of treatment, but antibiotics are now being used. We therefore examined the response of the various categories of lesions with respect to antibiotics treatment.

METHODS AND MATERIALS This study was conducted in two districts in the Eastern region of Ghana. Lesions were grouped according to WHO classification of lesions by size and site, namely: category I, II, III and III-multiple lesions. We recruited 68 laboratory confirmed Buruli ulcer patients. They were given the WHO recommended 8 week treatment of a combination of streptomycin and rifampicin. Clinical history and physical examination performed to assess the form and severity of the disease and to rule out other systemic diseases. Wounds were dressed on daily basis and were observed bi-weekly for reduction in size. The longest diameter of lesions were measured and photographed in a standardised manner in order to monitor changes in sizes.

FINDINGS The average duration of primary wound healing for the various categories of Buruli ulcer is 29 (14–84) days for category I lesions; 52 (42–168) days for category II lesions and 65 (42–188) days for single lesions in category III. The average duration for category III-multiple lesions was 43 (28 â€' 139) 139 days. Using one way analysis of variance for the categories of lesions there is a significant difference between the categories and duration of healing with a P-value = 0.003.

CONCLUSIONS Early detection and treatment of Buruli ulcer lesions with the recommended antibiotic regimen significantly improves the healing of wounds.

1.3-096

Evaluating decontamination methods for effective isolation of *Mycobacterium ulcerans* from Swab specimens

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INTRODUCTION The isolation of *Mycobacterium ulcerans* (MU) the etiological agent of Buruli ulcer from swab specimens remains a challenge. Specimens from BU lesions are usually contaminated with faster growing bacteria inhibiting the cultivation and isolation of *Mycobacterium ulcerans* for diagnostic and research purposes. The growth inhibition of these contaminants can be achieved when processed with useful decontamination agents. We investigated effective decontamination methods for the cultivation and primary isolation of *Mycobacterium ulcerans* from swab specimens of suspected BU cases.

METHODS AND MATERIALS We used three decontamination methods made up of 0.5% Cetyl pyridinium-chloride with 1% sodium chloride, an effective decontamination agent for the isolation of *Mycobacterium tuberculosis* (MTB) in addition to two known decontamination agents for MU isolation; 5% oxalic acid and Petroff. Eighty-two swab specimens from suspected BU cases recruited from the Akuapem South and Suhum Kraboa-Coaltar districts were processed with these agents. They were then inoculated onto tubes of antibiotic incorporated and antibiotic free egg based Lowenstein-Jensen media in duplicates. The media tubes were incubated at 32°C and examined weekly for a maximum of 10 weeks. An effective decontamination implies a growth inhibition of contaminants.

RESULTS Each experiment was conducted in duplicates and therefore, a total of 164 tubes were used for each decontamination method and media condition. Oxalic acid on antibiotic incorporated media had a total contamination rate of 21% (34 tubes), oxalic acid without antibiotics with a contamination rate of 49% (80 tubes), CPC-NaCl + antibiotics contamination rate of 13% (22 tubes), CPC-NaCl- antibiotics 44% (72 tubes), Petroff + antibiotics 46% (75 tubes) and Petroff â€antibiotics 71% (116 tubes) contamination.

CONCLUSIONS We conclude that 0.5% cetyl pyridinium chloride - 1% sodium chloride decontamination inoculated on antibiotic

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incorporated L-J media had a significantly lower contamination rate than that of standard 5% oxalic acid decontamination rate.

1.3-097

Risk profile for transmission of *Mycobacterium ulcerans* in Akuapem south and Suhum Kraboa Coaltar districts, Ghana

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Introduction Buruli ulcer (BU) disease is a chronic debilitating skin disease caused by *Mycobacterium ulcerans*. It is associated with areas where the water is slow-flowing or stagnant. Unfortunately the exact way of getting the disease and the development of the disease through human activities is unknown. We carried of risk profile assessment and mapped the distribution of the disease along the Densu river.

Methods and Materials We conducted a case–control study and spatial mapping in Akuapem South and Suhum- Kraboa-Coaltar districts of the Eastern region of Ghana to identify risk factors for BU and its spatial distribution along the Densu river. We carried out an active case search for Buruli ulcer patients in the study area to identify the cases and matched them with a community control of the same sex and 5 year age ranged. Structured questionnaire on host related, demographic, environmental and behavioural factors were administere. With e-trex Garmin Geographical positioning system machine receiver, we marked the location of the case and important attributes of the community.

Logistic regression model was used to determine the risk factors that were associated with BU at 95% confidence interval with P-value set at 0.05.

Results We interviewed 112 confirmed buruli ulcer cases and 112 community controls. Risk factors identified to be associated with BU were: Low educational background, being married, living in a mud house, presence of wetland, insect bite in water, wearing short pants while farming. Bed nets, washing of clothes and wearing clothes covering the limbs protect against BU. Spatially, there was clustering of BU patients downstream of Densu River.

Conclusions Wearing clothes that cover the limbs during farming OR = 3.04, P-value = 0.01 and use of bednets protect against Buruli ulcer disease. There is clustering of cases of buruli ulcer in areas where the river was most contaminated.

1.3-098

Identification of some recent clinical isolates of Kala-Azar from India and Bangladesh by RAPD-PCR, ITS-PCR and ITS1-RFLP methods: a pilot study

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Introduction Historically Kala-azar (KA) in India is caused by *Leishmania donovani*. The has been challenged by several authors claiming *L. tropica* and *Leptomonas* sp. may be associated with the disease. In the present study, we characterized recent nine clinical isolates of KA and PKDL patients with the help of Random Amplified Polymorphic DNA (RAPD) analysis, PCR-amplification of the Internal Transcribed Spacers (ITS) of the rRNA operon and ITS1-RFLP, followed by sequencing and aligning of the ITS1 amplicons.

Methods and Materials Nine clinical isolates from VL and PKDL patients were collected in the year 2006–2010, of which three were from Bangladesh. RAPD PCR analysis was done using random primers. The amplification of ITS regions of the rRNA operon was carried out. The amplified ITS and ITS1 regions were digested with restriction enzymes. The ITS1 amplicons were sequenced and aligned for the homology analysis.

Results Amplification of genomic DNA of all clinical isolates along with WHO reference stains, DD8 (*L. donovani*) and K27 (*L. tropica*) showed that T5 was matching exactly with K27 while banding patterns present of rest clinical isolates including Bangladeshi KA isolates, were all very similar to DD8. Amplification and digestion of ITS1 amplicon with the restriction enzyme HaeIII revealed the band patterns corresponding to DD8 for all eight isolates examined while T5, was again very similar to K27. Sequencing and homology alignment supported the above observations.

Conclusions This pilot study revealed that *L. donovani* is not the only agent causing Indian KA giving credence to the single report claiming *L. tropica* is also responsible for the disease. The observations thus emphasized the need for rigorous systematic typing of the clinical isolates of Indian KA as proper drug regimen against the disease demands appropriate identification of the parasites.

1.3-099

Ancylostoma duodenale infection, an important cause of severe anemia and iron deficiency in Malawian pre-school children, revealed by multiplex real-time PCR

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Introduction Hookworm infections are considered as a major cause of anemia and iron deficiency in children in the tropics. Although hookworm species and load of infection are known to be associated with disease burden, these parameters are often not assessed due to the limitations of the traditionally used diagnostic methods. The aim of this study was to evaluate the prevalence of the hookworm species and their infection load and assess the relative contribution towards the development of severe anemia and iron deficiency in pre-school children in Malawi, using a real-time PCR assay.

Methods and Materials *Ancylostoma duodenale* and *Necator americanus* DNA loads were determined by multiplex real-time PCR in 830 fecal samples of Malawian pre-school children participating in a severe anemia etiology case–control study.

Results and Conclusions Hookworm infections were found in 34.1% (86/252) of the cases and 27.0% (156/578) of the controls, with an overall prevalence of *A. duodenale* and *N. americanus* of 26.2% and 4.9%. Moderate and severe *A. duodenale* infections increased the risk for severe anemia with adjusted odds ratio of respectively 2.49 (CI 1.16–5.33) and 9.04 (CI 2.52–32.47). The risk for iron deficiency was increased in children infected with *A. duodenale* (adjusted odds ratio's of 3.63 (1.18–11.20), 16.98 (3.88–74.35) and 44.91 (5.23–385.77) for low, moderate and high intensity *A. duodenale* infections, respectively. The prevalence of *A. duodenale* was found to be surprisingly high in Malawian pre-school children and intensity of infection was significantly associated with both severe anemia and iron deficiency. This powered study illustrates the need for quantitative screening of species specific hookworm infections. Real-time PCR is a very capable tool to answer this need and proves to be a powerful

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diagnostic tool in epidemiological studies to monitor anemia and iron deficiency in children living in resource poor settings.

1.3-101

Tolerance and feasibility of nifurtimox-eflornithine combination therapy (NECT) for second stage *T. b. gambiense* hat in north-east DRC

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INTRODUCTION Nifurtimox-Eflornithine Combination Therapy (NECT) has recently been approved in DRC for the treatment of second stage *T. b. gambiense* Human African Trypanosomiasis (HAT). We report here the result of the initial 12-month use of NECT in the Haut and Bas Uélé regions, north-east DRC.

METHODS AND MATERIALS Second stage HAT patients were hospitalized in Doruma and Dingila Hospitals and treated with NECT (Eflornithine 400 mg/kg/day IV for 7 days with Nifurtimox 15 mg/kg/day oral for 10 days), unless contra-indicated. Demographic and medical characteristics of the patients were recorded in Epitryps software. Clinical adverse-events were monitored during treatment and recorded on pharmacovigilance forms prepared by the WHO. We performed a retrospective analysis of data collected in Epitryps and the pharmacovigilance forms.

RESULTS Three hundred and seventy-one patients with second stage HAT were diagnosed and treated in 2010. Among these, 367 patients received NECT, including 93 children (≤15 years). The case fatality rate was 0.3% (n = 1) and relapses were rare (n = 3). Drug-Related Adverse Events were reported in most patients although severe or life-threatening (grade 3–4) AE occurred in <10% of cases (convulsion, psychosis and nausea/vomiting being the commonest). Gastrointestinal AE were the most frequently reported AE, including in children who, overall, tolerated NECT well. Treatment was completed in almost all patients and the mean hospitalization duration was below 2 weeks.

CONCLUSIONS Treatment of second stage HAT with NECT was safe, well-tolerated and effective in a MSF-supported treatment centre located in a remote and unstable area of north-east DRC. Tolerance was particularly good in children. Treatment of nausea and vomiting to prevent malabsorption of nifurtimox and good nursing care to prevent catheter-induced local or generalized infections are both crucial. The good safety profile and improved feasibility of NECT should facilitate the gradual switch from melarsoprol-based to eflornithine-based therapy in all endemic countries.

1.3-102

Risk factor analysis in Indian visceral leishmaniasis

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INTRODUCTION India is one of major foci of Visceral Leishmaniasis (Kala-azar). Our study address two problems; first, Risk factors playing role in Multicase VL family is unclear and second, Propagation of disease in endemic area has not been studied.

METHODS AND MATERIALS The case–control study was conducted over 50 km^2 area of nine Blocks of Muzaffarpur districts in endemic region of Bihar. Global Positioning System [Garmin (e-Trex vista)] was used to see the distribution of families. We had enrolled 136 Multicase familes (two or more than two subjects were VL diagnosed between 2000 and 2009) and 136 individually matched control families (with no history of previous VL and matched on caste, sex, age group and same geographical region). All families (cases and controls) were unrelated (no blood relation) and subjected to a structured interview for micro environmental factors using a predesigned program. SPSS 16.0 software was used for the analysis.

RESULTS We found no significant associations between Education, Consumer Items, Communication, Transportation, Source of Water reservoir, Tree-Number and Landed property while Cattle number, Cattle variety, House-Hold structure, Plantation, Water lodging within 10 m of house, Sleeping on ground, Bednet use (preventive measures) and Monthly income (social perspective) were statistically significant. Spatial analysis of Multicase families showed that all families were distributed in nine blocks (out of 14 blocks) of Muzaffarpur districts. The Cases vs. Time gap analysis showed that in small area (Village of Kanti Block); within 200 m, 16 Multicase families was present and disease had been present in that area for 27 years (1980–2007).

CONCLUSIONS This is the first study on multicase families which reflects the role of several risk factors in disease progression and suggest control strategies in endemic area (like better housing, use of bednets, sanitation, insecticide spraying).

1.3-103

Differential expression of toll-like receptors in murine peritoneal macrophages *in vitro* on treatment with synthetic peptides containing B epitopes as vaccine candidates against *Fasciola hepatica*: a practical approach using a touchdown PCR

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INTRODUCTION The liver fluke *Fasciola hepatica* is an important global helminth pathogen of humans and livestock that releases a large number of molecules that are critical to inducing Th2/Treg immune response in its mammalian host. Toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a key role in immune modulation on helminth infection. In a previous work, 12 peptides from *F. hepatica* proteins containing B epitopes were designed using a bioinformatical approach, synthesized and purified as potential vaccine candidates against this parasite. Here, a touchdown PCR (TD-PCR) is developed for analysis of the differential expression of TLRs (1–9) in murine peritoneal macrophages *in vitro* on treatment with these new peptides.

METHODS AND MATERIALS Peptides 1–12 (10 μg/ml) and LPS (10 μg/ml) treatment was given for time intervals of 6 and 12 h on murine peritoneal macrophages *in vitro* (cell line J774.2). Thereafter, total RNA from macrophages was isolated, reverse transcribed (RT-PCR) and amplified using specific primers for TLRs 1–9 murine sequences by a common described PCR and by a novel optimized TD-PCR, consisting in 16 cycles with successive annealing temperature decrements of 1.0°C every two cycles from 65–58°C. The expression of housekeeping gene GAPDH was checked for each set of amplification experiment. Amplified cDNA was visualized in agarose gels stained with ethidium bromide.

RESULTS Rather than use multiple sets of annealing temperature parameters for amplification of each TLR sequence, the optimized TD-PCR allowed us to amplify multiple TLRs tested just in a single amplification assay. Results showed that peptides 1–4 treatment for 12 h clearly decreased TLR-6 expression.

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CONCLUSIONS The optimized TD-PCR resulted more specific, sensible, repetitive and less time consuming than common individuals PCR assays for each TLR sequence amplification in differential expression evaluation on treatment with peptides containing B epitopes.

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1.3-104

Undernutrition prevalence and associated factors among school age children in Amhara state, Ethiopia

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BACKGROUND Child undernutrition is a leading cause of mortality in Libokemkem and Fogera, two provinces of Amhara, Ethiopia. In order to propose effective nutrition and public health strategies it is important to have reliable information on the nutritional status of the population and the underlying factors affecting it. The aim of this study was to estimate the prevalence of undernutrition in school age children, and to identify the risk factors associated with it.

METHODS A cross-sectional survey using a multi staged stratified cluster sampling was conducted. Four hundred and fifty-eight children aged 4–15 years were surveyed. The survey included a socio-demographic, health and dietary questionnaire and anthropometric measurements, from which nutritional indicators based on the WHO references, were calculated. In order to define undernutrition two indicators were used: Body Mass Index for Age Z-score <-2 for wasting and Height for Age Z-score <-2 for stunting. Logistic regression models were used to identify associated risk factors.

RESULTS Overall prevalence of wasting and stunting was 23% and 35.7%, respectively. In the multivariate analysis, the risk factors associated with wasting were child's increasing age (OR = 1.3 per year; P < 0.001), being male (OR = 2.5; P = 0.005) and not consuming products of their own cattle in the household (OR = 2; P = 0.038). The factors associated with stunting were child's increasing age (OR = 1.2 per year; P < 0.001), living in a house with no bed nets (OR = 2; P = 0.003) and with poorly conditioned walls (OR = 2.4; P < 0.001).

CONCLUSIONS There was a high prevalence of undernutrition in the school age children of this area of Ethiopia. Results suggest that living conditions and health related behaviour are associated with stunting while the factors associated with wasting are related to gender and household dietary habits. This project was financially supported by the UBS Optimus Foundation and the Red de Investigación Cooperativa en Enfermedades Tropicales (RICET).

1.3-106

Mucosal vaccination using non-pathogenic lactic acid bacteria as a strategy to prevent morbidity and mortality caused by visceral leishmaniasis

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Visceral leishmaniasis (VL), a vector borne disease caused by protozoan parasites of the genus *Leishmania*, is responsible for significant morbidity and mortality worldwide and constitutes a major public health problem particularly in poorer countries. There is also recognised risk that the disease may become more prevalent in Europe as a consequence of global warming and migration. Treatment is prolonged and expensive and has been complicated by the emergence of drug resistant *Leishmania*. While attempts to develop an effective vaccine have been shown to be feasible, there is no vaccine in active clinical use. The past decade has seen increasing interest in the use of dietary lactic acid bacteria (LAB) as mucosal vaccine delivery vehicles and a number of prototypes have demonstrated efficacy in experimental models of infectious diseases. Furthermore, some strains of LAB have been shown to promote T helper 1 or mixed T helper cellular responses to expressed or co-administered antigen, which are considered advantageous in vaccination strategies targeting VL. The goal of this project is therefore to develop a new immunoprophylactic paradigm for preventing VL. We have bioengineered harmless *Lactococcus lactis* (an LAB) to express conserved immunogenic antigens from *Leishmania infantum*, the causative agent of VL in Europe. Using animal models, we have been able to confirm the immunogenicity of these vaccines when administered as mucosal vaccines and are now testing their efficacy against parasitic challenge. If successful, this program of research has enormous potential to create a new inexpensive path to preventing VL disease and other leishmaniases that is amenable to large-scale vaccination programmes in populations who are at risk. There may also be scope to expand this approach to target other neglected diseases.

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1.3-107

Ivermectin mass drug administration program to treat endemic scabies and strongyloidiasis in a remote aboriginal community in northern Australia

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BACKGROUND In many Aboriginal communities in northern Australia 70% of children are clinically diagnosed with scabies before they reach 1 year of age and a high percentage of adults and children in the same communities are sero-positive for *Strongyloides stercoralis*. As both parasitic infections can be treated with ivermectin and mass drug administration (MDA) programs are recommended when helminth infections are ≤20% and scabies >5%, we hypothesised that an ivermectin MDA would reduce the endemic prevalence of these two infections.

METHODS A population census for prevalence and MDA was conducted at months 0 and 12 to be followed by cross sectional surveys for disease acquisition and treatment failures at months 6 and 18. Scabies was diagnosed clinically and strongyloidiasis by faecal microscopy and/or agar plate culture or by serology. Participants were administered a stat dose of 200 μg/kg ivermectin unless pregnant or their weight was <15 kg. All participants received one dose of ivermectin and those diagnosed with scabies and/or strongyloidiasis were given a 2nd dose after 2–3 weeks.

RESULTS There were 1011 (81%) participants enrolled from 127 (80%) houses and 7 (78%) surrounding homelands. Scabies prevalence reduced from 4% at month 0 to a point estimate of 1.8% at month 6 and strongyloidiasis from 21% to 6% over the same period. At month 6, disease acquisition of scabies was 1% and strongyloidiasis 3%, with treatment failures of 11% and 16%

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respectively. The 2nd population census and MDA#2 is currently underway and preliminary results will be presented at the congress.

CONCLUSION These results are preliminary data from a study in progress however early indications for the success of the study are encouraging. A rigorously evaluated MDA has potential global ramifications for changing public health policy for the control of endemic scabies and strongyloidiasis.

1.3-108

Characterization of the immune response to *Leishmania* in blood donors from Madrid (Spain)

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Human visceral leishmaniasis (VL) caused by *Leishmania infantum*, is endemic in the Mediterranean basin. Clinical cases represent only a fraction of those infected and the percentage of asymptomatic individuals is underestimated. The aim of the present study was to quantify the prevalence of *Leishmania* infection among healthy individuals in the Madrid area and for the first time, verify the nature of immune responses that are elicited. For that purpose, the parasitological and immunological status of 261 blood donors from Madrid was characterized. We carried out: ELISA using soluble leishmanial antigen (SLA) for the detection of Leishmania-specific IgG, IgG isotypes and IgM; IFAT; SLA stimulated cellular proliferation assays (CPA); peripheral blood lymphocyte phenotyping by flow cytometry; and parasite detection in blood by real-time PCR. Levels of IL-6, IL-10, TNF-a and IFN-g were quantified using supernatants from CPA with positive antigen-specific responses (stimulation index >2). All individuals were negative by serological and parasitological techniques. Of the 261 donors studied, 11 (4.2%) presented a lymphoproliferative response to SLA. IFN-g levels found in positive responders were significantly higher when compared to the non-responder group. Three of the positive donors also presented high levels of IL-10 and TNF-a. Peripheral blood lymphocyte phenotyping indicated similar percentages of T and B cells in both groups, but the NK cell proportion in the responder group was approximately half that of the control group. In conclusion, our study has shown that as much as 4% of blood donors in the Madrid area elicited specific cellular response to *L. infantum*, which is associated with a high production of IFN-g. The usefulness of these immunological markers will be further evaluated in order to identify cryptic/asymptomatic infection in endemic areas. Rapsodi Project was supported by EC Commission, 7 Frame Program.

1.3-109

Knowledge about chagas disease among migrants from endemic areas settled in Spain

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INTRODUCTION Chagas disease (ChD) is endemic in Latin-America, but migration has expanded its geographical limits. Spain is the most affected country in Europe.

MATERIAL AND METHODS A questionnaire designed after a qualitative research (nine in-depth interviews with Bolivians) was used to measure level of knowledge about ChD among migrants from endemic areas. The questionnaire is made up of six questions about the disease and ten about epidemiological data. From December-2007 to July-2010, 492 migrants from endemic areas filled in the questionnaire before a talk about ChD in non-clinical settings, mainly NGOs of Madrid, Spain.

RESULTS Participants had an average age of 33, and 303/492 (61.6%) were women; 361/491 (73.4%) came from Bolivia, 62/491 (12.6%) from Ecuador and 31/491 (6.3%) from Peru; 122/473 (25.8%) came from rural areas. 267/469 (56.9%) had an educational level of secondary school. 241/420 (57.4%) knew ChD is caused by a parasite; 301/421 (71.5%) transmitted by a vector. Nevertheless, 294/419 (70.2%), 226/419 (53%) and 314/394 (79.7%) answered 'No' or 'I don't know' when asked whether ChD is transmitted from mother to child, through blood transfusion, or through solid organ transplant, respectively. 239/411 (58.2%) knew ChD can involve the heart; but only 164/408 (40.2%) believed that it is possible being asymptomatic and infected by *T. cruzi*. 394/487 (80.1%) stated not having performed the diagnostic test previously; and 345/428 (80.6%) showed their willingness to undergo the test.

CONCLUSION Migrants from Latin America living in Spain are not well informed about Chagas disease and its ways of transmission. More than 75% of those surveyed were unaware of vertical transmission, although the majority of them were women of child-bearing age. This highlights the need to improve health education activities tailored to migrant population from Chagas endemic areas in order to inform them about the disease, and where to go for diagnosis and follow-up.

1.3-110

Treatment modalities for visceral leishmaniasis (Kala-azar) under field routine program conditions

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Since 1989, Médecins Sans Frontières (MSF) has provided medical humanitarian assistance of Visceral Leishmaniasis in Sudan, Ethiopia, Uganda, Kenya, Somalia, India, and Bangladesh. Most of these areas are characterized by extreme isolation, insecurity, and poverty. Between 1989 and 2010 MSF treated over 98,200 patients with significant improvements in treatment outcomes, due to operational research, resulting in- early diagnosis (RDTs), new (safer) drugs and treatment regimes, improved treatment of opportunistic infections and complications. In southern Sudan MSF used Sodium Stibogluconate and Paromomycin (SSG&PM) short course combination therapy during an epidemic situation in a remote field setting in order to increase patient turnover to decongest treatment capacity, to reduce the risk of outbreaks of opportunistic infections in the treatment centres, and to improve treatment outcomes. A retrospective analysis of 4263 primary VL patients treated between 2002 and 2005 showed that in remote field settings 17 days of SSG&PM combination gives better survival and initial cure rates than 30 days of SSG monotherapy. Use of this combination has also been supported by recently completed phase III trials in the region. Other combinations like SSG with AmBisome® or AmBisome® with miltefosine are currently being explored and are urgently needed in East Africa. In Bihar, India, MSF started treating patients in 2007 under routine programme conditions, using liposomal amphotericin B (AmBisome®) at a total dosage of 20 mg/kg divided in 4 days, as well as comprehensive supportive care. In 3 years, MSF treated over 6000 VL cases with an initial cure rate of 98.7%, default rate 0.8%, and death rate 0.5%, demonstrating that liposomal amphotericin B is

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extremely safe and effective. Key challenges remain as liposomal amphotericin B is still expensive and requires cold chain. In order to improve the feasibility of VL treatment in India, implementation of new regimens recently developed in phase III using single dose liposomal amphotericin B (10 mg/kg) and combination therapies (with miltefosine, paromomycin, and liposomal amphotericin B) remains a priority.

1.3-111

Toxocara seropositivity and atopy in Cuban children with asthma

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INTRODUCTION Toxocariasis is one of the most common parasitic infections worldwide. *Toxocara* seropositivity rates tend to be higher in asthmatic individuals. Moreover, Toxocariasis has been proposed to promote the development/exacerbation of asthma in atopic individuals. We tested the latter hypothesis in Cuba, a country where both asthma and toxocariasis are widely prevalent.

MATERIAL AND METHODS In a cohort of Cuban school-aged children (n = 1011) we investigated the association of *Toxocara* seropositivity and atopic status with asthma. *Toxocara* seropositivity was diagnosed with ELISA, other helminth infections by stool examination and atopy by allergen skin prick test. Asthma was diagnosed by questionnaire: 1. asthma; ever been diagnosed asthmatic by a physician, and 2. current wheeze; according to the ISAAC questionnaire. Data were analyzed using multiple logistic regressions using either 'asthma' or 'current wheeze' as outcome variable. Level of significance was set at $P < 0.05$.

RESULTS In total, 38.7% of the children were *Toxocara* seropositive. Prevalences of 32.9% and 21.7% were found for asthma and current wheeze, respectively.

Using 'asthma' as outcome variable and atopy, *Toxocara* seropositivity, sex, age and helminth infection (*Ascaris lumbricoides*, Trichuris trichuria and hookworm) as independent variables, we found a significant effect of atopy and of *Toxocara* seropositivity. A negative association was found between the interaction of atopy and *Toxocara* seropositivity and asthma, but not significant ($P = 0.08$). In the model with 'current wheeze' as outcome variable, only a significant effect of atopy was measured.

CONCLUSION Using the definition of asthma confirmed by a physician, our data corroborate observations of higher *Toxocara* seropositive rates or atopy in asthmatic children. However, the observed effect of the interaction between atopy and *Toxocara* seropositivity on asthma tends to be the opposite of what was previously hypothesized. Finally, results show that the definition of asthma influences its associations with toxocariasis. Possible explanations for our findings will be presented.

1.3-112

Community-based activities for chagas disease and *Trypanosoma cruzi* screening of at-risk migrants in Spain

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INTRODUCTION Chagas disease (ChD) is endemic in Latin-America, but migration has expanded its geographical limits. Spain is the most affected country in Europe.

MATERIAL AND METHODS Framed in a culturally tailored community-based health education program for migrants settled in Spain called New Citizens, New Patients, ChD's activities started in December 2007, with an at-risk population approach. It is run by a multidisciplinary team of physicians, intercultural mediators and a psychologist. Main activities: – design of a culturally tailored brochure about ChD; – talks delivered in non-clinical settings and spread of information through migrant-oriented media; – targeted screening of ChD using a rapid immunochromatographic test (ICT), Operon Simple Chagas WB's that was offered to participants after the talks; and – referral of confirmed *T. cruzi* infected patients to our Chagas clinic.

RESULTS From December-2007 to July-2010, 487 migrants from Latin-America were informed about ChD through 44 talks given to groups, mainly in NGOs and migrants associations in Madrid. Median age was 32 years; 299 (61.4%) women; 257 (52.8%) women of child-bearing age; 350 (71.9%) from Bolivia. From May-2008 to December-2009, 276 (78.4%) participants from endemic areas (76.4% from Bolivia) were screened for *T. cruzi* infection with the ICT and the result was then confirmed by standard serologic methods. Forty-four (15.9%) were confirmed positive cases. All of them came from Bolivia (75% from Cochabamba and Santa Cruz regions). Seroprevalence rate in Bolivians was 20.9%. Eleven (25%) of those infected with *T. cruzi* were pregnant women. 70.5% of confirmed positive patients went later to the Chagas clinic.

CONCLUSION A holistic approach including delivery of information, tailoring of activities to the target population, collaboration with NGOs, and *in situ* screening can help reduce the public health problem of ChD in non-endemic countries. Early diagnosis can also lead to an improvement in the quality of life and prognosis of patients.

1.3-113

Rule-based modeling to simulate exposure to toxocariasis in children

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INTRODUCTION Toxocariasis is a widespread but neglected zoonotic disease. The use of validated serodiagnostic tests has provided much-needed insight in the distribution of human exposure to *Toxocara* but randomly selected data at population level are scarce. Here, we present the development of a simple rule-based model, combining a set of rules from several sources of information, to simulate *Toxocara* seropositivity (as a measure for exposure) in children.

MATERIALS AND METHODS Risk behavior, environmental contamination with *Toxocara* spp. eggs and seasonality were used to simulate antibody acquisition. This was coupled to three scenarios of antibody loss, related to the immune status of the individuals (primary, secondary and chronic challenge). Country-specific data were used to estimate the selected parameters for simulation. Validation was conducted by comparison of simulated and measured *Toxocara* seropositivity rates in Cuban schoolchildren.

RESULTS Results are the outcome of 100 iterations over a period of 200 years. The simulated proportion of *Toxocara* seropositive children within the first scenario of antibody loss (primary challenge) was 0.41% (CI 95%: 0.38–0.44), and approximated the measured proportion in Cuban schoolchildren (n = 1011) of 0.39 (CI 95%: 0.36–0.42). The simulated figures for secondary and chronic exposure, i.e. 0.48 (CI 95%: 0.45–0.52) and 0.51 (CI 95%: 0.45–0.52), respectively, were significantly higher. Cross

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validation was performed by using the same approach with data from Brazil, the Netherlands and Poland.

CONCLUSION By using a simple rule-based model we were able to successfully predict exposure to Toxocariasis in Cuban children. Since only little specific information is required, the model can be easily applied to other settings, or extended by adding biological rules. We will discuss the development of the model and the outcome of the simulations and show how such a step-wise approach can contribute to our understanding of the transmission dynamics of toxocarias.

1.3-114

The role of CD44 glycoprotein and selected cell adhesion molecules as prognostic markers in human alveolar echinococcosis

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INTRODUCTION Over the past years, alveolar echinococcosis has become an emerging infection in Poland and other countries of Central-Eastern Europe. Infection with *E. multilocularis* in humans takes the form of a severe and chronically progressive disease, which develops gradually and insidiously, infiltrating adjacent tissues by continuity, as well as other internal organs by haematogenic and lymphatic routes. Irregular space-occupying lesions in the liver, with a tendency to the formation of distant metastases in the lungs and central nervous system, make a differential diagnosis difficult, because of its clinical similarity to an advanced stage of slowly growing liver malignancy.

AIM OF THE STUDY To improve on the clinical evaluation of cases with alveolar echinococcosis (AE) and to determine the risk of parasite spreading to neighbouring or distant organs and tissues by the detection of selected glycoproteins and cell adhesion molecules involved in the tumorigenesis process.

MATERIALS AND METHODS Eighteen Polish patients (10 females, eight males), aged 18–67 years (average age 49.8 years), with liver alveolar echinococcosis were included in the clinical study. *E. multilocularis* infection was diagnosed by: (i) the detection of specific IgG serum antibody against the Em2plus antigenic complex, (ii) the presence of specific IgG antibodies anti – 16 and 18 kDa antigens using the reference Western blot technique, and confirmed by: (iii) the histopathological examination of liver sections obtained during surgery, demonstrating the typical PAS-positive alveolar structure of the metacestode, with a proliferation of fibrotic tissue, necrosis and calcifications. The clinical evaluation of the advancement of AE cases was carried out according to the international PNM (parasite-neighbouring organs-metastases) classification. Patients with malignant neoplasms (n = 78) of the liver (primary hepatocellular cancer, systemic dissemination or distant metastases to the liver) and gastrointestinal tract tumours (n = 41), and extra-hepatic proliferative diseases (n = 37), as well as healthy blood donors (n = 37) constituted the comparative control groups. Serum concentrations of the soluble 80–90 kDa subunit of the CD44 glycoprotein (sCD44std), human endothelial leucocyte adhesion molecule-1 (E-Selectin), vascular cell adhesion molecule-1 (VCAM-1), and human platelet endothelial cell adhesion molecule-1 (PECAM-1) were analysed by Enzyme-Linked ImmunoSorbent Assays.

RESULTS Serum concentrations of sPECAM-1 in AE patients ranged from 44 to 73 ng/ml (mean 55.8 ng/ml) and were significantly higher than in patients with liver and gastrointestinal tract cancers (P = 0.007) and in healthy individuals (P < 0.000001). Levels of sVCAM-1 in AE patients ranged from 440 to 2380 ng/ml (mean 884.4 ng/ml) and were comparable to those in cancerous patients (P = 0.7) but statistically higher than in healthy controls (P = 0.002). Similarly, values of sE-Selectin in serum samples from patients infected with *E. multilocularis* reached 30–134 ng/ml (mean 64.5 ng/ml) and were significantly higher than in the healthy donors group (P = 0.01). Serum concentrations of the sCD44std molecule in AE cases (524.7–5599.2 ng/ml, mean 795.8 ng/ml) were similar to those in patients with hepatocarcinoma (P = 0.09) but lower than in metastatic liver malignancies (P = 0.004).

CONCLUSIONS (i) The sCD44 glycoprotein, and sPECAM-1, sVCAM-1, sE-Selectin cell adhesion molecules may be useful as early predictive markers of active, tissue infiltrating alveococcosis and helpful in the evaluation of the risk of metacestode spreading through the circulation; (ii) High serum levels of soluble subunits of the CD44 and PECAM-1 cell adhesion molecules were strongly related to the progression of alveolar echinococcosis, the presence of distant metastases to the lungs or the central nervous system, and the fatality of patients' prognosis.

1.3-115

On the hunt for new diagnostic antigens for human African trypanosomiasis

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INTRODUCTION Human African Trypanosomiasis (HAT) is a prevalent disease in many parts in Sub-Saharan Africa. HAT is a parasitic infection caused by two species, *Trypanosoma brucei* gambiense and *T. b. rhodesiense*. Clinical diagnosis is not sufficient as symptoms from other endemic diseases are similar, i.e. Malaria. In addition, some patients do not show any symptoms until the parasites have invaded the brain; this is the case for a large proportion of diagnosed patients. Catching this disease early can save brain damage and enable safer drugs to be administered, however current diagnostic tests require lab based equipment, trained personnel and have varying degrees of sensitivity and specificity. New approaches are needed; to first identify new diagnostic biomarkers, and second, to find a more suitable platform for the test. Our aim is to identify new diagnostic antigens for *T. brucei* species for use in a lateral flow test.

METHODS AND MATERIALS Sera were obtained from patients infected with *T. b. gambiense* and control patients. Antibodies were purified from the sera and coupled to sepharose beads. The immobilised antibodies were added to *T. brucei* cell lysate, allowing the antibodies to bind to their complementary antigen, after which, the columns were washed and eluted, breaking the antibody-to-antigen bond. The antigens were identified by mass fingerprinting and amounted to 20, most of which have not been described as potential diagnostic antigens. Selected antigens were cloned, expressed and purified from *E. coli*.

RESULTS Recombinant antigens were validated by screening 170 clinically assessed human sera, both *T. b. gambiense* and *T. b. rhodesiense* first and second stage with matched controls, in a chemiluminescent ELISA. A lead protein antigen is currently being adapted for lateral flow format.

CONCLUSIONS The results show diagnostic antigens able to discriminate between infection and control serum.

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1.3-116

Simulium damnosum wolbachia (Wsdam) genomes harbor WOcauB2/B3-like bacteriophage

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INTRODUCTION Recent experiments transferring the *Wolbachia* strain wMelpop into the disease vector Aedes aegypti showed the transformed vector displays a range of disease refractory traits and opened the possibility that this technique could be adapted for a plethora of vector-borne disease problems. One potential obstacle, however, for broadening the use of this technology to further vector-borne disease systems is the existence of native *Wolbachia* populations, which are common among disease vectors and which often harbor lysogenic phage. The potential transfer of *Wolbachia* phage from native *Wolbachia* strains to deliberately-introduced disease refractory strains could influence the effectiveness for *Wolbachia*-based disease control, but little is known about how freely phage can transfer between *Wolbachia* strains.

METHODS Multi Locus Sequence Typing of *Wolbachia* isolated from the Onchocerciasis vector *Simulium damnosum* has identified a *Wolbachia* strain that is distinct from all other previously described strains. To test how freely *Wolbachia* phage exchange between genetically distinct strains of *Wolbachia*, we screened a *Simulium damnosum* BAC library with Wsdam genes to isolate large Wsdam genomic fragments (approximately 150 kb) and then used a combination of Roche-454 and Sanger sequencing to survey the Wsdam genome for lysogenic phage. Bioinformatic analysis of the resultant sequences identified two phage integrations: one an apparent full length phage and one an apparent partial integration. The sequence and structure of these phage integrations both show very high levels of similarity to the WOcauB2/B3 phage known to infect *Wolbachia* isolated from *Ephestia kuehniella*.

CONCLUSIONS These results have two key implications for *Wolbachia*-based disease control planning: (i) there is a high risk that phage in native vector populations will be able to infect foreign *Wolbachia* introduced for disease control; (ii) WOcauB2/B3-phage currently being developed for the genetic manipulation of *Wolbachia* could potentially be used to affect a very broad range of *Wolbachia*, possibly even filarial *Wolbachia*.

1.3-117

Sera reactivity of leishmaniasis using different *Leishmania* antigens

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INTRODUCTION Serological methods using *Leishmania* recombinant or total antigens may show cross-reactivity between different clinical forms of leishmaniasis and other diseases. In this study, we evaluated the sera reactivity from patients with cutaneous leishmaniasis (CL) and visceral leishmaniasis (LV) against total *Leishmania* antigens.

METHODOLOGY Twenty-two sera from patients with cutaneous and 12 sera with visceral leishmaniasis, confirmed by parasitological or PCR methods were used to evaluated reactivity following serological.

METHODS ELISA and IFI, using total antigen from *Leishmania* major-like in house; and DAT, using lyophilized *Leishmania infantum* antigen (Institute of Tropical Medicine in Antwerp, Belgium).

RESULTS ELISA test was positive in 16 (72.8%) sera from CL patients and positive in 11 (91.67%) in sera from VL patients. IFI was positive in 6 (27.3%) CL patients and 10 (83.3%) VL patients; DAT was negative in 21 (95%) sera from CL and positive in 11 (91.6%) sera from VL.

DISCUSSION Only one CL patient who presented DAT positive had co-infection AIDS and disseminated cutaneous leishmaniasis, presenting CD4 count below 100 cells/mm^3 and the only VL patient who showed DAT negative it was a patient with AIDS and severe immunosuppression who developed VL after contact with an endemic area. These data suggest DAT is a good method for diagnosis of visceral leishmaniasis however in immunocompromised patients may be false positive and false negative.

CONCLUSION Our results confirm that ELISA and IFI using antigen of *Leishmania* major -like shows reactivity with sera from CL and VL patients, it is not possible to distinguish the infection at sites of occurrence both clinical forms. The DAT using antigen from *L. infantum* is a method with high sensitivity for VL and high specificity in patients with CL.

1.3-118

'They need the business perspective!' The use of private veterinarians for the control of zoonotic trypanosomiasis in northern Uganda

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INTRODUCTION The Stamp Out Sleeping Sickness (S0S) campaign is a public-private partnership initiated in 2006 to prevent the spread of Rhodesian sleeping sickness in northern Uganda. Initially an emergency intervention based on the mass curative and prophylactic treatment of cattle, SOS has since pioneered a business-oriented, market-driven model to the community-based control of sleeping sickness supporting veterinarians to conduct sensitisation, maintain drug shops and organise networks of para-veterinarians. Through field research conducted on the SOS-model, this paper will evaluate the barriers and opportunities of using a market-based approach to the control of zoonotic trypanosomiasis in East Africa.

METHODS AND MATERIALS A variety of structured and semi-structured interviews, focus group discussions and participant observations were conducted with farmers, village leaders, para-veterinarians, hospital staff, private veterinarians, SOS partners and district officials over a 4 week period in 2010. These methods were used in conjunction with the review of sales books and hospital records.

RESULTS Despite low income and disease knowledge among farmers, insecticide coverage is between 10% and 40% of the cattle population. With growing but fragile businesses, the SOS-supported veterinarians have increased access to animal health products but have largely abandoned sensitisation efforts revealing a tension between business and public health concerns. Para-veterinarians earn good salaries but face problems with transport, equipment and seasonal demand. While district officials are sceptical of relying solely on the veterinarians, recommending a larger role for central government, hospital records show that reported cases of sleeping sickness have remained at equilibrium

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since 2006. Regardless, a number of para-veterinarian networks show the potential of the SOS-model.

CONCLUSION The method of insecticide-treated cattle allows the possibility of using a market-driven model for the control of zoonotic trypanosomiasis. However the tension between public and private goods together with the nature of vector and parasite requires a concerted and sustained effort on education and inter-sectoral collaboration.

1.3-119

Interlaboratory comparison of leishmania-PCR methods towards a common protocol to be used within the rapsodi consortium (European Commission FP7)

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RAPSODI (www.fp7_rapsodi.eu) is an EC granted research project integrated by partners from Leishmania-endemic countries in four continents. The aim of the project is to develop a human vaccine candidate against leishmaniasis and all the associated procedures required for the subsequent clinical trials. The use of common protocols will allow the comparison of results among the different partners. A common PCR method would be desirable to assess: (i) the parasitic burden on asymptomatic and symptomatic individuals, (ii) the changes in the parasitic load defining the infection outcome evolution, (iii) the monitoring of the vaccine efficacy at the parasitological level, (iv) the causative species of *Leishmania* in different settings. And this in the different scenarios of the project: (i) Peru, cutaneous (CL) and mucosal leishmaniasis (ML), (ii) Tunis, CL and visceral leishmaniasis (VL), (iii) India, PKDL and VL, (iv) France and Spain, VL. For this purpose, we settled up a workshop on which a panel of samples including DNA from different *Leishmania* species ($N = 8$) and *Trypanosoma cruzi* and DNA from human specimens ($N = 94$) from patients with different clinical conditions (CL, ML, VL), and healthy individuals was assayed. This panel of samples was tested in double blind using different PCR approaches (conventional and Real Time) and targeting different regions of the *Leishmania* genome (SSUrRNA, mpi, cpb, hsp70, kDNA minicircles and ITS-1 region). It turned out that a Real Time PCR, using SYBRGreenI, targeting kDNA minicircles presented the highest sensitivity on clinical specimens for all partners (though using different primers for Old World and New World *Leishmania* species). ITS-1 PCR RFLP was successfully adapted in all laboratories involved and showed to be suitable for species identification. However, in a New World scenario the combined use of mpi, cpb and hsp70 PCRs + RFLP turned out to be crucial for *Viannia* subgenus species identification.

1.3-120

Socio-cultural research on the neglected zoonotic diseases in Africa: a review of the literature and outline for a future research agenda

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INTRODUCTION A number of endemic zoonoses in Africa (anthrax, bovine tuberculosis, brucellosis, cysticercosis, echinococcosis, leishmaniasis, rabies and zoonotic trypanosomiasis) have recently been termed 'neglected zoonotic diseases' (NZDs) calling attention to their effect on poor, marginalised communities, underestimated burden and low prioritisation. The NZDs present unique control challenges as they involve issues at the animal-human-ecosystem interface where they impose a dual burden on communities, compromising livelihood status through reductions in livestock health while causing human morbidity and mortality. Following a number of contemporary review articles on the existent social science literature on tropical diseases, this paper will review published work on socio-cultural factors relevant to the spread and control of the NZDs.

METHODS AND MATERIALS A review of work published on the NZDs in the last 30 years was conducted using PUBMED together with a search on Google Scholar. Follow-up searches based on bibliographical information were pursued while relevant journals were purposively searched.

RESULTS Studies touching on socio-cultural factors are largely conducted by veterinarians as an appendage to epidemiological field studies and to a much lesser extent economic analysis. Aside from a few notable exceptions, the limited number of knowledge, attitude and practice (KAP) surveys appear the extent of direct engagement but are unaware of their methodological limitations. Studies into gender dynamics, belief and value systems, traditional knowledge, risk factors, health education, local control techniques, decision making processes, societal organisation, health systems, market structures and human-livestock-ecosystem interaction relevant to the NZDs are sparse and limited.

CONCLUSION Understanding socio-cultural factors are fundamental to designing and implementing effective control programmes to combat the NZDs. Presently there are a lack of social scientists working on social and cultural issues relevant to tropical livestock and zoonotic disease control generally and the NZDs in particular. Any future research agenda should appreciate the importance of socio-cultural studies complementing scientific inquiries and technical interventions.

1.3-121

Amplification and identification of DNA of *Leishmania* (Viannia) *Leishmania braziliensis* by PCR in salivary secretion in patients with American cutaneous leishmaniasis

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Due to the scarcity of parasite in clinical samples of patients with American Cutaneous Leishmaniasis (ACL) and the low sensitivity of traditional techniques is not always possible to diagnose disease. Modern technologies aimed at the genomic identification as Polymerase Chain Reaction (PCR) are an alternative, in view of the possibility of the presence of parasites in body fluids. We evaluated the possibility of DNA detection by PCR leishmaniotic in the salivary fluid of patients with the diagnosis of ATL before establishing the therapeutic regimen. When using b1/b2 primers specific to *L. (V.) braziliensis*, the fragments generated by amplification totaled 103 pb. Positive results were determined in samples of saliva from a patient with the cutaneous form of the disease and two with the mucosal form. For the first time it was possible to amplify *Leishmania* DNA in the saliva of patients with ATL, identifying the species present. Saliva has various advantages that indicate the possibility of its use as substrate in PCR for diagnosis of ACL. Further research is needed with a larger number of participants in order to standardize the type of collection, verify the sensitivity and specificity of the test and thus demonstrate the usefulness of salivary fluid as a noninvasive substrate to be used for

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diagnosis of disease through PCR collaborating complementarity of classical diagnostic methods of ACL.

1.3-122

Infection by calodium hepaticum in a rural area of the Amazon: rare event or misdiagnosis?

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INTRODUCTION *Calodium hepaticum* (syn. *Capillaria hepatica*) is a nematode of ubiquitous distribution present in the liver parenchyma of various mammals. Human infection is considered rare and occurs after the ingestion of non embryonated eggs present in the liver of animals (spurious infection) or embryonated eggs dispersed in the environment (true infection), the latter mechanism causing liver disease.

METHODS AND MATERIALS In a survey in August 2009 in a rural community with low levels of sanitation (Presidente Figueiredo, Amazonas, Brazil), stool samples of 194 individuals were analyzed by spontaneous sedimentation. Investigation of the transmission of *C. hepaticum* and feeding habits was also done. Differences between groups were evaluated using Fisher exact tests.

RESULTS Ten cases [5.1% (1.8–8.5) CI 95%] of spurious infection were identified. In one household the source of infection was determined by the finding of *C. hepaticum* eggs in the liver of a wild pig (*Tayassu* spp.) consumed as food. 57.5% of this population consume liver of hunted animals and the risk of spurious infection is 10-times higher in people eating this organ [10.9% vs. 0% (P = 0.03)].

CONCLUSIONS The prevalence of spurious infection by *C. hepaticum* in populations consuming game meat could be higher than previously known. Furthermore, the absence of specific signs and symptoms that facilitate disease diagnosis and the unawareness of the nematode by many laboratory technicians, indicates that this diagnosis may be underestimated. These results suggest that it is necessary to evaluate the effect of *C. hepaticum* as an agent of disease in tropical rural communities.

1.3-123

The epidemiology of cannine leishmaniasis in the dog population of Amudat district in Uganda

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Visceral Leishmaniasis is an important public health disease amongst the Pokot pastoralists in Amudat district in Uganda. A cross sectional study was performed in all the villages of Amudat district using the dip stick method and microscopic examination of lymph node biopsies. A total of 1245 dogs from 124 'Manyatas' were tested sequentially for *Leishmania* antibodies using the direct agglutination test or rK39 anti-gen-based dip sticks and microscopic examination of lymph node smears. Prevalence of 14.1% was recorded in female dogs whiles 28.1% were recorded in the male dog's population; while prevalence of 12.6% for females and 25.7% for male using the microscopic smear examinations were recorded. *Leishmania* prevalence was seen to vary according to area and grazing stategy. Age, sex, geographical location and history of migrations were found to have independent effects on the seroprevalence. This study establishes that canine leishmaniasis is endemic in dog populations owned by pastoralist communities in Uganda. The implications of these findings with respect to the epidemiology and control of canine leishmaniasis in Karamoja are discussed.

1.3-124

The spatial distribution characteristics of visceral leishmaniasia cases in Kashi, Xinjiang, China

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Visceral leishmaniasis (VL) has remained a public health problem in western China. In recent years, hundreds of cases were reported every year and there was mortality from VL in some areas. Meanwhile the endemic areas of VL have gradually enlarged. VL used to be limited to several areas in western China. The cases reported from Xinjiang, Gansu and Sichuan provinces accounted for 97.8% of all VL cases in China. The aim of the research is to describe the spatial distribution characteristics of VL in China in order to provide evidences for developing a VL control strategy.

METHODS We selected three administrative villages in highly endemic areas, Kashi, Xinjiang according to the reported data based on Infectious Diseases Report System, and did a household survey. Every family member who was more than 10 years old was interviewed with a questionnaire. The geographic position of each household was located by GPS. Data was analyzed by using multivariate statistical methods and geographic statistical methods.

RESULTS AND CONCLUSIONS The test of goodness of fit binomial distribution proved that distribution of VL cases, were not the binomial distribution $X^2 = 53.23$; $P < 0.0$. It showed that the distribution of cases was not random. Runs test (a kind of statistics method) results showed that if the number of families with VL patients in a group ismore than 5, then the distribution of patients along the canals is not random. There were 113 such households accounting for 63.84% (113/177). Scanning statistics analysis was done with Possion model to carry out spatial aggregation. Three clustering areas were identified. Furthermore, specific location, scope and relative risk (RR) were described and identified.

CONCLUSION The distribution of VL shows family aggregation and spatial aggregation. These characteristics are very helpful to develop appropriate control strategies and measures for VL in Xinjiang.

1.3-125

Failure to associate serum Th1/Th2 cytokine profiles with symptomatic and asymptomatic human *Leishmania* (L.) *infantum* chagasi-infection in Amazonian Brazil

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INTRODUCTION The immune response of human infection by *Leishmania* (L.) *infantum chagasi*, the causative agent of American visceral leishmaniasis (AVL), has been regarded with great interest in viewing of its role on the clinical-immunological spectrum that result from this interaction. Thus, the serum Th1/Th2 cytokine profile of symptomatic and asymptomatic infection was evaluated by Capture Enzyme-Linked Immunosorbent Assay (ELISA), aiming to better understand its immune response.

MATERIAL AND METHODS One hundred and sixty-eight serum samples from endemic areas of AVL in Pará state (Barcarena municipality), Amazonian Brazi were analyzed by ELISA. Definition of serum samples was based on the clinical-immunological profiles prior identified by using the indirect fluorescent antibody

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test (IFAT) and delayed-type hypersensitivity (DTH), both with *L. (L.) i. chagasi*-antigen: Asymptomatic Infection, AI = DTH+/++++/IFAT- (n = 84); Symptomatic Infection, AVL = DTH-/IFAT++++ (n = 8); Subclinical Oligosymptomatic Infection, SOI = DTH-/IFAT++++ (n = 6); Subclinical Resistant Infection, SRI = DTH+/++++/IFAT++ (n = 39), Indeterminate Initial Infection, III = DTH-/IFAT++ (n = 31). ELISA assays were carried out to recognize the serum Th1 (IFN and IL-2) and Th2 (IL-10) cytokines profiles.

RESULTS Among 84 samples of AI profile, 7 (8.3%) were positive for INF 9 (10.7%) for IL-10 and only 1 (1.2%) for IL-2; amongst 39 of SRI, 3 (7.7%) reacted for INF and 2 (5.1%) for IL-10; amongst 31 of III, only 1 (3.2%) showed reaction for INF amongst eight of SI (AVL), only 2 (25%) were positive for IL-10 and, amongst six of SOI, 2 (33%) reacted for INF.

CONCLUSION These results did not confirm an association between the clinical-immunological profiles of human *L. (L.) i. chagasi*-infection and the serum Th1/Th2 cytokines profiles, mainly regarding the asymptomatic AI, SRI and III profiles; with respect to symptomatic SI (AVL) and SOI profiles, there was confirmed IL-10 in 25% of AVL and INF in 33% of self-healing SOI cases.

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1.3-126

Health seeking behavior and utilization of health services by schistosomiasis patients in a poor rural community in Brazil

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Schistosomiasis causes a significant economic burden in endemic populations. Morbidity control through integration of health care systems is considered a potentially sustainable and cost effective approach. A questionnaire survey was carried out among 1228 individuals to investigate health seeking behavior and utilization of schistosomiasis diagnostic and treatment services in relation to socioeconomic status, social networks, signs and symptoms and actions taken against the symptoms during the period 2003–2006. In 2007, stool exams were performed using the Kato/Katz method. Univariate analysis and odds ratios between 2003 and 2006 revealed the following factors as being asociated with significantly lower for utilization of diagnostic services: being male, age <34 years, monthly per capita income <60 Reais, not owning a car/motorcycle, household receiving government assistance, having more than 0.80 persons per room and *S. mansoni*-infected persons with or without symptoms. All these variables except age, gender and government assistance remained significantly associated in the multivariate model. Only 24.5% of the persons self-reporting to a health facility between 2003 and 2006 obtained a stool test from the local health center or other health facilities and 138 of the 197 (70.0%) persons, experiencing symptoms suggestive of schistosomiasis within 30 days prior to the 2007 survey, used home remedies. The analysis individual records for the 12-month period prior to 2007 survey showed that only four of the 179 patients with symptoms suggestive of schistosomiasis received praziquantel, the others being treated symptomatically for soil-transmitted helminths. Only 18.3% of the persons who obtained a stool test and half of those who were treated obtained these services through the local health center. The others used health facilities outside the study area. This study confirms recent reports of low access and utilization of schistosomiasis diagnostic and treatment services in other endemic areas in Brazil.

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1.3-127

Correlation between tissue parasitism and expression of inos and CD3+ T cells in the cutaneous lesions developed experimentally in *Cebus apella* monkey by *Leishmania* (L.) *amazonensis* and *Leishmania* (V.) *braziliensis*

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Cebus apella monkey is susceptible to experimental infection by different species of New World dermotropic *Leishmania* sp. and is considered a suitable animal model for studying cutaneous leishmaniasis. The aim of this study was to correlate the parasite burden with the CD3+ T cells and iNOS+ expression during the evolution of cutaneous lesions in *C. apella* developed by *L. (L.) amazonensis* and *L. (L.) braziliensis* inoculation. Ten specimens of monkey were intradermally inoculated with 3×106 promastigotes in six different spots on the tail, two groups of five animals inoculated with each *Leishmania* species. Skin biopsies were collected at 30, 60, 90, 120, 150 and 180 days post-infection (PI) for immunohistochemical staining using as primary antibodies anti-Leishmania, anti-CD3 and anti-iNOS. A quantitative analysis of the immune-stained cells was done in each section using an image analysis system. A gradual increase on the parasitism was observed in the cutaneous lesion until the 60th day PI. After this period the parasitism decrease so pronounced that in the 180th day PI there were not found parasites in the healed lesions. The amount of parasites was smaller in *L. (V.) braziliensis* than in *L. (L.) amazonensis* infection. High densities of stained CD3+ T and iNOS+ cells were observed at 30th and 60th days PI in *L. (L.) amazonensis* infection followed by a progressive decrease. Nevertheless, the CD3+ T and iNOS+ cellular densities were higher in *L. (V.) braziliensis* infection since 90th day PI, reflecting a more lasting and efficient cellular immune response, which was related to decreased parasitism from the 90th day PI. Our results suggest an efficient activation of the cellular immune response of *C. apella* monkey, with the subsequent activation of dermal macrophages and NO production, which is directly related to the reduction of parasite burden and the infection healing in the skin of *C. apella* infected by both *Leishmania* species.

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1.3-128

Relationship between cellular immune response and dendritic cells in the experimenta, cutaneous leishmaniasis of BALB/c mice due to *Leishmania* (L.) *amazonensis* and *Leishmania* (V.) *braziliensis*

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The role of Langerhans (LC) and dermal dendritic cells (dDC) in the development of cellular immune response is still contradictory in leishmaniasis. Thus, we evaluated the potential of these dendritic cells in modulating the Th1 and Th2 immune responses in the skin of BALB/c mice experimentally infected with *L. (L.) amazonensis* (La) and *L. (V.) braziliensis (Lb)* parasites. At 4th and 8th weeks PI, skin biopsies were collected to determine the parasite load and CD207+, CD11c+, CD4+, CD8+ and iNOS+

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cellular density. IFN-g, IL-4 and IL-10 cytokines profile in draining lymph node cell cultures were also evaluated. At 4th week PI, it was noted a higher CD207+ and CD11c+ cell densities in La than in Lb infection. Already, at 8th week PI, there was an increase of both CD207+ and CD11c+ cell densities in Lb infection which were higher than BALB/c mice infected with La. The CD4+ and CD8+ cell densities had similar profiles at 4th weeks PI; both were higher in La infection. However, at 8th weeks PI, a significant increase of both cell densities was observed in Lb infection, especially CD8+ cell density. The iNOS+ expression was higher in Lb infection, at 4th and 8th weeks PI, compared to La infection. In contrast, the parasite load was higher in BALB/c mice infected with La, at 4th and 8th weeks PI, than that in Lb infection. The cytokine profiles clearly showed a higher and progressive IFN-g expression in Lb infection than in La-infection, however the expression of IL-4 and IL-10 were higher in La than in Lb infection either at 4th or at 8th weeks PI. These results represent strong evidence on the role of *Leishmania* sp. in modulating LCs and dDCs for preferentially stimulating a Th1 or Th2 immune response.

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1.3-129

Schistosomiasis transmission: a preliminary study in a rural village in Minas Gerais state

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Rural electrification programs have been initiated in many countries in the South during the last two decades. The major objectives of the program are poverty reduction, increase of overall socioeconomic development at the national level, create small industries in rural areas to reduce rural-urban migration, and enhance activities in several sectors, including agriculture, health, education and tourism. The objective of this study is to provide information on potential risk of schistosomiasis associated with the installation of electric pumps in natural water bodies in Virgem das Graças rural area in Brazil within the context of Brazil's rural electrification program – Luz para Todos (Light for All). The epidemiological studies carried out in this area since 2001 reported the decline in the *S. mansoni* infection rate from 57.7% in 2001 to 26.5% in 2009. Thirty-eight of the 144 households in the Virgem das Graças, a community endemic for schistosomiasis, owned wells with electric pumps and 130 households had storage tanks (caixas dágua) in 2009, representing a sharp increase since 2001, 2 years before the implementation of the rural electrification program. Piped water is used for all household purposes and 16 of the 38 households with electrified wells shared water with neighbors. The potential for schistosomiasis transmission, through the use of well water, is indicated by the presence of *B. glabrata* in some wells, including *S. mansoni*-infected snails recovered during earlier malacological surveys, the presence of *Biomphalaria* in tanks, and other researched documents indicating the survival of cercariae for considerable distances in turbulent water. We conclude that electricity has impacted significantly on the access to water by the population; however, the potential of transmission of schistosomiasis by the use of well water still needs further studies and the use of an experimental sentinel mouse model will be fundamental.

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1.3-130

High frequency of asymptomatic *Giardia intestinalis* infections in Rwandan children and association with malnutrition

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The effects of *Giardia intestinalis* on childhood health in high-endemicity countries are not well understood. This particularly applies to submicroscopic infections, detected by PCR only. In a combined community and health facility based survey among 583 children <5 years of age in southern highland Rwanda, intestinal protozoa and helminths were assessed in stool samples by three rounds of microscopy and by selected PCR assays. *G. intestinalis* was detected by light microscopy in 19.6% but three times more often by PCR (62.8%). In 492 community children, *G. intestinalis* prevalence differed greatly between villages (range, 27–87%; P = 0.008) and increased from 34% in infants to 81% in children 4 years and older (P < 0.0001). Independent of age, breastfeeding was negatively associated with *G. intestinalis*. Gastrointestinal symptoms were not increased in infected community children, except for abdominal distension (6% vs. 2%). However, clinically assessed severe malnutrition was observed in 4.3%, 11.9%, and 19.2%, of children without, with submicroscopic and with microscopic infection, respectively (P = 0.0005). This was paralleled by significantly reduced weight-for-age z (WAZ)-scores and an increased proportion of children with WAZ <-2 at microscopic infection. Adjusted for residence, socio-economic factors and intestinal co-infections, microscopic *G. intestinalis* infection was significantly associated with increased odds of severe malnutrition whereas submicroscopic infection showed a weak respective trend only. In 91 children attending health facilities, no influence of *G. intestinalis* (prevalence, 46%) on health parameters was discernible. Conventional microscopy grossly underestimates *G. intestinalis* in the study area where almost three of four infections are below the detection threshold. These submicroscopic infections appear to be of limited overt significance but likely contribute to transmission. The association of microscopic *G. intestinalis* infections with severe malnutrition illustrates the relevance of this otherwise unimposing neglected infectious disease.

1.3-131

Enhancement of the protective effct of Sj23 DNA vaccine against *Schistosoma japonicum* infection by a novel vaccine delivery vector- PAMAM-Lys

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OBJECTIVE To develop PAMAM dendrimers as a novel vaccine delivery vector for DNA vaccine of schistosomiasis japonica and evaluate its enhancement of protective effect against *Schistosoma japonicum* infection.

METHODS Lysine was used to modify 4.0G PAMAM, and the modified product PAMAM-Lys was synthesized. Fifty BALB/c mice were divided into four groups randomly. In control group, PJW4303 group, PJW4303-Sj23 group and PAMAM-Lys/PJW4303-Sj23 group, each mouse was immunized by injection intramuscularly with 100 ¦Ìg of PJW4303, 100 ¦Ìg of PJW4303-Sj23 DNA, 100 ¦Ìg of PAMAM-Lys/PJW4303-Sj23 respectively, for three times at 2-week intervals. Four weeks after the 3rd immunization all mice were challenged with (40¡À1) cercariae by abdominal skin penetration. At 45th day post-infection, mice were sacrificed and perfused, and the number of recovered worms and

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hepatic eggs were counted. The blood was collected from the tail vein of all mice 2 days before the 1st immunization and infection respectively. Serum was prepared for detection of IgG, IgG1 and IgG2a. Two weeks after the 3rd immunization, the spleen cells of two mice from each group were cultured and the supernatant was collected for detection of IL-2¡¢IL-4 and IFN-Ã.

RESULTS The levels of specific antibodies of the mice immunized with PAMAM-Lys/pJW4303-Sj23 were significantly higher than those of the mice immunized with naked DNA vaccine pJW4303-Sj23 ($P < 0.05$) ¡£In pJW4303-Sj23 group and PAMAM-Lys/pJW4303-Sj23 group the ratio of IgG2a/IgG1 were 1.95 and 3.15 respectively. In contrast to the control, IL-2 and IFN-Ã of mice in pJW4303-Sj23 and PAMAM-Lys/pJW4303-Sj23 group were augmented, while IL-4 was unchanged. Both PAMAM-Lys/pJW4303-Sj23 and pJW4303-Sj23 vaccination provided, with comparison to control group, 46.44%, 35.33% of worm reduction rate and 61.95%, 43.61% of hepatic eggs reduction rate respectively.

CONCLUSIONS The lysine-modified PAMAM-Lys is an excellent vector. PAMAM-Lys may enhance the immunoreactivity of DNA vaccine, and increase the protective effect of Sj23 DNA vaccine against schistosoma japonicum infection.

KEYWORDS *Schistosoma japonicum*, Sj23, DNA vaccine, dendrimer, PAMAM

1.3-132

Case seriers of 100 patients with loiasis seen at a single centre

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INTRODUCTION Loiasis is a human filariasis endemic in West-Central Africa where it is one of the most common reasons for medical consultation. *Loa loa* is transmitted by flies of the genus *Chrysops*. The adult worms living in subcutaneous tissues or fasciae produce microfilariae with a diurnal periodicity. The typical clinical manifestations are Calabar swellings and the subconjunctival passage of adult worm.

METHODS AND MATERIALS We reviewed retrospectively all cases of loiasis diagnosed at the Centre for Tropical Diseases from 1993 to 2010. Case definition was based either on detection of blood microfilariae (Knott) or presence of the Calabar swelling plus eosinophilia. Serology was done with an ELISA detecting *Acanthocheilonema vitae* antigens (Bordier Affinity Products SA, Switzerland).

RESULTS One hundred patients met the case definition. Having Calabar swellings was inversely associated with positive microfilaremia in immigrants and travellers/expatriates. Spleen hypoechogenic lesions were recorded in 8.2% of our patients.

CONCLUSIONS Comparing immigrants from endemic countries and travellers/expatriates, we observed that non-endemic population showed more frequently Calabar swellings, high eosinophilia (>3000) and positive serology, while immigrants had higher rate of eyeworm sign, microfilaremia and hypergammaglobulinemia.

Table 1 Loiasis: main clinical and laboratory findings

	Total	Immigrants	Others	*P**
Males	57 (57%)	21 (65.6%)	36 (52.9%)	0.234
Mean Age, years	45.5 (11–75)	33.5 (21–60)	51.1 (11–75)	< 0.001
Years of exposure	19.02 (0.08–60)	28.3 (14–60)	14.6 (0.08–42)	< 0.001
Positive microfilaremia	34 (34.7%)	19 (59.4%)	15 (22.7%)	< 0.001
Eosinophils > 3000/µl	27 (27%)	3 (9.4%)	24 (35.3%)	0.007
IgE > 1000/ml	29 (29%)	11 (34.4%)	18 (26,5%)	0.418
Hypergamma-globulinemia	30 (33.3%)	16 (64%)	14 (21.5%)	< 0.001
Positive anti-filaria serology	59 (73.8%)	12 (57.1%)	47 (79.7%)	0.045
Calabar swelling	77 (77%)	16 (50%)	61 (89.7%)	< 0.001
Eye worm	25 (25.3%)	13 (40.6%)	12 (17.9%)	0.015
Other symptoms†	71 (71%)	21 (65.6%)	50 (73.5%)	0.09

*Chi square MH or Fisher exact.
†Spleen lesions, urticaria, itching, arthropathy, linfoadenopathy, effusions, neurologic signs.

1.3-133

Evaluation of rapid serological diagnostic tests for infection with *Trypanosoma cruzi*/chagas disease

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Chagas disease is one of the main public health problems in Latin America . More than 10 million people are estimated to be infected with *Trypanosoma cruzi* and more than 40 million are at risk of infection of the disease. In recent decades, Chagas disease is no longer confined to the Latin American region. Cases have been reported in the USA, Canada, more than 15 European countries and in the Western Pacific region, mainly Japan and Australia. This can be explained by population movement and mother-child transmission. During the chronic phase, currently diagnosis is made by detecting circulating IgG antibodies through serodiagnostic tests based on immunological targets and different principles, such as ELISA, IFA or IHA methods. Alternatively, Rapid Diagnostic Tests (RDT) can be used. The ideal RDT should be inexpensive, not requiring external equipment, reagents or refrigeration, with high sensitivity and specificity, and easy to use.

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In its initial phase, this study aimed to evaluate commercialized RDTs through a multicentre study, including the Region of the Americas, Europe and Western Pacific. Ten National Reference Laboratories, selected according to their diverse geographical (representing different disease endemic and non-endemic areas), evaluated the 11 commercialized RDTs currently available, focusing on their ease of use-use, sensitivity, specificity, cross-reaction with other specific infections/diseases, as well as performance related to different geographical areas and degree of agreement between laboratories. Knowing the performance of the RDTs with serum, in laboratory conditions, will enable the authors, in a later phase, to test the best RDTs on whole blood in field conditions.

1.3-134

Production and characterization of antigens of *Fasciola gigantica* and its evaluation in the immunodiagnostic of human fascioliasis (Cape Verde)

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The diagnosis of human fascioliasis is made mainly by serological methods, due to the clinical polymorphism and the low sensitivity of parasitological methods. However, these methods have some limitations, so the improvement of immunological tests, including the use of homologous antigens, could be a useful approach to achieve higher specificity. This study aimed at the production and evaluation of somatic and dislipidized antigens of *F. gigantica* in the immunodiagnosis of fascioliasis by micro-ELISA and Western blot (WB), compared with those of *F. hepatica*. Sera from 111 individuals from Cape Verde and from 67 patients living in Portugal, with confirmed or clinically suspected fascioliasis were tested. The antigens were produced from *F. gigantica* adult worms collected from cattle in Santiago Island (Cape Vert). The two somatic antigens of Fasciola species showed similar sensitivity (100%) for IgG antibodies but specificity was superior with *F. gigantica* antigen (95.2%). Like the somatic antigen, dislipidized antigen demonstrated a sensitivity of 100%; however the specificity was higher (95.2%) with the antigen of *F. hepatica* compared to that of *F. gigantica*, (90.5%). For detection of *Fasciola* IgM antibodies, the sensitivity and specificity were 97% and 90.5%, respectively, to somatic antigens of both parasites whereas dislipidized antigens showed similar specificity (90.5%) but different sentivities:94% (*F. gigantica*) and 83% (*F. hepatica*), The antigenic fraction of 24 kDa detected in WB was common in four antigenic preparations for either IgM or IgG, while a polypeptide of 57 kDa was present only on *F. gigantica* antigen, suggesting to be specific to this parasite. These findings lead us to conclude that the characterization of these fractions could be relevant to the development of methods with higher specificity and reproducibility, as well as for serological differentiation between *Fasciola* species, which could significantly improve serodiagnosis of human fascioliasis.

1.3-135

Randomly amplified polymorphic DNA (RAPD) polymerase chain reaction assay for comparison of *Schistosoma mansoni* strains sensitive and resistant to praziquantel

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INTRODUCTION Schistosomiasis is a chronic disease caused by a trematode of the genus Schistosoma that not only kills hundreds of thousands of people each year but is also associated with high morbidity . Drug-therapy has been used as the major weapon for fighting schistosomiasis due to the fact that this type of intervention is safer, cheaper and produces quicker impact in the prevalence of the disease. Praziquantel (PZQ) is overwhelmingly the drug of choice for the treatment of schistosomiasis showing no significant side effects. However, this drug has some limitations concerning its therapeutic mode of action and more and more cases of drug resistance or increased tolerance have been reported in recent years. At IHMT we have been passaging continuously, for several years, the reference strain of *S. mansoni* BH, sensitive to 40 mg/kg of PZQ, through *Biomphalaria glabrata* snails and CD1 mice. Over the past 2 years, while maintaining a constant drug pressure we were able to select a line of *S. mansoni*, derived from the BH strain, which is resistant to roughly 70 mg/kg of PZQ (almost 2× higher than the sensitive strain).

MATERIALS AND METHODS We extracted DNA from pools of *Schistosoma mansoni* parasites, from different life stages (adults and cercaria) and divided into three groups: (i) sensitive to PZQ; (ii) resistant to 60 mg/kg of PZQ; (iii) resistant to 70 mg/kg of PZQ. RAPD-PCR reactions were performed with 10 different primers Negative controls were included in all reactions.

RESULTS AND CONCLUSIONS All RAPD primers produced multiple amplification products. Some RAPD profile differences were observed between strains with distinct PZQ sensivity levels, but not between different life stages (adult vs. cercariae) or between female and male parasites. Bands associated with resistance/sensitivity will be isolated and sequenced to identify regions associated with the resistance phenotype.

1.3-136

Fascioliasis on Madeira Island: Snails' intermediate hosts

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Fascioliasis is a zoonotic disease caused by helminth parasites of the genus *Fasciola*, which are transmitted by fresh water snails of the family *Lymnaeidae* and have cattle as definitive hosts, humans being accidental hosts. Madeira Island is located off the coast West Africa, possessing an abundance and variety of water streams, as well as previous evidence of fascioliasis, although it is not considered endemic. We conducted a study to identify Lymnaeid snails currently present in the Island and to determine the bioecological characteristics of their habitats. Snails were collected in two visits (July and November 2009) to the Madeira Island, morphologically identified and counted. In all water collections we recorded the pH and water temperature and collected a water sample to determine its physico-chemistry characteristics. One thousand four hundred and nine snails were collected, of which

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874 were *L. truncatula* (29/53 locations) and the remainder *Lymnaea natalensis, Physa acuta, Ancylus sp, Helisoma sp* and other species of operculated snails belonging to the sub-class *Prosobronchiata* (22 locations). The water collections were predominantly permanent, with a substrate composed of rock, clay and decaying organic matter, and 'emergent' vegetation, which was scarce in water or on the shore. The water was colorless and odorless with low sun exposure. The mean of pH values ranged from 6.0 to 8.0. The maximum water temperature was 7°C lower in November. Bioecological parameters of water bodies with and without detected *L. truncatula* will be discussed. The high prevalence of *L. truncatula* in Madeira Island may be relevant in terms of human and animal health because most cattle on the is imported from the Azores Archipelago, an endemic area for fascioliasis. Thus, it is urgent to implement surveillance measures in order to prevent the parasite from becoming endemic.

1.3-137

Molecular characterization of the first record of *Lymnaea natalensis* Krauss 1848 (Mollusca, Pulmonata, Lymnaeidae) on Madeira Island

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The fascioliasis is a zoonotic disease caused by a liver fluke of the genus Fasciola (Trematoda, Fasciolidae), which are transmitted by fresh water snails of the family Lymnaeidae. In addition to the risk posed to the human population, it causes economic losses in cattle. The two species responsible for the disease are *Fasciola gigantica* Cibbold, 1856 and *Fasciola hepatica* Linnaeus, 1758, the latter the only species found in Portugal. The Madeira Island is located off the coast of West Africa, has a tropical climate and an abundance and variety of water streams. Although there is previous evidence of fascioliasis in the island, it is not considered endemic. We conducted two malacological surveys in the island to estimate the risk of establishment of *F. hepatica* in the island from imported infected cattle. Twenty-two water collections were visited in July and 31 in November 2009. One thousand four hundred and nine snails were collected, of which 133 were *Lymnaea natalensis* (from six locations distributed throughout the island), the first record of this species in the Madeira Island. The classification was based on morphological characters of the shell and the radula of molluscs. The main habitats were small streams in runoff slopes. DNA was extracted, and nuclear ribosomal ITS and mitochondrial COI and 16S genes were amplified and sequenced, which confirmed the morphological classification. The finding of *L. natalensis* in this island is of particular importance, given that this species acts as intermediate host of *Fasciola gigantica* in several regions of Africa and Asia. Its wide distribution throughout the island and the constant flow of people from countries were fascioliasis by *F. gigantica* is endemic, warrants special attention and vigilance to avoid the possible establishment of this disease.

1.3-138

First isolation of *Leishmania major* from *Mastomys* sp and *Taterrilus* sp in Ouagadougou (Burkina Faso)

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INTRODUCTION Ouagadougou city was recognized as a focus of cutaneous leishmaniasis in April 2000. *Leishmania major* MON74 was the only strain isolated in this focus. We conducted a prospective study to determine the animal reservoir which would provide the evolution of the parasite between the vector and the man.

METHODS AND MATERIALS Rodents were caught in eight areas of the town during 1 year (from November 2005 to October 2006). All the rodents were killed with ether. After physical examination checking for superficial lesions, the spleen was removed. Smears were realized from the lesions observed and the spleen removed. Amastigotes were checked at light microscopy. Cultures on NNN media were realized for promastigotes. PCR completed the diagnosis series.

RESULTS AND CONCLUSION A total of 101 rodents were trapped: 26 *Tatera* sp, 24 *Mastomys* sp, 10 *Rattus* sp, nine *Praomys* sp, eight *Taterillus* sp, seven *Nannomys* sp, three *Crycetomys* sp, one *Stochomys* sp, one *Phuromys* sp and 12 rodents not identified because they lost their labels. The microscopic examination and the culture were negative for all the 101 rodents. But one *Mastomys* sp, one *Taterillus* sp and one unidentified rodent were found naturally infected by *Leishmania major* by PCR. *Mastomys* sp, and *Taterillus* sp would be the reservoir hosts of the Cutaneous leishmaniasis of Ouagadougou.

KEYWORDS Cutaneous leishmaniasis, *Leishmania major*, rodents, *Mastomys* sp, *Taterillus* sp, Burkina Faso

1.3-139

Mobile phones: combating neglected tropical diseases on the go

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INTRODUCTION Tanzania's health care system is overwhelmed with huge volumes of clients seeking care and run with a handful of qualified staff. This East African nation is ravaged by non infectious and infectious diseases including the Neglected tropical diseases lymphatic filariasis, soil transmitted helminthiasis, schistosomiasis, onchocerciasis and trachoma, most of which are nonexistent in the developed world. Inefficient service delivery mechanisms resulting from poor record keeping and reporting mechanisms further hamper proper planning and decision making.

METHODS AND MATERIALS The Tanzania Neglected Tropical Diseases (NTD) control program has successfully piloted Mass Drug Distribution (MDA) to over 9000 at risk people using mobile phone technology synergized with web and desktop applications. Forty community drug distributors (CDDs) were trained and equipped to use mobile phones to conduct house-to-house census, and later distribute Ivermectin and Albendazole to the eligible population. The exercise run parallel with the existing/routing paper based census, drug distribution and reporting mechanism.

RESULTS The CDDs were able to quickly adapt to QWERTY mobile phone keyboards, learned the mobile application and conducted the census while uploading the data in real time-via internet- to the central server. With the data in time, the district, regional and national office could calculate drug need and allocate supplied accordingly. Mass drug administration was conducted with coverage report live updates in the central server and via the web. This allowed early intervention decision making by relevant authorities.

CONCLUSION Mobile phones provide user-friendly, timely and efficient mechanisms to monitor and evaluate neglected tropical diseases control activities-e.g mass drug administration- at the village and sub-village level. In resource limited setting, they provide a viable solution to data collection and reporting woes of NTD control programs. The Tanzania experience could be shared in the developing world.

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Regulatory T cell profiles in the clinical-immmunological spectrum of American cutaneous leishmaniasis caused by *Leishmania (leishmania) Amazonensis* and *L. (viannia) braziliensis*

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INTRODUCTION T cell CD4+ CD25+ Foxp3+ (Treg) is a subpopulation of CD4+ T cells with an important role in the induction and control of inflammatory response. Recent reports have shown a major role of Treg Foxp3+ cells in the control of immune response against *Leishmania*. This study aimed to evaluate the Treg cell profiles through the Foxp3 expression in the skin lesions of different clinical forms of American cutaneous leishmaniasis (ACL) caused by *L. (V.) braziliensis* and *L. (L.) amazonensis*, addressing a better understanding on the role of Treg cells in the immunopathogenesis of ACL in Brazil.

MATERIAL AND METHODS Thirty-one patients were examined: anergic diffuse cutaneous leishmaniasis (ADCL): 6; borderline disseminated cutaneous leishmaniasis (BDCL): 6, both by *L. (L.) amazonensis* (DTH-); localized cutaneous leishmaniasis (LCL) also due to *L. (L.) amazonensis* with DTH- (8) and DTH+ (5) and, LCL due to *L. (V.) braziliensis* with DTH+ (6). Paraffin-embedded biopsies were submitted to immunohistochemistry using the primary antibody anti-Foxp3 (SC-28705), 1:500 dilution. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400×) in section by using an image analysis system (Zeiss).

RESULTS The comparison of Treg cellular density in the clinical-immmunological spectrum of ACL showed a progressive increase in FoxP3+ cells from the central LCL (DTH+) caused by *L. (V.) braziliensis* to the polar forms, ADCL and BDCL (DTH-) caused by *L. (L.) amazonensis*, as follows: (ADCL DHT-[636] > BDCL DHT-[487] > LCL/La DTH-[321] > LCL/La DTH + [278] > LCL/Lb DTH + [354]).

CONCLUSION The differences observed in FoxP3 expression in the broad ACL spectrum suggest an important role of Treg cells in the genesis of these different clinical forms, possibly by the control of the immune response mediated by effector T cells. Supported by: LIM50/HC FMUSP; FAPESP (06/56319-1)

1.3-141

Evaluation of rK39 immunochromatographic test with urine for diagnosis of visceral leishmaniasis

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INTRODUCTION Demonstration of parasites in tissue smears remains the gold standard for the diagnosis of visceral leishmaniasis (VL), however, it is invasive, risky and requires technical expertise which is not always available in the field conditions. Preliminary observation indicates the presence of rK-39 antibody in the urine of VL infected patients. Urine samples are noninvasive, acceptable to most population especially children and the ease of collecting urine makes it extremely attractive alternative for blood or sera in the diagnosis of VL. This study evaluates commercially available rK39 Immunochromatographic (ICT) strips with urine samples for diagnosis of visceral leishmaniasis (VL).

METHODS AND MATERIALS Freshly collected urine and serum samples of 280 parasitologically confirmed VL patients and 66 healthy endemic controls, 48 non-endemic healthy controls and 45 disease controls were tested with rK39 strips.

RESULTS The sensitivity of rK39 in urine samples were 96.4% while the specificity was low varying from 66.7% in endemic healthy controls (EHC), 77.08% in non endemic healthy controls (NEHC) to 62.2% in other diseases, while using serum, sensitivity was 100% whereas the specificity 100%, 92.4% and 95.55% for respective controls.

CONCLUSION In its present format, the ICT strip cannot be used for the diagnosis of VL using urine samples.

1.3-142

Identification of *M. leprae* DNA in household contacts resident in Governador Valadares – MG, Brazil

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INTRODUCTION Leprosy is a disease with long incubation period. The diagnostic is essentially clinical and it is assumed that some individuals who live with patients may be infected even though, they are asymptomatic. Thus the hidden prevalence is an obstacle to leprosy control. Addressing this problem, the validation of a diagnosis method to identify infection with *M. leprae* early is extremely. Prior knowledge of the genome of *M. leprae* made possible the amplification of DNA to identify bacilli in samples. Thus, the technique of real-time PCR can be an alternative for the diagnostic of leprosy in symptomatic and asymptomatic household contacts.

OBJECTIVE To evaluate the real-time PCR technique as a tool to identify *M. leprae* DNA in samples of dermal shave from index cases of leprosy and household contacts with sub-clinical infections.

METHODS We collected 111 samples of lymph from the earlobe of index cases of leprosy, which had already started specific treatment, and their household contacts. Real time PCR was done to amplify fragments Rlep that could be present in such samples collected from individuals.

RESULTS Twenty-eight individuals presented *M. leprae* DNA. However, 17 individuals were considered index cases and 11 individuals were household contacts who had no clinical manifestation.

CONCLUSION Real-time PCR test seems a promising tool for early diagnosis of leprosy.

1.3-143

Intracellular cytokines from household leprosy contacts residents in Governador Valadares, Minas Gerais, Brazil

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INTRODUCTION The cell-mediated immunity represents an important factor in controlling infection by *Mycobacterium leprae*. It is known that Th1 cells from paucibacillary patients produce higher levels of IFN-g, while Th2 cells from multibacillary patients produce higher levels of IL4 and IL10.

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METHODS Paucibacillary and multibacillary patients and their household contacts diagnosed in 2010 by the Municipality Health Department of Governador Valadares, Minas Gerais, Brazil (CREDEN-PES), and also healthy individuals as negative controls were included in this study. Peripheral blood was collected and processed at the Immunology Laboratory of the Vale do Rio Doce University. Intracellular cytokines (IL10PE, IL4PE and IFN-gPE) analyses were performed after *Mycobacterium leprae* (ML) specific antigen and mitogen (PMA) stimulation. In addition, surface markers (CD3FITC, CD4PERCP, CD8PE, and CD19PE CD56FITC, were included.

RESULTS Preliminary results by flow cytometer EPICS XL-MCL BEKMAN, showed that there was a proportion of IL10 (0.14%), IL4 (0.08%) and IFN-g (0.76%) producing cells from a paucibacillary patient after ML antigen stimulation, while his household contact showed IL10 (0.17%), IL4 (0.11%) and IFN-g (0.33%). On the other hand the proportion of cells producing intracellular citokynes from multibacillary patient was different. There was higher frequency of IL10 (0.39%), and IL4 (0.3%) and lower frequency of IFN-g (0.35%) producing cells in comparison with the paucibacillary patient. Interestingly, the cells from multibacillary household contacts showed higher frequency of intracellular cytokines IL10 (0.44%), IL4 (0.34%) and IFN-g (0.43%), than paucibacillary household contact cells. Furthermore, from immunophenotyping data, we observed higher frequency of CD3+ (65.76%), CD8+ (31.75%) and CD56+ (6.96%) cells from multibacillary patients, differently we notice higher frequency of CD4+ (45.03%) and CD19+ (9.39%) cells from paucibacillary patients.

CONCLUSION The detection of intracellular cytokines from blood leucocytes in household contacts may help to indicate the possibility of existing subclinical infection.

1.3-144

Prevalece of bovine brucellosis in Sindh, Pakistan

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INTRODUCTION Bovine brucellosis is important zoonoses. Though endemic in neighbouring countries like India, Afghanistan and Iran and with a huge livestock population, a little is known about brucellosis in Pakistan. This study focuses on the prevalence of bovine brucellosis and associated risk factors in Sindh province of Pakistan, the second largest province in terms of human and livestock population.

MATERIALS AND METHODS A three stage cluster sampling scheme was used to collect 2600 serum samples from randomly selected districts, villages and buffaloes/cattle from Sindh for routine serosurviellance of Rinderpest during 2008–2009. Data was collected on species, sex, age, location and type of husbandry (rural or peri urban dairy farms). The rose Bengal plate test was used for detection of antibodies against *Brucella* organisms. The odds ratio (OR) and Chi square (χ^2) test was used for data analysis.

RESULTS The overall seroprevalence of brucellosis in Sindh province was estimated as 14% (CI. 95%: 13–15%). The prevalence estimates ranged from 2% to 21% for the seven districts studied. Karachi district has the highest (21%: CI 95%; 18%-23%) and Badin had the lowest prevalence (2%: CI 95%; 1–4%). No association of species, sex, age or location of the animals was found with being seropositive for brucellosis. The odds of brucellosis were 3.66 (95% CI 1.758–7.647) times higher in peri urban dairy farms than rural farms: the production system is strongly associated ($P < 0.001$) with the risk of brucellosis in these animals.

CONCLUSION Peri urban dairy farms are main suppliers of milk to major cities of Sindh. Such a high prevalence in these dairy animals constitutes a potential threat to public health and agriculture based economy of this region. A control strategy and awareness campaign is highly recommended.

1.3-145

Impact of *Schistosoma japonicum* infection on collagen-induced arthritis in DBA/1 mice: a murine model of human rheumatoid arthritis

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The hygiene hypothesis suggests that helminth infections prevent a range of autoimmune diseases. To investigate the effects of *S. japonicum* infection on collagen-induced arthritis (CIA), male DBA/1 mice were challenged with unisexual or bisexual *S. japonicum* cercariae 2 weeks prior to bovine type II collagen (CII) immunization or at the onset of CIA. *S. japonicum* infection prior to CII immunization significantly reduced the severity of CIA. ELISA (enzyme linked immunosorbent assay) showed that the levels of anti-CII IgG and IgG2a were reduced in prior schistosome-infected mice, while anti-CII IgG1 was elevated. Splenocyte proliferation against both polyclonal and antigen-specific stimuli was reduced by prior schistosome infection as measured by tritiated thymidine incorporation (3H-TdR). Cytokine profiles and CD4+ T cells subpopulation analysis by ELISA and flow cytometry (FCM) demonstrated that prior schistosome infection resulted in a significant down-regulation of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β and IL-6) and Th1 cells, together with up-regulation of the anti-inflammatory cytokine IL-10 and Th2 cells. Interestingly, the expansion of Treg cells and the reduction of Th17 cells were only observed in bisexually infected mice. In addition, prior schistosome infection notably reduced the expression of pro-inflammatory cytokines and receptor activator of NF-κb ligand (RANKL) in the inflamed joint. However, the disease was exacerbated at 1 week after infection when established CIA mice were challenged with bisexual cercariae. Our data provide direct evidence that the Th2 response evoked by prior *S. japonicum* infection can suppress the Th1 response and pro-inflammatory mediator and that bisexual infection with egg-laying up-regulates the Treg response and down-regulates the Th17 response, resulting in an amelioration of autoimmune arthritis. The beneficial effects might depend on the establishment of a Th2-dominant response rather than the presence of the eggs. Our Results suggest that anti-inflammatory molecules from the parasite could treat autoimmune diseases.

1.3-146

Demographic, clinical and diagnostic profile of post Kala-azar dermal leishmaniasis (PKDL) cases in Bangladesh

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The dermatologic manifestations of *Leishmania* donovani usually followed by visceral diseases (Kala-azar) is known as PKDL. The condition is prevalent in South-east Asia and some parts of Africa with diverse epidemiologic and clinical pictures. The present study documented socio-demographic, clinical and findings of available diagnostic procedure of 89 Bangladeshi PKDL cases attending the Microbiology Department of Mymensingh and Shaheed Suhra-

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wardy Medical College, for parasitological diagnosis. Mean age of the study subjects was 21.53 ± 13.32 years. Most cases (79.5%) were 6–30 years old. Male and female ratio was almost similar (1.07:1). Only 13.3% of cases came from middle class families; the rest was poor with per capita incomes of <2 US Dollars/day. By occupation, most were students of primary and secondary schools, being the predominantly affected age group. More than 85% cases had no knowledge about consequences, transmission, agent and treatment of the disease. 67.5% of cases had used herbal or medicinal ointments. Mymensingh appeared to be the highest endemic district being Trishal as with highest case burden Upazila. More than 15% of cases had no history of previous Kala-azar or of its treatment. Family history of Kala-azar was not found in 55.6% of cases. Mean period between past Sodium Antimony Gluconate (SAG) therapy and development of skin lesion was found to be 22.75 ± 17.22 months (Maximum 96 and minimum 08 months). Concerning the morphologic types of skin lesions, the following chronology was found: Macular 67.5%, maculopapular 26.5% and maculo-papulo-nodular 6.0%. Maximum duration of the diseases was noted in a nodular case (385 months) and the minimum in macular disease (01 month). Mean duration yielded as 20.41 ± 50.91 months. Lesions were mostly distributed over face, trunk, back and extremities (67.4%). Over 97% had retained sensation of touch and prick. Change of colour of the lesions while exposed to sun-light and itching was noted in 85.5% and 13.2% cases respectively. As diagnostic test, rK39 based ICT and LD body in skin smear was positive in 92.8% and 67.5% cases respectively. In 03 ICT negative cases, LD body was detected. No L body was detected in 44.7% Macular lesions, whereas all nodular and papular cases showed LD body of varying grades in slit skin smears (WHO rating scale was followed).

1.3-147

Leishmania mixed infection in great gerbil populations of endemic areas of zoonotic cutaneous leishmaniasis in central Iran

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INTRODUCTION Zoonotic cutaneous leishmaniasis (ZCL), a neglected tropical disease, is a major public health problem in many rural districts of Iran, in 17 out of the 31 provinces. In the current study, natural *Leishmania* infection rate and seasonal variation of the infection in great gerbils, *Rhombomys opimus*, populations of two endemic fuci of ZCL in Iran was investigated.

METHODS AND MATERIALS The investigation was conducted from October 2006 to October 2008 in two endemic fuci of ZCL in Esfahan Province, central Iran. Regardless of having any obvious lesions, skin smears were prepared from the ear lobes of the animals. A Nested PCR assay was designed and used for detection and identification of *Leishmania* species from skin samples of rodent reservoirs.

RESULTS Of the 111 investigated great gerbils, 3 (2.7%) specimens were infected with *L. major*, 1 (0.9%) with *L. gerbilli* and 39 (35.1%) with *L. turanica*. We also found mixed natural infections with *L. major* and *L. turanica* in 18 (16.2%) specimens of the rodents, *L. major* and *L. gerbilli* in 1 (0.9%) and 2 (1.8%) were infected with all three species. 94.6% of the infected gerbils showed no cutaneous leishmaniasis lesion on their ear lobes. The highest *Leishmania* infection rates were observed in fall in both ZCL foci. Statistically significant difference was observed in *Leishmania* infection rates among different seasons ($P < 0.0001$).

CONCLUSION It is concluded that *L. major*, *L. gerbilli* and *L. turanica* circulate in the population of *R. opimus* in central part of Iran. *Leishmania turanica* was the dominant species in the population of great gerbils. *Leishmania major* infection usually accompanied with *L. turanica* in naturally infected gerbils with the highest rate in fall. It is recommended that the role of *L. turanica* in the epidemiology and transmission of ZCL should be considered carefully.

1.3-148

Drug discovery initiative at the university of Dundee: hit-to-lead development for hat and generation of chemical tools

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Neglected tropical diseases threaten the lives of millions of people in developing countries and are a major obstacle to human and economic development. Human African Trypanosomiasis (HAT), caused by *Trypanosoma brucei* subspecies, is one of the most neglected diseases: available treatments are old, toxic, and difficult to administer; they are not efficacious against all parasite species or disease stages and drug resistance is an increasing problem. The need for new drugs with novel mechanism of action has been addressed in the last decade by product development partnerships (PDPs), in collaboration with industry and academia. The Drug Discovery Unit (DDU) at the University of Dundee is at the forefront of research and development of drugs for tropical diseases. Protein kinases are well validated drug targets for a variety of human diseases with many inhibitors under development or in the clinic. Although the *T. brucei* kinome has been annotated and there is evidence of essentiality of some of the member of this family, their physiological role remains unclear. Here we report the chemical validation of Glycogen Synthase Kinase 3 (TbGSK3 short; Tb927.10.13780) as a drug target in *T. brucei*. TbGSK3 was screened against a focussed kinase library using the KinaseGlo assay method. Further repurchase and synthesis of novel compounds yielded 10 validated chemical series against TbGSK3 short. In particular two series showed anti-proliferative activity against the parasite: GSK3 07 series, further investigated with a phenotypic approach for its off-target effects, has yielded leads compounds for HAT and GSK3 09 series has been demonstrated to act 'on target' by both chemical proteomic and genetic methods. This work elucidates how Drug Discovery initiatives in academic settings contribute to the development of new drugs and provide the academic community with chemical tools for probing novel biology.

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1.3-149

Energy blackout against leishmania

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BACKGROUND Leishmaniasis, a neglected disease affecting 12 million people, lacks an effective treatment. Unicellular organisms in exponential growth -e.g. infection-or proliferating cells from multicellular ones-either physiologically or pathologically (as in cancer)- tend to metabolize glucose to lactate. This is a way to not to exhaust the C needed for macromolecular synthesis and biomass generation. Thus, ATP and metabolic intermediates production in many eukaryotic unicellular parasites is based at least to some extent on aerobic glycolysis. Cancer diagnosis takes advantage of the analog Warburg effect using glycolytic blocking agents, which are also being evaluated as anticancer drugs. The objective of this study is to assess glycolytic blocking agents for the treatment of leishmaniases and other bioenergetic similar parasitic diseases.

METHODS Cytotoxicity of different concentrations of the glycolytic blocking agents 3 bromopyruvate (3-BrPA), a new antitumour agent, was evaluated using resazurin (alamar blue) and Giemsa, on macrophage cell lines U937 and DH82, from canine and human origin respectively, on *Leishmania infantum* promastigotes, and on *L. infantum* amastigotes infecting macrophages.

RESULTS Toxicity for macrophages begun at 75 ìM 3-BrPA. A concentration of 9 ìM 3 BrPA induced a 40% growth inhibition on *L. infantum* promastigotes at 24 h. A concentration of 30 ìM 3-BrPA induced a 50% decrease in intracellular parasite load, both on macrophage cell lines U937 and DH82.

CONCLUSIONS These preliminary results show glycolytic blocking agents as promising new antileishmanial agents, to be used either alone or as adjuvants to existing treatments.

1.3-150

Chagas disease in European countries: present challenge in surveillance and information system

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OBJECTIVES To estimate the expected and observed prevalence of *Trypanosoma cruzi* infection, the annual incidence by congenital transmission and the estimated rate of underdiagnosis of Chagas disease cases among Latin-American immigrants in the nine European countries with major prevalence of Chagas disease.

METHODOLOGY Ecological study of aggregate data by country. Formal and informal data sources were used to estimate the population from endemic countries resident in Europe in 2009, diagnosed cases of Chagas disease and annual births from mothers originating from endemic countries. The expected and observed prevalence and the incidence of congenital transmission were calculated using the rates of infection according to endemic country. The index of underdiagnosis was calculated as the ratio between observed and expected prevalence.

RESULTS By 2009, 4290 cases were diagnosed compared with an estimated 68,000–122,000 expected cases. The expected prevalence rate per 1000 resident immigrants from endemic countries was higher in non regular (28–54) than in regular (18–28) immigrants, while the overall observed prevalence rate was 1.3 cases. An estimated 65–122 babies with congenital Chagas disease are born annually in the study countries. The annual incidence rate of congenital transmission per 1000 pregnancies in pregnant women from endemic countries was 1–2 cases per year. The index of underdiagnosis of *T. cruzi* infection was 94–96%.

CONCLUSIONS Chagas Disease is a present public health challenge in the nine European countries. Urgent measures need to be taken to prevent new cases of congenital transmission and to detect and take care the existing cases.

KEYWORDS Chagas disease, *Trypanosoma cruzi* infection, Europe, epidemiological information, prevalence, congenital transmission

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TLR signals regulate parasite and T cell invasion in the brain of *Trypanosoma brucei brucei*-infected mice

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INTRODUCTION Invasion of T cells and trypanosomes into the brain parenchyma is a major pathogenetic event in African trypanosomiasis. We have previously shown that T cells, the T cell derived cytokine IFN-*f*× as well as the IFN-inducible chemokine CXCL10 promote the infiltration of T cells and parasites into the brain across the blood-brain barrier and have suggested that T cells pave the way for parasite invasion. Since signaling from innate immune receptors are required to activate and determine the quality of T cell responses, we here investigated whether signals emanating from these receptors control the accumulation of T cells and parasites in the brain parenchyma.

METHODS AND RESULTS Infection studies in gene knockout mice demonstrated that TLR/MyD88-mediated signaling is required for systemic parasitemia control, antigen-specific T cell activation as well as for T cell and parasite penetration into the brain. Among different TLR deficient mice studied, an essential role for TLR9 in T cell penetration into the brain was identified. TLR-MyD88 signaling mediated increased levels of IFN-*f*Ò *f*nand TNF-*f*Ñ as well as of IFN-*f*×, CXCL10 and iNOS transcripts in the brain of infected mice. Both, TNFR1 and IFN-*f*Ñ/*f*ÒR*f*n signaling promoted T cell and trypanosome neuroinvasion. IRF3, which stimulated IFN-*f*Ò secretion in a MyD88-independent manner, also contributed to T cell invasion. TLR2 and TLR9 in synergy restricted the parasite load within the brain parenchyma. Studies in radiation chimeric mice showed that non-hematopoietic and hematopoietic cells in the brain controlled parasite densities in a MyD88-mediated manner. Neither IFN-*f*Ñ/*f*Ò*f*z TNF-*f*Ñ nor iNOS-derived NO alone contributed to parasite control in the brain.

CONCLUSIONS Altogether, our findings show that TLR signaling controls the systemic *T. b. brucei* infection regulates the penetration of T cells and parasites, but controls survival of the latter in the brain through distinct mechanisms.

1.3-152

Genetic diversity and diagnostics development for trypanosomatid protozoa

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INTRODUCTION The trypanosomatid protozoan species *Trypanosoma cruzi* and *Leishmania donovani/L. infantum* are, respectively, agents of Chagas disease and visceral leishmaniasis (VL), which remain major international health concerns. Comparative analyses of genes encoding antigenic epitopes theoretically provide an opportunity for (i) devising serological assays for intra-specific genetic lineages putatively linked to different eco-epidemiologies

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and clinical presentations and (ii) developing further currently available species-specific serological tests that have some limitations in sensitivity or specificity. We have begun to explore lineage-specific serology to identify an individual's history of exposure to different *T. cruzi* lineages, and also to analyse the molecular diversity of antigens used in a lateral flow assay for diagnosis of VL.

METHODS AND MATERIALS Chagas disease: Amino acid sequence comparisons of selected surface antigens across a panel of reference biological clones encompassing the known *T. cruzi* genetic lineages (TcI-VI) revealed lineage-specific sequences. Synthetic peptides based on lineage specific sequences were applied in ELISA serology. Visceral leishmaniasis: Species representation of antigens used in a commercial lateral flow diagnostic assay with differing regional sensitivities was investigated by comparative analysis of genetic diversity of *L. donovani* strains circulating in East Africa and India.

RESULTS The identification of TcII and TcVI–specific responses to corresponding synthetic peptides with sera from chronic chagasic patients and experimental animal sera demonstrates the proof-of-principle of a genomic approach to lineage-specific serology in Chagas disease. Amino-acid level diversity was identified in East African and Indian *L. donovani* strains, confirming the value of such an approach, in conjunction with epitope mapping, for improving the efficacy and applicability of promising serological tests.

CONCLUSIONS The increased availability of comparative genomics allows both highly focused and rational approaches to the development of novel diagnostic tools for trypanosomatid and other infectious diseases.

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1.3-153

In vitro adaptation of *T. b. gambiense* to different mammal sera: translational approach to epidemiological role as reservoir of domestic fauna

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African sleeping sickness due to *Trypanosoma brucei gambiense* is widely accepted to be an anthroponotic disease. However, in previous works we have reported the occurrence of *T. b. gambiense* in goat and sheep blood obtained from Equatorial Guinea. Despite long-standing control strategies, complete elimination of human cases was never accomplished in this focus, suggesting the presence of other possible reservoirs for *T. b. gambiense*. To address whether *T. b. gambiense* is able to proliferate in different reservoirs, we analyzed, under controlled *in vitro* conditions, the growth of the parasite in several animal sera such as cattle, swine, sheep and goat, and compared with growth in human serum. We show that *T. b. gambiense* (ELIANE strain) is able to grow *in vitro* in all different mammal sera tested. In order to understand the behaviour of the parasite in other hosts, we screened the antigenic variation, in these adapted lines. We developed one monoclonal and one polyclonal antibodies raised against the originally (LiTat 2.1) and a newly obtained (LiTat 3.1) expressed VSG respectively, as tools to detect the changes of the VSG during the adaptation process. All the sera-adapted cell lines consistently exhibited different VSG variants, including LiTat 3.1. By double immunofluorescence (áLiTat 2.1/áLiTat 3.1), no double expresser cells were detected suggesting that the new VSGs were already present in the original cell line and positively selected during adaptation process. To better understand if factors associated to VSG may play a role in such adaptation, analysis of some of the expression-site associated genes (ESAG) was performed. Clustering analysis of the expressed ESAG3, showed an association between the genotypes and the sera. Importantly, VSG switching was not associated with the loss of human serum resistance, confirming this is a stable feature. These findings suggest that *T. b. gambiense* retains the capacity to infect humans after being maintained in local fauna.

1.3-154

Clinical and epidemiological aspects influencing the outcome of human visceral leishmaniasis in Governador Valadares, MG, Brazil

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Visceral leishmaniasis (LV) is one of the six endemic diseases with priority in the world. It has gained great importance, in special with its urbanization, as well as its lethality. The clinical evolution is diverse, assuming serious and lethal forms. Governador Valadares (GV) reported the first case in 2008 and non clinical and epidemiological factors were identified previously. This descriptive study analyzed demographic, clinical, laboratory and epidemiological variables from LV patients living in GV that evolved to cure or death in 2008–2010 period. All cases were from urban zones, 76.5% were male (P = 0.032) and majority in the age ranging between 0 and 10 years in both sexes and between 41 and 50 only for males. 44.4% of the individuals looked for health services within 2 weeks of symptom onset, and only 30.3% had diagnosis in the first attendance. The cases showed symptoms and clinical signals characteristic of LV. The treatment of choice was Glucantime®. 88.5% had evolved to cure. Men (71%) presented greater adverse reactions than the feminine. There were 11 deaths in this period, being 72.7% male and 27.3% female. Co-morbidity existed in 72.7% (n = 8) of these; four had hypertension and diabetes. We observed correlation (P = 0.051) between age and comorbidity and presence of higher score in age groups over 20 years. Hemorrhagic signs was the principal complication, being present in 10 of 11 deaths. We concluded that clinical and epidemiological characteristics point to males as a more susceptible group and that the delay in diagnosis, comorbidities associated with the use of Glucantime® and presence of complications are possible risk factors relevant to poor prognosis of the disease.

1.3-155

Identifying phenotypes involved in resistance to *Schistosoma mansoni* infection in inbred mice

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INTRODUCTION Schistosomiasis is a disease with a strong genetic component influenced by socioeconomic and ecological factors. Nevertheless, epidemiological genetic studies have identified genetic regions involved in the degree of susceptibility/resistance in individual infection as well as in the degree of hepatic fibrosis. Experimental mice crosses provide a useful tool to identify genetic loci involved in the schistosomiasis pathogenesis. The aims of this study are (i) to identify the degree of susceptibility to schistosomiasis among BALB/c, C57BL/6J, FVB/NH, CBA/2J and DBA/2J inbreed strains and (ii) to carry out a F1 hybrid between the most

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divergent strains in order to characterize the immunophenotypes that correlates with the susceptibility of schistosomiasis disease in mice.

METHODS AND MATERIALS Mice were infected with 150 *S. mansoni* cercariae, complete blood counts and immunophenotype were determined at 0, 3, 6, and 9 weeks post-infection. Nine weeks after cercariae exposure, animals were perfused and worm recovery was assessed.

RESULTS The large number of granulomas, decreased numbers of eosinophils and basophils in the acute phase of infection and the decreased number of monocytes, neutrophils and B lymphocytes associated with a less effective humoral immune response are phenotypic characteristics associated with increased susceptibility to experimental infection with *S. mansoni*.

CONCLUSIONS C/57BL6 was identified as the most resistant strain to develop schistosomiasis and CBA/2J as the most susceptible strain, whereas F1CBAB6 hybrid showed an intermediate level of susceptibility to *Schistosoma mansoni* infection. Financial support: CIETUS (USAL).

1.3-156

Immunodominant linear epitopes of *Leishmania* histones for the diagnosis of American tegumentary leishmaniasis

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INTRODUCTION Leishmaniasis is a neglected disease responsible of cutaneous (CL) and mucosal (ML) leishmaniasis in the New World. Highly conserved proteins such as histones and acidic ribosomal proteins (ARP) of *Leishmania* can induce the production of specific antibodies in mammal hosts. Our aim is to assess the diagnostic value of synthetic peptides of *L. infantum* core histones and ARP and *L. peruviana* H2B histone (H2BLper) in a new serological diagnostic method for CL.

METHODS Sera from 21 CL, 20 ML and 10 negative endemic controls (NEC) were challenged against recombinant histones H2A, H2B, H3 and H4 and ARP P0, P2a and P2b from *L. infantum* using FAST-ELISA. A total of 71 overlapping peptides were assayed for epitope mapping with a pool of most reactive sera. Selected immunodominant peptides plus H2BLper were assayed with individual sera from 48 CL, 23 NEC and 13 patients with other pathologies by ELISA. Cut-off values were determined as the mean of NEC+3-fold S.D. Protein sequence homology was also analyzed.

RESULTS Recombinant proteins were recognized by most of CL and ML sera and 10 peptides were identified as immunodominant by pooled sera. Individual CL samples can be identified by the combination of three peptides from the C-region of H2A and P2a from *L. infantum* and H2BLper with a sensitivity of 92%, especificity of 83%, Positive Predictive Value of 88%, Negative Predictive Value of 88%. Positive likelyhood ratio of 5.5 and negative likelihood ratio of 0.1 reflects the combination effectiveness to detect CL patients by serology.

CONCLUSIONS Our results indicate that peptides from C-region of H2A and P2a from *L. infantum* and H2BLper can be used in the design of a new serologic diagnostic method for CL in rural areas. Specificity and sensibility can be improved by the addition of an extra peptide to ensure accurate diagnosis.

1.3-157

Immunological profile of asymptomatic leishmaniasis individuals after visiting endemic areas in Peru

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Cutaneous (CL) and mucosal (ML) leishmaniasis are endemic diseases in Peru. People who visit endemic areas could be exposed to the parasite but would not necessarily present clinical manifestation. Because host immune response plays a central role in determining the outcome of *Leishmania* infection, we investigated the cellular and humoral immune response in asymptomatic leishmaniasis subjects (ASY), in order to assess immune factors contribution to disease outcome. Sera and blood were collected from individuals with CL (7), ML (12) and those who visited endemic area but without any sign of the disease (28). Eight healthy subjects (HS) that never visited endemic areas were included as control. ASY were defined as individuals without disease who showed T-cell proliferation against *Leishmania* antigens. They represented 54% of those who visited endemic areas (SI median = 4.70). ASY presented low levels of IFN, TNF; and IL10 (medians = 18.60, 6.83 and 31.50, respectively), but a remarkably low IFN/IL10 ratio (median = 0.32) ($P < 0.05$, when compared with HS). ELISA for IgG isotypes showed that IgG3 and IgG1 were detected in 40% and 6.7% of ASY, respectively. These levels of cytokines and IgG isotypes were considerably lower than corresponding values from CL/ML patients ($P < 0.01$). Exacerbated pro-inflammatory response were found in CL and ML (Medians: SI = 77.60 and 22.20; IFN = 3259.48 and 4673.70; TN = 66.30 and 155.90; IFN/IL10 ratio = 61.27 and 64.30 respectively). IgG1 and IgG3 were detected in most of the CL/ML samples. Immune response in ASY was characterized by both, moderate cell proliferative response and production of IFN and TNF when compared with CL/ML, despite similar IL10 production in these groups. Furthermore, it is interesting to note the presence of IgG3 and absence of IgG1 in ASY, whereas both are present in CL/ML patients. This fact might suggest that other factors different than IL10 could be involved in the modulation of Th1 response in ASY.

1.3-158

Arginase, arginine uptake and their role in *Leishmania* (L.) *amazonensis* physiology

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Leishmania (L.) *amazonensis* uses arginine to synthesize polyamines to support its growth and survival. Arginase activity also regulates the arginine availability, modulating the immune Th1/Th2 balance. The arginine uptake by the parasite is important to supply the enzyme. The glycosomal location of arginine indicates a possible traffic of the arginine in the cell to provide the substrate for enzyme function. We showed the presence of two gene copies, arranged in tandem, that code for the arginine transporter (AAP), with similar ORFs. However, their 5′ and 3′ UTRs have distinct regions allowing the RT-PCR quantification of transcripts in different physiological conditions. Accordingly, the 5.1 AAP3 mRNA presents three times more molecules in relation to the 4.7 AAP3 mRNA along the promastigote growth curve. Promastigotes starved for 4 h followed by supplementation or not with arginine (400 μM) presented similar 4.7 AAP3 mRNA copy-numbers compared to the control parasites. However, the 5.1 AAP3 mRNA

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copy-numbers increased in the starved parasites but not in ones supplemented with arginine ($P < 0.05$). These results correlate with increases in amino acid uptake. The same starvation experiment was performed using a *L. (L.) amazonensis* null knockout for arginase (arg-) and two other mutants complemented with the arginase ORF with (arg-/ARG) or without the glycosomal addressing signal (arg-/arg SKL). The arg- and the arg-/arg SKL mutants, with no arginase activity, did not show the same behavior as the wild-type (WT) parasite or the arg-/ARG mutant, an indicative that the internal pool of arginine may be important for controlling its uptake.

1.3-159

Specific detection of *schistosoma* DNA in stool and urine samples illustrates shortcomings of microscopy in population based surveys

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INTRODUCTION Detection and quantification of *Schistosoma* eggs in stool and urine samples by microscopy is considered to be the gold standard to identify target populations for schistosomiasis control. Moreover, despite its acknowledged poor sensitivity, microscopy is commonly used for monitoring control measures such as mass drug administration. Recently, a sensitive and highly specific real-time PCR has been developed for the detection and quantification of Schistosoma genus DNA in clinical samples which could serve as a diagnostic alternative. In the present study we evaluated the performance of this PCR in an automated high throughput set-up in order to detect and quantify Schistosoma DNA in stool and urine samples collected during population based surveys in six different geographical regions.

METHODS In total almost 2.900 stool and urine samples, collected in Ghana, Senegal, Mozambique, Madagascar, Kenya and Tanzania were analyzed by multiplex real-time PCR. Qualitative and quantitative findings were compared with stool and urine egg counts performed in the field.

RESULTS Schistosomiasis prevalence determined by real-time PCR was found to be substantially higher than microscopic examination of a single sample. In particular in low transmission areas many additional cases were demonstrated. The overall sensitivity of PCR differed from region to region and was found to be strongly related to quantity and quality of microscopy performed, as well as procedures used for sample collection. Egg counts were found to be highly correlated with the cycle threshold value of the PCR, reflecting Schistosoma DNA concentrations.

CONCLUSION Our findings confirm real-time PCR to be a specific and highly sensitive tool to determine prevalence and intensity of Schistosoma infection in stool and urine samples and illustrate the diagnostic power of real-time PCR in population based surveys aiming to compare transmission patterns between different regions and to monitor control measures.

1.3-160

Expression of insulin-like growth factor (IGF)-I in the skin, liver, spleen and popliteal lymph nodes in the immunopathogenesis of visceral leishmaniasis

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Pathology in canine visceral leishmaniasis (VL) is similar to human VL, and in Brazil it is caused by protozoan *Leishmania* (Leishmania) *chagasi*. During infection, the insulin-like growth factor-I (IGF-I) plays a role in the development of leishmaniasis. In this study we evaluated the participation of IGF-I in the development of the infection and the pathogenesis of LV in six dogs naturally infected with *L. (L.) chagasi* from the endemic area in Northeastern Brazil, diagnosed by the finding of the parasite in lymphoid organs. The serum levels of IGF-I, in the infected animals were reduced compared to the control group. Using real time PCR, the analysis of mRNA expression of IGF-I in the skin, spleen and liver revealed higher levels in the infected dogs when compared to control animals. In contrast, popliteal lymph node in the infected animals showed lower expression of IGF-I when compared to control animals. The liver of dogs showed a higher mRNA expression of IGF-I when compared to other organs. In animals with more severe clinical manifestations of VL there was a notable decrease in the expression of IGF-I in the skin, spleen and lymph node, except the liver. In conclusion the animals with VL had a higher expression of IGF-I in skin, spleen and liver which suggests that the expression of IGF-I is related to the establishment of leishmanial infection.

1.3-161

Current epidemiological profile of visceral leishmaniasis in people's Republic of China

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BRIEF INTRODUCTION Visceral leishmaniasis (VL) is still an important public health problem and transmitted in six provinces/autonomous regions in western China with three types of endemic areas, including anthroponotic VL (AVL), mountainous sub-type of zoonotic VL (MST-ZVL) and desert sub-type of zoonotic VL (DST-ZVL). An outbreak of DST-ZVL occurred in 2008 in Xinjiang. It is essential to analyze epidemiological characteristics of the disease to adapt the control policy. In this study we establish the epidemiological profile of the VL in China.

METHODS AND MATERIALS The study retrospectively reviewed VL cases notified in the period of 2005–2010 through the National Diseases Reporting System. Data were tabulated, diagrammatized to analyze the spatial, temporal, and demographic distribution of the cases.

RESULTS Totally 2450 VL cases were notified with a mean of 408 cases per-year and 61 counties/cities identified as endemic area between 2005 and 2010, while a mean of 263 cases per-year were reported with 43 counties/cities as endemic area in the period of 1990–1999. 97.71% of cases concentrated in Xinjiang, Gansu and Sichuan provinces. An outbreak of DST-ZVL occurred in Jiashi County of Xinjiang between August 2008 and March 2010 with 441 cases found, and the incidence rate increased 20-folds compared to the average incidence rate. About 94.7% of the total cases occurred among infants <2 year-old. The cases start to appear in August and until February of the next year, with a peak in December. While in AVL and MST-ZVL, the cases were identified

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in all months with a peak in April, and with 5.32% and 30.24% of cases occurring among infants <2 year-old, respectively.

CONCLUSION Both the number of VL cases and of the endemic counties/cities are increased, even an outbreak occurred in the period. Differential control measures must be taken against incidence increase and endemic area spread.

1.3-162

Assessment of the disease burden and health inequities due to chronic schistosomiasis Japonica in two endemic counties

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OBJECTIVE To measure health inequalities due to chronic schistosomiasis japonica in two endemic counties based on years lived with disability (YLD).

METHODS The epidemiological data came from the thematic research carried out in two schistosome-endemic counties in lake areas between 2004 and 2005. The specific prevalence rates by subgroups such as age group, gender and endemicity were calculated. According to the age-specific disability weight obtained in the thematic research, the years lived with disability (YLDs) and the rates of YLD of stratified samples by age and gender, and the total YLDs of the two counties were calculated. Concentration index and Gini index, along with concentration curve and Lorenz curve, were applied to identify and quantify health inequalities either between subgroups.

RESULTS Compared to the non-endemic areas, chronic schistosomiasis brought an average extra of 4.398 and 1.505 years per 1000 person to population in two counties, respectively. Age concentration index was 0.395 of females and 0.380 of males, with no obvious difference between sexes. And more than 60% of YLD was contributed by those aged ≥45 years who accounted for about a third of total population in both sexes. Gini index was 0.666 and 0.451 for counties respectively, and 60% of YLD was contributed by the high endemic villages.

CONCLUSION Concentration index and Gini index could quantify the magnitude of health inequalities well. The priority of morbidity control should be given to those aged ≥45 years and lived in high endemic villages.

1.3-163

Leishmania (Viannia) species identification on clinical samples from cutaneous leishmaniasis in Peru: assessment of a PCR step-wise approach

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INTRODUCTION Cutaneous Leishmaniasis (CL) is a vector-borne disease, affecting up to 1.5 million persons annually in tropical and sub-tropical regions worldwide. In most endemic areas like Peru, several *Leishmania* species coexist. There, five species have been reported, where *L. (Viannia) braziliensis*, *L. (V.) peruviana* and *L. (V.) guyanensis* are the most prevalent. Species identification is relevant because different species differ in clinical outcomes, with respect to development of mucocutaneous leishmaniasis or treatment failure with pentavalent antimonials in patients with *L. (V.) braziliensis*. Different PCR targets are used to identify *Leishmania* (*Viannia*) species but methodological consensus is lacking. We present an algorithm based on three PCR assays that can discriminate between *L. (Viannia)* species that cause CL and can simplify species identification procedures.

METHODS AND MATERIALS We analyzed 80 cultured field isolates to construct the optimal algorithm for species identification and its performance was assessed using 70 filter paper lesion impressions from PCR-confirmed Peruvian CL patients. Specimens were subjected to the species identification algorithm: mpi PCR specific for *L. (V.) peruviana*, cpb PCR-RFLP for *L. (V.) braziliensis*, and hsp70 PCR-RFLP that identifies *L. (V.) guyanensis* and *L. (V.) lainsoni*.

RESULTS *Leishmania* species were unambiguously identified in 79/80 cultured field isolates, and in 53/70 filter paper specimens. Determination of PCR detection limit in targets demonstrated that small amounts of *Leishmania* DNA down to 1 pg could be detected. Progression through the algorithm reduced the number of specimens analyzed by approximately 30% after each step.

CONCLUSION This algorithm is specific for *Leishmania* (*Viannia*) species identification and optimized time and cost. It should be useful as a consensus strategy for clinical decision-making regarding patient management; this concept should be applied in Old World Leishmaniasis and in other pathologies, especially in public health settings where resources are limited.

1.3-164

Molecular characterization of *Clonorchis sinensis* tetraspanin 2 extracellular loop 2

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BRIEF INTRODUCTION Clonorchiasis caused by the infection with *Clonorchis sinensis*, a Chinese liver fluke is endemic in the Far East. A *C. sinensis* cDNA CsTSP2 encoding the protein similar to tetraspanin 2 (TSP-2) was found by EST analysis. TSPs are a family of eukaryotic membrane spanning proteins thought to anchor multiple proteins to one area of the cell membrane.

METHODS AND MATERIALS CsTSP2 cDNA was sequenced and aligned with those of schistosomes. The sequence was analyzed by SignalP and TMHMM prediction algorithm. To generate recombinant protein of CsTSP2 extracellular loop 2 (EC2), amplified cDNA was introduced to bacterial expression plasmid vector pEXP5 NT/TOPO. The recombinant protein was purified by Ni-NTA affinity chromatography under denaturing condition. Antigencity of the recombinant protein was examined by immunoblot.

RESULTS AND DISCUSSIONS Multiple sequence alignment of CsTSP2 revealed over 40% of identities with those of schsitosomes. The CCC, PXSC and CG motives characteristic to EC2 region of TSP-2 were well conserved. SignalP and TMHMM prediction of CsTSP2 showed similarity to TSPs of schistosomes. PCR amplified EC2 of CsTSP2 (CsTSP2EC2) was subcloned into pEXP5 NT/TOPO bacterial expression plasmid vector. Recombinant CsTSP2EC2 protein fused with 6× His tag was generated and purified by using Ni-NTA affinity chromatography under denaturation condition. The purified recombinant CsTSP2EC2 protein reacted with the sera from human infected with *C. sinensis*. Considering biological role of tetraspanin, it might be a probable vaccine against *C.*

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sinensis. Protective effects of immunizing CsTSP2EC2 will be further studied.

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Contribution of community health workers in the control of buruli ulcer in the Ngoantet area, Cameroon

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BACKGROUND AND OBJECTIVES Buruli ulcer (BU), a skin disease, causes consistent distresses and incapacitation, especially to children's academic performance, absenteeism and loss of productivity have been attributed to this infection consistent among affected populations. In order to improve the management of the infection at the community level. In the framework of the study of the morbidity related to the infection by *Mycobacterium ulcerans* and improving the management of the infection, we have conducted a transversal and descriptive study among some Community Health Workers (CHWs) from the 20th of April till September 20th 2010.

METHODS The present investigation was a cross-sectional descriptive study, designed to assess the participation of CHWs to the early diagnosis and treatment of BU. We used a questionnaire with open, semi-open and close ended questions related to the performance of BU control activities. The data was analyzed by the software Epi-info version 3.4.1. D. and Excel 2003 version.

RESULTS The study focused on 51 CHWs of Ngoantet II health area. The reference rate was 95%. Most of the suspicious cases (91.5%) referred were confirmed by health workers. Most CHWs (78.4%) declared that they had identified at least one presumptive case of BU infection.

CONCLUSION We conclude that the CHWs can play a key role in scaling up BU control activities. The study confirmed the role of home visits and inspections in the early detection and treatment of BU.

1.3-166

Association between water related factors and active trachoma in Hai district, northern Tanzania

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INTRODUCTION The developments of effective primary prevention interventions require a comprehensive approach addressing how risk factors are embedded and interdependent in different cultural settings. Some of the water variables such as amount of water and time/distance to water sources are related. A survey was conducted to assess the association of water related factors, general hygiene and active trachoma in school age children in Hai district, Northern, Tanzania.

METHODS AND MATERIALS This was a case control study design in which households with two or more children aged 1–9 years with active trachoma follicular (TF) were the case households and households with children 1–9 years of age, all without active trachoma were the control households.

RESULTS The findings indicated that families using more than 60 l of water on daily basis were less likely to have active disease compared to households collecting less amount of water. Households members who reported to use <2 l of water for face washing were more likely to be trachomatous (OR = 5.12, 95% CI:1.87–14.6, P = 0.001). The number of preschool children in the household was also associated with active trachoma; for each additional preschool child in their household the risk of trachoma increased 2.46 folds.

CONCLUSIONS This information enhances the development of a more focused and informed of social factors related to trachoma.

1.3-167

Taenia solium seroprevalence in immigrant and foreign adopted children in florence, Italy

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INTRODUCTION Epidemiological and clinical impact of neurocysticercosis in Europe is poorly known and probably underestimated. According to published data of the Italian ICD-9-CM reporting system, cysticercosis has been the main diagnosis at discharge in 265 cases from 1999 to 2005 (26% in pediatric patients). Immigrants from low resources countries are the most affected and adopted children are a vulnerable part of this population.

METHODS AND MATERIALS From 2001 to 2010, subjects evaluated at the Center for Immigrant and Foreign Adopted Children, Meyer Hospital, Florence Italy, were tested for seroreactivity to *Taenia solium* using EITB-test (Enzyme-linked immunoelectrotransfer blot) performed in the laboratory of the Tuscany Reference Centre for Tropical Diseases, Careggi Hospital, Florence-Italy.

RESULTS A total of 1090 children were screened. The mean age of screened children was 5 ± 3 years. According to the continent of provenience, 371 (34%) children were Asiatic, 340 (31.2%) Latin American, 248 (22.8%) African, 12 (1.1%) European. The most frequent nationalities were Ethiopian 156 (14.3%), Indian 129 (11.8%), Brazilian 99 (9.1%) and Colombian 95 (8.7%). One thousand and seventy-nine tests were negative and 11 (1%) positive. Positive Results were found in six Latin American children (two brothers from Colombia, and one child each from Peru, Chile, Bolivia, Costa Rica), three Ethiopian children, one unspecified African child and one Indian child. Positive children resulted to be older than negative ones, with a mean age of 8 ± 3.7 vs. 5 ± 3.3 years (P = 0.045). Neither association with nationality nor continent provenience were statistically significant.

CONCLUSIONS Although these data are not accompanied by clinical data and cost-benefit analysis of a screening programme based on detection of *T. solium* antibodies, immigrant and foreign adopted children from all continents seem at risk for neurocysticercosis.

1.3-168

Burden of Kala-azar in highly endemic villages of Gedaref state, Sudan

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INTRODUCTION Since January 2010, Médecins Sans Frontières provides care to Kala-azar (KA) patients at Tabarak Allah Health Centre in Gedaref State, the main endemic foci in Sudan. We aimed to retrospectively measure the incidence of KA over 1 year in 36 villages around Tabarak Allah.

METHODS From the 5th of May 2011, we conducted an exhaustive door-to-door survey in 36 villages. Demographic data were collected at the household level. All individuals with fever for at least 2 weeks, history of KA diagnosed in the previous year or with

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skin lesions suspect of post-kala-azar dermal leishmaniasis (PKDL) were referred to medical teams for clinical examination. New clinical KA suspect (defined as fever for at least 2 weeks with one or more of the following: splenomegaly, lymphadenopathies or history of wasting) were laboratory confirmed by a rk39 rapid test or, if negative, by the direct agglutination test (DAT). Incidence rate was calculated by summing the new KA cases detected during the survey to the KA cases reported at population level during the last year (including deaths possibly due to KA), divided by the mid-year population.

PRELIMINARY RESULTS By May 30 (survey ongoing until June 10), 9598 households were screened in 29 villages. KA treatment was reported last year by 765 subjects, and six out of 182 febrile patients were confirmed with KA. Overall the incidence rate was 13/1000 per person-years, varying from 0/1000 to 34/1000 between villages. Seventeen percent of the population reported a past history of KA. Among KA patients treated last year, 25% (130/525) reported past or present PKDL.

CONCLUSIONS The incidence of KA was high in villages of eastern Gedaref State last year and a high proportion of the population reported KA in the past. An active case-detection approach at household level resulted in few newly diagnosed KA cases.

1.3-170

Analysis of multilocus sequence typing for identification of *Leptospira* spp. isolates in Brazil

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Leptospirosis is a neglected infectious disease caused by spirochetes from the genus *Leptospira* and has now been identified as an emerging infectious disease. It constitutes a major public health problem in developing countries, with outcomes ranging from subclinical infections to fatal pulmonary haemorrhage and Weil's syndrome. Since certain serovars are often associated with specific mammalian hosts and with the symptoms and severity of the disease, the identification of serovar permits the prediction of sources of infection; thereby the spread of the disease can be controlled. Our goal is to evaluate the discriminatory power of MLST compared to serotyping.

MATERIAL AND METHODS A total of 101 *Leptospira* isolates was tested by MLST and by traditional serotyping with 23 standard group-specific rabbit antisera.

RESULTS MLST divided the isolates into four sequence types while serotyping classified in six serogroups. The serotyping revealed that 90 isolates belonged to the serogroup Icterohaemorrhagiae (89.11%), five to Canicola (4.95%), two to Ballum (1.98%), two to Autumnalis (1.98%), one to Pomona (0.99%) and one to Sejroe (0.99%). MLST identified 90 isolates as ST17, one isolate as ST58, six isolates as ST37 and two isolates as ST27. Two isolates identified by serotyping as Ballum failed to generate products by MLST method. The serogroups Pomona (one isolate) and Canicola (five isolates) produced the same ST. A significant finding of this study was that both methods identified Icterohaemorrhagiae as the predominant serovar in the state of São Paulo in all years.

CONCLUSION One disadvantage of the MLST assay was its inability to distinguish among some isolates at the serovar level. This study shows that with Brazilian isolates the serogrouping method is more affordable, easier to perform, may provide information similar or better than that obtained using more laborious and expensive genotyping methods based on DNA sequencing.

1.3-171

Evaluation of IgM dot blot with glycolipoprotein from pathogenic and saprophytic leptospires for serodiagnosis of human leptospirosis

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INTRODUCTION Leptospirosis is one of the infectious diseases that is widespread and can be deadly. Thus, the development of rapid and specific diagnostic tools which can achieve early detection of the disease before complications occur are deemed highly desirable. The aim of this study was to develop a simple, specific, rapid and inexpensive test for early diagnosis of human leptospirosis.

MATERIALS AND METHODS A total of 180 serum samples from 90 patients diagnosed with leptospirosis were analysed by IgM Dot Blot with glycolipoprotein (GLP) antigen from *Leptospira interrogans* serovar Copenhageni and *Leptospira biflexa* serovar Patoc. The results were compared with those obtained with microscopic agglutination test (MAT), the gold standard reference serological method. Serum samples from 108 healthy blood bank donors selected randomly and screened negative by the MAT test were used as negative controls to establish the specificity of the assay. The IgM Dot Blot was inspected visually by two experienced independent observers who were blind to all information.

RESULTS The specificities of both GLP-based assays were 100% with serum samples from negative control. With serum samples from patients with acute leptospirosis, sensitivity was 76.6% with IgM Dot Blot Copenhageni and 90% with IgM Dot Blot Patoc. With serum samples from patients in convalescence, sensitivity was 100% with both GLP-based assays.

CONCLUSIONS This IgM Dot Blot proved to be efficient for serodiagnosis of leptospirosis during all phases of illness and could be a good alternative method for the early diagnosis of leptospirosis. Also, the test is suitable for identifying a large number of samples and, hence, reducing the death rate of patients with leptospirosis. Thus, it could be used as an initial screen for leptospiral infection in all laboratories, with subsequent confirmation by MAT.

1.3-172

Factors associated with failure in treatment for cutaneous leishmaniasis in an indigenous community from Brazil

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INTORDUCTION Xakriabá is an indigenous community living in a reserve located in southeast Brazil. From June 2008 to December 2010, 89 cases of American cutaneous leishmaniasis (ACL) were identified in this community. Sixty patients were followed for up to a year after treatment with pentavalent antimony. The present study aimed to evaluate the risk factors associated with therapeutic failure.

METHODS Patients were treated with 15 mg of pentavalent antimonial (SbV)/kg/day given intravenously for 20 days. Clinical examination and individual interviews were conducted using precoded questionnaires to investigate socio-demographic characteristics, clinical history and personal habits. Electrocardiographic and biochemical exams were performed before and during the treatment.

RESULTS After the 20th day of treatment, 24 patients (40.0%) presented lesions completely epithelized while in 36 (60%) the

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lesions were partially epithelized. These groups were compared by demographic characteristics, co-infections, number, size, aspect and local of lesions, time of onset of symptoms, presence of bacterial infection, use of home or commercial medicines, induration size, interruption of treatment, results of diagnostic tests and profile of hsp70 gene sequencing of *Leishmania braziliensis* isolates. The characteristics associated with complete epithelization evaluated after the 20th day of treatment were: presence of lesions partially epithelized in the first clinic examination (OR = 6.5, CI 95% = 2.0–21.7) and bacterial infection (OR = 0.1, IC95% = 0.0–0.7). Ninety days after treatment 44 (73.3%) patients were cured. Cured and not cured patients were compared, and associations were observed between treatment failure and having had ACL in the past (OR = 8.2, CI 95% = 1.5–46.3), use of home medicine (OR = 10.7, CI 95% = 1.6–71.9), and time of onset of symptoms. The greatest efficacy was observed in patients presenting ACL treated up to 8 months after the onset of symptoms (OR = 6.3, CI 95% = 1.1–36.7). One year after treatment six relapses were identified.

CONCLUSION Risk factors could help local physicians to improve the health system routine and develop more successful ACL treatments.

1.3-173

Experience in leprosy patients attending in a rural reference leprosy centre in southern Ethiopia during 7 years

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OBJECTIVE To assess the epidemiology of hospital admission in leprosy cases at rural referral hospital over a 7 years period from 11 of September of 2002 to 10 of September of 2009 according of gender.

METHODS A retrospective data collection using admission leprosy registers and hospital cards in a rural private mission hospital.

RESULTS Over the 7-year period, 782 patients with leprosy were admitted, 64.4% were male and 35.6% were female. The median age was 40 years (range: 4–80 years). Patients male [median 44, interquartile range (IQR): 27–57] were older than female (median 35, IQR: 23–50) (P < 0.001). Only 3.5% patients were under 15 years, and 34.5% were between 15 and 34 years. Number of admission varied from 92 to 143 per year. The patients were admitted from the out patients clinic (OPD) in 92.8% of cases and from the field in 7.2% [male (8.7%) vs. female (4.3%); P = 0.03]. Seven hundred (89.7%) cases were multibacillary (MB) leprosy [male (92.3%) and female (84.2%); P = 0.001], and 80 (10.3%) were paucibacillary (PB) leprosy [male(7.5%) and female (15.6%)]. Six hundred and fifty-eight (84.1%) cases were previous leprosy patients, and 97 (12.4%) were a new leprosy cases [male (10.5%) vs. female (15.4%); P = 0.05]. From 658 old previous leprosy patients, the admission for a MB were more frequent than in male [399/441 (92.5%)] than in female (191/227 (84.1%) (P = 0.001). The main diagnosis was ulcer (55.4%) [male (58.4%) vs. female (47.1%), P = 0.002] follow by reversal reaction (9.7%), neuritis (8.8%), osteomyelitis (7.8%) and erythema nodosum leprosum (5.6%). The median of days of admission was 65.5 days (range, 1–690 days). The patients improved in 84.2%. The mortality was 2.4% and 4.6% were referral to a national leprosy hospital.

CONCLUSION This study detected epidemiology differences of leprosy patients according of the gender admitted in our hospital is different. Ulcer is the main cause of admission.

1.3-174

SYBR green-based quantitation of *Leishmania* parasite load in lesion biopsies from Peruvian patients with tegumentary leishmaniasis

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American tegumentary leishmaniasis (ATL) is a major public health problem in several areas of Latin America. It is characterized by a significant clinical pleomorphism, which has been related to both the infecting *Leishmania* species and the human immune response. Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) are the main clinical forms of ATL. Real-time quantitative PCR techniques have been utilized for the detection, identification and quantification of New World *Leishmania* species; however, the available techniques generally use expensive labeled probes or lack adequate sensitivity, depending on the target and amplification method used. In this study, we developed a SYBR Green-based real-time quantitative PCR (qPCR) assay to evaluate the *Leishmania* parasite load in Peruvian patients with CL and MCL. Our assay targets the *L. (Viannia)* minicircle kinetoplast DNA (kDNA) that is present at about 10,000 copies per parasite. The assay has a linear detection range of 50,000–0.005 parasite DNA equivalents per reaction. Thirty four lesion biopsies from confirmed CL (n = 14) and MCL (n = 20) patients were analyzed, among which the parasite numbers ranged from 1 to 144,000 per μg of DNA. Patients with CL had significantly higher parasite loads (median 648 parasites/μg DNA) than patients with MCL (median 31 parasites/μg DNA) (P = 0.02, Mann–Whitney test). This finding is consistent with earlier observations reported by others, based on histopathology and microscopy of stained tissues. Differences in parasite loads between CL and MCL could reflect the distinct immune responses reported for these clinical forms and warrant further investigation. Our kDNA qPCR assay is highly sensitive and affordable for its implementation in resource-limited settings. It promises to be a useful tool in ATL for studying host-parasite interactions and could be used to guide chemotherapy follow-up and prognosis of disease outcome.

1.3-175

Immunologic mechanisms involved in the pathogenesis of glomerulonephritis in experimental visceral leishmaniasis

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INTRODUCTION Pathogenesis of glomerulonephritis (GN) present in visceral leishmaniasis (VL) is poorly known. Since in dog and hamster, we previously observed proliferative GNs with CD4+ T cells, IgG deposit and a decreased apoptosis, here we proceeded studying in mouse VL.

METHODS BALB/c mice were infected intraperitoneally with 20 million purified *Leishmania (L.) chagasi* amastigotes. Kidney samples were taken at different times post-infection (PI), the histopathology analyzed and the tissue homogenate used for cytokine protein (capture ELISA) and RNA (real time PCR) evaluation.

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RESULTS We observed glomerular hipercellularity with mesangial proliferation from seven through 30 days PI, increased amount of glomeruli expressing CD4+ T and CD8+ T cells at 7 days PI. Since in mice the infection with *L. (L.) chagasi* is self controlled, we prolonged the antigen stimulation with rechallenge (same inoculums) when we observed a progressive increase of glomeruli expressing CD4+ and CD8+ T cells. The deposit of IgG in glomeruli was seen in the initial phase, but C3b deposit was absent. Further we observed expression of cleaved caspase-3 that increased progressively in glomeruli from 7 through 15 days PI. We also observed lower expression of TNF-*f*Ñ in animals with 7 and 15 days PI. The expression of TGF-*f*Ò was significantly high in 15 days PI and considerably higher level of IL-1£] at 7 days PI.

CONCLUSIONS The data suggest important participation of CD4+ and CD8+ T cell infiltration in GN in murine VL, and the contribution of TGF-£] and IL-1£] to the development and/or modulation of the inflammatory process. The presence of cleaved caspase 3 suggests the occurence of an active apoptotic process that contributes to the control of hypercellularity in glomeruli in mice. In the pathogenesis of GN in VL, IgG, T cells, different cytokines and apoptotic process participate in different time period or sequence.

1.3-176

Cytokine and nitric oxide production induced by synthetic peptides designed by bioinformatical tools as new subunit vaccine candidates against *Fasciola hepatica*

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INTRODUCTION Fasciolosis is a human emergent helminth disease in some areas in the world. Nitric oxide (NO) is a reactive molecule that plays an important role in host defense against pathogens and there is a close relation amongst Th cells and nitric oxide in disease. T cells activation as the result of a stimulus with a specific antigen leads to the secretion of Th1, Th2 and Treg-type interleukins which mediate various aspects from the immune response. We assess the NO production in macrophage cultures and the cytokine profile in splenocytes using 24 new synthetic peptides.

MATERIAL AND METHODS Twenty-four peptides containing T or B epitopes were designed and chemically synthesized. NO production was measured *in vitro* after treatment with T and B containing epitopes peptides (1–100 μg/ml) in naive and LPS pre-stimulated macrophages culture supernatants (cell line J774.2). Eighty-four 6-weeks old female BALB/c mice were immunized with 10 μg of each peptide formulated in the ADAD vaccination system using a natural immunomodulator on day 0. Two booster doses were administered on days 15 and 30 respectively. Two weeks after last immunization mice were euthanised and the splenocytes were isolated and cultured for 72 h. Cytokines were measured in cell culture supernatants by flow cytometry.

RESULTS Nitric oxide was not produced by any of the peptides in naive macrophages. In LPS pre-stimulated macrophages there was an inhibition in NO production by nine peptides containing B epitopes and an increase in NO production by four peptides containing T epitopes. A cytokine profile of IL-2, IL-10, TNF-IFN and IL-17 was induced in splenocytes by four B peptides and three T peptides.

CONCLUSION All peptides induced cytokine profiles Th1-like, but seven of them showed cytokines associated to Th2, Treg or Th17 responses.

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1.3-177

Effects on memory T cells in BALB/c mice splenocytes immunized with new subunit-based chemically synthesised vaccine candidates against fasciolosis

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INTRODUCTION Fasciolosis is an emerging helminth disease that impacts significantly both veterinary and human health worldwide and vaccination is considered an alternative in progress for control . The immunological relationship amongst liver flukes and their mammalian hosts is being studied in order to achieve an effective vaccine. Stimulation of B and T memory cells are involved in the generation of long-lived immunological protection, which leads to prevent infection or reduce the severity of disease. The objective was to assess the changes induced in T cell subsets by 12 new synthetic peptides in BALB/c mice using the Adjuvant Adaptation (ADAD) vaccination system.

MATERIAL AND METHODS According to a bioinformatical approach, we designed and synthesized 12 peptides derived from *F. hepatica* proteins containing T epitopes. Forty-two 6-week-old female BALB/c mice were used. Three mice per group were immunized with 10 μg of each peptide formulated in ADAD with two immunomodulators: the hidroalcoholic extract from *Phlebodium pseudoaureum* (PAL) or the synthetic aliphatic diamine AA0029 on day 0. Two boosters were done on days 15 and 30 respectively. Two weeks after last immunization mice were euthanised and the splenocytes were isolated. T lymphocytes CD4, CD8, CD27, CD197 and CD62L were measured using a flow-cytometry technique with a panel of monoclonal antibodies.

RESULTS We observed that using AA029 there was a reduction in CD27 subset by one peptide derived from cathepsin and reduction in CD197 subset by three peptides derived from amebapore and NADH proteins. Using PAL we found an increase of CD197 subset with seven out of 12 peptides as well as in CD62L cell population with six peptides.

CONCLUSION Most of the peptides containing T epitopes stimulate the CD197 and CD62L subsets related with the generation of long-lived immunological memory when are immunized using the immunomodulator PAL.

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1.3-178

Scaling-up the control of visceral leishmaniasis in Somalia

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INTRODUCTION Visceral leishmaniasis (VL) is considered to be endemic all over Somalia. In 1932, the first cases were described from Middle Shebelle region. Since 2005, most of the cases are located within Bay and Bakool regions. Insecurity poses a major challenge to implement control activities across the country. Children <5 years old are the most affected age group. Non-governmental organizations are the main implementers of control activities. The affected communities have shown increased interest in utilizing the medical services aimed at diagnosing and treating the disease.

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METHODS AND MATERIALS Literature review, data received from the health facilities and reports from coordination meetings among partners.

RESULTS The first three VL cases were described in Middle Shebelle between 1932 and 1934. During 1963–1964, 12 cases were reported from the same region. An outbreak occurred in 1952 in North West Somalia. Since 1995, Bay and Bakool regions have been the areas reporting most of the VL cases. From the beginning of 2010 up to April 2011, 296 cases were treated from Bakool and Bay regions. The availability of data for the whole country is rather limited due to inaccessibility issues and insecurity. In May 2011, WHO and NGO partners, in collaboration with other UN agencies, will scale up the control activities for VL by updating treatment guidelines, strengthening the surveillance system and ensuring that medical and nutritional supplies are made available.

CONCLUSION The current activities to control VL in Somalia need better coordination and concerted efforts among the NGOs and UN partners. However, a number of NGOs recently renewed their interest in supporting the affected communities due to the availability of medical supplies and other resources to effectively control the disease. Restricted access of health providers to many endemic areas remains a major challenge.

1.3-179

Toxoplasma gondii: molecular typing of Spanish human isolates by multigenic analysis

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Toxoplasma gondii is an important opportunistic pathogen that causes severe disease in immunocompromised patients and congenital cases. *T. gondii* has an unusual clonal population structure consisting of three clonal lineages (types I, II and III), the current studies are aimed to determine its relationship with the pathogenesis and epidemiology of the disease. To establish the genotypes of strains associated with human toxoplasmosis, was applied a genotyping method based on the analysis of multiple markers directly from clinical samples. Three reference strains, RH (type I), MC-49 (type II) and C56 (type III), and 40 samples from immunocompetent and immunocompromised humans patients with toxoplasmosis (eight amniotic fluid, 15 cerebrospinal fluid, 10 vitreous fluid, one urine, five brain biopsy, and two pulmonary samples) were analyzed. We proceeded to amplificate SAG1, SAG3, GRA6 and BTUB genes by nested-multiplex-PCR and the 3′ and 5′ ends of SAG2 gene by nested PCR. Subsequently, the amplification products were purified and analyzed by RFLP, digested with their corresponding restriction enzymes (Sau96I, HaeII, CfoI, Sau3AI, NciI, MseI, BsiEI and TaqáI). Finally, for visualization and definition of amplification and restriction patterns, samples were electrophoresed on agarose gel and capillary electrophoresis. Multiplex analysis of these five unlinked markers was able to distinguish all three common genotypes and also detected mixed genotypes. The developed method, whose amplification and restriction patterns have been defined, allowed us the characterization of *T. gondii* directly from clinical samples without prior isolation in mice or cell culture, and even allowed the identification of the three major genotypes and atypical or recombinant strains. It was possible to establish the genotypes on 83% of samples studied. Type II was the most prevalent in immunocompetent and immunocompromised humans patients.

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1.3-180

Updated phylogenetic scheme based on the complete mitochondrial protein-coding sequence for common parasitic flatworms

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Precise taxonomic understanding of zoonotic platyhelminths is crucial due to their role in emergence and reemergence of diseases in public health. The entire coding portion of the mitochondrial genome has now been sequenced for 35 species of parasitic flatworms. This has enabled us to prepare an updated phylogenetic scheme for these species using the complete protein-coding sequence. The mitochondrial genome of parasitic flatworms has 12 protein-coding genes (cox1-3; nad1-6; nad4L, atp6 and cob) and nad4L overlaps nad4 by 37 or 40 bp. Nucleotide and translated amino acid sequences were used for phylogenetic analyses, revealing unexpected placements for several species. For example, *Opisthorchis felineus* is more closely allied with *Clonorchis sinensis* rather than with its congener, *O. viverrini*, supporting several published opinions that these taxa belong in a single genus. Intra- and inter-specific pairwise difference comparisons indicate that inter-specific divergence is very slight (<10%) between *Taenia saginata* and *T. asiatica*, and in the Asian schistosomes. Pairwise differences among the fasciolids is about 25%, but even higher in many cestode and trematode taxa, but does not exceed 40%. Updated mtDNA data for common parasitic flatworms provide rich and valuable sources of genetic markers for species diagnosis/identification, studies in biogeography, phylogeny, genetic variation/hybridization of genotypes, population genetics and taxonomy/systematics.

1.3-181

Efficacy of the tubercidin anti *Leishmania* action associated with an inhibitor of the nucleoside transport

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INTRODUCTION Research for new antileishmanial drugs has been motivated by the development of resistance of the parasites acquired to drugs used as well as toxicity for leishmaniasis treatment. A rational strategy for therapeutic exploitation of parasitic diseases can be based on the identification of essencial differences between the parasite and its mammalian host. An important pathway is the purine metabolism. *Leishmania* and other protozoans are unable to synthesize purine nucleotides and must salvage them from the host, unique characteristics that are the basis for the susceptibility to parasites front to purine analogues. Tubercidin (TUB), a purine toxic compound was described as a potential antiparasite agent by inhibition of purine transport for *Schistosoma*, *Plasmodium* and *Trypanosoma*. TUB is also associated with severe host toxicity. This toxicity can be reverted with the association of nitrobenzylthioinosine (NBMPR), an inhibitor of the purine nucleoside transport, specifically selective to the mammals cells.

MATERIAL AND METHODS We evaluated the association of TUB-NBMPR in *L. (L.) amazonensis (La)*, *L. (V.) braziliensis*, *L. (L.) chagasi* and *L. (L.) major* promastigotes and in the interaction of La and the mammalian cells.

RESULTS Comparing the Results of the antimonial (20 μg/ml) with TUB treatment (2 μM/ml) in *Leishmania* promastigotes we

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observed a greater efficiency of TUB with cellular growth near zero. This effect was maintained with the NBMPR (4 μM) association. These results were also observed in La amastigotes. In the evaluation of the TUB-NBMPR association in macrophages infected with La we confirmed the toxicity of TUB (2 μM/ml) in mammalian cells with destruction of the cytoplasm of the macrophage. On the other hand, macrophages remain intact when the same infected cells were treated with TUB (8 μM/ml) in association with NBMPR (16 μM/ml), demonstrating that NBMPR was capable to protect the cells from the TUB toxicity, and decrease in 31% in the association index macrophages – La. In *in vivo* analysis, the mice group infected with La and treated with TUB-NBMPR (5–15 mg/kg) by 7 days had 100% of survival and control of the lesion size when compared with the control group or with the NBMPR inhibitor group. The group treated with only TUB died in the third day of the treatment.

CONCLUSION These data suggest as a promising target for leishmaniasis treatment the purine pathway that can be selective toxic only for the parasite.

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1.3-182

Enterobiasis in Timis county, Romania: a 13-year retrospective study

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INTRODUCTION Intestinal parasitic infections are characterized by high prevalence especially among children. The aim of the present study was to evaluate the prevalence of Enterobius vermicularis infection in Timis County, Western Romania.

METHODS AND MATERIALS Information regarding enterobiasis reported by family doctors between January 1994 and December 2006 was retrospectively investigated . Epidemiological data were extracted from the Statistics Department database of the Timis County Public Health Authority. Population was grouped into four convenient categories by age: 0–1 year, 1–14 years, 15–64 years and ≥65 years.

RESULTS During the 13-year period, 70,001 cases of *Enterobius vermicularis* infection were reported. 59.4% of the cases were from urban areas of the county. Of the total number of cases with enterobiasis, 50,535 (72.2%) were aged 1–14 years, 16,480 (23.5%) were aged 15–64 years, 1588 (2.2%) were ≥65 years and 1398 (2.0%) were aged 0–1 year. Higher prevalence of the disease was observed in urban areas among all ages: 0–1 year (66.1%) 1–14 years (58.7%), 15–64 years (61.3%), and ≥65 years (57.6%). Although *Enterobius* infections continued to be reported every year, a decline in prevalence was observed over the past 9 years of the studied period, from 8415 total cases in 1998, to 5729 cases in 2002 and to 2824 total cases in 2006.

CONCLUSIONS *Enterobius vermicularis* infection occurs at all ages but is more frequent in children aged 1–14 years. The prevalence of the disease was higher in the urban areas of the county. Enterobiasis is reported annually in Timis County and represents an important public health problem.

1.3-183

Prevalence of soil transmitted helminthiasis on school age children in south Cameroon

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INTRODUCTION Soil transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichura* and Hookworm) are a public health problem in south Cameroon. Lack of access to clean water, inadequate sanitation and insufficient hygiene practices are directly related with the transmission of STH.

METHODS AND MATERIALS Twenty-six schools were studied in the Subdepartment of Bengbis, in South Cameroon, being the total number of school in rural Bengbis. The study was designed as a cross-sectional observational study using clusters. Thirty-two children from 5 years old to 12 years old attending every school were selected. A total number of 756 children were included. GPS coordinates were taken for each school. Water and sanitation facilities were described and categorized to determine whether they were adequate according to UNICEF standards for water and sanitation in school. For every child one stool sample were examined by Ritchie modified technique. *Ascaris lumbricoides*, *Trichiuris trichura* and Hookworm were studied in each sample by two different microscopists. Weight, height, abdominal distension, edemas, and MUAC were analyzed using the ANTHRO and ANTHRO PLUS WHO softwares in order to estimate malnutrition. KAP surveys were conducted to establish knowledge, attitudes and practices related with intestinal parasites, and hygiene. Parasitological results, school, water and sanitation facilities were mapped using Geo media professional software.

RESULTS Prevalence of STH among SAC children in Bengbis is 63.8%. 42.59% of the SAC (CI 95%, 30.06–46.11%) presented *Ascaris lumbricoides* infection, 47.61% (CI 95% 44.05–44.17%) presented *Trichiuris trichura* and 9.79% (CI 95%7.67–11.90%) presented Hookworm infection, while 40% presented two or more of these infections. Only 5% of schools had adequate access to water and sanitation facilities.

CONCLUSION Water, sanitation, hygiene education and deworming (known as the WASHED framework) are the basic elements of a comprehensive strategy to fight against intestinal parasites. The WASHED framework will be implemented on the schools on the area and this study will serve as a baseline to evaluate the parasitological impact of the intervention over time.

1.3-184

The analysis of canine visceral leishmaniasis involvement in the tourist town of Embu das Artes -SP, Brazil

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INTRODUCTION Nowadays one of the biggest problems about leishmaniasis is the lack of a specific diagnosis capable to differentiate *Leishmania* species . This particular technique should be rapid and reliable for prevention, control of the spread, and treatment support. Since 2002, several cases of autochthonous canine visceral leishmaniasis (CVL) were detected in the tourist town of Embu das Artes, located around São Paulo, one of the largest cities in the world. Concerned about the spread of the disease in humans, an inquiry was created in order to investigate

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some aspects involved in the transmission and epidemiology of CVL in that touristic town.

METHODS We performed: (i) a profile study of Embu das Artes town, (ii) canine clinical analysis, collecting blood and tissue samples (spleen, liver, lymph node, skin with and without lesion) of 26 dogs euthanized presenting seropositive for leishmaniasis, (iii) direct parasitology tests, culture of isolated parasites and PCR with primers directed to kDNA (kinetoplastDNA), to evaluate canine infection, (iv) clinical examination of children among 4–10 years with potential risk of infection, (v) comparison with previous study that examined sandflies collected in the area.

RESULTS Clinical evaluation of the human population and the epidemiological analysis of sandflies showed the absence of human disease and the main vector involved (*L. longipalpis*). The inquiring of the canine population, based in serological tests, revealing that within a population of 2453 dogs, 66 dogs were positive for CVL (2.69%), while 26 dogs were sacrificed. From the 26 dogs euthanized, 22 (84.6%) were positive for direct parasitological test, while 21 (80.77%) had positive cultures in at least one of the collected samples. PCR analysis showed that spleen samples are the most sensible 92.30% (24/26), followed by skin lesions and lymphnodes samples, both with 88.46% (23/26) of positivity. Blood samples, despite less invasive, showed low positivity 80.77% (21/26).

CONCLUSION The survey in Embu das Artes town showed a different pattern of transmission for the CVL since the disease was confirmed in the canine population, but was not shown the classic vector or human disease. This suggests that a different vector should be involved in the transmission. The comparison of the canine clinical with the PCR Results of spleen tissue (24/26), showed that the two dogs negative for PCR had no typical signs of CVL, suggesting false positive Results of serology and unnecessary euthanasia.

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1.3-185

Development of a rapid lateral-flow immunochromatographic assay to detect krait (*Bungarus caeruleus* and *Bungarus walli*) snake venom

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Snake bite envenoming is one of the most neglected diseases and a major cause of morbidity, mortality and disability in many subtropical and tropical countries. In regions where specific treatment is available, lack of diagnostics complicates clinical management and delays therapeutic interventions. In this context, envenoming by kraits (genus *Bungarus*) -a common cause of fatal respiratory paralysis in South and Southeast Asia- poses a particular problem: Krait bite patients often lack definitive signs of envenoming for hours until irreversible damage to the peripheral nervous system has occurred. Once paralysis sets in, antivenom treatment appears to have little or no effect. Such patients would especially benefit from the availability of rapid diagnostic tests. Here, we report on progress in developing a lateral flow assay (LFA) as a user-friendly and rapid method to detect krait bite envenoming. To manufacture the LFA, we applied control line (rabbit anti-mouse) and test line IgG (equine anti-B. caeruleus) onto backed nitrocellulose membranes and blocked dried membranes with casein buffer containing surfactants. Pre-treated membranes, sample and adsorbent pads were fixed on an adhesive card which was then cut into test strips. During 5 min pre-incubation, various concentrations of venom were mixed with equine anti-B. caeruleus IgG conjugated with colloidal gold in wells of a microtitre plate. Test strips were placed into the wells and test results were read out visually after 20 min. The test strips showed a lower limit of detection for *B. caeruleus* venom of so far 500 ng/ml and strong cross-reactivity with *Bungarus walli* venom, suggesting utility for rapidly detecting the venoms of these two medically highly important wide-ranging krait species of South Asia. Buffer composition and concentrations of the capture and detection antibodies influenced sensitivity. Further optimization of the assays to increase sensitivity and adaptation to a test cassette are in progress.

1.3-186

Effect of heat shock stress on the expression of the virulence factor GP63 in *Leishmania major*

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Leishmania parasites must survive and adapt to different environments, from the vector to the final host. Their gene expression is regulated at the post-transcriptional level, where the 3′ UTR regions play a fundamental role. Heat stress affects mRNA stability in parasite heat shock proteins, nevertheless, this heat stress has not been assayed in virulence factors. *In vitro* drug testing is usually made at random, not understanding what processes are being disturbed by promising compounds until long lasting investigations. Then, standardized models for evaluation of drugs that possibly modify gene expression in parasites could be stablished. The objective of this study was to evaluate the effect of temperature in the gene expression of the surface protein GP63 in *Leishmania* major. According to GeneDB, there are four copies of gp63 in the *Leishmania* major genome, with main differences at the 3′ UTR. Primers have been designed to discriminate them at mRNA level. Promastigote cultures, both in logarithmic and stationary phase, were exposed to 24, 34 and 37°C for 3 h prior to RNA extraction. Reverse transcription was performed with OligodT to ensure post-transcriptional information, subsequently polymerase chain reaction (PCR) was performed. The PCR products were observed by agarose gel using ethidium bromide. No difference was noticed in expression of gp63 genes 1, 2 and 4 at different temperatures in both phases. However, the expression of gene number 3 shows trends of variation. Effect of temperature remains unclear for expression of gene number 3. Further investigations on factors modifying gp63 number 3 expression need to be pursued. An *in vitro* model for testing drugs and compounds that specifically impair natural gene expression in *Leishmania* could be developed in this context.

1.3-187

Ultrasensible real time PCR for the clinical management of visceral leishmaniasis (VL) in HIV infected patients

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BRIEF INTRODUCTION The clinical course of VL in HIV infected patients is characterized by frequent relapses . Due to this, secondary prophylaxis and a close follow up is mandatory. Molecular diagnostic methods such as PCR have been increasingly used for the diagnosis of VL. To try to evaluate the usefulness of a nested PCR in the follow up of HIV infected patients with VL, was the objective of our study

METHODS AND MATERIALS A prospective study of patients with VL and HIV co-infection was conducted. Episodes of VL were classified

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as relapsing or non relapsing episodes. A relapsing episode was defined when a subsequent episode occurred after initial cure. A non relapsing episode was considered if at the end of the study there was no evidence of relapse. CD4 cell count, HIV viral load and *Leishmania* PCR values were measured every 3 months.

RESULTS Thirty-seven VL episodes were analyzed in 16 HIV patients. Twenty-five were considered relapsing episodes. No significant differences regarding to initial CD4 lymphocyte count and parasite load were observed between both groups at the moment of diagnosis. Those episodes that relapsed had a lower increase in the number of CD4 lymphocytes and showed a scarce variation in the parasite load than those who did not. The best PCR cut off to predict relapse using a ROC curve was found at 12 month point time. A cutoff value of 30ct measured at this moment had a sensitivity that reached 100% (CI 95% 0.77–1), a specificity of 90.9% (CI 95% 0.63–0.98) a positive likelihood ratio of 11 (CI 1.7–71) and a negative likelihood ratio of 0. Two consecutives negative PCRs as parasitological cure condition had a negative predictive value of 92.3%.

CONCLUSIONS To incorporate an ultrasensitive quantitative *Leishmania* PCR in monitoring HIV-infected patients suffering from a VL may be useful.

1.3-188

The role of the dermal dendritic cells in the immunogenesis of the Th1/Th2 immune responses in American cutaneous leishmaniasis due to *Leishmania* (V.) *braziliensis* and *Leishmania* (L.) *amazonensis*

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The T cell immune response is crucial for the control and resolution of *Leishmania* infection and it is known that dermal dendritic cells have an important role in T cells activation since they promote the first contact between the parasite antigen and the host immune system. The purpose of this study was to evaluate the role of the dDCs in the development of Th1 (INF-γ) and Th2 (TGF-β) immune response in ACL caused by *L. (V.) braziliensis* and *L. (L.) amazonensis*. Paraffin biopsies from 26 patients from different clinical forms of ACL were subjected to immunohistochemistry, five of Anergic Diffuse Cutaneous Leishmaniasis (ADCL) and five of Borderline Disseminated Cutaneous Leishmaniasis (BDCL) and eight of Localized Cutaneous Leishmaniasis (LCL) caused by *L. (L.) amazonensis*, besides eight of LCL caused by *L. (V.) braziliensis*. Anti-Factor XIIIa, anti-TGFb1 and anti-IFN-g were used as primary antibodies, and Novolink polymer to develop de reaction. The immunostained cells were evaluated using an image analysis system (Zeiss). The density (cells/mm2) of Fator XIIIa$^+$ cells in the lesions of ACL patients was higher in ADCL(897) and BDCL(664) compared to LCL/*La*$^{DHT+}$(266) and LCL/*Lb*$^{DHT+}$(358). The expression of IFN-g$^+$ cells, decreased from the central form (LCL/Lb^{DHT+}) towards to polar form: LCL/Lb^{DHT+}[559] > LCL/La^{DHT+}[510] > LCL/La^{DHT-}[382] > LCDB/La^{DHT-}[291] > LCAD/La^{DHT-}[180]. Concerning to TGFβ1$^+$ cells, a progressive increase in expression from the LCL/Lb^{DHT+} towards to hyposensitivity pole (ADCL and BDCL) was observed: ADCL/La^{DHT-}[1534] > LCDB/La^{DHT-}[944] > LCL/La^{DHT-}[600] > LCL/La^{DHT+}[562] > LCL/Lb^{DHT+}[492].

Our findings suggest that the role of dDC in the immunogenesis of Th1/Th2 in ACL seems to be strongly influenced by intrinsic factors (specific antigens) of the *Leishmania* spp. involved, which showed to be capable of signaling the immune response modulated by the dDCs, directing the response to the Th1 [*L. (V.) braziliensis*] or the Th2 [*L. (L.) amazonensis*] immune responses.

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1.3-189

hsp83 gene analysis in species of subgenus Viannia

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Species of the subgenus *Viannia* are responsible for American cutaneous and mucocutaneous leishmaniasis . Within the subgenus so far nine species have been described, most of which are associated with cutaneous lesion, variation in the disease pattern to mucosal leishmaniosis involves only *L. (V.) braziliensis*. On the other hand, *Leishmania* species coexist in the same geographic areas, making identification difficult at the time of application and subsequent monitoring of treatment patients. In work done by our group have shown the heat shock protein of 83 kDa (hsp83) gene is useful to distinguish subgenus but do not know its capacity to identify species. The aim of this study was to analyze a fragment of the hsp83 in their ability to differentiate species within the subgenus Viannia. The analysis used was restriction fragment polymorphisms of 944 pb fragment of hsp83 gene. Four ATCC strains and 21 species of *Leishmania* isolated from patients were used. The isolated strains were previously characterized by RAPD and MLEE. The patterns of digestion with Hae II were the same to *L. (V.) braziliensis*, *L. (V.) guyanenesis and L. (V.) panamensis*, only *L. (V.) lainsoni* presented a different pattern. *L. (V.) peruviana* strain L677 on the other was different to the other two strains of the same species. Enzymes Xho I and Ksp I have the same restriction pattern for all species except the L677 strain that has no target for these two enzymes. The analysis of 944 bp fragment of hsp83 gene show the phylogenetic distance of *L. (V.) lainsoni* with respect to other species of the subgenus. The results indicate the need for further comprehensive analysis of the hsp83 gene for possible polymorphisms. This work was supported by funds provided by Agencia Española de Cooperación Internacional (AECID) under the PCI program.

1.3-190

Chagas Portfolio: recent advances in drug development

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After 40 years of limited progress in research and development (R&D) for Chagas disease, significant changes in the preclinical and clinical landscape have occurred over recent years. Since 2009, new compound classes have been identified and four new clinical trials on Chagas have started or are about to start, including the evaluation of a new class of compounds (E1224, a pro-drug of ravuconazole and posaconazole). This is the result of renewed interest from academic centers, the private sector and the establishment of product development partnerships. DNDi's strategy on Chagas disease focuses on the better use of existing treatments through new formulations, therapeutic switching and combination therapy in the short and medium term, and the development of new chemical entities in the long term. Priority setting is done through consultation with the Chagas Platform, including stakeholders such as physicians, regulators, and patient representatives, as well as local and global public health organizations. To address an urgent and immediate need in the field, DNDi and LAFEPE (a public pharmaceutical laboratory in Brazil) have collaborated in the development of a pediatric

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formulation of benznidazole for an easy and more adapted treatment of children with planned product launch in 2011. Also, DNDi has licensed E1224 from Eisai Pharmaceuticals for Chagas disease indication in endemic countries. A Phase II proof-of-concept clinical study in Bolivia evaluates E1224 in adults with chronic indeterminate Chagas disease. As a mid-term project, azole compounds are evaluated in combination with benznidazole or nifurtimox. Lastly, lead optimization consortia have been set up by DNDi. New candidates from DNDi discovery pipeline are now starting to move forward towards preclinical and clinical development. Continued efforts and investments are essential to ensure a timely delivery of new treatments for patients suffering from this long neglected disease.

1.3-191

Emerging zoonosic diseases: human dirofilariosis

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Heartworm (*Dirofialaria immitis*) infection is the cause of a severe disease in dogs and cats and of pulmonary dirofilariosis in humans. Different culicid species act as vector for this parasitosis (*Culex sp*., *Aedes sp*. and *Anopheles sp*.). Epidemiological studies to date have shown that heartworm is a global disease. It is now accepted that where there exists canine heartworm there is pulmonary dirofilariosis. A retrospective review of pulmonary dirofilariosis does not indicate an actual distribution of the infection, because it only provides information in areas where there is interest in parasites and/or good diagnostic tools are available to detect nodules. Human infections are mostly asymptomatic and often go unnoticed. However, in some cases benign lung nodules may be confused with carcinoma or other causes of lung nodules. The objectives of this study were to determine the seroprevalence of *D. immitis* in the human population of two areas where infection is proving to be emerging in other subtropical and study hyperendemic reactivity to *D. immitis*, taking into account that the function of the intensity of transmission and this may be different in each area. We analyzed a total of 710 human serum samples: 300 for individuals residing in the island of Gran Canaria, 320 were from individuals residing in Rostov na Donu (Southern Russia) and 90 individuals residing in Austria. The latter samples did not correspond to the general population and from a selection of patients with clinical suspicion of heartworm disease. The results showed a seroprevalence of 13.33% on the island of Gran Canaria, 32.18% in Rostov na Donu and 42.22% in Austria.

1.3-192

Scan the epidemiologic surveillance system and control of zoonoses of Buenos Aires city between 2005 and 2010

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INTRODUCTION The epidemiologic surveillance of zoonoses is of the utmost importance in public health. The system of health surveillance and disease control provides information about the occurrence, distribution and determinants of disease for interventions at community level. The proper functioning of the system involves the timely notification, the case study and proper implementation of control measures.

OBJECTIVE To scan the epidemiologic surveillance system and control of zoonoses of Buenos Aires city between 2005 and 2010.

MATERIALS AND METHODS A descriptive study was conducted. We analyzed reported assisted or studied cases of zoonoses between the years 2005/2010 in Buenos Aires city and studied types of zoonoses, residence, warning mechanism, effector notifier and case closure.

RESULTS Five hundred and eighty-five notifications were analyzed. One in four was a resident of CABA. 69.2% were non-resident. 5.3% stated no residence. Sixty percent were reported through clinical surveillance, 35% for laboratory monitoring and 5% by both routes. 93.8% of cases were reported by public effectors and 6. Two percent by effectors downstream. Forty-seven percent of cases were confirmed, discarding 9.1%, and 43.9% remained in the study.

CONCLUSIONS The surveillance and control of diseases in the city of Buenos Aires includes the reporting of zoonoses. It reports more cases of non-residents than residents. The main effector reporter was the public and this raises the need to involve private effectors and social security surveillance system. Notifications were received, mostly by one-way monitoring, demonstrating the lack of integration of clinical surveillance and lab surveillance. In some cases surveillance could not be completed properly due to lack of information or assistance and this leed to lack of community control and prevention, key tasks of surveillance.

1.3-193

Baseline study population can help immunological search to identify efficacy of antigen vaccine candidate in different endemic conditions

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OBJECTIVE Assessments of the recombinant antigen vaccine candidate for schistosomiasis are usually carried out using mice or sera from infected and uninfected individuals identified in endemic areas. This study aims to identify and characterize endemic communities at risk of infection, establishing a baseline population for future evaluation of immune response to Sm31 antigen.

METHOD We selected two periurban communities (CB and MQ) in southeastern Brazil, and evaluated secondary data relating to surveys of interventions, in 1990–2007 in these communities. In 2009, two surveys were conducted which consisted of stool examination in a random sample of the population and structured and pre-coded interviews for evaluation of risk factors.

RESULTS The prevalence at the time of the first intervention in both areas was 67.7% and 59.8%. There was a significant reduction (2, P = 0.00) in the two districts over the years. The estimated prevalences in the survey were 12.5% and 31.9%, representing a relative reduction of 81.5% in CB and 47.0% in MQ. We identified a higher ratio between infected and uninfected in CB (1:2) when compared with MQ (1:7). The two communities had different risk factors. In CB, the chance of infection was higher in males (OR = 0.25 CI: 0.08–0.70) and lower in individuals who had treated water (OR = 0.27/CI = 0.08–0.79). In MQ the chance of infection was lower in households that used water tank (OR = 0.41 CI: 0.19–0.84) and it was found association with other helminth co infection (OR = 3.59/CI: 1.45–8.86).

CONCLUSION Through a historical cohort was identified 35 individuals resistant to infection that have never shown positive results for *S. mansoni* and 38 susceptible individuals, even treated, were re-infected at least twice during the period.

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1.4 Vector Borne Diseases

1.4-001

Arthropod transmitted gastrointestinal helminthiasis: controversy between rare reports in humans and common vector populations

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There are several helminthic infections for which transmission must be taken place by different types of arthropods as biological vectors including *Hymenolepis diminuta*, *Dipilidium caninom* of cestodes, *Moniliformis moniliformis* and *Macracanthorhyncus hirudinaceous* of acanthocephalans and for nematodes *Gongylonema* sp. and *Rictularia* sp. Different members of Coleopterans, roaches and fleas act as intermediate hosts. Based on worldwide distribution of vertebrate reservoirs and biological vectors for all these zoonotic examples, the rarity of reports in human populations is under debate. In Iran for instance, over the last four decades along with gradual decline of common intestinal parasites from a prevalence rate of 70% for some, to <0/2% for the same parasite such as *Ascaris lumbricoides*, the available records of above mentioned worms number <5. Human gongylonemiasis was reported once; *Rictularia* sp. and/or *M. hirudinaceous* in humans, not at all. In slums the mentioned parasites are as plentiful as rats and pest arthropods. Are there any strong factors explaining host specificity, immunological claim, behavior and culture, or some other reasons like clinical and laboratory ignorance engaged in?

1.4-002

Emerging and reemerging infectious diseases: a call to the conscience of states leaders face to the issue of climate change

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There is a close link between climate change, human migration and the emergence of infectious diseases. Climate change will cause changes in ecosystems and will force millions of people to leave their homes to escape the rising sea level and droughts in the next decades. Each of these consequences is a risk factor for the emergence and reemergence of infectious diseases. Furthermore, human migrations are a great way to spread these diseases in our global village. Beyond the impacts on health, emerging and reemerging infectious diseases will have serious consequences on the global economy. In a context where the negotiations to find a solution to the issue of climate change are stalling, this paper is to remind states leaders that this issue is not only a concern for future generations, but the threat, through the risk of emergence of infectious diseases, is more current than future.

1.4-003

Epidemiological position of leishmaniasis in south of Iran

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Leishmaniasis is a native disease in south of Iran. Its epidemiology has changed ver the past 20 years. In the mid-1990s, rural cutaneous leishmaniasis in the cities was a matter of concern. Urbanization caused humans to enter rodent habitats. Most of these were young couples with newborn babies. This, and the increase in nonimmune people (as a result of new births) caused epidemic rural leishmaniasis in the city. The increase in the acceptance of university students in different fields of study at the end of the 1990s brought more nonimmune immigrants. The hot weather in Larestan keeps them from wearing enough clothes at home or wherever they rest. During these years, seasonal rainfall (summer) made suitable places for sand flies to grow. This plus nonimmune immigrants and created another epidemic. Hygiene and development of hygiene training and treating contaminated persons, to some extent, could control the disease. The resurgence of disease during the last few years has been the result of new births and better laboratory diagnosis. Lack of correct diagnosis caused, contaminated persons to act as a source of infection in the region. Retraining personnel and improving the diagnostic methods can control the communicable diseases in region.

1.4-004

Quality of life in filarial lymphoedema patients in Colombo, Sri Lanka

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INTRODUCTION Lymphatic filariasis (LF) is an important global public health and socio-economic problem. It affects 120 million people in over 80 countries, of whom about 14 million suffer from lymphoedema or elephantiasis of legs. Although LF does not cause immediate mortality, the associated severe morbidity has resulted in it being recognized as the second leading cause of disability worldwide.

METHODS AND MATERIALS The quality of life (QOL) was assessed in 141 filarial lymphoedema patients and 128 healthy individuals in the Colombo district of Sri Lanka. Information was gathered by administering the validated translated version of the WHO 100-item QOL questionnaire (WHOQOL-100), which ascertains an individual's perception of QOL in the physical, psychological, level of independence, environmental and spiritual domains, as well as the general QOL. There is no documentation of the WHOQOL-100 having been used in filarial lymphoedema patients prior to this study.

RESULTS Healthy controls had a better QOL in all domains as well as in the overall general QOL, when compared to patients with lymphoedema. Several facets such as pain and discomfort, sleep and rest, activities of daily living, dependence on medication and treatment, working capacity and social support were significantly affected by the acute adenolymphangitis (ADL) attack/s patients had suffered. The environmental and spiritual domains were significantly affected by the maximum grade of lymphoedema.

CONCLUSIONS The significant difference in the QOL as perceived by patients suffering from filarial lymphoedema and apparently healthy individuals reiterates the importance of morbidity control in patients already affected by filarial lymphoedema.

1.4-005

Kinetic of dengue virus NS1 protein in dengue confirmed adult patients

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INTRODUCTION Early laboratory diagnosis of acute dengue virus infection is still a need for clinical management and epidemiological surveillance. In this work, we evaluated the NS1 protein as a possible early marker of dengue infection using serum samples collected from confirmed adult patients with primary and secondary dengue infection.

METHODS AND MATERIALS A total of 209 serum samples collected among day 2 and day 7 of fever onset from 71 patients were tested by MAC-ELISA for IgM detection, ELISA Inhibition Method for

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IgG detection and Platelia NS1 capture antigen (BioRad) for NS1 detection.

RESULTS The 83.3% of primary cases and 96.4% of secondary cases were NS1 positive. The kinetic of NS1 protein showed the highest values in OD mean ratio or in percentage of positives between days 2 and 4.

CONCLUSIONS The results obtained in this study show the utility of the NS1 protein as a virological early marker of dengue infection. Prospective studies should be carried out to confirm its utility as a prognostic marker of severe illness.

1.4-006

Prevalence of canine visceral leishmaniasis in domestic dogs (*Canis lupus familiaris*) in Palestine

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Dogs (*Canis lupus familiaris*) are considered the main domestic reservoir for *Leishmania infantum* parasites causing human visceral leishmaniasis (VL). In Palestine, the disease mainly affects children with average annual incidence 3.02/100,000. This study aimed to investigate the prevalence of canine VL in Palestine.

METHODS The disease prevalence was established using ELISA, in-vitro cultivation and ITS1-PCR. In total, 215 dogs were tested. The examined dogs were from different Palestinian districts including Al-Khalil and Bethlehem in the south, Ariha (Jericho) in the center and Jenin, Salfeet, Qalqilia and Tubas in the north.

RESULTS Of the 215 tested dog sera, 16 (7.4%) were seropositive. ITS1-PCR showed that 23/215 (10.7%) were positive for *Leishmania* DNA. Three different dog samples produced promastigotes in culture, of which one was ELISA and ITS1-PCR negative. The VL cases were distributed as follows: 30.6% from Al-Khalil, 19.4% from Jenin, 16.7% from Salfeet, 5.6% each from Ariha (Jericho) and Bethlehem as well as 11.1% each from Tubas and Qalqilia. Among the 36 VL infected dogs, 29 (80%) were males and 9 (20%) were of local breed. The average age of the VL-infected dogs was 3.3 year ranging from 1 to 10 years. CPB-PCR revealed that the causative agent was *L. infantum*. The overall prevalence using the in-vitro cultivation, ELISA and ITS1-PCR was 16.7% (36/215).

CONCLUSIONS Canine VL is present in all Palestinian districts included in this study which poses a threat to the Palestinian public. *L. infantum* is the causative agent. Results agree with previous studies done in specific regions in Palestine. Jenin and Al-khalil districts were the most prevalent for CVL infections which is in agreement with the high prevalence of human VL cases.

KEYWORDS ITS1-PCR, CPB-PCR, ELISA, *L. infantum*

1.4-007

Noninvasive molecular diagnosis of human visceral leishmaniasis

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INTRODUCTION Visceral leishmaniasis is a vector borne disease which affects 0.5 million people around the world per annum. Its accurate diagnosis and definitive cure still requires attention. The gold standard procedure of diagnosis of symptomatic VL is parasite demonstration in spleen biopsy, which carries risk of intra abdominal hemorrhage. Bone marrow aspirates are often done but due to its low sensitivity this highly painful procedure fails to make it better choice for diagnosis. The detection of *Leishmania* DNA by polymerase chain reaction (PCR) in splenic, bone marrow or blood samples is an important advance in molecular diagnosis of Visceral Leishmaniasis (VL). We for the first time introduce use of noninvasively obtained buccal swab samples in molecular diagnosis of VL.

METHODS In this study we performed PCR from buccal swabs; multicopy rRNA (Small Subunit Unit, SSU) gene was used as the target region for amplification of *L. donovani*. The PCR assay was optimized and sensitivity was determined in 307 subjects including 148 parasitological confirmed VL patients, 39 healthy controls from non endemic region, 92 endemic healthy controls and 28 subjects of different diseases such as malaria, tuberculosis etc.

RESULTS The results were encouraging, buccal swab samples were positive in 123 out of 148 patients (sensitivity 83.11%, 95% CI, 76.25–88.29). The developed assay was 100% specific as none of the non endemic healthy control samples amplified. The specificity in healthy controls of endemic region was 86% (95% CI, 77.31–91.55) and in different disease group it was 92.85%.

CONCLUSION Molecular diagnosis using buccal cells provide new tool for absolutely specific, highly sensitive and easy diagnosis for all type of symptomatic VL cases. This assay can also open new prospects for epidemiological studies in endemic population.

1.4-008

Prevalence of the microflora in the gut of *phelebotomus* and their relationship with *Leishmania major in vitro*

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BACKGROUND *Leishmania* parasites reside in the gut system of sandflies through which they are transmitted to human. Leishmaniasis is a worldwide problem for which no satisfactory drug or vaccine is available at present. This pilot study was designed to investigate the possible antagonistic effects of isolated bacteria from the gut system of sandflies against Leishmania; aiming to find clues for new strategies in controlling Leishmaniasis.

METHODS Bacterial isolates of; four Enterobacteriaciae, one Psoudomonas, and three Bacillaciae obtained from gut system of 53 locally caught sandflies 'Phlebotomus' were cultured individually and in combination with *Leishmania major* (MRHO/SU/59/P) in a modified RPMI medium (omitted antibiotics) under various conditions. The population of each microbe was estimated by microscopic counts after different incubation periodes.

RESULTS All tested bacterial strains were able to stop the growth of *Leishmania* completely under all tested conditions, even when the number of bacterial cells was much lower than the parasites at the start of co-cultures, or when the pH value was kept almost constant by increasing the buffer capacity of medium. The filterate of 24 h old bacterial cultures did not prevent parasits growth.

CONCLUSION Various factors including the type of microflora of the gut system of sandflies, may play some roles on *Leishmania*. In our study, none of the insects were infected with parasite, therefore, the antagonistic effects of isolated bacteria against *Leishmania* could be justified, but the actual mechanism is unclear. From our data, it seems that the role of competition or pH and long lasting bacterial metabolites for such serious antagonistic effects could be ruled out. We are planning to look into the effects of some short lived metabolites such as oxygen radicals in the near future.

KEYWORDS gut bacteria, *Leishmania*, antagonism

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1.4-009

Identification of main biting midge species and detection of arboviruses in Korea

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INTRODUCTION Culicoides biting midges are known to transmit several diseases caused by viruses. We undertook this study to identify main biting midges and to detect the arboviruses from those.

MATERIALS AND METHODS Culicoides biting midges transmitting arboviruses in three cattle farms were collected in Tongyeong city in Gyeongnam province and Gangjin city in Jeongnam province and Goyang city in Gyeonggi province, from May to October, 2010. Adult Culicoides biting midges were collected once a week by using a light trap. The trap was set between 4:00 and 5:00 p.m. and collected the next morning in 9:00. Collected samples were sent to National Veterinary Research and Quarantine Service (NVRQS) and were sorted into species based on the identification keys of Kitaoka (1984) and were stored at -80¡É until virus detection. Samples were pooled based on collection date and species. Arboviruses in Culicoides biting midges were detected by RT-PCR.

RESULTS A total of 21,297 Culicoides biting mideges were collected. *Culicoides punctatus*, *Culicoides arakawae*, *Culicoides oxystoma*, *Culicoides maculatus*, *Culicoides japonicus* were 87.9%, 7.7%, 3.9%, 2.5%, 0.8%, rerspectively. *Culicoides punctatus* was most frequently collected all three provinces and proved to be the most abundant *Culicoides* spp. in Korea. Identified midges were used for detecting arboviruses. Akabane virus, Aino virus, Chuzan virus and bovine ephemeral fever virus were detected from only *Culicoides punctatus* and *Culicoides arakawae* by RT-PCR.

CONCLUSIONS This study is just start but will elucidate the role of Culicoides biting midges in the transmission of bovine arboviruses in the future. So far, *Culicoides punctatus* is most frequently found in the cattle farms in Korea. However, it remains to isolate the arboviruses from Culicoides biting midges and to control and prevent outbreaks of arbovirus diseases in Korea.

1.4-010

The therapeutic effects of lowsonia inermis and cedrus libani on *Leishmania* major promastigotes: an *in vitro* study

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Leishmaniasis is parasitic disease caused by different species of protozoan parasites belonging to the genus Leishmania. In this study, *Leishmania* major (MRHO/IR/75/ER) promastigotes were cultured at 23–25°C in Brain Heart Infusion (BHI) medium supplemented with 10% heat- inactivated fetal bovine serum (FBS) and penicillin and streptomycin, then by using a MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the biological activity of lowsonia inermis and *Cedrus libani* methanol extracts (0.07, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10 mg/ml) on *L. major* promastigotes was investigated. All experiments were repeated at least three times. *Cedrus libani* methanol extract did not show activity while lowsonia inermis methanol extract inhibited the growth of promastigote forms of *L. major in vitro* after 72 h. of incubation and had a 50% inhibitory concentration (IC50) of 1.25 mg/ml. The methanol extract of *Lawsonia inermis* (henna) seems be a promising antileishmanial agent. Further experiments are needed for isolation of active fractions and identification of the active components of methanol extract.

1.4-011

Seroprevalence of human dirofilariasis on the island of gran canaria, Canary Islands-Spain

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Dirofilaria immitis is the causal agent of canine and feline heartworm disease, a vector-borne transmitted disease from temperate, semitropical and tropical areas of the world. The existence of canine dirofilariasis implies a risk for human populations living in an endemic area in which, the parasite can cause pulmonary dirofilariasis, characterized by the appearance of solitary benign pulmonary nodules, which can be confused with lung cancer. The aim of the study was to update the current seroprevalence and distribution of human dirofilariosis on Gran Canaria. Three hundred random serum samples from humans (121 men and 179 women) taken in 2011 in a local hospital were analyzed. The sample reflects the distribution of the human population throughout different areas of Gran Canaria. Human samples were analyzed by ELISA to detect specific anti-*D. immitis* IgG antibodies using adult *D. immitis* somatic antigens (DiSA). Total seroprevalence in humans was 13.3% (40/300). There were no significant differences among men (17+/121, 14.04%) and women (23+/179, 12.84%). When age was considered, the highest seroprevalence was found in individuals between 20 and 60 years, followed by that observed in individuals younger than 20 years; the lowest seroprevalence was found in the oldest section of the population (>60 years). There were significant differences between the three groups ($P < 0.05$). Current human seroprevalence is similar to that found in 2010 (12%). This demonstrates that Gran Canaria, an endemic area of canine heartworm disease, is a potential risk for the resident human population. The seroprevalence observed in human populations living in each area shows a high correlation with canine prevalences, which confitrms the key epidemiological role played by the canine hosts. These results should alert physicians to pulmonary dirofilariosis in the differential diagnosis of pulmonary nodules.

1.4-012

Temporal evolution and impact of climate factors on the incidence of *L. major* cutaneous leishmaniasis in central Tunisia

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INTRODUCTION Old world Zoonotic cutaneous leishmaniasis (ZCL) is endemic with epidemic peaks in the Middle East and North Africa. Like other vector born diseases, ZCL is highly sensitive to environmental and climate factors. However, no study has addressed the temporal dynamics or the impact of climate factors on the risk of ZCL among humans.

MATERIALS AND METHODS The seasonality of ZCL incidence was assessed by using a box diagram, Fisher and Kruskal-Wallis tests. The intraannual cycles of ZCL was checked by the partial autocorrelation function. We used X-12-ARIMA algorithm to adjust for seasonality and measure the inter-epidemic period in years of monthly incidence data from 1991 to 2007 in Sidi Bouzid, Tunisia. Prediction of ZCL incidence was based on the procedure

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of Box and Jenkins for univariate time series within the concept of ARIMA process (Autoregrssive Integrated Moving Average). The patterns of association between climate variables and ZCL were assessed by the binomial negative GAM and GEE models adjusted for linear trend and seasonality.

RESULTS We confirmed seasonal variation of ZCL with cycles ranging from 4 to 7 years and the best model for forcasting was ARMA(1.1). Besides, we showed that for increase of 1 mm in the mean rainfall lagged by 12–14 months and 1% in humidity lagged by 2 months, ZCL incidence raised by 1.8% and 5.0% respectively.

CONCLUSIONS Historical observations and random noise lagged by 1 month can almost perfectly predict the incidence of ZCL including its distribution, trend and seasonality. The Higher rainfall would increase the density of chenopods, a halophytic plant that constitute the exclusive food of *Psammomys obesus* and the humidity in the summer and fall would lead to higher vector density during the same season. These findings pave the way for early warning models.

1.4-013

Anti-leishmanial effects of artemisinin in comparison with glucantim on *Leishmania major in vitro*

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INTRODUCTION Cutaneous leishmaniasis (CL), a zoonotic infection caused by *L. major* and *L. tropica*. This disease is still one of the health problems in the world, region and Iran. Although artemisinin (qinghaosu) is widely used as anti-malarial agent, it is also demonstrated its anti-promastigote activities and an inhibitory effects on *Leishmania* proliferation.

METHODS In this study, artemisinin was diluted in the methanol and promastigotes of *L. major* were treated with four concentrations (61.5, 125, 250, 500 μM) of artemisinin and compared with those treated with glucantim and untreated groups. The number of promastogotes in each well was counted using a haemocytometer slide at 24, 48, 72 h after being harvested.

RESULT Artemisinin inhibited the parasite proliferation at doses of 250 and 500 μM at 48 and 72 h in culture medium. The related doses of 61.5 and 125 μM presented the same effect at 24, 48 and 72 h of cultivation. Moreover, glucantim inhibited parasite multiplication at doses of 125, 250 and 500 μM at 48, 72 h.

CONCLUSION Artemisinin at concentration of 500 μM is strikingly potent against leishmania, inhibiting the growth of *L. major* promastigotes after 72 h.

KEYWORDS artemisinin, glucantim, Leishmania, promastigote

1.4-014

Application of killed *Leishmania* vaccine's efficacy with imiquimod as adjuvant in inhibition of visceralization of *L. major* in BALB/c mice

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INTRODUCTION KLV have been applied for its immunogenicity in human and mice model. IMQ as adjuvant is inducing humoral and cellular immune responses during leishmaniasis. In this study, both KLV and IMQ were applied in order to investigate the inhibition rate of L major replication and visceralization in mice.

METHODS Promastigotes of *L. major* were harvested from culture, counted and used to infect Balb/c mice. Primarily, mice were injected with KLV/IMQ, and then infected by *L. major* intradermally with 2 × 106 promastigotes. Six weeks after infection, a small nodule appeared leading to a large lesion and visceralisation. Effects of KLV/IMQ, physiopathological changes, lesion size, delay of lesion formation, proliferation of amastigotes inside MQs and detection of amastigotes in target organs were also studied.

RESULT Analysis of body weight, rate of hepato/splenomegaly, and survival rate indicated no significant differences among experimental groups. Neither KLV nor IMQ represented cytotoxic effects on the host, but they partly increased lesion size; and impressed number of amastigotes inside MQs. Application of KLV/IMQ decreased visceralization in liver and induced liver, spleen and plasma NO. Application of IMQ solely decreased visceralization in lymph nodes, but KLV/IMQ presented no effects in concentrations of plasma Cu/Zn and it increased liver SGOT and SGPT.

CONCLUSION Unlike topical application of IMQ, injectable IMQ presented no ameliorative affects on CL. IMQ efficacy may be associated with route, dose and number of injections, which require more investigations.

KEYWORDS vaccine, *Leishmania* major, adjuvant, imiquimod, NO, CRP

1.4-015

Chikungunya and dengue epidemic in Madagascar

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INTRODUCTION In October 2009 an increase of fever cases at the Eastern coast was noticed and in the beginning of February 2010 the Institute Pasteur diagnosed Chikungunya virus (CHIKV) infection in a patient from the Eastern coast. The focal point of the epidemic was around Mananjary, where the epidemic reached its peak in February and abated in March. Here we report the retrospective assessment of reported clinical features and serological markers of CHIKV, dengue virus (DENV) and Rift Valley fever virus (RVFV) infections in pregnant women presenting for routine follow up visits at six different geographical locations in the highlands an the coastal areas of Madagascar.

METHODS AND MATERIALS Several antenatal clinics on different heights on and above sea level, where pregnant women present for routine pregnancy follow up were visited between May and July 2010. A venous (EDTA-) blood sample was taken for serological screening for anti-CHIKV-IgG anti-DENV-IgG and anti-RVFV-IgG antibodies. Samples from Mananjary were additionally screened for anti-CHIKV-IgM antibodies.

RESULTS AND CONCLUSION The 2009/10 arboviral outbreak in coastal south-eastern Madagascar was a combined CHIKV/DENV outbreak with CHIKV infections predominating. The anti-CHIKV-IgG seroprevalence was 45% in Mananjary and 23% in Manakara, both at the south-eastern coast. The corresponding anti-DENV-IgG seroprevalence was 17% and 11% respectively. Seroprevalences in altitudes between 450 and 1300 m were low (0–3%). With more than a third of all pregnant women affected in the epicentre the penetrance in the population was high. Data from higher elevation levels suggest that the epidemic did not spread upwards and inbound, but remained restricted to the coastal areas around the epicentre. Joint pain and stiffness was reported by 78% of the seropositives from the coast; 21% did not report any

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symptoms. CHIKVinfection was associated with body weight (P = 0.001, test for trend).

1.4-016

First report of natural infection of *Mustela nivalis* Linnaeus, 1776 with *Leishmania major* in Tunisia

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INTRODUCTION Zoonotic Cutaneous Leishmaniasis, caused by *Leishmania major*, has been recently emerged in new foci, posing a public health problem in Tunisia. Several rodents have been identified as reservoir hosts of parasites.

MATERIAL AND METHODS A weasel was trapped in Sidi Bouzid (Central of Tunisia), using an unabated pincer trap. Microscopic examination and *in vitro* culture assay were carried out, from the cutaneous lesion for *Leishmania* detection. Isoenzyme charactarization was used for *Leishmania* species identification.

RESULTS We confirmed for the first time the natural infection of least weasel: Mustela nivalis Linnaeus, 1776 by *Leishmania* major zymodeme MON-25.

CONCLUSION This finding justifies further research on larger samples of this animal to verify its role as a potential reservoir host for Cutaneous Leishmaniasis in Tunisia.

1.4-017

Leishmania major infection among rodent reservoir hosts of zoonotic cutaneous leishmaniasis in central Tunisia

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INTRODUCTION Zoonotic Cutaneous Leishmaniasis in Tunisia is caused by *Leishmania* major MON-25. Many ecological studies of the reservoir hosts identified three rodent species carrying *Leishmania major*: *Psammomys obesus* with a major part in amplifying the transmission, *Meriones shawi* and *Meriones libycus* with a role to propagate the parasite because of their common migration, thus increasing the distribution of the parasite. A longitudinal study was undertaken between November 2008 and March 2010, in the focus of cutaneous leishmaniasis of Central Tunisia, to evaluate the role of *Psammomys obesus* and *Meriones shawi* as reservoir hosts for *Leishmania major* infection.

MATERIAL AND METHODS Rodents were captured using unabated pincer traps and wire-mesh cage traps. The weight of the desiccated eye lens was used for the age determination. In addition with clinical manifestations, parasitological and serological assay were carried out for *Leishmania* detection.

RESULTS Four hundred and seventy-two *Psammomys obesus* and 167 *Meriones shawi* were captured. Prevalence of *Leishmania major* infection was 7% vs. 5% for culture (P = NS), 19% vs. 16% for direct exam of smears (P = NS) and 20% vs. 33% (P = NS) for Indirect Immunofluorescence Assay Technique among *Psammomys obesus* and *Meriones shawi* respectively. The peak of this infection was in winter and autumn and increased steadily with age for the both species of rodents. The clinical exam showed that depilation, hyper-pigmentation and severe oedema of the higher edge of the ears were the most frequent signs observed in the study sample (all signs confounded: 79% for *Psammomys obesus* vs. 68% for *Meriones shawi;* P = NS). However, the lesions were bilateral and seem to be more destructive among *Meriones shawi* compared to P*sammomys obesus*. Asymptomatic infection was approximately 40% for both rodents.

CONCLUSION This study demonstrated that *Meriones shawi* play an important role in the transmission and the emergence of *Leishmania major* cutaneous leishmaniasis in Tunisia.

1.4-018

Zoonotic cutaneous leishmaniasis in central Tunisia: a cross-sectional survey on *Leishmania major* infection prevalence and its risk factors

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INTRODUCTION Zoonotic Cutaneous Leishmaniasis (ZCL) due to *Leishmania* major (*L. major*) is still a serious public health issue in Tunisia. The control of leishmaniasis depends on knowledge of the magnitude of the disease and the burden exposure to it. The aim of this study was to estimate the prevalence and evaluate risk factors associated with *L. major* infection in old and new foci in an endemic area for ZCL in central Tunisia.

MATERIALS AND METHODS A cross sectional household survey was carried out between January and May 2009 on a sample of 2686 healthy individuals aged between 5 and 65 years living in the governorates of Sidi-bouzid and Kairouan. We determined the prevalence of *L. major* infection using the Leishmanin Skin Test (LST). Risk factors of LST positivity were identified by logistic regression procedure.

RESULTS The overall prevalence of LST positivity was 57% (95% CI: 53–59). The prevalence of *L. major* infection was significantly higher in old foci (99%; 95% CI: 98–100) than in new foci (43%; 95% CI: 39–46) (P ≤ 10–3). Multivariate analysis of LST positivity risk factors showed that household's characteristics had no significant effects. However, yearly income, age, the nature of the foci (old/new), personal and family history of ZCL are determinants of positive LST results. Adults over the age of 50 years had the highest risk. Subjects from the old foci boosted the risk of LST positivity by more than one hundred times (Adjusted OR = 122.22; 95% CI: 9.52–378.00) compared to those from emerging ones.

CONCLUSION The results update the current epidemiologic profile of ZLC in central Tunisia. This information is useful for implementation of future control strategies to prevent epidemic peaks and geographic spread of the disease.

1.4-019

Visceral leishmaniasis rapid assessment in Bhutan

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Bhutan had never reported kala-azar till 2006. In response, the IMRC verified nine cases and identified Phlebotomus argentipes (Bhattacharya, 2010). A rapid assessment was carried out in April 2011 in Mongar, Trashigang and Tsirang Districts, including leishmanin skin testing (LST), clinical record reviews, questionnaires, sandfly trapping, and parasite identification from bone marrow slides. Consent was obtained from community leaders and individuals. Three communities were studied, Trashigang where 4-year-old boy had kala-azar 18 months earlier, Tsirang where a 55-year-old man had kala-azar 5 months earlier, and a market-town in Tsirang with no reported cases. LSTs were placed for 429 individuals and read for 398 (93%), of WHO 43 (10.8%) were positive. In Trashigang village, the LST positivity was 18.5% (40/

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216), whereas in Tsirang only 3 (4%) of 71 tested and none of 108 individuals from the market-town. Respondents who reported sleeping under a bednet were significantly less likely to have a positive LST (8% vs. 15%, $P < 0.05$). In Tsirang village and market-town, 100% and 90% of respondents reported sleeping under a net compared to 35% of those in Trashigang. *P. argentipes* were trapped in the Trashigang village. *L. donovani* was identified in four out of five clinical isolates through PCR amplification and sequencing of the HSP70 gene rending two clusters. The assessment demonstrated that (i) local transmission occurred in both villages but circulation was likely limited in Tsirang by usage of nets, (ii) the presence of *P. argentipes* permits *L. donovani* transmission in the hills; (iii) several parasite introductions likely occurred from India, this supported by the different clusters and the non response of the Trashigang patient to antimony. The high LST positivity and sparse number of cases suggest that asymptomatic carriers may be playing a role in transmission. Bhutan should be considered endemic and included in the regional Kala-azar Elimination Programme.

1.4-020

An insight into the proteome of *Phlebotomus argentipes* by a 2D approach

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BRIEF INTRODUCTION Sand fly saliva is known to play an important role in host infection outcome after an infective bite. Knowledge on *Phlebotomus argentipes* saliva, established vector of kala-azar in the Indian subcontinent, is scarce. Immunogenicity of *P. argentipes* saliva has already been described. However, specific antigens that contribute to these immunogenic properties are unknown. This work focuses on the identification of antigens present in *P. argentipes* saliva.

METHODS AND MATERIALS Salivary glands from reared female *P. argentipes* specimens were obtained through dissections under a microscope. Protein extracts from salivary gland contents were separated by 2-DE using 15 cm IPG-strips of different pH range (3–10, 4–7, 7–10 and 7–11) and 15% polyacrylamide gels. Silver stained spots were analyzed by MALDI-TOF/TOF. Sera from immunized animals were obtained from hamsters repeatedly exposed to the bite of uninfected *P. argentipes*. Levels of saliva IgG in these pooled sera were tested by direct ELISA. Western blot experiments were done to detect specific antigens among *P. argentipes* salivary proteins.

RESULTS *P. argentipes* salivary proteomic map consisted of at least 20 spots located between 10 and 60 kDa. According to their isoelectric points, spots were mostly distributed around pH ranges: 5–6 and 9–10. Pooled sera of immunized hamsters showed elevated anti-saliva IgG levels. Proteins such as SP10, SP01, SP17, SP07, SP05 among others were recognized by western blot conducting to the identification of specific antigens.

CONCLUSIONS To our knowledge, this is the first attempt to establish a proteomic map of *P. argentipes* salivary proteins. Combination of 2-DE and western blot techniques has permitted to identify specific antigens present in *P. argentipes* saliva. These results contribute to a better understanding of sand fly saliva and its role in immune response.

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1.4-021

Genetic variability of Colombian *Trypanosoma cruzi* I populations based on cytochrome B and SSU rDNA genes sequences

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BRIEF INTRODUCTION Chagas disease is a complex systemic disease caused by the parasite *Trypanosoma cruzi*. This parasite displays a high genetic variability evidenced by six Discrete Typing Units (DTUs). *T. cruzi* I (TcI) is the most prevalent DTU circulating in Colombia and five genotypes based on the intergenic region of mini-exon gene (SL-IR) have been proposed related to transmission cycles of Chagas disease. TcI has gained paramount clinical importance due to recent reports showing cardiomyopathy is mainly linked to this DTU. The aim of this work was to corroborate the previously proposed genotypes using mitochondrial and nuclear DNA regions in a well-characterized set of TcI Colombian clones.

METHODS AND MATERIALS Seventy TcI Colombian clones isolated from humans, vectors and reservoirs from different geographical regions were used. The clones were characterized as TcI using the SL-IR and the 24S rDNA regions. The cytochrome b (Cytb) gene and SSU rDNA V7-V8 were sequenced. The sequences were aligned in ClustalW and concatenated to construct a Maximum Likelihood (ML) tree and a median-joining haplotype network in Network 2.0.

RESULTS AND CONCLUSIONS According to the topology of the ML tree of the concatenated sequences, three genotypes related to the transmission cycles of Chagas disease were observed. Genotype 1 related to human infections, Genotype 2 related to peridomestic clones and Genotype 3 related to sylvatic clones. These genotypes are in accordance to those observed using the SL-IR region suggesting the high intraspecific variability displayed by this DTU and the need to pursue new studies across the American continent with the aim of elucidating the genetic epidemiology of this DTU.

1.4-022

Real time PCR quantification of Colombian *Trypanosoma cruzi* I and II stocks in *Rhodnius prolixus* and BALB/c mice

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BRIEF INTRODUCTION *Trypanosoma cruzi* is the ethological agent of Chagas disease, a complex and endemic zoonotic pathology linked to the American continent. *T. cruzi* is considered a highly variable parasite divided into six Discrete Typing Units (DTUs) showing various host-vector-parasite interactions. The main insect vector of *T. cruzi* in Colombia is *R. prolixus* which has been found mostly infected with TcI. The aim of this study was to evaluate the parasite-vector-host interactions determining the role of *R. prolixus* and Balb/c mice in the possible selection of two Colombian *T. cruzi* I and II stocks.

METHODS AND MATERIALS *T. cruzi* I stock CG and *T. cruzi* II stock VS were characterized by the intergenic region of mini-exon gene. One hundred and eighty 4th instar *R. prolixus* were artificially infected and separated in three groups (TcI, TcII and TcI-TcII). The samples from faeces from this triatomines were collected in the 15, 30, 60 and 90 days after infection and the parasitemia was quantified using a real time PCR assay. The mice were infected with TcI, TcII and TcI-TcII and the blood samples were collected every 18 days and further quantified using a real time PCR assay. The values of quantification were statistically analyzed to observe plausible interactions.

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RESULTS AND CONCLUSIONS The real time PCR quantification showed that TcI and TcII coexist in mice and triatomines as well. The results showed that there is not any type of selection favouring any DTU growth dynamics in the stocks analyzed under the experimental conditions. These results suggest that there are other interactions that are probably generating *R. prolixus* to be mainly infected with TcI and further research is needed in this field.

1.4-023

Multilocus PCR-RFLP profiling in *Trypanosoma cruzi* I highlights an intraspecific genetic variation pattern

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BRIEF INTRODUCTION Chagas disease caused by the parasite *Trypanosoma cruzi* is considered an important problem in public health. This parasite is highly polymorphic and subdivided into six Discrete Typing Units (DTUs). *T. cruzi* I (TcI) is the most prevalent DTU circulating in Colombia and recently associated to heart alterations. This DTU has been divided into five genotypes related to the transmission cycles of Chagas disease using the intergenic region of mini-exon gene. The aim of this work was to develop a multilocus PCR-RFLP analysis to corroborate the genetic diversity of this DTU.

METHODS AND MATERIALS Seventy well-characterized TcI clones isolated from humans, vectors and reservoirs were analyzed. Ten genomic regions were amplified (1f8, Gp72, Gp63, SAPA, ITS, H1, H3, HSP60, HSP70, GPI) and the products were digested with restriction endonucleases. The restriction patterns were analyzed and genetic distance dendrograms by each genomic region, and a consensus dendrogram were constructed.

RESULTS AND CONCLUSIONS From the ten regions used, only four showed to be polymorphic according to the restriction patterns (1f8, H1, SAPA, HSP60). In the consensus tree two groups were determined, the group 1 associated to sylvatic clones and the group 2 associated to peridomestic and domestic clones. This corroborates the previous reported genotypes and the high genetic diversity displayed by TcI. This is the first time that a multilocus coding genomic regions strategy is developed in this DTU suggesting a high intraspecific variation and showing once again the need to unravel the molecular epidemiology of this DTU.

1.4-024

Molecular and phylogenetic analysis of the trypomastigote small surface antigen (TSSA) gene within Colombian *Trypanosoma cruzi* I populations

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BRIEF INTRODUCTION Chagas disease is a systemic pathology caused by *Trypanosoma cruzi*. The high genetic variability of *T. cruzi* has allowed classifying this parasite into six different DTUs (Discrete Typing Units). Recent reports show the enormous genetic variability within TcI using different molecular markers. New markers are needed to confirm this genetic variability with the purpose of establishing subdivisions within this group. The analysis of TSSA gene could help to confirm this premise due to recent reports of positive selection evidenced in the six DTUs of *T. cruzi*. The objective of this work was to screen for genetic variants across a well characterized set of TcI clones from Colombia using the DNA sequences of TSSA gene.

METHODS AND MATERIALS Fifty clones isolated from humans, vectors and reservoirs from different regions from Colombia were selected. They were characterized as TcI using the intergenic region of mini-exon gene. The TSSA gene was sequenced and aligned using ClustalW. Maximum Composite Likelihood (MCL) trees were constructed and haplotypes networks were generated in Network 2.0. According to the DNA sequences, the protein sequences were predicted to generate the secondary structure to develop analysis of positive selection and structure.

RESULTS AND CONCLUSIONS The presence of polymorphisms observed in the TSSA region did not show any association between the transmission cycles of Chagas disease based on the MCL tree. The haplotype network showed a high degree of genetic variability evidenced in 13 different haplotypes where association with transmission cycles could be accomplished. The analysis of secondary structure using the TSSA gene sequences allowed us to infer different peptide folding structures within TcI populations whilst some substituted residues associated to O-glycosilations. This work is the first attempt using TcI populations to help researchers in the generation of lineage specific serology tests based on this gene.

1.4-025

Infected dogs and vectors in an area of transmission of chagas disease in western Mexico

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Chagas disease is one of the most important zoonoses in Mexico, where is considered that more than two million of people are infected by *Trypanosoma cruzi*, with annual increases of 71,000 cases. Even when infected rates of people are known in many areas of Mexico, related rates of infected vectors and infection in dogs (one of the most important reservoir hosts) remain unknown.

OBJECTIVE To determine infection rates of vectors and dogs in Western Mexico.

METHODS In an area of the South of the state of Jalisco, with five small villages with 3% of infected people by *T. cruzi*, we collected triatomines and sampled dogs. More than 50 human dwellings were manually searched once a month for 1 year in order to collect domestic triatomines. Peridomestic areas were inspected with the aid of modified Noireau traps. Dogs were analyzed for *T. cruzi* infection by Western Blot analysis and ELISA.

RESULTS The infection rate for the only triatomine species (*Meccus longipennis*) found in the area was 53.2% (n = 94). Seven (5.3%, n = 131) dogs tested positive for *T. cruzi* by both analyses.

CONCLUSION The high infection rate with triatomines is incompatible with that detected on domestic dogs. Apparently, even when many triatomines are infected by *T. cruzi*, their peridomestic habitat reduces vector-host reservoir contact, leading to few infected dogs. However, special attention has to be paid to domestic dogs, since they usually act as a bridge between sylvatic/peridomestic and domestic cycles of *T. cruzi*, which could represent a higher risk of disease to human populations in the study area.

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1.4-026

Schistosomiasis status in national surveillance sites of Hunan province in 2009

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OBJECTIVE To determine schistosomiasis prevalence in 16 surveillance sites of the Dongting Lake region.

METHODS In accordance with the national requirements for case finding, an the investigation was carried out by the professional staff. IHA was applied to find cases among human and then Kato-Katz was used to examine the positive cases, and hatching test was used to find positive of cattle.

RESULTS The average human prevalence was 1.18%, which was less than that in 2008 (3.0%). The average cattle infection rate was 9.39%, and as high as 28.57% in some villages. The cattle infection rate was <5% in 12 surveillance sites that took up 75% of all the surveillance sites.

CONCLUSION The average human prevalence declines continuously in the national surveillance sites in 2009. Cattle still play an important role in the transmission of schistosomiasis in the Dongting Lake region. The chemotherapy to cattle and snail control in highly potential transmitted areas in the lake beach are the main control measures.

KEYWORDS schistosomiasis, surveillance, prevalence, quality control, Hunan province

1.4-027

Learning to draw cartoons: an educational intervention to improve communication skills of students involved in *Aedes aegypti* control in Cuba

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INTRODUCTION In Cuba, all urban high schools are involved in dengue vector control. This is done through 'BELCAa' (Brigadas Estudiantiles de Lucha Contra el Aedes aegypti). The brigades involve all high school first grade students 12–13 years old and are supervised by teachers and local vertical control program staff. Every 2 weekends these groups conduct home visits in neighborhoods to control potential breeding sites and to mobilize the population for *Aedes aegypti* prevention. The objective of this pilot research project was to strengthen students' communication skills on vector control by increasing their drawing abilities.

METHODS The project was implemented early 2009 in one school in San Antonio de los Baños municipality, province of La Habana. It consisted in conducting four weekly 2 h workshops with a group of 18 BELCAa students to increase their technical knowledge on vector control on the one hand and their drawing skills on the other hand. The workshops conducted by teachers in art were based on the pedagogical model of Popular Education. The drawings were exhibited in public spaces and qualitatively assessed on content, artistic quality and overall presentation by scientists working on dengue, artists from the community and the population respectively.

RESULTS Two hundred and fifty drawings underlined by educational messages were elaborated. The students increased their communicational and artistic abilities and their knowledge on *Aedes aegypti* control activities. The drawings were widely accepted by scientists working on dengue and the population. The capacity-building process increased the motivation of the students involved, improved their outreach activities and offered lessons for BELCAa training at municipal level.

CONCLUSIONS The project strengthened the students' communications skills on *Aedes aegypti* control. Local health authorities have shown interest in the intervention and its potential is currently being tested in other municipalities and schools of the province.

1.4-028

Efficacy of radiofrequency ablation in treatment of localised cutaneous leishmaniasis caused by *Leishmaniasis tropica* in India

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BACKGROUND Localised cutaneous leishmaniasis (LCL) is a wide spread protozoal infectious disease caused by *Leishmania* parasite. LCL is endemic in the Bikaner, India and causative agent being *L. tropica*. In search of a well tolerated, effective therapy with good compliance, we used radiofrequency heat therapy (RFH) and compared it with twice weekly intralesional sodium stibogluconate (SSG).

METHODS One Hundred fresh established cases of CL were included in the present study. Alternate patient were categorized into two groups, Group A and B of 50 each. GroupA patients were treated with RFH (50°C for 30 s) once. Group B patients were given seven, twice weekly intralesional SSG in dosage of 50 mg/cm^2 of lesion. Lesions were evaluated at 6th, 8th, 10th 12th, 16th, 20th and 24th weeks. RFH and intralesional SSG injection were well-tolerated.

RESULTS Complete cure rate of lesions at 6th, 8th, 10th, 12th and 20th weeks were 24%, 42%, 50%, 82% and 98% in group A and 30%, 44%, 56%, 76% and 92% in group B respectively.

CONCLUSION Both modalities are effective and well-tolerated. Intralesional injections of SSG are painful, cause localized edema, requires several visits, whereas RFH is a ruggedized, non invasive, painless, battery operated method, requires single session, cosmetically more acceptable. So, RFH is better alternative to intralesional injections of SSG in resource poor country like India.

1.4-029

Determination species of cutaneous leishmaniasis by its-PCR in the locations of infection Golestan province in Iran

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INTRODUCTION AND OBJECTIVE Golestan province is an important endemic foci for Cutaneous leishmaniasis in Iran. Diagnosis of leishmaniasis can be made on the basis of clinical and epidemiology data and parasite size. The aim of this study was to determine the epidemiological status of Cutaneous leishmaniasis and predominant species based on molecular methods in order to recognize accurate species and reservoir control for patients treatment in Golestan province.

MATERIALS AND METHODS This discriptive analytical study was performed on the 63 patients who had suspected ulcers of leishmaniasis and referred to the laboratory of rural health center from 2004 to 2007. For patients a questionnaire including demographical and clinical information was filled. Direct examination of the lesion carried out also the lesion was cultured in N.N.N culture medium. The promastigote DNA was extracted from cultured and a PCR was developed to amplify a fragment

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containing the internal transcribed spacers of the ribosomal DNA, with primer design ITS1 and ITS2.

RESULTS The species of *Leishmania* in Torkmen strain was *major* and Sistani strain people was *tropica* but in Fars people the frequency of *Leishmania major* was 33.3% and *Leishmania tropica* was 66.7%. The most infected area of *Leishmania major* was Gonbad townshipe. *Leishmania major* was seen in both sexes but in men more than women (57.1%, 42.9% respectively, $P < 0.05$).

CONCLUSION This study showed that the predominant species in Golestan province is *Leishmania major*. Patients had rural cutaneous leishmaniasis.

KEYWORDS cutaneous leishmaniasis, Golestan province, ITS-PCR, species

1.4-030

Preclinical and clinical development of Sanofi Pasteur recombinant tetravalent dengue vaccine

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Sanofi Pasteur is engaged in the development of a tetravalent dengue vaccine (TDV) comprising four recombinant, live, attenuated viruses, (CYD-1–4) based on the YF17D vaccine virus. Extensive pre-clinical characterization was conducted based on a benefit/risk evaluation and mitigation strategy. Pre-clinical studies demonstrated that the CYD viruses are genetically and phenotypically stable, non-hepatotropic, less neurovirulent than YF17D and do not infect mosquitoes by the oral route. The vaccine induced controlled stimulation in human dendritic cells, and was immunogenic in monkeys. Early clinical trial data led to the selection of the vaccine formulation and a 3-dose, 0–6–12 month regimen. Eleven Phase I/II trials are completed, and >6000 individuals aged 12 months–60 years have received at least one dose of TDV. The observed reactogenicity and safety profile in humans is comparable to that of licensed control vaccines. Observed viraemia is transient and low, and does not increase after the first dose. After three doses of TDV, between 67% and 100% of vaccinees are seropositive (PRNT50 titre 10) to all four serotypes. Immune responses are higher in 2–5 years old children than in older children and adults, and are higher in those previously exposed to dengue or other flaviviruses, than in the naïve. These findings led to the initiation of the first efficacy proof-of-concept study in 2010, and plans for 2011 to start two large efficacy trials involving more than 30,000 children (2–16 years old) in Asia and Latin America. Assuming continued successful outcomes and based on the positive results of this first efficacy study we can envisage the availability of this vaccine in high disease burden countries in the next few 3–5 years.

1.4-031

False-positive reactivity of latex agglutination test for kala-azar (Katex) without urine sample boiling in auto-immune patients

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INTRODUCTION Visceral leishmaniasis (VL) is usually fatal if untreated. Diagnostic latex agglutination for Kala-azar (KAtex) is an easy, inexpensive, field applicable test. Its main drawback is a boiling step to remove false positivity. We surveyed false positivity results of KAtex without boiling urine in autoimmune patients.

METHODS Ninety-two VL negative urine samples from auto-immune patients (systematic lupus erythromatous (SLE), rheumatoid arthritis (RA), scleroderma, auto-immune vasculitis, vitiligo, pemphigus and wagner cases) and 20 urine samples from healthy people were collected. All urine samples were checked by KAtex after boiling for 5 min. After that, false positivity rate of the test was determined when repeating the test without the boiling process. Following result analysis, sera from all cases checked for rheumatoid factor (RF).

RESULTS All samples represented negative results with KAtex with boiling (100% specificity). Without boiling, 20% positivity showed in healthy cases. False-positive reactivity was more in most disease groups except Vitiligo than healthy group. But significant difference was only represented in RA group ($P < 0.05$). Twenty-five cases of all had RF positive results and 13 out of 25 RF positive cases (52%) showed positive results for KAtex (without boiling step) which was significant too.

CONCLUSION Inflammatory basis of auto-immune diseases resulted in production of different factors and anti-bodies by immune system. Secretion of them or their bi-products from kidneys with normal or impaired function may result in cross-reaction in diagnostic tests on urine samples. Rheumatoid factor or its metabolism products may play a role in false-positive reactivity of KAtex. So RF should be considered in attempts for modification of KAtex for boiling process removal.

1.4-032

Association between nutritional status and severity of dengue in children in Dr. Sardjito hospital Yogyakarta

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BACKGROUND Dengue hemorrhagic fever (DHF) can lead to high morbidity and mortality if not treated properly and promptly. Obese and overweight children with DHF are predicted to be at higher risk of severe dengue than those with normal or subnormal weight. Identifying risk factors for the development of shock in patients with DHF can increase the awareness of clinicians to perform a close monitoring.

OBJECTIVE To determine the clinical risk factors for DSS, particularly in obese and overweight children.

METHODS This case control study was conducted on DHF and DSS patients admitted to pediatric ward of Dr. Sardjito Hospital Yogyakarta from June 2008 until the sample size needed is achieved. The subjects were patients aged <18 years who fulfilled WHO criteria (1997). The exclusion criteria were diagnosed as dengue fever or other viral infections and incomplete data.

RESULTS There were 342 patients met the study criteria; 116 (33.9%) patients of DSS as cases group and 226 (66.1%) patients of DHF as control group were identified. Univariate analysis showed that there was association between severity DHF and obese (OR = 1.882, 95% CI 1.009–3.509, P = 0.065), infection type (OR = 0.820, 95% CI 0.412–1.631, P = 0.694), hematocrit increasing >25% (OR = 3.415, 95% CI 2.064–5.650, P = 0.000), platelet count <20.000/µl (OR = 1.947, 95% CI 1.200–3.158; P = 0.010), and inadequate of fluid management (OR = 9.107, 95% CI 1.126–73.656, P = 0.022). By logistic regression analysis, it was found that hematocrit increasing >25% is a risk factor for DSS (OR = 2506; IK 95%: 1122–5593), whereas obesity was not significant as risk factor for DSS (OR = 1025; IK 95%: 0.318–3.306).

CONCLUSION Hematocrit increasing >25% is the risk factor for severity of dengue, and obesity is not a significant risk factor for DSS.

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KEYWORDS dengue hemorrhagic fever, dengue shock syndrome, risk factors

1.4-033

Anatomopathological behavior of artificial oral *Trypanosoma cruzi* infection in ICR line mice

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BRIEF INTRODUCTION Chagas disease caused by the parasite *Trypanosoma cruzi* is considered an important problem in public health. The main route of infection is vectorial by reduviidae bugs but oral infection has gained paramount importance. There are recent several outbreaks of oral infection in Brazil, Venezuela and Colombia. The course of this infection leads to severe cardiomyopathies and in some cases to sudden death. The aim of this work was to observe the anatomopathological behavior of *T. cruzi* infection by comparing artificial oral route and intraperitoneally infection in experimental animals.

METHODS AND MATERIALS It was selected a *T. cruzi* strain isolated from an oral outbreak in Colombia (MHOM/CO/09/NCh) characterized as TcI using the intergenic region of Mini-exon gene. Twenty mice were infected by oral route and intraperitoneally with neat concentrations. After infection a blood sample was collected every 2 days to observe parasites, after 30 days they were sacrificed and organs were collected for histopathological analyses.

RESULTS AND CONCLUSIONS It was possible to observe the parasitemia in mice inoculated orally on the tenth day and intraperitoneally from day 26. The inflammatory involvement was observed in all organs of the two routes of inoculation. 77.7% of mice inoculated both intraperitoneally and orally were positive for pathological findings. The parasite was found in all tissues including the digestive tract, but to be effective oral infection requires greater parasite concentration. This is the first attempt to understand the mechanism of oral infection in mice showing the relevance and recent importance of this route of infection in epidemiological and clinical terms.

1.4-034

Molecular characterization of *Trypanosoma cruzi* stocks isolated from oral infection outbreaks in Colombia

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BRIEF INTRODUCTION Chagas disease is a complex zoonoses caused by the parasite *T. cruzi*. This parasite comprises heterogeneous populations evidenced in six Discrete Typing Units (TcI-TcVI). There are various routes of infection, the vectorial route being the most frequent. Outbreaks of Chagas disease due to oral route infection have gained importance in the last years. Reports in Brazil, Venezuela and Colombia show the need to obtain more information about the epidemiology of these outbreaks. In 2009, there were two oral outbreaks in Colombia and five stocks were isolated from these patients. The objective of this work was to elucidate the molecular epidemiology features of this isolates with the purpose of obtaining more information about the transmission dynamics of this route of infection.

METHODS AND MATERIALS The five stocks (LJVP, EH, LER, LCV, XcH) were cell-cloned by limiting dilution and on average 5–10 clones were obtained per stock. The molecular characterization was performed using the intergenic region of mini-exon gene (SL-IR) and the D7 divergent domain of rDNA. The clones were submitted to direct sequencing of the SL-IR, cytochrome b and SSU rDNA regions to observe the genetic variability among the clones.

RESULTS AND CONCLUSIONS According to the results, all the clones were characterized as *T. cruzi* I but it was observed the presence of genotypes Ia and Id. When the direct sequencing DNA analysis was performed, the clones from stocks LER, LCV and XcH showed a high degree of divergence including mixed TcI genotypes among them. These results corroborate the pattern of contaminative transmission between cycles of Chagas disease in oral infection outbreaks in Colombia. More studies towards the understanding of the molecular epidemiology of this route of infection are needed.

1.4-035

Significant increase in imported dengue infections in 2010 and an overview of 10 years of dengue surveillance in Belgium

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The expanding geographical distribution of its vector and the increasing mobility of travellers leads to an increase in the number of imported dengue infections. Surveillance of dengue infections in Belgium started in 2001. Clinical samples were obtained from travellers presenting at the outpatient clinic of the Institute of Tropical Medicine (ITM) Antwerp, Belgium or were submitted by Belgian laboratories to the Central Laboratory of Clinical Biology of ITM for the diagnosis of dengue. Testing was performed by serology (immunofluorescence or ELISA). In 2010 a real time PCR with four serotype-specific primer sets and probes was used to determine the serotype in viraemic samples. In 10 years 5905 samples from 5129 returning travelers were investigated. Dengue infection was diagnosed in 490 patients (9.6%). Most of the infections occurred after a stay in South-East Asia. In recent years the number of cases coming from Africa increased to reach 12% in 2010. In 2010 the number of cases augmented with 143% to 129. The most represented age group was 20–29, the mean age was 38 years. In 29 of the 129 samples (22.5%), RNA could be amplified. Dengue 1 was the predominant serotype (15/29, 51.7%). The increase of infections was especially obvious in August and September, with 55 (42.6%) of the cases diagnosed during these 2 months. Thirty percent of the patients diagnosed in August visited the French Antilles where an intense dengue transmission was ongoing at that time. Dengue fever is an increasing problem in import medicine. As in several other European countries a peak incidence of dengue was seen in Belgium in 2010.

1.4-036

Blackflies (Diptera: Simuliidae) associated with dams in Algarve-southern Portugal

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Construction of dams can be associated with an increase in insect borne diseases owing to modifications of ecology of the region involved and post-construction development of agricultural areas and, in some cases, of villages. The objective of this study was to identify blackflies associated with Bravura and Odeleite dams in Algarve – southern Portugal. Immature stages were collected directly in their breeding sites. The adults were obtained in a laboratory. Species identification was based on morphology. The identification of larvae, pupae and adults showed that *Simulium ornatum* complex members are present in both dams. Taking into account that *S. ornatum* members can be vectors of Onchocerca spp. infecting animals and were susceptible to *Onchocerca*

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volvulus in experimental conditions, the results of the present study will be used for future impact studies of these dams concerning vector-borne diseases.

1.4-037

Leishmaniasis and immunosuppressive therapy: a switch in clinical expression?

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INTRODUCTION Immunosuppressive therapy can result in significant clinical benefits in rheumatoid diseases. A wide range of immunosuppressive agents are presently known. It has been reported that immunosuppressive therapy increases the risk of opportunistic infections, as mycobacteria, fungi and parasites.

METHODS AND MATERIALS We present three cases of cutaneous and mucocutaneous leishmaniasis in patients under immunosuppressive therapy.

RESULTS The first case was a 42-year-old man from Spain, affected by an ankylosing spondylitis, who had been under treatment with infliximab for 3 years. He exhibited multiple skins lesions in exposed areas, diagnosed by biopsy as cutaneous leishmaniasis. *Leishmania infantum* was identified by PCR. Infliximab therapy was interrupted and intravenous liposomal amphotericine B (L-AmB) was administered. The ulcers disappeared completely after a total dose of 20 mg/kg of L-AmB. The second case was a 53-year-old man from Spain, who had been under treatment with infliximab for 2 years for a psoriatic arthritis. He presented a mucocutaneous leishmaniasis involving nasal mucosa and bone marrow. The specie identification by PCR over biopsy of mucosa and bone marrow revealed *L. infantum*. As infliximab could not be interrupted, 40 mg/kg of L-AmB (total dose) was administered in ten doses. The patient completely recovered. The third case was a 39-year-old man from Paraguay, affected with Wegener's granulomatosis, who had been under treatment with ciclofosfamide during 6 months. He displayed a mucocutaneous leishmaniasis involving nasal mucosa. Specie identification of *Leishmania* was not possible. He initially received intravenous L-AmB, but due to a recurrence in the first month, treatment was switched to pentavalent antimony and recovered. Subsequent prophylaxis with pentavalent antimony was started.

CONCLUSIONS We provide evidence that latent leishmaniasis can be reactivated in patients under immunosuppressive treatment. Moreover, the immunosuppressive treatment can modulate the clinical expression of leishmaniasis.

1.4-038

Larvicidal activity of oximes

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INTRODUCTION It has been reported that quinones and derivatives present toxic activity against mosquito larvae *Aedes aegypti L.* Therefore, these facts led us to investigate the larvicidal potential of 14 oximes derived from quinones against *Aedes aegypti* L. (Culicidae) larvae.

METHODS AND MATERIALS Third-instar larvae were used in the experiment. The concentration ranges were determined by a previous curve concentration-response with 20 larvae. A 20,000 ppm stock solution was prepared using each compound (20 mg/ml), Tween-80 (10% v/v), DMSO (30% v/v), and natural mineral water (60% v/v). The stock solution was used to make 20 ml water solutions ranging from 10 to 500 ppm. Twenty larvae were collected with a plastic Pasteur pipette to a graduated cylinder, the volume was completed to 20 ml with mineral water and the larvae transferred to disposable cups. Adequate volumes of the stock solution were transferred to empty disposable cups, followed by the addition of previously separated larvae. A mortality count was conducted 24 h after treatment. Controls were prepared with Tween-80 (0.1 ml), DMSO (0.3 ml), and water (19.6 ml). Three replicates were used for each concentration and the control.

RESULTS All the oximes were found to have larvicidal effect. The [1,4]-benzoquinone oxime was the compound that exhibited the lowest potency, CL50 = 121.181 ppm, while 2,6-dimethyl-[1,4]-benzoquinone oxime tosylate was the most bioactive, CL50 = 9.858 ppm.

CONCLUSIONS The presence of lipophilic groups in the unsaturated ring increases the larvicidal activity. The authors are grateful to FAPITEC and CNPq for providing financial support.

1.4-039

Resistant isolates of anthroponotic cutaneous leishmaniasis to glucantime and genetic diversity of *Leishmania tropica* MDR1 gene in Bam, south-eastern Iran

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BRIEF INTRODUCTION Pentavalent antimonials are the first-line drugs for treatment of cutaneous leishmaniasis (CL), but resistance is increasing. In Iran, *Leishmania tropica* and *L. major* are two causing agents of CL and Bam is one of the old and well- known foci of anthroponotic CL (ACL) in Kerman province, south-eastern Iran. The objective of the present work was to identify the resistant isolates to glucantime (meglumine antimoniate) in patients with CL and to determine genetic diversity of *L. tropica* MDR1 gene.

METHODS AND MATERIALS This study was conducted in 2010. Skin scrapings were taken for direct smear preparations, culture media and Nested – PCR for species identification and sequencing for genetic diversity of *L. tropica* MDR1 gene. A massive earthquake has recently occurred in Bam and the number of CL cases has sharply increased to epidemic proportions.

RESULTS Of 2126 cases with CL, 235 patients (11.1%) were resistant against glucantime. All 51 randomly selected isolates were *L. tropica*. Of 20 sequenced isolates nine isolates (Gen Bank Accession No.: HM854717, HM854718, HM854719, HM854720, HM854721, HM854722, HM854723, HM854724, HM854725) were genetically different from standard species.

CONCLUSION Although several mechanisms of multi-drug resistant strategies are exerted by the parasites, a causal link between MDR1 and resistance to glucantime has already been demonstrated. This study confirms the high prevalence of point mutations in the *L. tropica* MDR1 gene, which are associated with glucantime resistant isolates. The emergence of mutant strains of *L. tropica* poses a serious threat to control efforts.

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1.4-040

A new endemic focus of visceral leishmaniosis (Kala-azar) in Kerman province, southeast of Iran

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BRIEF INTRODUCTION Visceral leishmaniasis (kala-azar) is an endemic disease in some areas of Iran. A cross- sectional descriptive study was conducted for sero-epidemiological survey of visceral leishmaniasis (VL) in Baft district, Kerman province, Southeast of Iran.

MATHERIAL AND METHODS Blood samples were collected from children 12 years old and 10% of adult population in Sothern villages of Baft with a multi-stage random cluster sampling. In addition, blood samples were collected from 30 domestic dogs from the same areas. All the collected blood samples were tested by direct agglutination test (DAT) for detection of anti-*Leishmania* antibodies in both human and dogs using the cut-off values of 1:3200 and 1:320, respectively. Parasitological, molecular, and pathological tests were performed on infected dogs. Chi-square and Fisher exact tests were used to compare sero-prevalence rates.

RESULTS Of 1476 collected human serum samples, 23 (1.55%) showed *Leishmania* antibodies at titers of 1:800 and 1:1600, whereas 14 (0.95%) showed *Leishmania infantum* antibodies at titers of 1:3200. No statistically significant difference was found between males (1.18%) and females (0.69%) sero-prevalence ($P = 0.330$). Children of 5–8 years showed the highest sero-prevalence rate (3.22%). Seven of 30 domestic dogs (23%) showed *Leishmania* antibodies at titers 1:320. *Leishmania infantum* was identified in five infected dogs by nested – PCR assay.

CONCLUSION It seems that visceral leishmaniasis is endemic in southern villages of Baft district, Southeast of Iran.

1.4-041

Detection of *Borrelia* spp. in arthropods collected in state of Rio de Janeiro, Brazil

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INTRODUCTION Spirochetes of the genus *Borrelia*, etiological agents of borreliosis, are transmitted to humans and to domestic and wild animals by ticks of the genus Ixodes, *Amblyomma, Rhipicephalus* and *Dermacentor* in several regions of the World. In Brazil, despite the clinical and epidemiological evidence of the occurrence of a 'Lyme-like' disease in the states of Amazonas, Mato Grosso do Sul, Rio de Janeiro and São Paulo, to date, the agent was not characterized in Brazilian territory. The aim of this study was to investigate the presence the DNA of *Borrelia* burgdorferi sensu lato in arthropods collected at state of Rio de Janeiro, in Southeastern Brazil.

METHODS Arthropods were captured from animals and taken to the Laboratory of Hantavirosis and Rickettsiosis (LHR), Oswaldo Cruz Institute, FIOCRUZ, where they were kept at -20°C for further processing. All arthropods were taxonomically identified at LHR: 165 *Rhipicephalus sanguineus*, 198 *Amblyomma cajennense*, 38 *Ctenocephalides* sp., 07 *Rhipicephalus* sp., 17 *Amblyomma* sp., 15 *Anocentor nitens* and 03 *Pediculus humanus*. Ticks were washed out using solution of sodium hypochlorite and 70% alcohol, and subsequently, their DNA was extracted using QIAamp DNA Blood Mini kit (Qiagen), following the manufacturer's instructions. The polymerase chain reaction (PCR) was performed in different stages (nymphs, larvae and adults), using pairs of primers for *Borrelia* (OspA1/OspA2; p66-1/p66-2; Fla-1/Fla-2), which amplify different regions of the bacterial genome.

RESULTS AND CONCLUSIONS Four hundred and thirty-nine tick samples were submitted to molecular analysis and 38 (8.6%) were *Borrelia* spp. PCR positive: 16 *R. sanguineus*, 09 *A. cajennense*, and 13 *Ctenocephalides* sp. These preliminary results confirm the circulation of *Borrelia* spp. in population of ticks of genus *Amblyomma*, *Rhipicephalus* and *Ctenocephalides* at Rio de Janeiro. Further studies, including molecular identification of *Borrelia* spp., are necessary in order to define the presence and the importance of borreliosis in Brazil.

1.4-042

An adapted theoretical framework to assess implementation fidelity of participatory health strategies

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INTRODUCTION Fidelity of implementation of an innovation is the degree to which it is implemented as intended by its developers. Fidelity assures that, when disseminated in the health system, the innovation maintains its intended effects. Classical fidelity measures are adherence, exposure, quality of delivery, participant responsiveness and programme differentiation. However, what happens when the innovation is tailorable such as in participatory strategies? We propose an adapted theoretical framework for fidelity assessment of the latter.

METHODS We conducted a fidelity assessment of an evidence-based participatory strategy for dengue prevention that was implemented at a larger scale. We anticipated that the classical fidelity approach would be of limited use. We therefore opted to conduct a process-oriented evaluation of the strategy, to assess reinventions and their implications on fidelity. We confronted our methodology with the literature on fidelity research in order to develop the adapted framework.

RESULTS The requirements for fidelity studies of participatory strategies are discussed in the light of the integrated conceptual framework proposed by Caroll et al. for implementation fidelity. We underline the usefulness of fidelity research including for implementation of tailorable innovations. From Caroll's framework we retain outcome evaluation and components analysis, to determine which elements have to be implemented to maintain intended effects. However, we demonstrated the need to adapt the framework to reflect the tension between fidelity and reinvention in implementing participatory strategies. Proposed adaptations are: (i) a process-oriented participatory assessment of fidelity and reinvention using ad hoc measures instead of assessing fidelity through the measurement of adherence alone; (ii) take into account the effect of potential moderators on the balance fidelity-reinvention; (iii) the recognition that facilitation strategies influence moderators and therefore can improve implementation.

CONCLUSIONS The adapted framework permits a comprehensive assessment of implementation fidelity of tailorable innovations.

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1.4-043

First detection of *Leishmania infantum* in naturally infected phlebotomine sand flies in Torres Novas municipality, central region, Portugal

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Portugal is an endemic region for human and canine Leishmaniasis caused by *L. infantum* . This neglected disease is a zoonosis with the dog as the main reservoir and *Phlebotomus perniciosus* and *P. ariasi* are the proven vectors. Four foci are known but canine Leishmaniasis has been reported from several other areas in the country, for which sand fly species and their infection rates are unknown. The main objectives of this study were to identify the phlebotomine species, determine their *Leishmania* infection rates and the bioecological and vectorial transmission risk factors in the municipality. A survey was performed from June to November 2010 with CDC light traps in 275 biotopes covering the municipality 17 parishes. Simple and multiple logistic regressions were used to access risk factors for the presence of phlebotomine species. Nonparametric tests were used to compare densities. Screening of *Leishmania* DNA was done with ITS1-PCR and kDNA- PCR. A total of 1261 sand flies were captured. The two vector species comprised 81.9% (1032/1261) of the captures, and were present in all parishes. *L. infantum* DNA was detected in one *P. ariasi* gravid female, in June, which represents an infection rate for this species of 0.04 (1/25) and an overall sand fly infection rate of 0.0032 (1/315). High temperatures, low relative humidity, absence of wind, presence of pine trees, peridomestic animal shelters and swallow nests were identified as risk factors for sand fly presence. Dogs' *Leishmania* seroprevalence in the district (5–10% – OnLeish, 2010), the high abundance and wide distribution of proven vector species, together with the detection of *Leishmania* in a gravid female suggests that Torres Novas Municipality is a new focus of zoonotic leishmaniasis in Portugal. Furthermore, in this area, *Leishmania* vectorial transmission risk starts in the beginning of summer.

1.4-044

Incremental cost of dengue outbreak control in Guantánamo, Cuba

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INTRODUCTION We assessed the economic cost of routine *Aedes aegypti* control in an at risk environment without dengue endemicity and the incremental costs incurred during a sporadic outbreak.

METHODS The study was conducted in 2006 in the city of Guantanamo, Cuba. We took a societal perspective to calculate costs in months without dengue transmission (January–July) and during an outbreak (August–December). Data sources were bookkeeping records, direct observations and interviews.

RESULTS The total economic cost per inhabitant (p.i.) per month. (p.m.) increased from 2.76 to 6.05 USD in the respective periods. In months without transmission the routine *Aedes* control programme costed 1.67 USD p.i. p.m. Incremental costs during the outbreak were mainly incurred by the population and the primary/ secondary level of the health care system, hardly by the vector control programme (1.64, 1.44 and 0.21 UDS increment p.i. p.m., respectively). The total cost for managing a hospitalized suspected dengue case was 296.60 USD (62.0% direct medical, 9.0% direct non medical and 29.0% indirect costs). In both periods, the main cost driver for the *Aedes* control programme, as well as the health care system and the community, was the value of personnel and volunteer time or productivity losses.

CONCLUSIONS Intensive efforts to keep *Aedes aegypti* infestation low entail important economic costs for society. When a dengue outbreak eventually occurs, costs increase sharply. In depth studies should assess which mix of activities and actors can maximize the effectiveness and cost-effectiveness of routine *Aedes* control and dengue prevention.

1.4-046

Evaluation of the protective potential of a recombinant modified vaccinia virus Ankara (MVA) encoding rift valley fever virus (RVFV) glycoproteins in lambs

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BRIEF INTRODUCTION Rift Valley fever virus (RVFV) is a mosquito-borne virus widely distributed in sub-Saharan countries, Egypt and the Arabian Peninsula, causing disease in both human and livestock. RVF is considered an emerging threat for European countries due to globalization. It is desirable to improve virus detection as well as improving the current vaccines against RVFV in susceptible species.

METHODS AND MATERIALS Three groups of six lambs were used: one group was immunized subcutaneously once with rMVA-Gn-Gc, a second group was immunized with rMVA-gfp (vaccination control) and the last was inoculated with saline solution (infection control). Fourteen days later, all animals were inoculated with 105 TCID50 of the 56/74 virulent RVFV isolate. The vaccine efficacy was assessed in terms of clinical signs, body temperature, viremia and shedding by RT-qPCR and neutralizing antibodies by seroneutralitzation test.

RESULTS Few clinical signs associated with RVF were observed with the exception to one animal from the rMVA-Gn-Gc vaccinated group and two animals from the vaccination control group, which presented apathy and one each from two groups died. Both sheep showed the typical RVF hepatic lesion. Sheep from control groups exceed 40°C from day 2 until day 5 pc; meanwhile rMVA-Gn-Gc vaccinated animals showed only one peak of pyrexia at day 3 pc. RVFV RNA was detected in both nasal and bucal swabs from day 3 pc to day 7 pc in both control groups, while the immunized group with rMVA-Gn-Gc showed no viral secretion with the only exception of the lamb which died. Finally, the rMVA-Gn-Gc vaccinated group showed neutralizing antibodies earlier and with higher levels than vaccination controls group.

CONCLUSIONS The present study shows the partial protection of rMVA-Gn-Gc vaccine with a single dose. For to the safety of MVA vaccines, it could be further developed for humans in RVF endemic areas.

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1.4-047

Zoonotic visceral leishmaniasis: a recent emerging focus in Posadas, Misiones, Argentina

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INTRODUCTION A growing number of reports are drawing our attention to a worldwide spread of Leishmaniasis, due to changes in demographic and ecological factors . Particularly in Latin America, urbanization is a characteristic pattern in the epidemiology of zoonotic visceral Leishmaniasis (ZVL). A recent example is the urban focus of ZVL in the city of Posadas (Misiones, Argentina), which emerged in 2006 affecting humans and dogs.

METHODS AND MATERIALS In the second semester of year 2006 we conducted a parasitological and serological pilot survey by convenience sampling in 110 dogs from Posadas. In 2009, we conducted a seroprevalence study on a representative sample of 349 dogs that were obtained using a random sampling strategy. In order to estimate the cumulative incidence of the disease, an additional serological survey was conducted in 2010 in dogs that had tested negative in 2009. Blood and/or lymph node samples were obtained from all dogs. Seroprevalence and incidence analysis was performed by rk39 immunochromatography and IFAT. PCR analysis of blood and bone marrow was carried out targeting the SSUrRNA and the ITS-1 regions.

RESULTS AND CONCLUSIONS We found a seropositivity rate of 43.6% (68/110) and *Leishmania infantum* was identified as the causative species in 2006. Later in 2009, the observed seroprevalence was 35% (29.4–40.9%), overall, and 23.8% (50/210) in dogs that displayed no clinical signs suggestive of canine Leishmaniasis. We also observed that seropositive dogs were distributed throughout the city in a fairly homogeneous pattern. 14.2% (18/127) of the dogs had become seropositive in one year. These results indicate this focus of canine Leishmaniasis seems to be well established and poses a threat to the human population in Posadas. Notably, since 2006, 70 cases of human visceral Leishmaniasis have been diagnosed in the province, 42 of them in Posadas.

1.4-048

Effectiveness of insecticide treated curtains in a setting with low *Aedes aegypti* infestation levels and an intensive routine vector control programme

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INTRODUCTION Insecticide treated curtains (ITC) are novel but expensive *Aedes aegypti* control tools. Their effectiveness in settings with already low dengue vector infestation and an intensive routine vector control programme (IRVCP) has not been studied yet.

METHODS In 2009 we set-up a cluster randomized trial in urban areas of Guantanamo, Cuba. Twelve clusters (of 500 houses on average) were randomly allocated to two arms: IRVCP (with inspection of premises every 11 days, temephos application once a month and indoor and spatial spraying with cypermethrine each 7–22 days in function of epidemiological situation) and IRVCP + ITC. We used the House Index (HI: number of houses positive for *Aedes aegypti*/number of houses visited) as effectiveness measure. The design had 80% power to detect a reduction of 50%. We assessed the difference between two arms, with a random-effects negative binomial regression model. We additionally calculated the cost per household of implementing ITC.

RESULTS The initial ITC coverage was 98.4% (at least 1 ITM per household; the average was 2.33). After 18 month the coverage remained high (97.3%). There was no difference in average HI between control and intervention areas (RR = 0.93 95% CI 0.59–1.46). In both areas the average HI increased slightly, but not significantly, during the 18 months after ITC implementation (RR = 1.43 95% CI 0.81–2.50). ITC had no significant effect (RR = 1.15, P = 0.8). The cost per household of ITC distribution, on top of IRVP, was 6.03 USD.

CONCLUSIONS In an environment with low infestation and intensive routine vector control programme, ITC deployment does not seem to further reduce the *Aedes* burden.

1.4-049

Is dengue disease surveillance in Indonesia able to detect and predict outbreaks?

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BACKGROUND Dengue has been reported in Indonesia since 1968 and since then periodic outbreaks have emerged throughout the country. Development of early warning systems to predict outbreaks in Indonesia has been detailed in many studies, however, the elucidation of dengue disease surveillance in identifying and forecasting outbreaks has been the focus of only a few. In this qualitative study, we intended to fill the gap in knowledge by assessing the effectiveness of the dengue disease surveillance in Indonesia in detecting and predicting outbreaks.

METHODS AND MATERIAL The Conceptual Framework from CDC Working Group for assessing a Public Health Surveillance System was adapted. We undertook semi-structured interviews with 22 key informants with a distinct group of respondents from primary health center up to the national level involved in dengue surveillance to explore the structure, process, purpose, view, and difficulties faced by different respondents entailed in different parts of the systems. Data were recorded, transcribed, coded, and analyzed. Furthermore, we also analyzed the secondary data relevant to dengue surveillance.

RESULTS Outbreaks occurred in cycles of 5 years, with the cycles subsequently becoming irregular. The Incidence Rate has increased over the years despite Case Fatality Rate has successfully declined to <1%. As in the majority of endemic countries, dengue disease surveillance in Indonesia was still mainly passive, without laboratory support on confirmation of cases and serotype of virus, making it is ineffective to predict an outbreak. With current surveillance systems, Indonesia is able to detect dengue cases as well as to detect outbreaks. Nevertheless, an additional delay in preparedness planning and outbreak response was still observed.

CONCLUSION Dengue disease surveillance from Indonesia is not effective enough to predict an outbreak. Thus, interventions should be designed to involve active components of surveillance

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systems and the utilization of outbreak potential indicators systematically.

1.4-050

Detection of piroplasms in ticks infested on horses in Korea

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Piroplasms, which cause severe diseases in humans and animals, are transmitted by ticks. Our previous study reported the first infection of *Theileria equi* in horses reared in Korea. This study was performed to determine if ticks infested on horses carry equine piroplasms. Thus, the species of ticks on horses and the piroplasms potentially transmitted by ticks were identified. A total of 232 ticks were harvested from horses, including one larva, 43 nymphs and 188 adults. All the ticks harvested were identified as *Haemaphysalis longicornis*, based on the rectangular basis capitulum and the characteristics of scutum, festoons, eyes and palps, by microscopy. While *H. longicornis* is primarily a cattle tick, it is also able to bite horses as well as humans. Since ticks transmit piroplasms in humans and animals, ticks were assessed for piroplasms by PCR using primers specific to the 18S rRNA of piroplasms and ticks, respectively. Out of 232 ticks, 10 ticks (4.3%) were positive for piroplasms. Currently, the complete 18S rRNA genes are being sequenced to identify the species of piroplasms.

1.4-051

Molecular and phylogenetic analysis of tick-borne equine piroplasms in Korea

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Equine piroplasms, *Babesia caballi* and *Theileria equi* are tick-borne protozoan parasites with worldwide distribution. The seroprevalence of *T. equi* in two horses (*Equus caballus*) reared in Korea was first described using ELISA in our previous research. This study was initiated to screen out Korean horses infected by piroplasms using PCR for the detection of parasite and to assess phylogenetic variation among genes of piroplasms. From 2007 to 2010, a total of 224 horse blood samples, including the two horses seropositive to *T. equi*, were collected in four provinces of Korea and analyzed by PCR using primers specific to the 18S rRNA of piroplasms. Among 224 samples, only the two from the seropositive horses were positive to piroplasms by PCR (0.9%), suggesting a similarity in sensitivity and specificity between two diagnostic methods in this study. Sequencing of the complete 18S rRNA of *T. equi* in the two horses (GG-7 and GG-14) whose information was submitted to GenBank (accession nos. HM229407 and HM229408, respectively) showed 100% identity. Alignment of the complete sequences of *T. equi* 18S rRNA showed high degree of identity (98.6–99.8%) with GenBank databases of *T. equi*. The sequences of *T. equi* GG-7 and GG-14 were clustered together with *T. equi* isolates from Spain, Sudan and South Africa, indicating the possibility of a close epidemiological link among these isolates.

1.4-052

Studies on predatory efficacy of *Daphnia magnus* and mesocyclops species on different instars of *Aedes albopictus* larvae

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Larvivorous copepods of *Daphnia magnus* and *Mesocyclops sp.* were tested for their predatory, survival and mortality percentages under high and low nutrient levels against different instars of *Aedes* albopictus larvae. *Daphnia magnus* showed no significant difference in rate of survival and mortality under high and low nutrient input levels as compared to control, although its predation efficacy was affected by high nutrient levels as it preferred the alternative food material than the mosquito larvae. Similarly the survival, mortality and predation of *Mesocyclop*s sp. also remained unaffected by both nutrient levels. Both copepods voraciously predated over 1st instars *Ae. albopictus* larvae with predation percentage of 42% for *Daphnia magnus* and 80% for *Mesocyclops* sp. Only the *Mesocyclops* sp. predated 1st three instars of *Ae. albopictus* larvae under control conditions. Laboratory tests revealed that *Mesocyclops* and *Daphnia magnus* could be used as biological control agent of *Aedes albopictus*. Field conditions show that copepod abundance was related to mosquito population. Fresh water streams, channels and ponds that provide natural breeding sites for mosquito, also help in propagation of copepods. Breeding sites like tree holes and abandoned tires do not show much copepod population. Over experiments based on the release of both the copepods species are on way in the tree holes and automobile tires against the mosquitoes larvae belonging to any genera.

1.4-053

Investigation of patients presenting with tick bites in a military hospital in Turkey

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AIM The aim of this study was to evaluate the epidemiological, clinical and laboratory findings of patients admitted by tick bites.

METHODS Patients admitted to hospital because of tick bites between 2007 and 2008 were included in this study. Demographic, laboratory and clinical data were prospectively collected for each patient using a standardized questionnaire.

RESULTS During the study period, a total of 947 cases were admitted with tick bite. The mean age of patients was 22.8 years and 93.5% were male. The distribution of the cases as a professional was soldiers (92.9%), housewife (3.1%) and students (2.3%), respectively. Most of the cases were living or were made duty in areas of dense vegetation cover. The most common admission to hospital due to tick bites had been September and July, respectively. Ticks were detected mostly on extremities. Forty-five percent were removed by a doctor in the hospital, and the remaining 55% were removed by the patient himself or a relative. Four hundred and thirty patients had no complaints, no pathologic findings or laboratory abnormalities were detected. Abnormal laboratory findings were detected in 517 patients. Elevated creatine kinase and transaminase with prolonged prothrombine time were the most common findings. Fatigue and headache were the most common complaints. Seven cases were investigated in serum samples with serological and PCR analysis for CCHF but it was detected in only one case.

CONCLUSION The vegetation of Turkey has suitable environment for the survival of the most of the types of ticks. So tick bites are one of the major health problems in our country. To prevent tick borne diseases, educating the community on measures against tick bite plays a critical role.

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1.4-054

Surveillance of pathogenic *Leptospira* in rodents in the canary islands

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BRIEF INTRODUCTION Leptospirosis is an important emerging infectious disease that is widespread but under-diagnosed zoonosis. It is acquired by animal contact or water contaminated with urine of infected animals. Species such as mice (*Mus* species) and rats (mainly *Rattus norvegicus* and *Rattus rattus*) serve as the reservoirs, excreting *Leptospira* with the urine. Recently, *Leptospira* has been found in rodents from the Canary Islands (Spain). Therefore, the aim of this study was to determine the healthy risk in the archipelago.

METHODS AND MATERIALS The study was carried out in the Canary Islands, located in the SouthWest of Iberian Peninsula. A total of 218 rodents, 104 *R. rattus* and 114 *Mus musculus domesticus*, were captured in six islands (Tenerife, La Gomera, El Hierro, Lanzarote, Gran Canaria and Fuerteventura). Genomic DNA was extracted from the urinary bladders and the lipL32 fragment was amplified and sequenced.

RESULTS More than a quarter of the rodents studied were infected. Pathogenic leptospires were detected in all the studied islands and for both host species. Two different sequences were obtained. The BLAST analyses showed high similarity between the sequence obtained from *R. rattus* and *Leptospira interrogans* serovar Copenhageni. On the other hand, the sequence recovered from *M. m. domesticus* shown high homology with *Leptospira borgpetersenii*.

CONCLUSIONS These results are highly relevant from the public health point of view, due to the high density of the populations of rodents in the Canary Islands and their proximity to inhabited areas. Furthermore, it is well known that both *L. interrogans* and *L. borgpetersenii* can cause severe leptospirosis in humans. This high incidence found suggests a likely role for the rodents in transmission of human leptospirosis. Finally, this is the first finding of Leptospira in Fuerteventura and Gran Canaria.

1.4-055

Evaluation of rabies postexposure prophylaxis for animal exposures in military hospital in Turkey

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INTRODUCTION The aim of the study was to evaluate rabies suspected animals bite and post-exposure prophylaxis (PEP) practices in military hospital in Turkey.

METHODS Records of the patients applied to our hospital with suspected rabies contact between January 2005 and December 2008 were reviewed retrospectively.

RESULTS During the study period, 564 cases of suspected rabies contact were applied to our hospital. Most the cases were male (75%). Mean age was 27.7. Dogs were the most encountered with a number of 287 (50.8%) fallowed by cats 269 (47.6%), rats 6 (1.1%), a donkey (0.1%), and a rabbit (0.1%). All of the dog contacts were bite and most of the cat contacts (185) were as scratches. Only 20.7% (117) of the animals had an owner, and only 51 (9%) of them had a rabies vaccination certificate. The remaining 79.2% (447) of the animals (42.9% of dogs, 36.3% of cats) had no owners and they were assumed to be unvaccinated against rabies. The average time of admission to hospital patients was 13 h (0.5–130 h). In all cases, four doses of vaccine (0, 3, 7, 14, 28th day) were performed within the PEP. Anti-rabies immunoglobulin was performed with additional vaccine who suspected contact by an animal which is unvaccinated. None of the patients developed rabies disease.

CONCLUSION Considering the fact that a large number of animals had no owner and was not vaccinated against rabies, it is essential to establish an effective animal control to eradicate rabies disease in our country where rabies are still endemic.

1.4-056

Detection of symptomatic and asymptomatic canine visceral leishmaniasis by *Leishmania infantum* fucose-mannose ligand-elisa in an endemic area in Iran

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INTRODUCTION Visceral Leishmaniasis (VL) is caused by *Leishmania infantum* in Mediterranean basin and is an endemic disease in some parts of Iran.

MATERIALS AND METHODS Blood samples of sixty ownership dogs (≤3 years old) were collected from Meshkin-shahr district in Ardabil province, north-west of Iran where VL is a major health problem. All of the dogs were examined for clinical signs such as hair loss, cachexia and dermal wounds.Their serum samples were separated for serological assays (DAT and FML-ELISA) and buffy coats were collected for molecular evaluation. A Nested PCR was used for specific amplification of kDNA of *L. infantum*.

RESULTS Three of 60 dogs had Kala-azar symptoms and 57 dogs were asymptomatic. Two out of 60 (3.33%) serum samples were positive (antibody titer of ≥1/320) in DAT while seven out of 60 (11.66%) serum samples were positive by FML-ELISA. Nine of 60 (15%) buffy coat samples showed a band about 680 bp in PCR representing visceral canine Leishmaniasis. Three of 60 dogs had Kala-azar symptoms and were positive by PCR and FML-ELISA while two of these three dogs had antibody titer >1/320 in their serum samples.

CONCLUSION Considering the high performance of FML-ELISA, using of this serological method can be recommended for epidemiological surveys of CVL.

KEYWORDS FML- ELISA, canine visceral Leishmaniasis, DAT, PCR, diagnosis, Iran

1.4-057

A community empowerment strategy embedded in a routine dengue vector control programme: a cluster randomized controlled trial

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INTRODUCTION The non sustainability of vertically organized dengue vector control programmes led to pleas for changing the emphasis towards community-based strategies. The objective was to test the effectiveness of a community empowerment strategy for dengue vector intertwined with the routine dengue vector control programme.

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METHODS We conducted a cluster randomized controlled trial with 16 intervention and 16 control clusters in La Lisa, Havana City. The intervention included four components: organization and management, entomological risk surveillance, capacity-building and community work for vector control. In the control clusters routine activities continued without interference. Participation, knowledge, risk perception, practices related with Aedes aegypti control and Breteau indices (BI) were compared before and after the intervention. To capture the changes in Aedes aegypti larval densities we applied a semiparametric mixed model. The influence of the intervention on the BIs was evaluated by a generalized estimating equation model.

RESULTS In the intervention clusters the community participation score increased from 1.4 to 3.4. Good knowledge of breeding sites increased 21.6% and 11.7% in intervention and control clusters respectively. There were no changes in adequate Aedes aegypti control practices at household level in the control clusters, but in the intervention clusters adequacy increased 21.8%. At baseline the Breteau indices (BI) were comparable. They became different with the launch of the community-based dengue control activities in the intervention clusters. At the end of the study the BI had decreased 35% in the intervention clusters, against no changes in the control clusters.

CONCLUSIONS The empowerment strategy increased community involvement in dengue vector control and was effective in reducing levels of Aedes aegypti infestation.

1.4-058

Dengue fever imported to the Czech Republic: characterization of cases and evaluation of diagnostic methods

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INTRODUCTION Dengue fever is, after hepatitis A, the second most common viral infection imported to the Czech Republic. The aim of this study was epidemiological and clinical characterization of imported dengue cases and evaluation of diagnostic methods.

MATERIALS AND METHODS In a prospective study at Bulovka University Hospital in Prague 44 patients were diagnosed with dengue fever between January 2005 and May 2011. Acute infection was confirmed by serological methods using ELISA kits (Panbio Diagnostics) and positivity of anti-dengue IgM and seroconversion of anti-dengue IgG. Detection of viral RNA by RT-PCR (Shanghai ZJ Bio-Tech) and NS1 Ag (Bio-Rad Laboratories) were implemented into routine diagnostics in 2008.

RESULTS Nineteen males and 25 females with age median 31 years were investigated during the study period. Twenty-nine patients were hospitalized and 15 were treated as out-patients. The majority of cases were acquired in South and South-East Asia (38), especially in Thailand (17), Indonesia (8), India (6) and Vietnam (5). Only six cases were imported from Central or South America and none from Africa. Fever was the most common symptom (44), followed by rash (34), cephalea (27), arthralgia (24) and myalgia (18). In laboratory findings dominated leukocytopenia (median = 2300/μl), thrombocytopenia (98,500/μl) and elevation of aminotransferases (AST 1.66 μkat/l, ALT 1.59 μkat/l). RT-PCR was performed in 18 patients with acute dengue infection, 12 were positive and six negative. In seven serum samples were performed both RT-PCR and NS1 Ag detection: in four patients were positive both tests, only NS1 Ag was positive in one and only RT-PCR was positive in one, and both were negative in one patient also.

1.4-059

Japanese encephalitis vector mosquitoes in a highly endemic state of South India

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Japanese encephalitis (JE) is a zoonotic disease, with a complex life cycle involving pigs and ardeid birds and vector mosquitoes. JE has been known in India since 1955. JE is fast emerging as a disease of paramount public importance which has rapidly engulfed as many as 25 states/union territories of India. Japanese encephalitis is an important health problem in Andhra Pradesh (AP) and Kurnool district in AP was the most affected. JE virus has been recovered from several mosquito species (19 species) in different parts of India. The most important vectors are Culex tritaeniorhynchus and *Cx. vishnui* from which largest number of isolations have been made. A 4-year entomological study was carried out in Kurnool, south India, to identify the mosquito vectors of Japanese encephalitis (JE) virus. In total, 37,139 female mosquitoes belonging to five genera and eighteen species were collected from vegetation surrounding cattle sheds in villages and peri-urban areas at dusk. Mosquito species composition and pattern of JEV infection varied in peri-urban and rural areas. In peri-urban areas, *Culex gelidus* was the most abundant species, comprising 49.7% of the total catch followed by *Cx. tritaeniorhynchus* (44.5%). In rural areas, *Culex tritaeniorhynchus* was the most abundant species, comprising 78.9% of the total catch followed by *Cx. quinquefasciatus* (10.8%) and Anopheles subpictus (7.1%). *Culex gelidus* (1.1%). Light traps were also employed to collect a total of 24,709 mosquitoes with *Cx. gelidus* and *Cx. tritaeniorhynchus* predominating in peri-urban and rural sites respectively. Overall, 50,145 mosquitoes were screened for JE virus. Japanese encephalitis virus isolations were made from *Cx. gelidus* and *Cx. tritaeniorhynchus*. Based on high abundance and frequent JE virus infection, *Cx. tritaeniorhynchus* seems to be the most important vector whereas *Cx. gelidus* is probably a secondary vector in peri-urban areas.

1.4-060

Reproduction number, turning point and total number of cases during a dengue outbreak in playa municipality, Havana city

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INTRODUCTION The basic reproduction number, R0, is the most important quantity in the study of epidemics. Our objective was to estimate the R0, the turning point and the maximum predicted case number in a dengue outbreak in Havana.

METHODS The research was conducted in all Primary Health Care Areas of Playa municipality, Havana City. During the 2001–2002 dengue outbreak control actions were stepped up in three phases. The estimation of R0, and turning point was derived for each Health Area using the Richards model and we contrasted the cumulative case number with the estimates from the model.

RESULT We obtained an overall basic reproduction number of 3.8 (95%CI 3.1–4.5) for the whole municipality. The R0 by health area ranged from 2.6 to 7.7. At municipal level the turning point of

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the outbreak was 14.5 weeks (95% CI 14.3–14.7). It lay between 13 and 14 weeks for six of the eight health areas taken individually. It was not associated with R0 in the area or with the moment transmission started. However, the R0 were higher (R0 between 6.0 and 7.77) in areas with higher *Aedes* larval indices and lower (R0 between 2.6 and 3.80) in areas where transmission started after the second phase of control actions. Reported cumulative case number and their estimates from the Richard model were 1778 and 1725 (95% CI 1701.9–1748.7) respectively.

CONCLUSION The model used enabled us to estimate R0 and the turning point of the dengue outbreak by health area and predicted well the number of cases during the outbreak.

1.4-061

Is leishmaniosis present in Lleida province, Spain? Epidemiological data on human and canine leishmaniosis and its vectors

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The knowledge of leishmaniosis distribution in Spain is very scarce due to the focalized studies carried out, under reporting of human cases (HL) and the absence of official data on canine leishmaniosis (CanL). In the province of Lleida, leishmaniosis was considered not endemic. Because the province is located between the known foci of the north of Spain and the south of France, it was decided to carry out a study in the area. Bibliographic search was conducted on PubMed and ISI WEB using the keywords '(canine Leishmaniasis OR human Leishmaniasis) and (Lerida OR Lleida OR Catalonia OR Spain)'. The Butlletí Epidemiològic the Catalunya was consulted for HL. An entomological study was carried out in 340 stations covering the entire province. Also, for CanL a questionnaire was sent to the veterinarians of Lleida and a prospective study was carried out in the Pallars Sobirà (Pyrenees). Blood samples were obtained from 145 dogs and serology was done by ELISA. Bibliographic research did not allow finding any published case of CanL and only one of HL. The official cases of HL recorded from 1982 to 2010 (weeks 1–12) were 37. The two vectors, Phlebotomus ariasi and *P. perniciosus* were captured from 80 to 1630 m.a.s.l showing a different altitudinal distribution. Seventy-eight percent of the veterinarians answered that CanL were increasing in Lleida. The canine seroprevalence detected was 29%. The high seroprevalence observed in this limited area (Pallars Sobirà) might be explained by the fact that the dogs analyzed lived in periurban or rural kennels, favouring the transmission of the disease. Positive dogs were found from 500 to 1500 m.a.s.l in 12 of the 16 localities studied. The results show the presence of autochthonous leishmaniosis in Lleida. Data on the heterogeneous distribution is discussed.

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1.4-062

Genetic characterization and evolutionary history of chagas disease vector *Triatoma dimidiata* (Hemiptera: Reduviidae) based on the ribosomal its-2 marker

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INTRODUCTION *Triatoma dimidiata*, an important Chagas disease vector, is distributed in México, all Central America and up to Ecuador and Peru coasts. *T. dimidiata* presents extensive phenotypic, genotypic and behavioural diversity. It is able to colonize peridomestic and domestic habitats, and sylvatic populations acting as reinfestation sources; The ITS-2 ribosomal DNA has been selected for genetic characterization of different *T. dimidiata* populations through its geographical distribution.

MATERIALS AND METHODS One hundred and sixty-seven *T. dimidiata* specimens from 10 countries (Mexico, Guatemala, Honduras, El Salvador, Costa Rica, Nicaragua, Panama, Colombia, Ecuador, and Peru) were used. The DNA was isolated by standard techniques; the ITS-2 was amplified by PCR and sequenced by the Sanger method. DNA sequence analyses, phylogenetic reconstruction methods, and genetic variation approaches are combined to investigate the haplotype profiling, genetic polymorphism, phylogeography, and evolutionary trends of *T. dimidiata*.

RESULTS A total of 43 haplotypes were obtained and analyzed. Phylogenetic and population genetics analyses confirmed the existence of four groups, three of them proposed to be subspecies of *T. dimidiata*, named after *T. dimidiata* dimidiata for Central American populations, T. d. capitata for Colombian populations, and *T. d. maculipennis* for Mexican populations; and a fourth group proposed to be a different species (*Triatoma sp. aff. dimidiata*) confined to the Yucatan peninsula and specific locations in Chiapas, Guatemala and Honduras.

CONCLUSIONS *Triatoma dimidiata* populations follow different evolutionary divergences with the influence of geographical isolation. Phylogeographic analyses support their origin in a southern Mexican–northern Guatemalan ancestral form, and later spreading events. The very large intraspecific genetic variability found in *T. dimidiata* sensu lato has never been detected in a triatomine species before, furnishing a new frame to designing Chagas disease control strategies effective for local species and populations.

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1.4-063

An incidence hotspot, associated with ecological factors, during a dengue outbreak in Havana city

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INTRODUCTION Dengue spread in non-immune populations occurs in explosive epidemics. A better understanding of transmission patterns during outbreaks is essential to fine-tune surveillance and improve disease control.

METHODS We studied the spatial distribution of dengue cases and the relationship with mosquito vector and human density for the May 2001–February 2002 outbreak in Playa Municipality, Havana. The house block (a block has on average 50 houses) was the unit of analysis. Spatial clustering was assessed monthly and we used kernel density estimation to model the pattern of dengue spread. We calculated Breteau indices (BI: Number of positive containers per 100 houses) and used cross-lagged correlation methods to assess their correlation with dengue incidence. We analysed the correlation with human population density by means of the Moran index.

RESULTS Clustering of disease incidence in a geographically well defined 'hotspot' occurred during the whole epidemic. The incidence rate ratio within the hotspot, compared with the outside area, ranged between 2.86 and 3.98. Cross lagged (2 weeks) correlation between dengue cases and houses indices was 0.73 ($P < 0.05$). The Moran index was 0.07 ($P < 0.001$) The incidence hotspot was situated on the limits of four different health areas

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and regrouped house blocks with some of the highest population density and vector infestation levels.

CONCLUSIONS Adding a geographic component to the current surveillance system in Havana would permit to identify hotspots of dengue incidence during an outbreak. The dispersal pattern of *Aedes aegypti* could be used to guide vector control measure. These should be planned at municipality level and not independently by health area.

1.4-064

Cutaneous leishmaniasis in Nalut district, the Libyan Arab Jamahiriya: a clinicoepidemiological study and *Leishmania* species identification

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INTRODUCTION Cutaneous Leishmaniasis (CL) is an endemic disease in the littoral zones of the Mediterranean area, the Middle East, East Africa, especially in Libya but has not been fully documented. The present study aims to clarify the clinicoepidemiologic profile of CL and the molecular genotyping of the *Leishmania* species in Nalut district, Libya.

METHODS AND MATERIALS Two hundred and twenty-three CL patients were examined at the out-patient clinics of Nalut Hospital from March 2006 to February 2007. Cutaneous Leishmaniasis was diagnosed by clinical, microscopic, culture, polymerase chain reaction (PCR), and PCR-restriction fragment length polymorphism (PCR-RFLP) analyses.

RESULTS The disease was observed in all months of the year with the highest incidence rate from November to February. Fifty-nine percent of patients were younger than 20-years old. Noduloulcerative lesions, indurated ulcer, papuloulcerative lesions, and subcutaneous nodular lesions were observed in 170, 25, 15, and 13 patients, respectively. Two hundred patients (89.69%) had dry type lesions, 23 patients (10.31%) presented wet type lesions. One hundred and fifty-nine (71.34%) cases of 223 patients were confirmed positive for CL by presence of the amastigote form of *Leishmania* in stained Giemsa smear. One hundred and seventy (76.23%) cases were confirmed by demonstrating the promastigote form of *Leishmania* by culture in RPMI medium supplemented with 10% fetal bovine serum (FBS). On the other hand, PCR confirmed 203 (91.03%) cases as positive. Genotyping of *Leishmania* species by RFLP analysis revealed that *L. tropica* was the most common species in all ages and that *L. infantum* was the second under 20 years of age.

CONCLUSIONS Cutaneous Leishmaniasis is endemic in Nalut district, Libya. PCR was the most sensitive parasitic diagnostic test and *L. tropica* was the most common species.

KEYWORDS Cutaneous Leishmaniasis, *Leishmania* species, polymerase chain reaction-restriction fragment length polymorphism analysis

1.4-065

Successful treatment of rhodesian trypanosomiasis with pentamidine in a polish tourist returning from equatorial Africa

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INTRODUCTION African trypanosomiasis is a parasitic infection sporadically imported to Europe by tourists or immigrants returning from endemic areas of the tropics. A delay in medical diagnosis or the inappropriate treatment of the disease, which is usually rarely considered in the differential diagnosis of fever in a returning traveller, may be the cause of severe multi-organ injury or even a fatal clinical prognosis during an acute period of peripheral blood parasitaemia or in a chronic phase of meningoencephalitis. West African trypanosomiasis, which is circulating in a human environment, is characterised by a slowly progressing and chronic clinical course, while infection with *T. brucei* rhodesiense is a typical zoonosis with a recent onset, fulminant manifestatation of clinical symptoms and poor patient's prognosis. In this study, we present the first and an unusual case of East African trypanosomiasis imported to Poland by a patient coming back from a tourist trip to Uganda and Rwanda, and which was successfully treated with Pentamidine.

CASE PRESENTATION A 61-year-old Polish man was transferred from a regional hospital in an air ambulance and admitted to the Department because of high-grade fever and multi-organ dysfunction after a tourist trip to East Africa. The patient spent 16 days in Uganda and Rwanda and had returned 4 days prior to admission. He experienced a single tse-tse fly bite during a safari trip to the Queen Elizabeth National Park in Uganda. On admission, his clinical status was severe, with high fever of 41°C preceded by chills, low blood pressure, bleeding from the gums and oral mucosa, haemorrhages at the sites of vein puncture, numerous ecchymoses, fine-spotted skin rash, tachycardia, hepatosplenomegaly, dehydration, jaundice, dyspnoea, peripheral swelling and oliguria. On his left arm, there was a typical non-painful trypanosomal chancre with central necrosis and peripheral erythema. Laboratory examinations showed leucopoenia, thrombocytopenia, haemolytic anemia, hyperbilirubinaemia, and hypoglycaemia, elevated concentrations of creatinine and urea, high activity of aminotransferases, elevated inflammatory markers, hypoproteinaemia, proteinuria, abnormal clotting and bleeding times, low level of fibrinogen, metabolic acidosis, and electrolyte disturbances. Peripheral blood smears showed numerous *T. brucei* trypomastigotes, with a massive parasitaemia of 100,000/μl. *T. brucei* rhodesiense subspecies was finally identified by finding the characteristic SRG gene using a PCR technique and specific antibodies by ELISA in the peripheral blood. A severe clinical course of rhodesiense trypanosomiasis with renal failure, respiratory distress, clinically overt DIC syndrome, haemolysis, liver insufficiency and myocarditis was confirmed. There were no trypomastigotes in the cerebrospinal fluid. Intensive anti-parasitic and symptomatic treatment was immediately instituted, which included intravenous Pentamidine, plasmapheresis, oxygen therapy, blood preparations, catecholamines, fluid infusions, as well as haemostatic, hepatoprotective, anti-inflammatory, antipyretic and diuretic drugs. The final outcome was efficacious with no late sequelae.

CONCLUSIONS (i) Proper health education on threats posed by *Glossina* fly and the possibilities of their prevention are important for persons travelling to African areas with a high risk of *T. brucei* infection; (ii) African sleeping sickness should always be con-

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sidered in the differential diagnosis of fever in people returning from safari trips to national parks or nature reserves of sub-Saharan Africa.

1.4-066

Identification of cutaneous leishmaniasis agents in four geographical regions of Khouzestan province, Iran, using nested PCR

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INTRODUCTION AND AIM *Leishmania tropica* and *Leishmania* major are the main agents of cutaneous Leishmaniasis in endemic regions of Iran. The aim of this study was identification of cutaneous Leishmaniasis agents in Khouzestan province.

MATERIALS AND METHODS One hundred and forty-six samples were collected from the lesions of 146 individuals including 67 (59.59%) male and 59 (40.415) female with cutaneous Leishmaniasis referred to Iran- Zamin diagnostic laboratory and smeared on slides, stained with wright eosin methylen blue stain and examined microscopically and graded from 1+ to 4+. DNA was extracted from the slides and identification of cutaneous Leishmaniasis agents were performed using Nested PCR with the primers of CSB1XR, CSB2XF, LIR and 13Z.

RESULTS One hundred an thirty-eigth (94.5%) out of 146 cases of four regions was *L. major* and 8 (5.5%) were *L. tropica*. 57.97% of *L. major* cases were male and 42.03% were female. 87.5% of *L. tropica* were male and 12.5% were female. The majority of cases of *L. tropica* was from north (8.16%) and the lowest was from west (3.22%). 96.78% cases of *L. major* were from west of Khouzestan.

CONCLUSION *Leishmania major* is the main causative agent of cutaneous leishmaniasis in Khuzestan province southwest of Iran.

KEYWORDS Cutaneous Leishmaniasis, *Leishmania major*, *Leishmania tropica*, PCR

1.4-067

Ehrlichia spp. infection in sick dogs and theirs arthropods in espirito santo state, Brazil: serological and molecular study

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INTRODUCTION Ehrlichiosis is a tick-borne disease caused by organisms from the family Anaplasmataceae . In Brazil, species of the genus *Ehrlichia* such as *E. canis*, *E. chaffeensis* and *E. ewingii* are responsible for disease in domestic and wild animals. *Ehrlichia* canis is involved in canine infections, due to its worldwide distribution, a reflection of the primary vector, Rhipicephalus sanguineus. The main vector of *E. chaffeensis* is *Amblyomma*, although this agent has been also described in Dermacentor and *Rhipicephalus* ticks. The purpose of this study was to identify the specie of *Ehrlichia* in blood sample of sick dogs and theirs arthropods in Esp"rito Santo state, in Southeastern Brazil.

MATERIAL AND METHODS Sera and blood samples of three sick dogs were taken to the Laboratory of Hantavirosis and Rickettsiosis/FIOCRUZ, where serum samples were analyzed using a commercial indirect immunofluorescence assay – IFA for IgG (titer ¡Ý 64). DNA extracted from blood samples and ticks collected from dogs, after taxonomic identification, was submitted to the polymerase chain reaction (PCR) using pairs of primers for *Ehrlichia* that amplify part of the 16S rRNA gene, and for nested-PCR, using specific primers for *E. canis* and *E. chaffeensis*.

RESULTS AND CONCLUSIONS All dogs presented antibodies reactive to *Ehrlichia* sp. by IFA, with titers ranged from 4096 to 8192. Ehrlichial DNA was detected in all canine samples and nucleotide sequence of the amplicon generated (389 bp) showed 99% identity to the homologous sequence of the 16S rRNA gene of *E. canis* deposited at Genebank. Only two of the 15 *R. sanguineus* tick pools were analyzed and one of them was PCR positive. The nucleotide sequence of the amplicon generated (388 bp) showed, unexpectedly, 99% identity to the homologous sequence of *E. chaffeensis*. Studies to further evaluate these data are continuing and results will be made available as soon as possible.

1.4-068

Molecular detection of *Rickettsia lato sensu* in arthropods from Rio de Janeiro state, Brazil

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INTRODUCTION Rickettsiosis, a group of diseases widely distributed throughout the World, are zoonosis transmitted to humans by fleas, lice, ticks and mites. In Brazil, the Brazilian spotted fever (BSF), caused by *Rickettsia rickettsii*, has been reported, besides other rickettsiosis 'lato sensu' such as bartonelosis, Q fever and ehrlichiosis.

MATERIAL AND METHODS Arthropods collected from animals, humans and environment in several areas in Rio de Janeiro state were taken to the Laboratory of Hantavirosis and Rickettsiosis, where were kept at -20°C for further processing, after taxonomic identification. Among all collected arthropods, 48 were identified as *Rhipicephalus sanguineus*; 112, *Amblyomma cajennense*; 38, *Ctenocephalides felis*; 07, *Rhipicephalus* sp.; 11, *Amblyomma* spp.; 15, *Anocentor nitens* and 03, *Pediculus humanus*. Ticks were washed out using solution of sodium hypochlorite and 70% alcohol, and subsequently, their DNA were extracted using QIAamp DNA Blood Mini kit (Qiagen), following the manufacturer's instructions. The polymerase chain reaction (PCR) was performed in different stages (eggs, nymphs and adults), using pairs of primers for *Bartonella* sp., *C. burnetii*, *E. canis*, *E. chaffeensis* and *Rickettsia* spp.

RESULTS AND CONCLUSIONS Of the 234 arthropods samples submitted to PCR, 89 (38%) were positive for one or more agent; 70 (29.9%) amplified the fragment of 16S rRNA gene of *Ehrlichia* species, 06 (2.6%) amplified the fragment of the ompA gene of the spotted fever group *Rickettsia* species, 35 (14.9%) amplified the fragment of the htpAB gene of *C. burnetii* and 36 (15.4%) amplified the fragment of htrA gene of *Bartonella* sp. The positive PCR ticks samples were 07 *R. sanguineus*, 36 *A. cajennense*, 03 *Amblyomma* sp., 06 *A. nitens*, 03 *P. humanus* and 34 *C. felis*. The sequencing and phylogenetic study are underway, but the preliminary results confirm the circulation of the *Rickettsia* 'lato sensu' agents in several arthropods at Rio de Janeiro, Brazil.

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1.4-069

Sonographic evidence of ascites, pleuro-pericardial effusion and gall bladder wall edema may be used as non-invasive rapid diagnostic markers in subjects with probable dengue fever

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INTRODUCTION Dengue is fast becoming a global epidemic, however, radiographic findings of dengue fever (DF) have not yet been clearly elucidated with relation to clinical and serological findings. We analyzed ultrasonographic (USG) features of patients presenting with probable dengue fever during the outbreak of DF of 2006 in North India.

METHODS Case records of consecutive patients presenting to an urban tertiary care referral hospital in India with probable DF, satisfying the inclusion criteria were retrospectively included in the study. Ten individual sonographic parameters were reviewed vis-à-vis ascites, hepatomegaly, splenomegaly, gall bladder wall edema (GBWE), pleural effusion (right or left or both), pericardial effusion, pericholecystic collection, perinephric collection. Subjects who had GB wall thickness more than 3 mm as measured on ultrasound were identified as those positive for GB wall edema. The cases were then analyzed in view of their serological profile. Statistical analysis was done using SPSS version 11.

RESULTS One hundred and sixty-nine patients with mean age of 27.9 (+13.4) years were included in the study; male: female was 3:1. The mean platelet count of the subjects was 57.4×103/cm (+22). The most common ultrasonographic feature was ascites (126, 75%) followed by gall bladder wall edema (122, 72%), hepatomegaly (78, 46%), splenomegaly (66, 39%) and pericholecystic collection (63, 37%). Forty-eight (28%) subjects demonstrated evidence of pleural effusion on the right side, while 19 (11.2%) had bilateral effusion. None of the subjects had an isolated left pleural effusion. Twenty-seven (16%) subjects reported bleeding manifestations in the form of petechiae and 5 (3%) developed renal dysfunction. Presence of pleural and pericardial effusions was found to be specific while ascites and GBWE were identified as highly sensitive markers for seropositive primary DF.

CONCLUSION Ultrasonographic evidence of ascites, pleuro-pericardial effusion and gall bladder wall edema may be used as rapid non-invasive markers during outbreaks of dengue even before serological investigations become available and may not only indicate severity but also predict onset of bleeding in the form of petechiae or preempt renal dysfunction. The study findings can help optimize management especially in the resource restricted settings.

1.4-070

Culex theileri is a potencial natural vector of *Dirofilaria immitis* in Canary Islands, Spain

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Dirofilaria immitis is the causal agent of cardiopulmonary dirofilariosis (heartworm disease). Humans are accidental host in which it causes pulmonary dirofilariosis. The infection is transmitted by several species of culicid mosquitoes in different parts of the world that are frequently able to bite both humans and animals. In Canary Islands (Spain), dirofilariosis has been for years an endemic disease. A total of 1219 female mosquitoes were captured. The most abundant species was *Culex theileri* (52.26%) followed by *Cx. pipiens* (35.44%), *Anopheles cinereus hispaniola* (6.23%), *Culiseta longiareolata* (5.74%), and *Culex laticintus* (0.33%). PCR was applied for the detection of larval *D. immitis* DNA in mosquitoes. *D. immitis* DNA was observed in the abdomen of one *Cx. theileri* females. Prevalence of *D. immitis* was therefore 0.082% of the entire mosquito population and 0.17% in *Cx. theileri*. A molecular identification of *Cx. theileri*, the potential mosquito vector of dirofilariosis in this zoonotic focus in Canary Islands of Spain, has been made for first time based on sequences of the 18S rRNA gene, the second internal transcribed spacer (ITS2) of ribosomal DNA and the barcode region of the cytochrome c oxidase I (cox1) gene of mitochondrial DNA, allowing a broad mosquito molecular basis for future populations genetic analyses of this important vector species. Parasitological and entomological molecular Results suggest that *Cx. theileri* is a potential natural vector of *D. immitis* in Canary Islands, Spain and implies a Dirofilariois transmission complexity in Southern Europe markedly higher than that considered so far.

1.4-071

Chikungunya virus epidemic in Madagascar: infection associated with body weight in pregnant women

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INTRODUCTION In October 2009 an increase of fever cases at the Eastern coast was noticed and in the beginning of February 2010 the Institute Pasteur diagnosed Chikungunya virus (CHIKV) infection in a patient from the Eastern coast. The focal point of the epidemic was around Mananjary, where the epidemic reached its peak in February and abated in March. We report the retrospective assessment of reported clinical features and serological markers of CHIKV, dengue virus (DENV) and Rift Valley fever virus (RVFV) infections in pregnant women at six different geographical locations on different heights on and above sea level in Madagascar.

METHODS AND MATERIALS Antenatal clinics were visited between May and July 2010. A venous (EDTA-) blood sample was taken for serological screening for anti-CHIKV-IgG anti-DENV-IgG and anti-RVFV-IgG antibodies from 1244 women. Samples from Mananjary were additionally screened for anti-CHIKV-IgM and anti-DENV-IgM antibodies.

RESULTS AND CONCLUSION The 2009/10 arboviral outbreak in coastal south-eastern Madagascar was a CHIKV outbreak. The CHIKV-IgG seroprevalence was 45% in Mananjary and 23% in Manakara, both at the south-eastern coast. The corresponding DENV-IgG seroprevalence was 17% and 11% respectively. Only four women had RVFV-IgG antibodies. All DENV-IgM tests for samples from Mananjary were negative; the seroprevalence of anti-CHIKV-IgM was 27.5% (2–3 months after the outbreak). CHIKV-IgG-seroprevalences in altitudes between 450 and 1300 m were low (0–3%). More than a third of all pregnant women were affected in the epicentre. Data from higher elevation levels suggest that the epidemic did not spread upwards and inbound, but remained restricted to the coastal areas around the epicentre. Joint pain and stiffness was reported by 78% of the IgG-seropositives

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from the coast; 21% did not report any symptoms. CHIKV infection was associated with body weight (P = 0.001, test for trend).

1.4-072

Dengue virus infection in Tuscany, Italy: evaluation of ICT rapid test and clinical criteria for the diagnosis of acute dengue fever

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INTRODUCTION We investigated the performance of a rapid Dengue IgG/IgM test in patients with suspected acute dengue fever (ADF) comparing the Results with those obtained by gold standard testing with ELISA. Moreover, we evaluated in our survey which were the most reliable criteria in 2009 WHO Dengue classification as tools for suspecting the ADF diagnosis.

METHODS AND MATERIALS From January 2006 to December 2010, 191 serum samples of patients with suspected ADF were sent from 12 hospitals in Tuscany to the laboratory of the Reference Centre for Tropical Diseases to undergo serological testing with DENGUE IgG/IgM Combo Rapid Test (Cypress) and DENGUE IgM & IgG Capture ELISA (Panbio). ADF was confirmed by detection of Anti-Dengue IgM antibodies (ELISA). Symptoms and laboratory findings of patients were investigated retrospectively and reported on separate schedules. Patients with missing clinical and laboratory findings were excluded. WHO classification criteria for suspected dengue, was applied to our survey and parameters of the scheme were evaluated individually in terms of sensibility and specificity.

RESULTS One hundred and nine patients were enrolled. According to ELISA, the survey was split into Dengue (N = 36) and other febrile illnesses (N = 73) (OFI) group. The sensitivity and specificity of ICT test was 97% and 98.6% respectively: 35 of 36 patients in the dengue group tested positive vs. one of 73 in OFI. Furthermore, WHO classification scheme showed sensibility of 80.5% and specificity of 63.4% for the diagnosis of ADF. Among evaluated diagnostic parameters, leucopenia, thrombocytopenia, arthralgia/myalgia, nausea/vomiting, rash, and country of origin other than Africa proved to be significantly associated with ADF. Leucopenia and thrombocytopenia were most strongly associated with ADF diagnosis (OR 18.9 and 12, respectively).

CONCLUSIONS The rapid test appears to be extremely reliable. The WHO classification remains a very sensitive screening tool to suspect an ADF with a good specificity.

1.4-073

Identification of relapsing fever *Borrelia* persica and *Borrelia microtti* by diagnostic species-specific PCR based on flagellin (flaB) gene

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TBRF is an endemic disease in Iran, with 1415 confirmed cases from 1997 to 2006. The spirochete *Borrelia* persica accounts for most of cases, however, the epidemiological evidence for *B. microtii* relapsing fever is also strong. The high infection rates in *Ornithodoros erraticus* ticks and occurrence of the disease in areas where no report of *O. tholozani* ticks is available brings *B. microtii* to light as the second TBRF causative agent in Iran. This study aimed to identify the two most prevalent TBRF-causing species, *B. persica* and *B. microtii*, in Iran using species-specific PCR based on Flagellin (flaB) gene. *B. microtii* originated from *O. erraticus* ticks was collected from rodent burrows in Hesark, an area near Karaj city in Alborz Province, and *B. persica* was isolated from *O. tholozani* ticks that were from rural areas in Ardebil Province. The pathogenicity test confirmed the identity of both isolates; *B. persica* caused heavy infections in adult guinea pigs, however, *B. microtii* caused occult infections in adult guinea pigs heavy ones in adult mice. Molecular typing of the both species based on partial sequencing of flaB gene over 718 bp exhibited 88% homology (51 nucleotide difference and 33 gaps). Presence of 12% nucleotide difference between two DNA sequences enabled designing species-specific primers, which amplified a 376 bp fragment for *B. microtii* and a 200 bp one for *B. persica*. Phylogenetic analysis of the data grouped *B. microtti* with the African agents, very close to *B. duttonii* and *B. recurrentis*, however, *B. persica* appeared on a border between African and American agents. Application of specific–species PCR provides a sensitive and fast tool for identification and monitoring of prevalent TBRF *Borrelia* species in endemic areas of Iran.

1.4-074

Development of diagnostics for determination of V- and F1 antigens of *Yersinia pestis*

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Plague is the most dangerous bacterial illness humans ever encountered. *Yersinia pestis*, causative agent of plague, lurks in prolific natural rodent reservoirs, and outbreaks are constantly detected at various locations of the world. WHO data indicate 72 cases of plague in 2010: 55 in Africa, 13 in Asia, and four in America. There is urgent need in new diagnostics for efficient detection of plague pathogen. The aim of the present work was development of the mAb-based ELISA test for detection of *Yersinia pestis* capsular antigen F1, and raising mAbs to *Yersinia pestis* V-antigen in order to use them for development of yet another ELISA detecting the plague pathogen. In the prior work, we obtained a panel of hybridomas producing anti-F1 mAbs. The mAb combination, displaying the best sensitivity to the target, was determined in the two-site Western dot-blot. A pair of best performing mAbs suitable for indirect ELISA was thus selected. Control studies using recombinant F1 antigen indicated that the sensitivity of the two-site ELISA based on the selected mAb pair has detection limit of 4 ng/ml. The developed ELISA can thus be used for F1-based plague detection as a standalone of adjunct system. mAbs specific to the *Yersinia pestis* V-antigen were obtained according to the standard protocol, and screening of the primary hybridoma pool was done using indirect Elisa. Of mAbs analyzed, six demonstrated the detection limit of 2.5 ng/ml of V-antigen, specifically detected 10 strains of *Y. pestis*, and displayed no cross-reactivity with other *Yersiniae* and 20 other arbitrarily selected bacterial species. Based on mAbs specific to F1 andV-antigens, *Yesrsinia pestis* detection systems based on immuno-PCR and xMAP will be developed.

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1.4-075

Rapid response against pandemic influenza in tangerang Banten province, Indonesia in August, 2009

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Pilot Project of Avian Influenza Control and Pandemic Influenza Preparedness in Tangerang, Banten Province, Jakarta, Indonesia

On Saturday, August 8, 2009 at 10.28 am a staff of Tangerang District Health Services explained that during the last 3 days, the students of Islamic Boarding School Daar el Qolam (DEQ-P) (about 800) suffered from fever, deep cough and runny eyes and noses, and cases rose despite care from a physician from the nearest Public Health Center accompanied by a Nurse. Pilot Project of Avian Influenza Control and Pandemic Influenza Preparedness (PPAIC) as Influenza Field Investigator, equipped with PPE sets, anti viral drug of Tamiflu and Hank's solution medium. The same situation of ILI (Influenza like Illness) had occurred in Al Amanah Al Gontory-Pesantren (AAAG-P) in South Tangerang Municipality. The result of the field investigator together with the result of laboratory examination of collected specimens were: of 4.200 persons in DAAAG-P 400 were students. In DEQ-P there were 825 cases, attack rate = 19.6%; in DAAAG-P there were 60 cases, attack rate = 15%. Nasal and tracheal swab specimen collected were 95 and 18 respectively. All specimens were PCR tested and 100% positive of H1N1 virus. In the field there was no evidence of animal involvement of this virus of H1N1 transmission, but person-to-person transmission was significant. In DEQ-P 4.531 persons were treated with Tamiflu: 4.200 students, with 2.705 males and 1.495 females and 331 teachers and school staff. In AAAG-P 472 were treated: 400 were students, 297 were males and 103 were females, 95 teachers and AAAG-P staff. No death and treatment side effect was reported. Although the Case Fatality Rate (CFR) was zero but since Indonesia have AI flu H5N1 virus with CFR = 82% we are very worried about this virus; H1N1 virus may have a reassortment of type A or its subtypes and may mutate into a highly pathogenic form. The outbreaks occurred after students returned from their home town after vacation. We urge to intensify ILI surveillance.

1.4-077

Detection of CTX-M-1 and CMY-2 beta-lactamases among *Escherichia coli* isolates of healthy pets in Tunisia

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OBJECTIVE To detect extended-spectrum (ESBL) and AmpC beta-lactamases among faecal *E. coli* of healthy pets and to characterize the recovered strains.

METHOD Eighty faecal samples of healthy dogs and cats were inoculated onto MacConkey agar plates supplemented with cefotaxime (CTX, 2 μg/ml). Characterization of ESBL and AmpC genes and of their genetic environment was performed by PCR and sequencing. Detection of associated resistance genes, virulence factors, and phylogroup-typing were performed by PCR, and plasmid analysis by PBRT-typing. MLST and integron characterization were carried out by PCR and sequencing.

RESULTS ESBL-positive *E. coli* isolates were detected in 13 of 80 faecal samples (16.2%) and they contained the following genes (number of isolates): blaCTX-M-1 (8), blaCTX-M-1 + blaTEM-1c (1), blaCTX-M-1 + blaTEM-135 (1), blaCTX-M-1 + blaTEM-1b (3). The ISEcp1-blaCTX-M-1-orf477 structure was found in all cases. In one sample (1.25%), one AmpC-positive *E. coli* isolate was found, that harbored the blaCMY-2 and blaTEM-1b genes. Twelve different sequence-types (STs) were identified by MLST among the CTX-resistant isolates (number isolates/phylogroup): ST58 (2/B1), ST57 (2/D), ST398 (1/A), ST602(1/B1), ST1914 (1/A), ST1720 (1/A), ST1431(1/A), ST10 (1/A), ST155 (1/B1), ST345 (1/B1), ST539 (1/B1), and a new sequence-type (1/D). Nine plasmid replicon types were detected among the 14 CTX-resistant isolates, and all of them carried two or more replicon types. The plasmid IncI1 was detected in all the isolates. The fourteen isolates showed tetracycline resistance and they contained tet(A) +/- tet(B) genes. The class 1 integrase gene was found in eight of the CTX-resistant isolates. The virulence genes detected were (number isolates): fimA (13), aer (11), sxt, papGIII, papC, hly, cnf, and bfp (none).

CONCLUSION A high rate of healthy pets in Tunisia showed faecal carriage of ESBL-positive *E. coli* isolates, mostly of CTX-M-1 class. The domestic animals could be a reservoir of these resistant isolates, representing a problem in human health.

1.4-078

Presence of Phlebotomus pernicious, vector of Leishmania infantum, in Mallorca

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Although Mallorca is considered an endemic area of human and canine leishmaniosis in the Mediterranean region, there are few entomological studies on the sandfly fauna of the island and these were done a long time ago. In July 2008 sandfly captures were carried out in Majorca with sticky castor oil traps (20 × 20cm) according to the standardized methodology implemented in the EDEN project (EU). The sampling sites consisted of holes used to drain embankments or containment walls and were distributed in 77 grids covering the entire island (5 × 5 km square grid) and geocoded using a Tom-Tom wireless GPS. Data were filled in a form using Pendragon Forms v.5.0 software and a Palm Tungsten T5 PDA. 1935 traps were placed in 108 stations recovering 1706 of them (88.2%, 136.48 m^2). The sandflies collected were fixed firstly in alcohol 96° and then in alcohol 70° until their identification with a stereoscopic microscope and/or an optical microscope, on the basis of morphological characteristics. A total of 14,449 specimens were captured on the island. The sandfly fauna of Mallorca is composed of four species: *Phlebotomus pernicious* (number (*N*): 921 specimens, abundance (A): 6.4%, density (D): 6.75 specimens/m^2), *P. sergenti* (*N*: 25, A: 0.2%, D: 0.18), *P. papatasi* (*N*: 3, A: 0.02%, D: 0.02) and *Sergentomyia minuta* (*N*: 13,500, A: 93.4%, D: 98.92). Of these, *P. perniciosus* is responsible for the transmission of *Leishmania infantum* in the Mediterranean region. This species was captured around the entire island (79/108 stations, frequency 69.4%), from 5 m above sea level to 726 m a.s.l., although mainly below 400 m. A distribution map has been compiled and the results are discussed in relation to previous records on sandflies and the distribution of human and canine leishmaniosis on the island.

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1.4-079

In vitro effect of horse fat oil on *Leishmania major* lesions on mice

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INTRODUCTION Dermal Leishmaniasis is an endemic problem in 88 countries in the world with an annual exposure of 1.5 million people to this disease. Of this number, 90% live in Algeria, Iran, Iraq, Syria, and Saudi Arabia (old world form), as well as Brazil and Peru (new world form). Although the effects of oils extracted from animal sources in the improvement of skin lesions in general have been well established, studies on the effects of oils from horse fat on *Leishmania major* have been rather limited. This study is a primary assessment of the effects of oil produced from horse fat on Dermal Leishmaniasis (major) (MRHO/IR/75/ER) in Balb/c mice.

METHODS This study was performed on 30 Balb/c mice with ages of 6–8 weeks. Mice were randomly divided into two equal groups, one of which being the control with no treatment and the other treated with oil produced from horse fat. The treatment consisted of 0.1 g of horse oil applied two times a day for 20 days. Lesion size and number of amastigotes in the wound was subsequently assessed.

RESULTS This research showed that topical use of horse fat oil in Balb/c mice diminished the size of wound and reduced the number of amastigotes ($P < 0.05$).

CONCLUSION The hypothesis that the oil produced from horse fat improved wound of *Leishmania major* in Balb/c mice was supported by this experiment. However, further studies are required to establish better founded judgments of horse fat oil impact on *Leishmania major* in humans.

KEYWORDS Cutaneous Leishmaniasis, animal oil, horse fat oil, *Leishmania major*

1.4-080

Relationship between education, occupation, water storage and dengue hemorrhagic fever in Central Java

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BACKGROUND Wonogiri is one of the regencies in Central Java, Indonesia which contribute to the upsurge of Dengue Hemorrhagic Fever (DHF) cases. In 2009, IR of DHF cases were 3,64/10.000.- with a CFR of 0.52% distributed in 13 sub regencies, and 48 endemic villages. DHF control through cleaning, cleaning of mosquito breeding sites has been conducted in Wonogiri, but it has not been done simultaneously, while the existence of water storage containers allowing for breeding of *Aedes aegypti* mosquitoes are still found in the community. The Wonogiri sub regency is one of the DHF endemic sub regencies among 13 other endemic sub regencies in Wonogiri regency. Cases are distributed in all villages in Wonogiri.

OBJECTIVES To know the relationship between education, occupation and water storage based on Maya Index with the occurrence of DHF in Wonogiri regency in 2010.

MATERIALS AND METHODS This research is an observational analytical one, with a case control design. The number of cases was 138 cases and the control is similar to the number of cases (1:1). The number of total cases is 278 samples. The analysis was done using bivariate analysis with Chi-square test.

RESULTS In multivariate analysis, houses of cases with high risk as breeding sites for mosquitoes compared to control houses showed that the water storage based on high Maya index is the dominant risk factor, with the risk being 3.613 bigger to get DHF than in a community with a low Maya index. Education and occupation are not risk factors of DHF.

CONCLUSIONS Water storage tanks based on high Maya index is the dominant risk factor for the occurrence of DHF.

KEYWORDS Maya Index, water storage, dengue hemorrhagic fever

1.4-081

Leishmaniasis outbreak in Fuenlabrada, Madrid (Spain)

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INTRODUCTION The number of new cases of Leishmaniasis has been steadily increasing in the South of Madrid (Area IX, around 200.000 inhabitants) since 2009. The aim of this study was the description of Leishmaniasis cases diagnosed in the Hospital of Fuenlabrada in the last 7 years.

METHODS We retrospectively reviewed all Leishmaniasis cases diagnosed in our hospital from June 2004 to May 2011.

RESULTS From June 2004 to June 2009, 16 Leishmaniasis cases were diagnosed in our hospital. However, 71 cases were detected until the beginning of May 2011. Forty-four were visceral Leishmaniasis (VL) cases, 36 male (82%), mean age 46.5 years, 16 were immigrants (36%), 33 immunocompetent (75%), and 5 HIV+ (15%). Forty-three were cases of cutaneous Leishmaniasis (CL), 21 male (51%), mean age 44.4 years. All VL cases showed fever, pancytopenia and splenomegaly, except two HIV+ patients who had no fever. Ten patients had cervical lymphadenopathies (ganglionar Leishmaniasis) and were diagnosed by fine needle aspiration puncture and microscopy. Bone marrow aspirate was performed in 34 VL cases: 13 cases were diagnosed by microscopy; 5 (31%) of 16 samples were positive by culture; and PCR was positive in 21 of 22 samples (95%). *Leishmania* antibodies were detected by IFAT in 27 patients (100%). All CL cases were diagnosed by PCR on skin biopsy. Thirty eight VL patients received specific treatment, three were lost, two died before starting treatment and one ganglionar case resolved spontaneously. Five patients relapsed, two of them were HIV+. Thirty six patients were cured.

CONCLUSIONS We have detected such a marked increase of Leishmaniasis cases in our hospital in the last 2 years that has been considered as an outbreak. Indeed the leishmaniasis incidence rate in Madrid Autonomous Community was 0.56/100.00 in 2010 while it was 14.5/100.000 in Fuenlabrada just in the first 15 weeks of 2011.

1.4-082

Survey of ectoparasites (Fleas) of *Rattus norvegicus* captured in Tehran, Iran

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INTRODUCTION Rodents play an important role as host of ectoparasites and reservoir of different zoonotic diseases. The aim of this study was the morphological identification of ectoparasite (Fleas) of *Rattus norvegicus* captured in different areas of Tehran.

METHODS AND MATERIALS Rodents were captured using live traps during the study period in year 2009–2010. After transferring the rodents to the laboratory, they were identified and then among them the *R. norvegicus* were selected and then their ectoparasites

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were collected and mounted for species identification using appropriate systematic keys.

RESULTS AND CONCLUSION A total of 150 rodents were identified including *R. norvegicus* (%83), *Rattus rattus* (%11/7) and *Mus musculus* (%5/3). *R. norvegicus* were selected for this study because 92% were infested with ectoparasites. A total of 628 ectoparasites were collected comprising mites (320), lice (214) and fleas (93). Lice were selected for morphology. Two species of flea; *Xenopsylla cheopis* and *Nosopsylla fasciatus* were identified with higher index of *X. cheopis*. Among all arthropods collected, mites and fleas were the most and the least common, respectively. The data showed that the ectoparasites on some rodent hosts tend to prefer particular host body sites, and that some ectoparasite species sites may overlap owing to their inaccessibility to the host. These arthropods are important due to their role in plague, CCHF and typhus transmission. Monitoring of ectoparaiste infestation is important for preparedness and early warning preparation for possible control of arthropod-borne diseases.

1.4-083

Infestation rate of sheep and goat of ticks during winter 2011 in Jiroft of Kerman province/Iran

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INTRODUCTION Ticks play a significant role as a vector of patogenes of domestic animals and they are considered as the main vector for transmission of various diseases to human being. Kerman province including Jiroft city is one of the most important husbandry south region of Iran. This study was conducted to determine tick infection rate of sheep and goat in Jiroft.

METHODS AND MATERIALS Sampling was performed season in four village through during winter only sheep and goat were selected and tested for tick infection. After collection ticks were identified by morphological characteristic using stereomicroscope device.

RESULTS AND CONCLUSION Tick infection was detected of 33 sheeps and also of 84 goat. Infection rate was seen in sheep 17/33 (51.5%) and also in the goat 29/84 (34.5%). In this study 265 ticks were collected on sheep and goat which were classified in *Ixodidae* ticks family. They belong to three genera, *Hyalomma* (2%), *Rhipicaphalus* (96.5%) and *Haemaphysalis* (1.5%). *Rhipicaphalus* was the commonest genus found, although we identified several ticks which cause diseases in sheep, goats and humans.

1.4-084

Climate change and dengue: analysis of historical health and environment data for Peru

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INTRODUCTION Dengue, a mosquito-borne virual infection that is the most common cause of hemorrhagic fever globally, is rapidly spreading worldwide. An estimated 40% of the world's population are at risk for this disease that is transmitted by *Aedes* sp. mosquitos. The *Aedes* mosquito-Dengue virus lifecycle varies with temperature, and climate change may increase the risk of Dengue epidemics in the future. We examined whether changes in sea surface temperature (SST) along the Peruvian coast were associated with dengue incidence from 2002 to 2010. In Peru the effects of the El Niño cycle on weather conditions are pronounced, providing an ideal place to study fluctuations in climate and dengue incidence.

METHODS We used negative binomial models to examine the relationship between Dengue cases and changes in SST across regions of Peru. Spearman's rank test was used to determine the lagged SST term that was most correlated with Dengue incidence in each region. The negative binomial models included terms for tthe optimum lagged SST and a term for the trend of increasing Dengue incidence over the study period.

RESULTS The magnitude and sign of the correlation coefficient of dengue and SST varied between the 15 regions of Peru with Dengue cases. Nine provinces had positive correlations between the two while six had negative correlations. The optimum lag ranged from 0 to 6 months. In all of the regions lagged SST was a significant predictor of Dengue cases in the negative binomial model.

CONCLUSIONS The relationship between dengue and sea surface temperature in Peru appears to be significant across the country. Given the varied nature of the relationship between regions it is not possible to make accurate generalisations about this relationship in Peru. Accounting for additional climatic variables such as precipitation may help in improving the predictive model.

1.4-085

Diagnosis and identification of *Leishmania* spp. from Giemsa-stained slides, by real time-PCR in South West of Iran

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INTRODUCTION Diagnosis and Identification of *Leishmania* species is important for prognostic, epidemiologic, and therapeutic reasons. The aim of present study was describing a real-time PCR assay for the diagnosis and direct identification of *Leishmania* species on Giemsa-stained slides without cultivation in South- west of Iran.

MATERIALS AND METHODS Altogether, 102 Giemsa-stained slides were collected from different part of south west of Iran between years 2008–2011. All the Giemsa stained slides were examined under light microscope. After DNA extraction, Real-time PCR amplification and detection were conducted with fluorescent SYBR Green I. For identification, PCR products were analyzed with melting curve analysis.

RESULTS One hundred two archived slides from suspected lesion examined by microscopy and real time-PCR. The sensitivity of the real time-PCR for Gimsa-stained slid was 98% (96/102) vs. 67% (68/102) for microscopy. The melting curve analysis (Tm) were, 88.3 ± 0.2 C for *L. tropica* (MHOM/IR/02/Mash10), 86.5 ± 0.2 C for *L. major*(MHOM/IR/75/ER) and 89.4 ± 0.3 C for *L. infantum* (MCAN/IR/97/LON 49) respectively.

CONCLUSION This study is first report in use of real time-PCR for diagnosis and identification of *Leishmania* spp in iran. Up to now in Iran, the majority of identification of *Leishmania* species are restriction fragment length polymorphism (PCR-RFLP) of ITS1 and kinetoplast DNA. Our data showed that Giemsa-stained slides that were stored more than 3 years, could be use for *Leishmania* DNA extraction and amplification by real time-PCR. Compared to conventional PCR-RFLP, the real-time PCR is extremely rapid with Results, and does not need to gels to run and more samples can be processed at one time.

KEYWORDS *Leishmania*, Giemsa-stained slides, real-time PCR, Iran

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1.4-086

Methicillin resistance, virulence and genetic lineages of nasal *Staphylococcus aureus* isolates of healthy farm animals in Tunisia

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OBJECTIVE To study the carriage rate, methicillin resistance, virulence traits and genetic lineages of nasal *S. aureus* of healthy farm animals in Tunisia.

METHODS Nasal swabs of 201 healthy animals (75 bovines, 74 donkey and 52 goats) were obtained in different farms and in one big abattoir that receives animals from all Tunisia. Samples were inoculated into Baird Parker and ORSAB plates for *S. aureus* and methicillin-resistant *S. aureus* (MRSA) recovery, respectively. Isolates were identified by biochemical methods and nuc-gene PCR. Antibiotic susceptibility profile was determined by disk diffusion. The presence of lukF/lukS genes (encoding Panton-Valentine leucocidin, PVL), lukE-lukD, eta, etb and tsst1 were studied by PCR. *S. aureus* isolates were typed: spa and agr.

RESULTS Forty-five *S. aureus* were detected (one/sample) from the 201 tested samples (22.4%), representing 1.3% in bovines, 45.9% in donkey and 19.2% in goats. All *S. aureus* were methicillin-susceptible. Twenty different spa-types were detected among *S. aureus* isolates (t127, t1534, t1326, t1773, t1268, t1784, t701, t2484, t2420, t1736, t3043, t166, t1403, t091 and t4735) including five new spa-types (t7717, t7718, t7720, t7721 and t8449). The more frequent spa-types were: t127 (four isolates of donkey origin), t1534 (four isolates of goat origin) and t701 (three isolates of donkey). *S. aureus* isolates were ascribed to agr type I (32 isolates), II (3), III (8), IV (1) and non-typable (1). Virulence genes carried were: tsst1 gene (12 isolates of goat and donkey), PVL genes (three isolates of goats).

CONCLUSIONS The nares of goats or donkey frequently harbour methicillin-susceptible *S. aureus* and they could be a reservoir of toxin genes encoding PVL or TSST-1, with potential implications in public health.

1.4-087

Insecticide resistance mechanisms in *Aedes aegypti* populations from localities with high dengue transmission risk in Guerrero, Mexico

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Dengue is one of the most important vector-borne diseases worldwide and is a serious public health problem in Mexico. Most dengue control programs in disease endemic countries rely extensively on insecticides to control dengue vectors. In Mexico, organochlorines, organophosphates and a range of pyrethroids have been used to control the vector *Aedes aegypti*. Point mutations on the target site of pyrethroids and increased metabolic activity were studied as insecticide resistance mechanisms in *Ae. aegypti* populations from six localities in Guerrero state, where the chemical control of vectors has historically been intense and there is a high risk of dengue virus transmission. *Ae. aegypti* eggs were collected between October and December 2009 from 250 ovitraps placed throughout neighborhoods in each locality. F1 generation *Ae. aegypti* from these localities were exposed to pyrethroid, carbamate and organophosphate insecticides using WHO bioassays. Biochemical assays were performed on F1 non-exposed mosquitoes and PCR and Hot Oligonucleotide Ligation Assays (HOLA) on mosquitoes that survived pyrethroid exposure. Bioassays showed high pyretroid and moderate organophosphate resistance. Elevated esterase activity was detected in five out of six localities, while elevated glutathione S-transferase was found in all localities. HOLA assays detected the existence of the V1016I mutation on domain II (IIS6) of the sodium channel gene, with an allele frequency of 0.80 for the whole area. The Fisher test (P = 0.0002) indicated that the resistant phenotype and the genotype homozygous for this mutation are highly related. A second mutation, F1534C on the IIIS6 domain, was identified in six survivors of pyrethroid exposure from five different localities by a tetraplex PCR and confirmed by sequencing. This represents the first report in Mexico of this mutation. These Results demonstrate cross-resistance to DDT and pyrethroids by kdr, and different resistance levels to multiple insecticide classes in *Ae. aegypti* from Guerrero state in Mexico.

1.4-088

Surveillance for mosquitoes (Diptera: Culicidae) in Madeira and Porto Santo Islands

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BACKGROUND Mosquitoes are important as vectors of malaria, various forms of filariasis and numerous arbovirus, the best known being Dengue, Yellow Fever and West Nile Virus. In addition, mosquitoes can be considered a nuisance factor for human populations due to allergic reactions caused by their bites and the pain caused by them.

METHODS During 2009 oviposition traps and artificial containers were examined for presence of mosquitoes immature stages in the cemeteries of both Madeira and Porto Santo Islands. The fieldwork took place between January and August 2009 and 53 traps were placed for the eggs (oviposition) detection, 395 observations were made and potential biotopes prospected. Bioecological data were recorded for each breeding place. The larvae and pupae were mountained in slides and observed at the microscopy. Adults were observed in the stereomicroscopy (When maintained in entomological pins) and in microscopy (the structures of the genitalia).

RESULTS Five species have been identified: Aedes aegypti, Aedes eatoni, Culiseta longiareolata, Culex pipiens and Culex theileri.

CONCLUSIONS There was a prevalence of *Aedes* eatoni in relation to other species. The distribution of mosquitoes has diverged with altitude, being more abundant between 200 and 300 m. In general, the containers with capacity up to 0.5 l, in the sun, were preferred by the immature stages. *Ae. aegypti* and species of *Culex* are vectors of the pathogenic agents of Yellow Fever and Dengue and of lymphatic filariasis, respectively. So, these two species may be considered in the control measures.

KEYWORDS mosquitoes, Ilhas da Madeira e de Porto Santo

1.4-089

Report of the first case of eosinophilic meningitis due to *Angiostrongylus cantonensis* diagnosed in São Paulo, Brazil

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Angiostrongylus cantonensis is natural parasite of the arteries of the lungs of mice that may cause in humans a neurological form of

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eosinophilic meningitis. We report the first case of eosinophilic meningitis caused by *A. cantonensis*, in São Paulo city, Brazil. F.A.M.D. M, 11 years old, male, resident of Jardim Marquesa, south, São Paulo. History of present illness: A child was admitted to the pediatric emergency room of a hospital, complaining of a headache for 3 days without fever or other complaints. Epidemiological history: Presence of snails, rodents around the homes and direct contact with dogs and cats. Background medical history: Lesions of nettle rash 20 days ago. Without pathological stunting. Physical examination: Child awakens, acyanotic, anicteric and hydrated. Regular respiratory rhythm, preserved breath sounds without riles. Regular cardiac rhythm without murmurs, with good peripheral perfusion. Flaccid abdomen and painless on palpation superficial and deep, without visceromegalies. He was lucid, oriented, responding to verbal requests, preserved muscle strength, with isochoric, pupil fotorreagentes, terminal neck stiffness, and Glasgow 15. Complete blood count: Red cells-5.04/μl, hemoglobin-14.1 g/dl, hematocrit-42.5%; 12,400/μl leukocytes; Rods-1%-segmented 39%, eosinophils-14%, basophil-1%, lymphocytes-40%, monocyte-55%; Platelets-393.000/l; C-reactive protein, 0.05 mg/dl. CSF analysis, appear clear, comprehensive cytology, 160/mm^2 leucocyte; neutrophyls-5%, lymphocytes-50%, eosinophils-36%, monocytes-09%, Protein-42 mg/dl; Glicose-54 mg/dl; Cloreto-679 mg/dl; Lactate-16.2 mg/dl. Culture negative for any bacteria. CT of brain without changes. Lookup larvae and nucleic acids in CSF negative. In serum samples collected 5 days after the onset of symptoms, the EIA test to IgG *A. cantonensis* was negative and in CSF sample it was indeterminate. The patient showed seroconversion after 135 days of symptoms. CSF was not examined in this period. Serology is the gold standard for diagnosis of the disease. Therefore, it was confirmed the first report of eosinophilic meningitis by *A. cantonensis* in São Paulo, Brazil.

1.4-090

Early containment of dengue epidemics in Mesoamerica

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INTRODUCTION The Salud Mesoamérica 2015 Initiative (SM2015) is an innovative, regional, public-private partnership between the Bill and Melinda Gates Foundation, the Carlos Slim Health Institute (ICSS), the Government of Spain, the Inter-American Development Bank (IDB) and the Mesoamerican countries. The main objective of the plan for Dengue Prevention and Integrated Control in Mesoamerica is to reverse the actual ascending trend and decrease the incidence of dengue by 50% in the demonstration areas over the next 5 years.

METHODS/INTERVENTIONS The selected demonstration areas for this project are along the border between Guatemala (Jutiapa, Chiquimula, Zacapa, Izabal) and Honduras (Ocotepeque, Copán, Santa Barbara and San Pedro Sula). The population in this demonstration area is about 3,500,000 inhabitants. The proposal of intervention is based on the concept that interventions carried out during the early stages of transmission in the highest risk areas will minimize and contain dengue transmission within the region. The pillars of the proposal are: (i) Reorganization of the national programs to strengthen their strategic performance (ii) Improvement the sensitivity, specificity, representativeness and opportunity of the epidemiologic surveillance systems within the region. (iii) Improvement of the diagnostic laboratory capacity in the region. (iv) Ensuring proper clinical case diagnosis and management to maintain the case fatality rate for severe dengue below 1% by implementing an effective clinical management plan. (v) Improvement the effectiveness of integrated mosquito control over the 5 year project period. (vi) Development of capacity building interventions to upgrade skills and competencies of health personnel (vii) Evaluation of the impact of interventions designed to prevent dengue and improve vector control.

CONCLUSION The proposal is based on the concept that interventions carried out during the early stages of transmission in the highest risk areas will minimize and contain dengue transmission within the region. This project will provide evidence on whether it is possible to control dengue transmission across Mesoamerica with the current available tools.

1.4-091

Evaluation of repellency effect of essential oils of *Satureja khuzestanica* (carvacrol), *Myrtus communis* (myrtle), *Lavendula officinalis* l. and *Salvia sclarea* l. using standard who repellency tests

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INTRODUCTION We evaluated the repellency effect of essential oils of four plants.

METHODS AND MATERIALS Standard WHO repellency tests were used. 10%, 20% and 40% concentrations of *Satureja khuzestanica* (carvacrol), *Myrtus communis* (myrtle), *Lavendula officinalis* L. and *Salvia sclarea* L. were compared with traditional repellents (Deet, 50% diethyl toluamide) and 33% diethyl toluamide and positive control (pure ethanol, 99.6%). Different concentrations of essential oils of plants were produced separately. The essential oils were rubbed on forearms from elbow to wrist; the hand was protected by a glove. The whole forearm was exposed (45 s) in a mosquito cage with 50, 2 or 3-day-old unfed *An. stephensi* females, and the number of bites recorded. This procedure was repeated three times. Negative controls were naked hands and positive controls were hands rubbed with pure ethanol. All tests were repeated after 1, 2, 3, 4 and 5 h.

RESULTS Essential oil of *Satureja khuzestanica* had side effects on skin so it was omitted from tests. Negative and positive controls experienced the maximum of bites and users of essential oils and chemical repellents experienced the minimum of bites over time. The number of bites was reduced significantly with the negative ($P = 0.011$) and positive ($P < 0.001$) controls. In case of *Lavendula officinalis* L. ($P = 0.001$), *Salvia sclarea* L. ($P = 0.04$) and *Myrtus communis* ($P = 0.011$), the number of bites increased significantly over time.

CONCLUSIONS Essential oils of *Myrtus communis* (*myrtle*), *Lavendula officinalis L.* and *Salvia sclarea* L. have repellency effects on *An. stephensi* that are comparable chemical repellents. With increasing time the repellency effect of essential oils of plants was not reduced.

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1.4-092

First case of human Dirofilariosis in Tarragona (Spain)

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INTRODUCTION Dirofilariosis is a parasitic zoonosis transmitted by cullicid mosquito with a cosmopolitan distribution. Dogs and cats act as reservoirs being affected with the cardiac-respiratory clinical form (Dirofilaria immitis) or subcutaneous clinical form (*D. repens*). Human could be affected by immature forms of these species developing pulmonary nodules (pulmonary dirofilariosis) or subcutaneous nodules (subcutaneous dirofilariosis), respectively.

CLINICAL CASE Woman 64 years old origin from Amposta (Ebro Delta, Tarragona, Spain), area where prevalence of canine dirofilariosis is of 26%. She consults her physician because of a cutaneous nodule in the right paracilliar region. The rest of physic exploration and analytic exams are negative. Further to the surgical extirpation, pathologic exam evidenced some images corresponding to adult worms of Dirofilaria and confirmation of *Dirofilaria repens* was showed later on the study. Thoracic CT didn't demonstrate any pulmonary nodules. No previous travels reported. Frequent contact with dogs was referred by the patient. Serological study was carried out to detect the presence of IgG antibodies against *Dirofilaria*, but specie was not identified. PCR with specific primers was not done due to the inability of biopsy material for this test.

CONCLUSION *Dirofilariosis* should be introduced in the differential diagnosis of human pulmonary and subcutaneous nodules in canine endemic areas. It's confirmed the risk of infection by *D. repens* in people living in the Mediterranean peninsular coast.

1.4-093

Influence of intestinal parasitism and environmental conditions on childhood nutritional status in a periurban and rural area in La Paz, Bolivia

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INTRODUCTION Intestinal parasites and malnutrition cause high morbidity among children in developing countries. Mechanisms for the effect of parasitic infections in children include: (i) decreased appetite due to infection, (ii) decreased food intake, and (iii) depleted micronutrients. This can lead to a decreased growth rate, as well as decreased activity and school performance. This work considers the impact of intestinal parasitism and childhood environment on nutritional status in a periurban and rural area in La Paz, Bolivia.

MATERIALS AND METHODS: A total of 118 children 0–5 years of age from three periurban and rural areas in La Paz (Bolivia) were included. The sample enrolled was stratified to include 65% from a periurban area and 35% from a rural area. Data was collected on: environmental information, anthropometric measurements and one stool specimen for parasitological examination. Nutritional status was assessed by weight-for-height (WFH) and height-for-age (HFA). Data was analyzed using chi square tests, and logistic regression for multivariate analysis using the SPSS 18.0.

RESULTS The prevalence of malnutrition was 31.1%; malnutrition based on WFH was 11.3% (acute-malnutrition) and 19.8% based on HFA (chronic-malnutrition). The total prevalence of intestinal parasite was 65.1%, with a higher prevalence of Blastocystis hominis (41.5%), Giardia lambia (21.7%) and Hymenolepsis nana (19.8%). Intestinal parasites were more commonly found among children with malnutrition (63.1%) than those well-nourished (27%) (2 = 11.343, *P*-value = 0.010). The factors associated with malnutrition were: human waste disposal (other destination *vs.* septic tank) [OR: 4.52; (CI 95%:1.50–15.82)], home construction material (adobe material *vs.* brick) [OR: 3.19 (CI 95%:1.13–8.99)], and water treatment (no treatment *vs.* boiled water) [OR: 4.42 (CI 95%:1.77–11.03)].

CONCLUSIONS The prevalence of global malnutrition and intestinal parasitism in the rural Andean areas are relatively high and independently of the characteristics of the housing and the level of hygienic conditions, malnutrition is associated with intestinal parasitism.

1.5 Diarrhoeal Diseases

1.5-001

Diagnosis of *Clostridium difficile* from patients with diarrhea

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BRIEF INTRODUCTION *Clostridium difficile* is an important pathogen associated with outbreaks of pseudomembranous colitis and other intestinal disorders, such as diarrhea. In this study, the prevalence of *C. difficile*, from patients with diarrhea was investigated.
Methods and Materials

Enterotoxin and cytotoxin (toxin A and toxin B) of *C. difficile* on the patient's stool samples were detected by a double sandwich enzyme-linked Immunosorbant assay technique using a commercial kit (Premier toxins A & B; Generic Assays, Inc., Germany).

RESULTS AND CONCLUSIONS Out of 98 patients with diarrhea the results of *C. difficile* were positive for nine patients (9.2%) according to the results of *C. difficile* antigen kit. More recently, enzyme-linked immunosorbent assays (ELISAs) have been developed to detect toxin A and/or B in stool. These assays detect 100–1000 pg of either toxin and have a sensitivity of 66–94% and a specificity of 92–98%. Because of the rapidity of testing and ease of performance, ELISAs for toxin A and B are now used most frequently by clinical laboratories for diagnosis of *C. difficile* infection. Because toxin A negative, toxin B-positive strains of *C. difficile* may cause clinical illness, an ELISA for detection of both toxins in clinical specimens should be used.

1.5-002

Molecular investigation of *Cryptosporidium* spp. among children with diarrhea

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BRIEF INTRODUCTION At present 18 *Cryptosporidium* species are identified as valid but *Cryptosporidium parvum* and *C. hominis* are the common species in human. The aim of this study was to establish the genotypes of *Cryptosporidium* spp. among children with diarrhea using the TRAP-C2 gene.

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METHODS AND MATERIALS Fecal samples were collected from 1263 children <12 years with diarrhea. After determine the presence of *Cryptosporidium* oocysts by Ziehl-Neelsen acid fast staining, genomic DNA was extracted of positive samples and nested PCR-RFLP was performed to amplify the TRAP-C2 gene.

RESULTS AND CONCLUSIONS Out of 1263 samples, *Cryptosporidium* oocysts were found in 31 (2.5%) sample. RFLP analysis showed *C. parvum* in 25 (80.6%) isolates, *C. hominis* in 5 (16.1%) and mix infection pattern of both *C. parvum* and *C. hominis* in 1 (3.2%). In conclusion the use of TRAP-C2 primers could be sensitive enough to conduct a routine detection study. The nested PCR method using the TRAP-C2 gene sequence can be an alternative diagnostic method to identify human infected with *Cryptosporidium* and its genetic diversity.

1.5-003

Imported cholera case serogroup O1 from Thailand

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OBJECTIVES Two imported cholera cases in Slovenian citizens were identified in 1977 and 2005. Disease risk for tourists traveling to endemic countries is extremely low as long as basic preventive measures are respected. In January 2010 imported cholera case was detected in Maribor, Slovenia.

METHODS Clinical and epidemiological characteristics of cholera case are described as well as preventive measures that have been taken.

RESULTS Thirty-six years old healthy man developed acute onset of nausea, vomiting, abdominal cramps and watery diarrhea on January 25. Approximately 30 h later he sought medical care in a regional hospital with clinical signs of dehydration, but without abnormal laboratory findings. He was managed as an outpatient, treated for dehydration and sent home without antibiotics. Microbiological laboratory isolated *V. cholera* on January 30. Further testing identified the isolate as *V. cholera* serogroup O1, biotype El Tor and serotype Ogawa. Epidemiological investigation revealed that patient traveled in Thailand in small group of five tourists from January 9 to January 24. They were all without any health problems during travel and no others co-travelers developed clinical signs of illness after the travel. They spent the last day in Bangkok and two days previous in Phi Island. They consumed various types of food and drinks, including shellfish, fruit juices and drinks with ice in local restaurants. Duration of illness was 4 days. On February 1, *V. cholera* test was negative. No secondary cases occurred among family members or coworkers. All co-travelers were contacted and informed about the disease. Stool cultures taken from co-travelers tested negative for *V. cholera*.

CONCLUSION Imported cases of cholera are rare, but information how to prevent the disease is still very important for travelers to cholera endemic areas.

1.5-004

Study of *Giardia* and *Cryptosporidium* oocysts in drinking water (with surface water sources) of Hamadan, West Iran

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OBJECTIVES *Giardia* and *Cryptosporidium* are two important protozoa that have water-borne transmission basically. There are many reports of vast epidemics through drinking water contamination. Different methods developed and performed for parasite elimination from drinking water but much of them had not complete efficacy. Instant surveillance of drinking water is a necessitate measure to provide human health. This study evaluated the efficacy of treatment system of drinking water of Hamadan.

MATERIALS AND METHODS Samples of 400 l were taken from two major sources of drinking water (Ekbatan dam and Shahid Beheshti), before and after treatment, and six water storage as well. The water samples filtered through foam filter with 1 μm porosity. Concentrate materials backwashed from filters and samples were examined by fluorescent staining by a dual kit for *Giardia/Cryptosporidium*. Viability examined by eosin vital dye staining.

RESULTS Mean number of *Giardia* cysts in untreated water of Ekbatan dam was 2.5/100 l and *Cryptosporidium* oocysts were 1.5/100 l. Of these, 0.5 cysts and 0.25 oocysts per 100 l were viable. Mean number *Giardia* cysts and *Cryptosporidium* oocysts in this water after treatment were 0.25/l or 1 cyst per 400 l. None of the detected cysts or oocysts were not viable. Almost all water samples from six main water storage tanks of the city contained *Giardia* cysts and *Cryptosporidium* oocysts but they were not viable.

CONCLUSION Cysts and oocysts of two major intestinal pathogens exist in drinking water of Hamadan. Although water treatment did not eliminate parasites, detected cysts were not viable and probably infection transmission to humans is very low.

KEYWORDS Giardia, Cryptosporidium, drinking water

1.5-005

Detection of *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. enteropathogens by real-time multiplex PCR

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BACKGROUND Infectious diarrheas can be classified, based on their clinical presentation, into non-inflammatory and inflammatory diarrheas. Among cases of inflammatory diarrhea *Shigella* is the most common cause, followed by *Salmonella*, and *Campylobacter*. Because the time frame in which treatment choices must be made is short, there is a need for a rapid, sensitive, and inexpensive detection technique. The purpose of our study was to develop a multiplex real-time PCR procedure to simultaneously identify *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. enteropathogens.

METHODS We have analyzed 65 enteropathogenic strains: Campylobacter spp. (20), Salmonella spp. (15), and Shigella spp. (20), as well as other enteropathogens (10).

DNA was extracted by boiling the bacterial suspension, and was stored at -20°C until the PCR analysis. Primers were designed to amplify invA, ipaH, and 16SrRNA simultaneously in a single reaction to detect Salmonella spp., Shigella spp., and Campylobacter spp., respectively.

RESULTS 54/55 strains of the targeted enteropathogens, and 10/10 of the other pathogens were correctly identified using this approach. The multiplex real time PCR showed a specificity of 100% and a sensitivity of 98% as compared to standard culture. The melting temperatures™ for *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. were 82.96°C (±0.05), 85.56°C (±0.28) and 89.21°C (±0.24), respectively.

CONCLUSIONS This assay is a simple, rapid, inexpensive, and reliable system for the practical detection of these three enteropathogens in clinical specimens. It is practical for use in clinical settings in both developed and many underdeveloped areas.

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KEY WORDS multiplex real time PCR, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp

1.5-006

Protection against diarrhoea associated with asymptomatic giardiasis is lost with multi-nutrient supplementation: a prospective study among rural Tanzanian children

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BACKGROUND Asymptomatic infections with *Giardia lamblia* are common among children in developing countries, but the role of giardiasis as cause of diarrhea in such settings has been questioned. Impaired linear growth and cognition have been associated with giardiasis, presumably mediated by malabsorption of nutrients.

AIM In a prospective cohort study, we aim to compare rates of diarrhea in pre-school children with and without *G. lamblia* infection. In addition we assessed how micronutrient supplementation influenced the relationship between *G. lamblia* and diarrhea rates, and to what extent *G. lamblia* modifies the effect of supplementation on nutritional status.

METHODS Data were collected in the context of a randomized placebo-controlled trial with 2 × 2 factorial designs assessing the effects of multi-nutrients (with or without zinc) on morbidity. Children (*n*-612, aged 6–59 months and height-for-age *z*-score <1.5 SD) from a poor rural area were followed for at least 7.4 months after enrolment. Outcomes measures were episodes of diarrhea (any reported, or with >3 stools in the last 24 h) and fever without localizing signs, as detected by clinic based surveillance. *G. lamblia* was detected in stool samples by ELISA. Multivariate Cox regression analysis was used to compare disease rates between groups, and to assess interaction effects.

RESULTS Asymptomatic *G. lamblia* infection was associated with a substantial protection against diarrhea (HR 0.32; 0.15–0.66), but only so among children who did not receive multi-nutrients; no such protection was observed among children who received multi-nutrients (P-value for interaction between *Giardia* and multi-nutrients 0.03, after adjustment for age, HAZ-scores and distance to the dispensary).

CONCLUSIONS/INTERPRETATION Although causality of the *G. lamblia*-associated reduction in morbidity cannot be established, the data also show that multi-nutrient supplements neutralize this protection and are thus likely to influence the proliferation of virulence of *G. lamblia* or associated intestinal pathogens.

1.5-007

Trichinella spiralis paramyosin protects the tissue-dwelling nematode from being attacked by host complement

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INTRODUCTION Paramyosin is a thick myofibrillar protein found exclusively in invertebrates. Evidence suggests that paramyosin from helminths serves not only as a structural protein but also as an immunomodulatory agent. We previously reported that recombinant *Trichinella spiralis* paramyosin (Ts-Pmy) elicited a partial protective immunity in mice. In this study, the ability of Ts-Pmy to bind host complement components and protect against host complement attack was investigated.

METHODS AND FINDINGS In this study, the transcriptional and protein expression levels of Ts-Pmy were determined in *T. spiralis* newborn larva (NBL), muscle larva (ML) and adult worm developmental stages by RT-PCR and western blot analysis. Eexpression of Ts-Pmy at the outer membrane was observed in NBL and adult worms using immunogold electron microscopy and immunofluorescence staining. Functional analysis revealed that recombinant Ts-Pmy rTs-Pmy) strongly bound to complement components C8 and C9 and inhibited the polymerization of C9 during the formation of the membrane attack complex (MAC) rTs-Pmy also inhibited the lysis of rabbit erythrocytes (ER) elicited by an alternative pathway-activated complement from guinea pig serum. Inhibition of native Ts-Pmy on the surface of NBL with a specific antiserum reduced larvae viability when under the attack of complement *in vitro*. *In vivo* passive transfer of anti-Ts-Pmy antiserum and complement-treated larvae into mice also significantly reduced the number of larvae that developed to ML.

CONCLUSION These studies suggest that the outer membrane form of *T. spiralis* paramyosin plays an important role in the evasion of the host complement attack.

1.5-009

Challenges for vector-borne disease mathematical models-density-dependent host choice by onchocerciasis vectors

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The ability of mathematical models to predict intervention impact against vector-borne diseases will be affected by whether the proportion of bloodmeals taken on humans (the human blood index or HBI) depends on vector and host density. Current models of vector-borne diseases (VBDs) assume that the HBI is fixed in a particular environment and therefore predict that the case reproduction number (Ro) of these infections varies linearly with vector abundance. A non-linear relationship would imply that efforts to control VBDs by antivectorial measures could have unforeseen effects on the ability of the parasite to invade and persist in host populations. Five study sites in three regions of Ghana have been visited since 2009 in both the rainy and dry seasons, to obtain data on variation in blackfly and host densities and host choice by onchocerciasis vectors. Surveys for wild birds and mammals, households and their domestic animals were conducted. Blackflies were collected at host-independent (oviposition) and host-dependent (host-seeking) sites, assessed for parity status, and stored for molecular and morphological analysis for fly species-, Onchocerca- and past bloodmeal-identification. Daily biting rates by black flies ranged from 0 to 298 bites/person/day. In total 3617 flies were collected on oviposition traps; 127 in light traps; 2048 and 1455 in, human-baited and cow-baited traps, respectively, and 5875 by vector collectors. Detailed human, domestic and wild animal population censuses, bloodmeal analyses and Onchocerca infection prevalence and intensities will be presented and discussed in the context of vector and blood-host density and potential impacts on ivermectin treatment and challenges towards the potential elimination of onchocerciasis.

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Abstracts of the 7th European Congress on Tropical Medicine and International Health

1.5-010

Frequency of *Cryptosporidium* infection in cattle and buffalo in some rural regions of shoshtar, Khozestan- Iran

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INTRODUCTION *Cryptosporidium* circulates in domestic and wild animals; however, the significance of this fact for infection of humans is unclear.

MATERIAL AND METHODS Sixty-seven fecal specimens from cattle, buffalos and their calves were collected randomly. *Cryptosporidium* oocysts were identified by using Sheather's and formalin-ether Concentrate techniques and following by Ziel-Neelson modified staining.

RESULT 62.67% of samples were positive for *Cryptosporidium* oocysts by two methods.

DISCUSSION In view of the high rate of ruminant *Cryptosporidium* infection in the region, the project high- lights the importance of the domestic and wildlife animals as reservoir populations for humans. Further research is needed to give an overview of infection prevalence in humans and to study interaction between them and animals.

KEYWORDS *Cryptosporidium*, cattle, buffalo, frequency

1.5-011

Investigation of *Cryptosporidium* oocysts in elementary school children in Gorgan, North of Iran

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INTRODUCTION *Cryptosporidium* is one of the most common causes of diarrhea all over the world and can causes fatal disease in children and immune compromised people. The present study was undertaken to know the infection status of cryptosporidiosis among children of elementary school in Gorgan city, located in the north of Iran.

METHODS AND MATERIALS Stool specimens were collected from children, 7–12 years in age during a period of 6 months between October 2010 and March 2011. Fecal smears were prepared by formalin-ether sedimentation, and examined after modified Ziehl Neelsen staining. The smears were observed by light microscope (1000X) for the presence of *Cryptosporidium* oocysts.

RESULTS Cryptosporidium oocysts were observed in the specimens of 56 (6.9%) of 801 children. The prevalence rate of infection indicated a significant correlation (P-value < 0.5) between diarrheic (61%) and non diarrheic (39%) children.

CONCLUSIONS The results indicate that *Cryptosporidium* sp. infection is prevalent in schoolchildren in Gorgan. Since there are no effective drug therapies for cryptosporidiosis, prophylaxis is most important. So, the source of infection should be investigated and proper control strategies established. But, before that, an epidemiological survey is needed for the exact understanding of infection status of *C. parvum* all over the Iran.

1.5-012

Development and validation of a PCR assay for diagnostic of diarrheagenic *Escherichia coli*

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Escherichia coli is the most important etiologic agent of childhood diarrhea and represents one of the major public health problems. Diarrheagenic *E. coli* (DEC) are classified into six groups: enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAgEC), and diffusely adherent *E. coli* (DAEC). These groups of DEC are differentiated by the mechanisms of their virulence. The aim of the study was to elaborate the method for detection of virulence factors of DEC. Therefore, multiplex PCR assay based on end-point and real-time hybridization-fluorescence detection for determining and differentiation five groups of DEC in clinical material ['AmpliSens® Escherichioses-FRT (FEP)'] was developed. Target genes were selected as follows: eae for the EPEC; stx1, stx2 and eae for EHEC; aggR for EAgEC; LT gene for ETEC; ipaH for EIEC and Shigella spp. The sensitivity was estimated as 3*102 – 3*103 CFU per 1 ml/1 g of the feces. The specificity of PCR kit was confirmed by examination of human DNA as well as a DNA panel of the following microorganisms: 30 *E. coli* strains and 36 strains of other microorganisms. Sequence analysis of PCR positive samples did not revealed any nonspecific amplicon. To demonstrate the diagnostic usefulness of PCR kit, we examined stool specimens collected from patients of 0–14 years old admitted in Moscow children's hospital of infectious diseases. EPEC were detected in 196 of 2006 samples (9.77%), EAgEC – 121 samples (6.03%), EHEC – 27 samples (1.35%), ETEC – 24 samples (1.2%) and EIEC and Shigella spp – 21 samples (1.05%). In conclusion, PCR kit described here is suitable for routine diagnostic of diarrheagenic *E. coli* in clinical laboratories.

1.5-013

Diarrhea incidence and its treatment in a representative sample of Albanian children under 5 years old

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BACKGROUND Until recently diarrhea was a very frequent disease in Albania and a significant cause of infant mortality. During the last decade, the Albanian health system has put a lot of effort in child health and specificillay management of diarrhea cases.

METHODS Data are gathered in the framework of Albania Demographic and Health Survey 2009, using a representative sample of almost 9000 households. Closed questions on frequency of diarrhea and diarrhea with blood during two weeks preceding the survey as well as details on treatment were asked to mothers about their children under 5 years old (1550 cases).

RESULTS Incidence of diarrhea is much lower compared with the figures reported one decade ago; only 5.3% of children had diarrhea and 0.3% had diarrhea with blood. The incidence decreased with age. Sex, geography, household wealth was not statistically associated with increased risk. The only strong statistical association was mothers education; children of mothers with no education or only primary education had twice more risk to have diarrhea compared to children of mothers with secondary, professional or university education (P = 0.004). Sixty percent of children with diarrhea were taken to a health facility. Almost 40% of the cases were treated with antibiotics. Some bad practices remain: 26% of mothers reduced the amount of fluids given to children and in 29% of the cases the amount of food given was

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reduced. Knowledge of mothers about ORT is high (more than 80%), it is highest in Tirana and among highly educated mothers.

CONCLUSIONS Diarrhea incidence among Albanian children has decreased for a combination of factors including a fall in fertility rates and diarrhea control and management interventions in the health system. Despite the advances, more efforts are needed, especially in control of the antibiotic use and other bad practices.

1.5-014

Etiological investigation of diarrheal diseases in the suburbs of Nairobi, Kenya

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An etiological investigation of diarrheal diseases was carried out on the children of under 5 year of age in the surrounding area of Nairobi in Kenya from 2008 to 2009. Diarrheal stool samples were collected from those who visited health facilities complaining of diarrhea at least more than three times a day. Samples from healthy controls which correspond to the diarrheal cases were collected from children of same age and sex living in the same area after confirming no diarrhea for a week. All samples were examined for etiological agents causing diarrhea such as enteropathogenic *Escherichia coli*, *Campylobacter* sp and Rotavirus, as well as *Vibrio* sp., *Shigella* sp. and *Salmonella* sp. For the detection of enteropathogenic *E. coli*, multiplex PCR was applied. Isolation rates among the two groups were statistically analyzed by McNemar test. A total of 595 paired samples were analyzed bacteriologically and molecular biologically. Classical diarrheagenic pathogens like *Shigella* sp. *Salmonella* sp. and *Vibrio cholerae* were not isolated in this study. The detection rate of Rotavirus among diarrheal cases and control group was 169 (28.4%) and 1 (0.17%). Isolation rates of enteropathogenic *E. coli*, enterotoxigenic *E. coli* and enteroaggregative *E. coli* from diarrheal cases and control group were 14 (2.4%) and 16 (2.7%), 37 (6.2%) and 38 (6.3%), 88 (14.8%) and 111 (18.7%), respectively. McNemar test revealed that only Rotavirus is closely related with occurrence of. In the suburbs of Nairobi, a major causative agent of diarrheal diseases was Rotavirus and classical enteropathogens seemed to be no longer major diarrheagenic agents. The significance of enteropathogenic *E. coli*, enterotoxigenic *E. coli* and enteroaggregative *E. coli* as diarrheagenic agents seems unclear.

1.5-015

Prevalence of *Hymenolepis nana* in an area of high immigration

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Hymenolepis nana is the most common cestode in humans of the world, especially among children. It is widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene in temperate zones and tropical regions. In Spain, it is found in stools of foreign people (especially in children coming from Sahara for summer). The Poniente Hospital attends to a population with a high rate of immigration from developing countries; therefore the number of isolated parasites is high.

OBJECTIVE The purpose of this study was to carry out the prevalence of intestinal infestation in humans by *H. nana* over the last 10 years (2001–2010).

MATERIALS AND METHODS We performed a retrospective study of all patients with *H. nana* eggs over a 10-year period (2001–2010). Stool specimens were examined using Ritchie biphasic concentration method and microscopy examination.

RESULTS During a survey from January 1st 2001 to December 31(st) 2010 a total of 46,476 inpatients and outpatients, were evaluated in our Hospital. Eggs of *H. nana* were visualized in the faeces of 118 (0.25%) patients, 90 (76.3%) males and 28 (23.7%) women. 115 (97.4%) of foreign origin and 3 (2.5%) of Spanish origin. The mean age was 16.5 years (7 months–47 years) of whom 59 (50%) were children (<15 years), 54 (45.8%) were adults and 5 (4.2%) with unknown data. The prevalence in years was: in 2001 (0.33%), 2002 (0.04%), 2003 (0.04%), 2004 (0.17%), 2005 (0.13%), 2006 (0.30%), 2007 (0.55%), 2008 (0.29%), 2009 (0.23%) and 2010 (0.30%) Isolates in months in different years were: 001 (one case in May, one in June and one in October), 2002 (one case in July), 2003 (one case April), 2004 (one case in June, three in July, one in August and one in October), 2005 (one in January, one in April, one in June, three in July), 2006 (one in April, one in May, five in June, three in July), 2007 (one in January, two in may, 13 in July, three in August, three in October, one in November and two in December), 2008 (two in February, two in March, one in April, one in May, one in June, 12 in July, one in August, two in September, one in November), 2009 (one in January, two in February, three in March, one in April, one in May, three in June, three in July, one in August, three in October, one in November, one in December) and 2010 (three in January, two in February, two in April, one in May, six in June, five in July, two in October and one in November).

CONCLUSIONS There was a significant increase in the percentage of *H. nana* in our population due to the large increase of immigrants in recent years There is a large percentage of isolates in adults. The highest percentage of isolates occurred in the summer months coinciding with stays in the Sahara.

1.5-016

Analysis of the mechanisms of resistance to azithromycin in clinical isolates of *Escherichia coli* and *Shigella* spp. isolates from Lima, Peru

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INTRODUCTION Infant diarrhea is a serious problem in periurban areas of Lima, where diarrheogenic *Escherichia coli* and *Shigella* spp . showing high levels of resistance to selected antimicrobial agents, rank among the main etiological causes. Azithromycin (Azm) has been proposed as an alternative treatment. The aim of this study was to analyze the mechanisms of resistance to Azm in *Escherichia coli* and *Shigella* spp. isolates from Lima, Peru.

METHODS The Minimal Inhibitory Concentration (MIC) to Azm was established in 68 clinical isolates (59 *E. coli* and nine Shigella spp.) with an Azm halo disk diameter ≤15 mm. The role of efflux pumps was determined establishing the MIC levels in presence of Phe-Arg-β-Naphtylamide (PAβN) an efflux pumps inhibitor, while the presence of transferable mechanisms of resistance (ereA,ereB and ermB) and point mutations in rplD and rplV genes was determined by PCR and sequencing. Plasmids were classified within 18 Inc-groups by PCR-based replicon type.

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RESULTS The analyzed MICs of Azm ranged between 32 and >25 6 mg/l among the *E. coli* isolates and between 4 and 8 mg/l for Shigella spp. When PAβN was added the MICs levels decreased from 1 to 8 folds. The ereA (3.2%), ereB(37%) and ermB(6.5%) were only present in *E. coli*. In seven strains of *E. coli* the substitutions in rplV gene were found: L46-Q, K82-N, D94-H, K98-N, S101-T and I103-L. Further the substitution P80-S was found in two Shigella spp.. Both in *E. coli* and Shigella spp. isolates the most prevalent plasmids found were F and K (75% and 43% of isolates, respectively).

CONCLUSIONS PAβN-inhibible efflux pumps play a role in development of resistance. ereA, ereB and ermB were found in Azm resistant *E. coli*. The possible association between detected mutations in rplV gene and resistance to Azm may not be ruled out. Finally, the Azm seems to be a good alternative to treat diarrhea in Lima.

1.5-018

Prevalence of Giardia duodenalis genotypes from cattle in Qazvin Province, Iran

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Giardia duodenalis is a protozoan parasite commonly infected cattle and human. Some assemblages of this parasite have zoonotic importance. This study was undertaken to determine the prevalence of G. duodenalis genotypes in dairy and beef cattle in Qazvin province, Iran. During January to September 2010, two hundred faecal samples were collected from cattle between 16 days and 56 months old. The samples were subjected to DNA extraction after sucrose gradient purification. A fragment of the glutamate dehydrogenase gene (gdh) was amplified by nested PCR and analyzed by restriction analyses. The prevalence of G. duodenalis infection, ranged from 12.5% to 100%, with an average prevalence of 58.5% (117/200) among 12 farms. G. duodenalis was identified in 75 (58.1%) of 129 beef cattle and 42 (59.2%) of 71 dairy cattle. Restriction analyses revealed the presence of Assemblage A, B and E, G. duodenalis. Of the 117 isolates examined, 42 (35.9%) were typed as assemblage E, 16 (13.7%) as assemblage B, three (2.6%) genotype AII and one (0.85) genotype AI. The remaining 55 (47.0%) isolates were typed as mixed infections, double infections of E + AI, E + AII, E + B, AII + B, AI + AII was found in 33, five, nine, two and one isolates, respectively. Triple infections were revealed in four (E + B + AI) and one (E + AI + AII) isolates. Forty-six (61.3%) of 75 beef cattle and 29 (69.0%) of 42 dairy cattle harbored zoonotic assemblages of G. duodenalis. Host-specific parasite (assemblage E) infection was found in 36% of beef cattle and 31% of dairy cattle. Molecular analysis of the Giardia gdh gene showed the presence of zoonotic assemblage of the parasite in a majority of cattle (64.1%), suggesting that cattle should be considered as a potential source of human infectious and environment contamination also as possible reservoirs of infectious parasites by exposure to infected animals and their products, especially their faeces.

1.5-019

Evaluation of three diagnostic methods for Strongyloides stercoralis

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INTRODUCTION Direct diagnosis of Strongyloides stercoralis is based on identification of larvaes in faeces by microscopy or enriched culture, while indirect diagnosis detects IgG in the serum of the infected patient. However, there is not gold-standard method for the diagnosis.

OBJECTIVES Evaluation of microscopy and culture against serology for the diagnosis of S. stercoralis.

METHODS & MATERIALS One hundred and thirty suspected S. stercoralis patients attending our tropical medicine unit between January and December 2010 participated. Microscopic examination of fresh faeces and after concentration was performed in 128 patients. Culture of stools was made in 72 patients; plates were incubated during a week, and presence of larvaes was determined by tracks of bacterial colonies founded. IgG detection through ELISA was performed in 86 patients.

RESULTS In 60 (46%) patients a positive result was found by one of the methods, 70 (54%) were found negative. Serology gave 49 (75%) positive culture 25% and microscopy 22.6%. Concordance of techniques was evaluated: 8 (13%) patients were positive by the three methods; 10 (16.4%) were positive by microscopy and culture, and same number by microscopy and serology; 31 (51%) were only diagnosed by serology, and 1 (1.6%) only by microscopy. One limitation of the study is that culture and serology were not performed in the same number of patients than microscopy. All negative results by serology were also found negative by microscopy and culture, except one case that was considered as false negative of serology.

CONCLUSIONS Although serology was the as only diagnostic technique used for S. stercoralis, it detected a large number of cases. We recommend a combination of direct and indirect methods to reduce the number of false positive Results of serology because of cross reaction, and false negative Results of microscopy and culture.

1.5-020

Rifaximin susceptibility levels among diarrheogenic and commensal *Escherichia coli* from children under 2 years of periurban areas of Lima, Peru

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INTRODUCTION Antibiotic-resistance among diarrheogenic *Escherichia coli* (DEC) in periurban areas of Lima is a serious problem. Rifaximin (Rfx) is active against DEC with a CMI50 usually around 8 mg/l. The aim of this study was to analyze the *in vitro* activity of Rfx against DEC and commensal *Escherichia coli* in children <2 years.

METHODS We studied 120 strains of *E. coli*: 91 DEC and 29 commensal (non-DEC – from healthy children). Diarrheogenic character was established by multiplex Real-Time PCR. Minimal

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Inhibitory Concentration (MIC) was determined by dilution agar method in presence and absence of Phe-Arg-¦Â-naphthylamide(-PA¦ÂN), an efflux pump inhibitor.

RESULTS The CMI to Rfx ranged between 8 and >256 mg/l (DEC) and 32 and >256 mg/l (commensals). In the presence of PA¦ÂN the MIC decreased to values between 2 and 128 mg/l (DEC) and 2 and >256 mg/l (non-DEC). MIC50 was the same both for DEC and commensal isolates: 32 mg/l, decreasing to 4 mg/l in presence of PA¦ÂN. While DEC isolates presented a MIC90 of 64 mg/l decreasing to 4 mg/l when PA¦ÂN was added, the non_DEC presented a MIC90 > 256 mg/l and 128 mg/l in absence and presence of PA¦ÂN. Four DEC (4.4%) and 7 (24.1%) non-DEC presented a MIC ≥16 mg/l in presence of PA¦ÂN.

CONCLUSIONS Our MIC values were higher than those observed in other studies, suggesting selection pressure. This is also supported by the higher levels of resistance found among non-DEC isolates, which corroborate community pressure. In four DEC and seven commensal isolates PA¦ÂN-inhibitible efflux pumps do not explain the high MIC-levels, suggesting the presence of mutations in rpoB gene. The Results show the need to use Rfx with caution in the area of Lima.

1.5-021

Serum lipid profiles and eosinophilia among *Giardia* cyst passers

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BACKGROUND *Giardia* trophozoites attach to upper portion of small intestine and may present with severe damage affecting nutrient absorption. One of the main complications of giardiasis is lipid malabsorption and consequently infected people may lack the important caloric source and lipid soluble vitamins. This study aimed to assay serum lipid profiles and eosinophilia in *Giardia* cyst passers and compare with non- infected individuals.

METHODS Three hundred and fifteen peripheral blood samples of *Giardia* cyst passers confirmed by formalin- ether concentration were studied by cell blood cunt and colorimetric methods and compare with control.

RESULTS Eosinophilia >5% was observed in 18% of infected people but not in controls. Exception to high density lipoprotein (HDL) the cholesterol, triglyceride and low density lipoprotein (LDL) levels were statistically lower than those observed in the control groups ($P < 0.05$).

CONCLUSION This study revealed that there is a significant influence of *G. lamblia* on lipid levels and eosinophilia in infected individuals.

KEYWORDS *Giardia* cyst passer, lipid profiles, eosinophilia

1.5-022

Incidence of rotavirus diarrhea in a rural hospital in Southern Ethiopia

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INTRODUCTION Rotavirus is an important cause of diarrhea world-wide in children under 5 years old, with high morbidity and mortality especially in those younger than two. Most of the studies in developing countries are based in urban settings and big hospitals, with few epidemiological data from rural settings. We conducted a survey about the incidence of rotavirus diarrhea in children younger than 2 years old in a rural hospital in Southern Ethiopia (Gambo Rural Hospital) during July and August 2008.

METHODS AND MATERIALS We included children under 2 years old coming to the outpatients department with acute, non-bloody diarrhea (>3 loose stools per day). We tested the stool samples for rotavirus-adenovirus (VIKIA Rota-Adeno®; BioMerieux, France), leucocytes and parasites. Stool culture was not performed.

RESULTS Sixty-four children <2 years were attended, 56% were male and 44% were female. From 64 samples, 11 tests [17.2%, (confidence interval [CI] 95%: 9.3–29.1%)]: were positive for gastrointestinal viral infection: nine for rotavirus (14.1%, CI 95%: 7.0–25.5%) and two for adenovirus (3.1%; CI 95%: 0.6–11.8%). Four of our patients (6.1%; CI 95%: 2.0–16.0%) had >100 white blood cells in the stool sample suggesting a bacterial origin. We found five positive samples for parasite (7.8%; CI 95%: 2.9–18%): 3 (4.7%; CI 95%: 1.2–14%) for *Giardia lamblia*, 1 (1.6% IC 95%: 0.08–9.5%) for *Ascaris lumbricoides* and one for *Entamoeba histolytica*. Five of the nine patients with a positive rotavirus test were admitted (55.5%) and 34 of the other 55 patients needed hospitalization (62%) ($P = 0.5$).

CONCLUSION The prevalence of Rotavirus diarrhea is slightly lower than the Results obtained in other studies, and we did not find differences in hospitalization between rotavirus and non-rotavirus diarrhea in children under 2 years, but the sample size is not big enough to come to a conclusion about these differences.

1.5-024

Evaluation of elisa with figured antigen for diagnosis of *Toxoplasma* infection in human sera

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BACKGROUND *Toxoplasma gondii* is an obligate intracellular parasite that infects warm-blooded vertebrate as well as human. It can make a neurological manifestation and disseminated toxoplasmosis in newborns with congenital infection and immunocompromised patients. Diagnosis of toxoplasmosis is based on serological methods such as IFA and ELISA. This study was performed to evaluate the ELISA assay with figured antigen (formalized tachyzoites) in comparison with IFA

MATERIALS AND METHODS One hundred and ten referred serum samples in serology laboratory of School of Public Health, Tehran University of Medical Sciences were collected. For ELISA antigen preparation, 106 formalized tachyzoites of *T. gondii*, RH strain were coated in each well of microtiter plate. ELISA and IFA methods were performed for detection of *T. gondii*-IgG antibody.

RESULTS Seventy-one out of 110 cases were negative and 31 were positive with these two methods. Eight positive samples for IgG antibody with ELISA were diagnosed negative by IFA method. The agreement rate between these two methods was 92.4%.

CONCLUSION The advantage of using figured antigen is elimination of some time consuming process such as sonication and high speed centrifugation. Complementary studies for comparing figurate antigen with soluble antigen in ELISA assay is in progress.

KEYWORDS *Toxoplsma gondii*, figured antigen, ELISA, IFA

1.5-025

Epidemic study of cholera in the Far North region of Cameroon : the role of natural and social environment

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Cholera made an unforeseen appearance in the Far North region of Cameroon . Prompt medical intervention, health education and

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media awareness campaigns, epidemic cholera continued to spread throughout the Far North Region. In 2010 more than 6239 cases were recorded and 417 dead with a CFR of 6.68%. The question then was, what factors played a role in the cholera epidemic of Cameroon? The objective of the study was to investigate the possible role of the natural environment i.e. temperature, rainfall and humidity as the primary factors that influence cholera outbreaks in the Far North Region; on the basis of its uniqueness in climatic conditions as compared to other areas of Cameroon. The socio-economical and demographic factors was also considered as factors that enhanced the spread of the Cholera. As such, the exploration of the Cholera Database by the use of spreadsheet, statistical correlations and spatial mapping using GIS technology to investigated the relationships between the different variables enhancing the spread of cholera. The age groups/sex groups highly affected was determined.

KEYWORDS socio-economical, climate, poverty, epidemic cholera, GIS mapping

1.5-026

Detection and characterization of Cryptosporidium spp. and *Giardia lamblia* in faeces and drinking water in the metropolitan area of Tenerife (Canary Islands, Spain)

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Cryptosporidium spp and *Giardia lamblia* are protozoan parasites responsible for waterborne or food diarrheal outbreaks. In recent decades, these parasites have become very important because of their low infective dose and their resistance to conventional treatment for drinking water. Hundred samples of drinking water were analyzed from the cities of Santa Cruz de Tenerife and San Cristóbal de La Laguna and 108 stool samples from patients with acute gastroenteritis ceded by the Pediatric Emergency Department of the Children's Hospital of Tenerife, collected between 2007 and 2008. The samples were processed for the concentration of cysts and oocysts. We proceeded to the specific molecular detection for each parasite by the nested PCR technique. The amplified products of expected size were purified and sequenced. In drinking water samples, 3% of samples tested positive for *Giardia lamblia* while 4% were for Cryptosporidium spp. Sequence analysis confirmed they belonged to *C. hominis* species. About the stool samples, 3.7% were positive for Giardia lamblia and 2.8% for Cryptosporidium spp. After sequence analysis it was confirmed that two positives corresponded to *C. hominis* and one to *C. parvum*. In conclusion, we confirmed the presence of both protozoa in the drinking water and fecal samples analyzed. Also, the presence of *Cryptosporidium hominis* in the analyzed drinking water suggests a possible human and/or animal origin of this contamination. Finally, it was not possible to establish a correlation between the consumption of contaminated drinking water and acute gastroenteritis cases studied due to the lack of geographic data.

1.6 Bacterial diseases, pneumonia and respiratory infections

1.6-001

Etiology and antibiotic resistance patterns of community-acquired extended-spectrum beta-lactamase-producing gram negative isolates in Sanandaj

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OBJECTIVE The aim of this study was to determine the distribution and antibiotic susceptibility patterns of extended-spectrum beta-lactamase (ESBL) producing bacterial strains isolated from patients with community acquired infections at Sanandaj's two major hospitals in Iran.

PATIENTS AND METHODS The study took place at the Faculty of Medicine, Kurdistan University of Medical science, Sanandaj, Iran. We evaluated 158 g negative strains isolated from various clinical specimens. The double-disk synergy test was performed on the isolates for the detection of ESBL. These genes were confirmed by PCR methods.

RESULTS The majority of community-acquired ESBL types belong to CTX-M (10.76%) and SHV (10.76%). Resistance to ceftazidime and cefotaxime were 33.54% and 34.18%, respectively. Multiple resistances antibiotics were often associated with ESBL-producing organisms.

CONCLUSION This is the first report of prevalence of ESBL-producing isolates originating from the community in Sanandaj. ESBL-producing isolates type's especially CTX-M-producing gram negative bacteria are a rapidly developing problem in Iran. A heightened awareness of these organisms by clinicians and enhanced testing by laboratories, including molecular surveillance studies, is required to reduce treatment failures, to limit their introduction into hospitals and to prevent the spread of these emerging pathogens.

1.6-002

Determinants of antibiotics prescription in school children at Allada, South Benin

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INTRODUCTION Implementation of malaria rapid diagnostic tests (mRDT) has been repeatedly associated with an increase of antibiotics prescriptions. We aimed to study determinants of antibiotics prescriptions to schoolchildren by nurses in South Benin.

METHODS Data were collected in the setting of a prospective study on treatment of malaria restricted to parasitologically-confirmed cases. Children were included from February until June 2008. For each patient, sociodemographic characteristics, complaints, final diagnosis established by nurses and therapeutic prescriptions were collected. Assessment of malaria was performed with mRDT. Data were entered and validated with Epidata® software, and analyzed with STATA 10® software.

RESULTS One thousand six hundred thirty children were included. Fever was the first reason for consultation (57%), followed by the digestive symptoms (27%), respiratory symptoms (24%) and skin lesions (17%). A malaria diagnosis was made in 61% of the children attending for fever. Forty percent of children were prescribed an antibiotic (21% of children with a malaria diagnosis and 57% of children with a non-malarial-fever). We found a very significant association between an antibiotic prescription and a respiratory infection diagnosis [OR (IC 95%): 41.09 (24.34–

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69.33)], and to a lesser extent between an antibiotic prescription and a cutaneous infection diagnosis [OR (IC 95%): 5.78 (4.20–7.97)].

CONCLUSIONS The rational use of antibiotics in malaria endemic areas has become an even more critical issue, since the implementation of mRDT is boosting antibiotics prescriptions. Analyzing determinants of antibiotics prescription is a first step on the way to rationalize antibiotics prescriptions. We found that, by far, the diagnosis of respiratory infection is the main factor associated with an antibiotic prescription. Further clinical research studies are needed in order to develop algorithms aimed at selecting among children who complain with respiratory symptoms, those who should be prescribed antibiotics.

1.6-003

Fluorescence in situ hybridization (FISH) for the rapid diagnosis of melioidosis

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INTRODUCTION Burkholderia (B.) pseudomallei, a Gram-negative rod-shaped bacterium is the causative agent of melioidosis, a potentially fatal disease in tropical areas occurring in men and animals. Several cases of melioidosis imported to Europe have been reported. *B. Mallei* causes glanders, a similar zoonotic disease. The diagnosis of the acute or chronic infections still remains challenging and classical biochemical testing is time-consuming. Many efforts have been made to develop new molecular techniques for diagnostical use.

METHODS AND MATERIALS We could design directly 5′-labeled probes for the identification of the two highly pathogenic species *B. pseudomallei* and *B. mallei* as well as for the environmental strain *B. thailandensis* by fluorescence in situ hybridization (FISH). The combined use of these new probes was evaluated with reference strains and clinical isolates of the target species as well as with negative controls. Further evaluation was done with histopathological organ samples of experimentally infected mice.

RESULTS FISH correctly identified 100% of the tested *B. pseudomallei* (n = 11), *B. mallei* (n = 11) and *B. thailandensis* (n = 1), excluded 100% of all tested negative controls (n = 61) and allowed demonstration of *B. pseudomallei* infection in a paraffin-embedded spleen sample with high bacterial load.

CONCLUSIONS The presented method allows rapid and reliable identification and differentiation of *B. pseudomallei* and *B. mallei* from culture within 90 min thus providing a safe diagnostic tool and avoiding further biochemical differentiation of the BSL three agents. The low sensitivity of FISH is not relevant when suspected colonies are tested. This method has also proven to be useful for histopathological investigations but further evaluation has to be done in endemic areas with clinical materials, such as blood culture, sputum samples or pus.

1.6-005

What next measles control for Karnataka, India?

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INTRODUCTION Worldwide, measles still kills 400 people every day, more than 90% of them being under-fives. Three out of four deaths happen in India, The objectives of my work are to describe the epidemiology of measles in Karnataka and to identify ways to improve measles control in the state.

METHODOLOGY Weekly surveillance reports and outbreak investigation line lists for 4 years were collated and tabulated, and the spatio-temporal distribution of measles cases and outbreaks in the state synthesized. Through logistic regression multivariate analysis, I specified the contextual demographic and socio-cultural determinants of measles outbreaks and vaccination coverage in the state of Karnataka.

FINDINGS Measles surveillance data from 2006 to 2009 reveal that measles is indeed endemic in Karnataka, with frequent outbreaks. The notification rate of measles is 10.94 cases per 100,000 population per year. Seasonality of notified measles cases characteristically increases between November and April and decreases from May to October. There were 163 confirmed outbreaks in the state in the four years under study. Measles outbreaks were happening consistently in the northern part of the state and sporadically in the southern part: The duration of these outbreaks from the date of appearance of rash in a first case to last case was ranged from 7 to 120 days with median of 39 (q1 15: q3 63) days.

DISCUSSION Significant reduction in measles incidence and outbreaks can be attained only by adding a second dose to the routine vaccination scheme in the whole state and by conducting catch-up campaigns in northern Karnataka.

1.6-006

Gaps in the current knowledge of acute respiratory infections affecting children under 5 years in Morocco

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INTRODUCTION The burden of acute respiratory infections (ARIs) among Moroccan children remains presumably important despite progresses in treatment and preventive strategies. Little information is available regarding the epidemiology and the etiology of ARI in Morocco.

OBJECTIVES To describe the burden of ARI among children under 5 years of age in Morocco.

METHODS Pubmed®, Hinari®, Google Scholar®, official reports of the Moroccan Ministry of Health (MOH) was searched and publications from 1997 to 2011 reviewed. Search queries included: Respiratory Tract Infections, epidemiology, etiology, microbiology, mortality and Morocco.

RESULTS Ten published articles and reports were found. In 2008, ARIâ€™s remained the leading cause of death among children under five (17.2% among total under five deaths), constituting around half of the consultations at health facilities and a third of admissions in an urban pediatric teaching hospital. ARIs are managed according to the integrated management of childhood illnesses (IMCI) program since 1998. The two principal micro-organisms identified among hospitalized children with ARIs in Casablanca were Streptococcus pneumoniae (38%) and Haemophilus influenzae (Hib) (15%). In its preventive strategy, MOH introduced Hib vaccines into the national immunization programme (PNI) in 2007. The 13-valent vaccine against pneumococcus has been added in 2010. The national first line antibiotics prescribed are Amoxicillin (oral treatment, outpatients) and Ampicilline associated to Gentamicin (parenteral, inpatients). Studies of antibiotic resistance among isolates causing ARIS, showed a high and increasing rate of penicillin non-susceptibility among Streptococcus pneumoniae from 1998 to 2008 (21.4–43.3% increase). Viral respiratory infections remain poorly

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characterized, with the exception of the H1N1 epidemic episode of 2010, which affected 29% of children under five who attended the university hospital of Rabat.

CONCLUSION Efforts should be made towards the development of adequate surveillance programs to further clarify the epidemiology, etiology and sensitivity patterns of the different causes of ARI.

1.6-007

Trends in tetanus admissions in the paediatric age group at a Nigerian teaching hospital

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INTRODUCTION Tetanus continues to account for high morbidity and case fatality rate in the developing countries, in spite of programs recommended by the World health organization for its elimination. Recently, we observed a change in the trend of tetanus admissions among the paediatric age group at our hospital. This informed our decision to conduct this study.

METHODOLOGY Paediatric admissions at Ladoke Akintola University Teaching Hospital between the 1st January 2006 and 31st December 2008 with the diagnosis of tetanus were studied. Data was analyzed with SPSS 18 and statistical significance was set at $P < 0.05$.

RESULTS Of the total 1681 paediatric admissions, 30 (1.8%) had tetanus. Of the 878 neonatal admissions, 8 (0.9%) had tetanus, while 22 (2.7%) of the total 803 post neonatal admissions had tetanus. The greater 7 (2.3%) of the 305 neonates admitted in 2006 compared with the 1 (0.2%) of the remaining 573 babies admitted between the year 2007 and 2008 is statistically significant. 2 = 7.50, P = 0.01. The post-neonatal cases of tetanus admitted in the year 2006, 2007 and 2008 were four, 12 and six children respectively. Case fatality rates for neonatal tetanus (NNT) and post NNT in the present study were 87.5% and 18.2% respectively. Of the eight mothers whose neonates had tetanus, 5 (62.5%) were secondary school drop outs. Most of the 22 children had received no tetanus toxoid immunization in their first year of life and none received booster doses thereafter.

CONCLUSION Potential mothers of babies having tetanus, such as secondary school drop outs need to be identified ante-natally and vaccinated. Their babies should also receive good care post delivery. Completion of routine tetanus toxoid schedule in the first year and booster doses in the post neonatal age should be ensured.

1.6-008

Respiratory tract infections in Ashanti province in Ghana – extrapolating disease rates by linking hospital records to a probability model for hospital attendance

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Ghana, like other sub-Saharan countries, has high rates of respiratory tract infections (RTI). However, detailed data on the disease occurrence is lacking hampering the ability to plan health policy, service delivery, or control programmes. The aim of the current study was to extrapolate incidences for RTI in Ashanti province in Ghana using referral data from a local hospital. Since hospital surveys are not generalisable, the individual likelihood of a clinic visit was used to weight disease rates. Diagnoses from children visiting the Agogo Presbyterian Hospital during August 2007 to September 2008 were recorded. With a logistic regression model, based on a population study conducted within the hospital catchment area, the individual probability of clinic attendance was calculated and used to extrapolate the number of recorded cases. These figures were used to estimate cumulative incidences for children <15 years of age living in the hospital catchment area. The study showed that upper RTI was the most common respiratory diagnosis, with an incidence of 19,064 cases per 100,000 per year, followed by pneumonia with an incidence of 2496 per 100,000 per year. A seasonal trend could be seen with a peak in October and a second lower peak around April. All analysed diseases were most common in the first year of life. The study results are in line with estimates from comparable studies. Yet, the analysis also pointed to methodological issues biasing the results in different directions. For example, the extrapolated disease rates are likely to overestimate milder opportunistic infections that are more often observed in hospital attendees. Furthermore, the admission probabilities used do not consider differences between age groups. However, while these methodological challenges require further work, the applied approach presents an applicable solution to extrapolate data on disease occurrence if routine disease monitoring systems are lacking.

1.6-009

Diagnostic biomarkers discovery for respiratory infection and malaria in African children

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Malaria, bacterial pneumonia and viral pneumonia contribute substantially to the disease burden of children in developing countries. Clinical differentiation of these infections is complicated by overlapping symptoms of respiratory distress, fever and cough. Misdiagnosis (especially over-diagnosis of malaria) is thought to be frequent, resulting in children receiving incorrect or multiple treatments. This inaccuracy contributes to antimalarial and antibiotic drug resistance, increased morbidity and mortality, exposure to side effects of unnecessary medication, and increased medical costs. The RAPID project aims to identify host protein biomarkers in peripheral whole blood that reliably distinguish between these three infections in pediatric patients meeting the WHO-defined criteria for clinical pneumonia. Unbiased discovery using microarray mRNA profiling will identify transcripts of differential abundance between disease states of interest. The protein products of differentially expressed genes will constitute biomarker candidates that will be quantified using targeted mass spectrometry. In parallel, plasma chemokine and cytokine levels will be assessed using Luminex immunoassays. Novel biomarkers credentialed through this process could provide the basis of new rapid diagnostic tests of great practical utility in areas where laboratory facilities are scarce. Accurate classification of patients into clinical categories (malaria, bacterial pneumonia, viral pneumonia and healthy community controls) is based on detailed clinical profiling, including signs and symptoms of infection, chest radiography and a series of laboratory tests including complete blood counts, parasitology, RT-PCR viral antigen testing, blood culture, and a purpose-built PCR panel capturing the species most frequently responsible for bacterial pneumonia. Sample collection is currently underway at the Centro de Investigação em Saúde de Manhiça (CISM) in Mozambique and will begin at the Kenya

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Medical Research Institute (KEMRI), Centre for Clinical Research, Walter Reed Project, Kisumu, Kenya in the near future. Study design and preliminary results will be presented.

1.6-010

Q fever in the South area of Madrid in 6 years: the complex diagnosis of a little known disease

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OBJECTIVES To describe Q fever case-mix in the South of Madrid and compare them with patients in whom Q fever was suspected but ultimately rejected, trying to establish criteria to guide the differential diagnosis.

MATERIAL AND METHODS Prospective study of all consecutive patients with suspected Q fever in our hospital from 1 January 2005 to 31 December 2010. Case defined as patients with compatible clinical and serological confirmation by IIF and/or positive PCR to Coxiella burnetii in biological sample and Control as suspected case with alternate diagnosis. Bivariate analysis; Significance $P < 0.05$ (SPSS 15.0)

RESULTS $N = 44$, 24 cases (54.5%) and 20 controls (46.5%). Admitted 16 cases (70.8%) with a prolonged stay (between 2 and 33 days) and many medical checks (>3/year); No case died. Males (83.3%), Spanish (91.7%) and healthy (83.3%) no epidemiological clues; Low incidence (3–5 cases per year, peaking in 2007); The common presentation was fever syndrome (FUO criteria nine cases 37.5%), involving 14 patients, hepatitis (58.3%), pneumonia 2 (8.3%), pericardial effusion 2 (8.3%) and headache 8 (33.3%). No dermatological symptoms (87.5%) but a serious case debuted as erythema nodosum. Diagnosis confirmed by serology (IFA) and PCR in liver biopsy in three patients. Most with leukocytes, platelets and normal Hb and a not very high CRP (7.2 ± 7 (7)); Frequent abnormal liver profile with high GGT; Autoantibodies positivity in three cases (12.5%) and multiple ELISAs (including a false HIV positive), which complicated the diagnosis. Treatment with doxycycline in 16 cases (66.7%). At follow-up four patients (16.7%) had a typical serologically pattern of chronic infection, but they were asymptomatic. In TT echocardiography of 16/24 patients, we found three mild valve disease and two valvular endocarditis (only one per *C. burnetti*), four severe cases (FUO with symmetric polyarthritis and erythema nodosum; FUO and granulomatous hepatitis and synchronous papillary thyroid; FUO with granulomatous hepatitis, serositis, hemolytic anemia and GMN; endocarditis requiring aortic valve replacement.) When comparing cases and controls there were differences in age (younger cases $P = 0.003$) and FUO ($P = 0.01$), plus higher GGT and lower CRP without statistic significance.

CONCLUSIONS (i) Q fever is rare and its diagnosis is complex. (ii) Cases are healthy young Spanish men. (iii) The typical clinical courses is hepatic involvement, prolonged fever, absence of pancytopenia, not very high PCR and systemic symptoms in severe cases. (iv) Pneumonia, pericarditis and endocarditis are rare. (v) It is not possible to establish criteria that discriminate cases of suspicion. (vi) Confirmation of the diagnosis sometimes requires invasive studies, in our country liver biopsy is most appropriate.

1.6-011

Five-year evaluation of the frequency and antimicrobial susceptibility patterns of bacteria causing bloodstream infections in Iran

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Bloodstream infections (BSI) are a serious cause of morbidity and mortality worldwide. Emerging antimicrobial drug resistance among bacterial pathogens causing BSI can hinder optimal treatment and patient management decisions. In order to encourage the prudent use of appropriate antibiotics in our pediatric population at Children's Medical Center Hospital, Tehran, Iran, we studied the frequency and antibiogram patterns of blood culture isolates from January 2001 to December 2005. Of 25,223 blood cultures examined in this investigation, 2581 (10.23%) were positive for bacterial growth. The frequency of gram-positive bacteria isolated was 47.5% (1228 of 2581) and that for gram-negatives was 52.5% (1353 of 2581). The rates of oxacillin (methicillin) resistance for *S. aureus* and coagulase-negative staphylococci (CoNS) were 86% and 89%, respectively. About 45% of *S. pneumoniae* were resistant to trimethoprim-sulfamethoxazole and approximately 73% to penicillin. Among the gram-negative isolates, *P. aeruginosa* was most frequently isolated, representing 943 (36.7%) over 5 years. In addition, this pathogen showed extremely high rates of antimicrobial resistance. There were notable differences in the sequence of the five most common organisms isolated from blood cultures, which can help set priorities for focused infection control efforts. Our findings underscore the need to monitor blood culture isolates and their antimicrobial resistance patterns in order to observe trends that would impact appropriate treatment and infection control strategies for bacteremic children.

1.6-012

Diagnosis of *Helicobacter pylori* infection by invasive and noninvasive methods

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BACKGROUND AND OBJECTIVE Invasive and noninvasive tests have been developed for the diagnosis of this organism but all of the tests have their limitations and it might not be realistic in clinical practice, therefore it is important to know which test in a certain clinical setting gives the best possible outcome.

METHODS Eighty-nine patients (28 children, 61 adults) for upper gastrointestinal (UGI) endoscopy entered the study and noninvasive tests such as immunoassay for serological antibodies against *H. pylori* and detection of *H. pylori* antigen in feces were

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measured. The biopsies were utilized for histological examination, urease rapid test and PCR.

RESULTS The *H. pylori* statuses of 89 patients were evaluated by the positivity of urec PCR in biopsy specimens, 53 subjects of whom were *H. pylori* positive. Histopathology showed high overall performance in adults and children with sensitivity 100%, specificity 90%. Lower efficiency of stool antigen test, with a sensitivity, specificity, and accuracy of 82%, 64%, and 75%, respectively, was found with samples from children. However, the performance in adult patients was good, with a sensitivity, specificity, and accuracy of 91%, 79% and 85%, respectively.

CONCLUSIONS The RUT and histopathology, while being as accurate as the PCR of biopsy, are more cumbersome to perform. Commercial EIAs to detect *H. pylori* antibodies in serum have a lower accuracy, particularly in children. Nevertheless, in this study, histology and RUT of gastric biopsy specimens were found to be relatively reliable methods for Diagnosis of *H. pylori* Infection.

1.6-013

Bacterial pneumonia in HIV-SIDA

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INTRODUCTION In HIV -AIDS patients are many the risk factors involved in appearance of the bacterial pneumonia BP of different origin without to know the causal germ of respiratory picture .

METHODS Authors studied 85 HIV- AIDS patients diagnosed with BP according the clinical, radiological and laboratory criteria in the Pedro Kouri Tropical Medicine Institute between November 2007 and April, 2008.

RESULTS *S. pneumoniae* 40.5%, enterobacteria 28.3%, and no-fermented bacilli 13.5% were the more frequent identified. There was prevalence of subjects aged under 50 91.7%, smokers 65.9%, with a TDC4 lymphocytes count under 200-cel mm3 64.7% with a high-performance anti-retroviral therapy 54.7%. Risk factors of BP due to enterobacteria included age of 50 years.

CONCLUSIONS It was demonstrated that the etiological agents identified in sputum from HIV-AIDS Cuban patients presenting with BP are similar to those described by other authors at international level, being more probable that the ill persons aged over 50 and also malnourished have a BP due to enterobacteria than remainder microorganisms.

1.6-014

Impact of sea in chronic osteomyelitis caused by *Staphylococcus aureus*

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INTRODUCTION Osteomyelitis is a bone infectious disease, mainly caused by *S. aureus* that secrete exotoxins (SEA). Increased levels of pro-inflammatory cytokines such as IL-1, IL-6 and TNF released by the host may play a role in its pathogenesis.

METHODS We investigate the production of cytokines by SEA-stimulated cells (PBMC) and the levels of plasma NO, in chronic ostemyelitis patients. We showed that *in vitro* SEA-stimulation induced significantly higher levels of cell proliferation in osteomyelitis (OST) patients and non- infected individuals (NI). SEA-stimuli led to higher levels of TNF, IL-4 and IL-10 synthesis by PBMC from NI and OST as compared to non-stimulated cultures. Despite no impact of *in vitro* SEA-stimuli in the IL-6 production by PBMC from OST and NI, the levels of IL-6 in the supernatant of PBMC cultures from OST were always significantly lower as compared to NI. The plasmatic levels of NO were significantly lower in OST than in NI. We demonstrated that while higher levels of TNF and IL-4 with down-regulation of IL-6 are observed during early osteomyelitis episodes (1–4 months), increased levels of IL-10 and IL-2 with up-regulation of IL-6 are the hallmark of late osteomyelitis episodes (5–12 months). We categorized the study population as low or high cytokine producers, and showed that 5–12 months patients exhibit increased frequency of IFN IL-10, IL-6, and IL-2 cytokine-producers. And, 1–4 months patients showed a higher frequency of TNF-α and IL-4, after SEA stimulation.

CONCLUSION There is a distinct cytokine profile with dichotomic IL-6 dynamic during early and late chronic osteomyelitis episodes.

1.6-015

Disease control programmes and strengthening public health care services: experience from pandemic influenza preparedness in Southern Africa

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With the spread of avian influenza A H5N1 into Africa in 2006, and the appearance of human cases of H5N1 influenza in north and west Africa, national governments felt the need to increase preparedness for influenza pandemics. This came in the wake of increasing numbers of human H5N1 influenza cases with high case-fatality rates in South-East Asia. The experience of (re-) emerging infectious diseases, in other parts of the world earlier e.g. SARS, and also Ebola and novel arena virus hemorrhagic fever outbreaks in Africa contribute to a felt need to reinforce outbreak preparedness and response capacity. US Centers for Disease Control and Prevention (CDC) developed a multi-disciplinary rapid response team training curriculum based on CDC/WHO/OIE/FAO principles of outbreak response vis-à-vis avian influenza. With support of CDC, and later the InterAfrican Bureau for Animal Resources (IBAR), countries took on training of multi-disciplinary rapid response teams in avian and human influenza pandemic preparedness, including principles of general outbreak response and infection control. This served to strengthen public health capacity to respond to other outbreaks. Surveillance for severe and acute respiratory illness (SARI) was initiated at a number of sentinel Tertiary Referral Hospitals, for virological and clinical monitoring. Laboratory capacity for diagnosis of influenza viruses was strengthened, with support of the National Institute of Communicable Diseases in South Africa. With the onset of pandemic influenza novel H1N1 in 2009, experience from avian and human influenza preparedness programmes was activated. Surveillance systems, monitoring influenza viruses in circulation and SARI among presenting patients, were reviewed, as was infection control, in settings with limited resources. Communication messages were developed. Examples from a few southern African countries will be shared, illustrating positive health system strengthening effects of this disease control programme.

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1.6-016

Prevalence of HRV-C in rural aboriginal and non-aboriginal children: comparisons with urban populations

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Background A recently-identified group of human rhinoviruses (HRV), HRV-C, has been associated with more frequent and severe acute lower respiratory infection (ALRI) in children. Previous studies have reported that Aboriginal Australian children have more respiratory infections than non-Aboriginal (n-A) children. Therefore, we investigated the hypothesis that HRV-C infection will be more common in Aboriginal children from a rural community than in n-A children from a rural or urban setting.

Methods Specimens from five populations of children in rural (Kalgoorlie-Boulder) or urban (Perth) Western Australia were tested for respiratory viruses including HRV groups. HRV-C prevalence was compared between (i) rural Aboriginal children (n = 103), (ii) rural n-A children (rn-A) (n = 94), (iii) urban n-A healthy controls (un-Ah) (n = 44), (iv) urban n-A siblings of group v (un-As) (n = 45), and (v) urban n-A children with an ALRI (un-AALRI) (n = 232). For HRV strain identification, RNA was extracted and cDNA prepared and used for a 2-step PCR amplification of the 5′ non-coding region, followed by DNA sequencing and phylogenetic analysis.

Results HRV was identified in 23.6% rural Aboriginal, 16.5% of rn-A, 22.2% of un-Ah, 37% of un-As and 70.9% of un-AALRI children. Of the typed specimens, HRV-C was identified in 6.2% of rural Aboriginal, 5.1% rn-A, 0% un-Ah, 22.2% of un-As and 44% un-AALRI children. HRV was identified more often in rural Aboriginal than in rn-A children ($P < 0.05$). HRV-C was identified more often in rural Aboriginal and rn-A children than in un-Ah children ($P < 0.001$). HRV and HRV-C were more commonly found in un-As and un-AALRI children than all other groups ($P < 0.001$).

Conclusions Rural Aboriginal children have higher rates of HRV infection than rural non-Aboriginal children, but lower rates than children with a severe ALRI and their siblings. HRV-C was more common in rural than urban children, but much lower than in children with an ALRI and their siblings.

1.6-017

Viral etiology of community-acquired acute lower respiratory infections in children under 5 years in Ampasimanjeva district of Madagascar

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Background Acute lower respiratory infections (ALRI) are a leading cause of morbidity and mortality in children under 5 years worldwide. In Madagascar, the diagnosis is most commonly made empirically and consequently most of the patients are treated inappropriately.

Objectives This is a prospective study to identify the viral etiology and ecology of acute lower respiratory infections in children under 5 years living in a commune in the Southern part of Madagascar.

Material and Methods Three hundred twenty-five children <5 years old with ALRI at the health center of Ampasimanjeva were evaluated. Multiplex real-timePCR assay for detection of 17 respiratory viruses were used in nasopharyngeal swabs. Demographic, clinical and biological data were collected and compared.

Results A viral agent was identified in 73.23% (238 cases) of children with ALRI. In this population, RSV, human metapneumovirus and Rhinovirus, were the maindetected respiratory viruses. Interestingly, four cases were positive for Influenza A, H1N1 virus. Bocaviruswas found in eighteen children. In 68.49%, representing 163 out of 238 cases, a single virus was detected and viral co-detections were found in 31.51% (75 cases). A different seasonal viral pattern was observed among the identified pathogen.

Conclusion In this study, viruses were involved in two-thirds of children with acute lower respiratory infections and moreover, recent discovery viruses, which never been detected before in Madagascar, were identified. Viral co-detection was frequently detected in this population, representing one-third of positive cases. The viral circulation showed to be very different among viruses found showing possible epidemics emergence.

Keywords multiplex real-time PCR, etiology, respiratory viruses, Madagascar, acute lower respiratory infection

1.6-018

Knowledge and practice of rural mothers regarding acute respiratory infection of children in Bangladesh

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Introduction Acute respiratory infection of children is one of the public health problems in Bangladesh. Every year around 400,000 children under 5 years die of respiratory infections. The Acute Respiratory Infection (ARI) control programme of BRAC has been in operation for few years. So far, no independent evaluation has been conducted to explore how far the objectives of the programme have been achieved in terms of raising awareness among mother/caregivers about ARI, and changing health-seeking behaviour of mothers for appropriate and quick treatment.

Methods This study is a cross-sectional, population-based study comparing groups with or without ARI programmes. The study included 2800 mothers, 1440 children with ARI symptoms.

Result The level of awareness among mothers in terms of recognition of symptoms of ARI and its prevention remains inadequate in both programme and non-programme areas. The majority of the mothers heard about the community-based BRAC ARI control programme, but they were unaware about the detail activities of the programme. Significant proportion of mothers (60%) would not seek ARI treatment from BRAC in programme areas. Mothers preferred to go to the informal healthcare providers such as village doctors and drug sellers in both the programme and non-programme areas. During health-seeking, one-fourth of the mothers did not seek treatment, and one-fourth received spiritual treatment.

Conclusion Level of awareness in mothers was low. It is difficult to expect quick management of ARI to save life of children in community. Many mothers still sought treatment from unqualified providers. So, we conclude that many children were at risk of mortality due to ARI. BRAC ARI programme should look into the matter seriously to achieve the programme goal, and to reduce child morbidity and mortality.

What is the title of this subsection? the numbers switch to 1.0 – is that correct?

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1.6-019

Seroepidemiology of Letospirosis in mazandran, northern Iran, in 2010

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Leptospirosis is a zoonotic contagious disease of worldwide significance that infects both animals and humans and spread by infection with a bacterial pathogen called Leptospira. In human it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Leptospirosis is spread through contact with water, soil, vegetation or any part of a moist environment contaminated by urine or tissue of infected animals or humans. In the early stages of the disease, symptoms include high fever, severe headache, muscle pain, chills, redness of the eyes, jaundice, hemorrhages in the skin and mucous membranes, vomiting, diarrhea, and rash. In this study, 249 blood serum samples (213 men, 35 women) were collected from people suspected of having Leptospirosis according the World Health Organization guidelines for diagnosis and control of this disease and based on physician diagnosis. These samples were collected from 16 cities of the Mazandaran province (north of Iran) during the transmission season and took from April to October 2010. The Indirect Fluorescent Antibody Test (IFAT) was carried out at the Pasteur Institute of Iran, Amol Research center to detection the anti leptospira antibody. Results of this study showed that antibody against Leptospira were detected in 91 (36.5%) of 249 patients. Of these patients 59% were farmers, and High distribution rate of Leptospirosis was seen in patient more than 50 years old. It seems that in Mazandaran province the occurrence of Leptospirosis is high and is mostly distributed in warm months of the year (July and august). Therefore, it is important to increase attention about this disease among physicians and to find the best laboratory method for its diagnosis in infected patients in the north of Iran.

1.6-020

Virulence properties of *Klebsiella pneumoniae* strains from different clinical specimens

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Klebsiella pneumoniae is an opportunistic pathogen expressing various virulence factors; studies on its virulence have limitations regarding the narrow number of virulence factors investigated. The aim of our work was to investigate virulence properties of 54 strains from different clinical specimens. Hemagglutination tests were performed with human erythrocytes, serum resistance was assessed by plaque assay on agar, siderophores were checked using the cross-feeding bioassays, haemolysin was detected with sheep's blood agar, mucoviscosity was examined with string test and biofilm formation was determined y a crystal violet absorption assay. PCR was used to detect 16 virulence genes. Genes of adhesins FimH (type1 pili) and MrkD (type 3 pili) were present in 100% and 96.3% of isolates. Mannose-sensitive haemagglutination was observed in 94.5%, and mannose-resistant haemagglutination in 68.5%. kpn and ycfM genes of FimH-like adhesin and putative fibronectin-binding lipoprotein were detected in 63% and 94,4% of isolates. entB (enterobactin), iutA (aerobactin), irp1-irp2-ybtS-fyuA (yersiniabactin) and iroN (ferric-catecholates) genes showed prevalence of 100%, 5.5%, 46.3% and 3.7%, respectively. Siderophores phenotypic tests were in accordance with genotypic detection. Serum resistance was observed among 92.5% of isolates, whereas traT gene was detected in only 1.8%. rmpA gene, an enhancer of the colony mucoidy, was present in 3.7% of isolates, and hypermucoviscosity was showed by 9.2%. Another mucoviscosity-associated gene, magA specific to serotype K1 capsule was not found. Haemolysin was not detected (hlyA-) and similarly, cnf-1 gene was absent. Biofilm formation was observed in 90.7% of isolates. Thirteen combinations of virulence factors were noted, of which two were predominant, they include pili F1, F3-Enterobactin-Serum resistance-Biofilm formation (20/54) or pili F1,F3-Enterobactin-Yersiniabactin-Serum resistance-Biofilm formation (18/54). This study showed that *Klebsiella* strains are invasive, and there is no correlation between virulence profiles and infection location.

1.6-021

Analysis of the antimicrobial resistance gene expression in *Pseudomonas aeruginosa* clinical isolate by quantitative real-time-PCR in Malaysia

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BACKGROUND *Pseudomonas aeruginosa* has several resistance mechanism outcomes at the gene expression as demonstrated by the efflux Pumps, the chromosomal AmpC -lactamase and the outer membrane protein porin OprD. We employed real-time-PCR to investigate the expression of these genes in multidrug resistant *P. aeruginosa* clinical strains.

RESULTS Colistin showed the highest susceptibility to *P. aeruginosa* (93.1%) where as gentamicine showed 94.3% resistance followed by ciprofloxacin and ceftazidime exhibiting 92% resistance rate. in comparison Imipenem and Amikacine showed 73.9% and 65.9% in addition to Pipracilline/tazobactam 57.9% and Aztreonam 55.6%. The gene expression analysis of 88 *P. aeruginosa* clinical isolates demonstrated that 72.7%, 81.8% and 65.9% showed over expression of efflux pump as mexB, mexZ and mexY transcription mRNA intensities, correspondingly, compared to those of *P. aeruginosa* ATCC 27853. Additionally, 64.8% appeared over expression of lactamse gene representative in AmpC and association with 96.6% down regulation of out membrane porin of OprD coupled with down regulation of penicillin binding protein of PBP2 and PBP3 for 77.3% and 84.1% respectively.

CONCLUSIONS AmpC Over-expression in association with distinct mechanisms as Efflux pump systems and the porin down-regulation coupled of down-regulation of penicillin binding protein play a significant role in the multi-drug resistance phenotype among *P. aeruginosa* clinical isolates. In a previous study the most important mechanism of Imipenem nonsusceptible in *P. aeruginosa* is through downregulation of the gene for OprD porin alone. However, this study argues that the downregulation of PBP genes contribute to Imipenem resistance in *P. aeruginosa* clinical strains.

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1.6-022

The impact of the H1N1 influenzae a virus pandemic in a secondary referal hospital in catalonia 2009–2011

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BRIEF INTRODUCTION During 2009, the first influenza pandemic of the twenty-first century, due to the swine origin Influenza A virus (H1N1) 2009 virus, was declared. This study aimed to describe the characteristics of the patients infected with Influenza A virus (H1N1) during 2 years follow-up in our hospital. We determinate the prevalence, comorbility and features of H1N1 infected patients confirmed during this time.

MATERIAL AND METHODS Observational study of patients diagnosed of influenza since April 2009 until February 2011 at hospital setting. We got upper tract specimens for laboratory testing from a nasopharyngeal and throat swab from patients with suspected influenza. Influenza A virus (H1N1) infection was documented in all patients through Real-time PCR test. We report their clinical and epidemiological features, comorbidities, need for hospitalization, days of hospital admission, treatment and outcome by history taking reviewed; and we compared data obtained in both years.

RESULTS Fifty patients were diagnosed of influenza A (H1N1) during this time (21 in 2009 and 39 in 2010). There were not differences between sexes. There was high incidence in young adults (15–44 years old). The underlying risk conditions were respiratory disease (COPD or asthma), cardiovascular disease, obesity, diabetes and HTA. Clinical presentation was fever, dyspnoea, cough, chest pain, weakness; although the major clinical syndromes in admitted patients were influenza with respiratory insufficiency, pneumonia, and gastroenteritis. The major determinant of ICU admission was shortness of breath. Five patients required admission at ICU for mechanical ventilation and two died (80% during 2nd year). Oseltamivir and wide spectrum antibiotic was given in almost all patients.

CONCLUSION The majority of cases of Influenza A virus (H1N1) infection during years 2009–2011 were mild and self-limiting, but some people developed complications and others died, mainly during the second year. We need more data to understand pandemic influenza viruses' behaviour.

Miscellanea

1.0-001

Characteristics and malaria prevalence of migrant populations in malaria-endemic areas along the Thai-Cambodian border

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The rise of artemisinin resistant *Plasmodium falciparum* on the Thai-Cambodia border is an urgent public health threat. Little is known about migrant workers along the Thai-Cambodia border who are exposed to malaria in the course of their work and thus may contribute to the spread of artemisinin resistance. We conducted an employer-based survey of migrant workers in two provinces on the Thai-Cambodia border to explore socio-economic conditions, bednet ownership, and parasite prevalence among migrant workers. In total, 5371 migrant workers were enrolled, of which 56.9% were male and 43.1% female. Cambodians made up 69.0% of migrant workers, followed by migrants from Myanmar (20.7%). The remaining 10.3% were Mon or Laotian. Short term (<6 month) Cambodian migrants were primarily located in Chantaburi province, where they typically worked in orchards or on cassava farms. The majority did not speak Thai, and bednet ownership was low. The only cases of malaria, all *P. vivax*, were found in Chantaburi. In contrast, migrants in Trat province were primarily long-term residents (>6 months), both from Cambodia and Myanmar, engaged in rubber tapping, fisheries, and domestic work. Bednet ownership and Thai oral fluency were higher, though Thai literacy remained low. Migrants from Myanmar had higher mother tongue literacy than migrants from Cambodia. This study gives preliminary information regarding migrant populations on the Thai-Cambodia border, and will enable targeting of malaria prevention interventions to populations most at risk. The low Thai oral fluency and literacy suggest that an Behavior Change Communication (BCC) package for Cambodian migrant population groups would be most effective in the mother tongue. The low parasite prevalence and absence of *P. falciparum* in this sample are encouraging signs in the fight against artemisinin resistance in eastern Thailand.

1.0-002

Improvement of global access to life-saving medicines- facing the future

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In this research, with the main focus on HIV/AIDS, tuberculosis and malaria, materials for data collection were the literature, a global questionnaire and interview surveys with the main stakeholders: authorities, drug-developers and NGOs/foundations. The results of the surveys revealed the following determinants that contribute to the occurrence of drug pricing conflicts in Brazil, Thailand and South Africa in the past 10 years: governmental constitutional commitments to supply medicines to poor people, the existence of a local pharmaceutical industry capable of producing generic versions of patented medicines, and long histories of disease treatment programmes. The research documented the preferred approaches to increase global access to life-saving medicines for the next decade, which were found to be: public-private-partnerships, prevention measures, dedication of >0.5% of GNP to poor countries, and improvement of national healthcare/insurance system and improvement of the quality of medicines. The global financial and economic crisis was reported as the main roadblock to further increase access to life-saving medicines worldwide. As a conclusion, the most preferred approaches to increase global access to life-saving medicines for the next decade – as perceived by the key stakeholders – were integrated into a conceptual framework, which could enable country-level organizations to move beyond the conflict model mentality via 'Public-Private-Partnerships for gradual Self-Sufficiency and Sustainability (P3S3)'. Within this framework, rich countries should invest >0.5% of their GNP to help to alleviate poverty in poor countries. With these funds, national governments should implement programmes to expand implementation of disease prevention measures and improve national healthcare/insurance systems and the quality of the medicines involved. Public-private-partnerships should act as 'steering-and-controlling' organizations to guide the process and to minimise corruption. As such, the scope of public-private-partnerships could be extended beyond their traditional scope, which has been, so far: product-based, product-development-based, or systems/service-based.

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1.0-003

Eschar swabbing for diagnosis of mediterranean spotted fever (*Rickettsia conorii*)

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OBJECTIVES The utility of skin swab for diagnosis of rickettsial diseases was evaluated in a prospective study in Algeria, an endemic region for rickettsioses with four tick borne rickettsioses and one flea-borne spotted fever.

METHODS For each patient presented a picture of spotted fever with inoculation eschar, a dry sterile swab was realized. All eschar swabs were transported to Unité des Rickettsies, Marseille, France. DNA samples extracted from 200 µl solution of echar swab were tested by genus-specific quantitative (q)PCR for spotted fever group Rickettsiae, Bartonella, Coxiella burnetii, *Borrelia*, for universal 16S rRNA and actin gene.

RESULTS Clinical pictures and outcome for 39 patients are detailed. The mean duration of treatment was 3.5 ± 1.4 days; 97.4% patients recovered after antibiotic treatment. Twenty-six eschar swabs were positive for Rickettsial DNA by qPCR (26/41, 63.4%). The prevalence of *R. conorii* was 64%. The mean C_t value of actin gene was significantly lower for *R. conorii* negative samples (P = 0.0003). Four patients had swab and skin biopsy samples. Three of four patients were tested positive for *R. conorii* conorii specific qPCR on both swab and skin biopsy samples. The mean Ct value [±SD] of *R. conorii* conorii amplification by qPCR of three positive swab samples was 34.5 ± 3.56 and for three biopsy samples: 31.25 ± 4.59. Analysis of questionnaire sent to health professions and patients showed that they proffered to have an eschar swab to skin biopsy (P = 0.0001). qPCR for *Bartonella*, *Coxiella burnetii*, *Borrelia*, and for universal 16S rRNA was negative for all tested samples.

CONCLUSIONS The eschar swabbing can be competently an easy method to diagnosis of rickettsioses and a helpful tool for future epidemiologic and clinical studies. The detection of Mediterranean spotted fever using eschar swab is in accordance with those from other study realized on skin biopsy samples, as gold standard.

1.0-004

Mycobacterial lymphadenitis patients infected with HIV

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Mycobacterium tuberculosis is the most important etiological agent producing pulmonary as well as extrapulmonary infection. During these last decades, the increase in the incidence of infection due to other mycobacteria species is evident. Lymphadenopathy is the most frequent extrapulmonary presentation form of Mycobacterium Genera infection among HIV positive patients either in developed or underdeveloped countries. The aim of this work is to analyze the results obtained during January 2008 – December 2010 in our laboratory. Two hundred and ten tissue samples were studied; 190 (90.4%) samples were lymph node biopsied tissues and 20 (9.5%) samples were obtained by fine needle aspiration; 17 were from HIV-patients (8.1%) and 193 from HIV+ (91.9%). A total of 16 (7.6%) samples produced a positive culture for BAAR, four VIH-(25%) and 12 VIH+ (75%). Classification and identification for mycobacteria confirmed *Mycobacterium tuberculosis* in 13 of the cases (81.25%), and Mycobacterium avium-intracellulare in three patients (18.7%). The present study once again confirms that BAAR culture has more sensitivity and specificity than histopathologhic studies have. Lymphadenopathy in immunosuppressed patients should by studied for the presence of an BAAR coinfection where *M. tuberculosis* is still the agent most frequently found, nevertheless, other species of Mycobacteria may be causing infection and should be searched for. Our objective as National Reference Laboratory of Tuberculosis and Mycobacterial was to obtain the etiological characterization of Mycobacterium lymphadenopathy in clinically suspect patients.

1.0-005

Comparison of leishmaniasis laboratorial diagnosis methods in diagnostic centers in South Iran

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Leishmaniasis is one of the most important parasitic-skin disease in different of the world including in Iran. Leishmaniasis is considered as a Endemic disease in south of Iran, but this disease has reached 549 cases in 2010 from 67 cases in 2005.The increase of sensitive and nonimmune persons is considered as a factor of the outbreak. But the other point is the change of laboratorial diagnosis method of this disease in medical-hygienic centers of the city. The object of this research is the analysis of the effect of this factor. This was a qualitative study done with questionnaires and oral surveys at all 10 diagnostic centers. We evaluated the quality of the test and diagnostic personnel's knowledge. One of the most important diagnosis centers in the city changed the method of sampling. They took samples from the center of the sore. As a result staff was retrained in diagnosis. Although the increase in sensitive persons due to new births is an important factor in outbreak of cutaneous Leishmaniasis, there are some other factors: The attention of health care providers to the cases and better knowledge and efficiency have probably increased case finding. In other words, the lack of correct laboratory diagnosis can increase the amount of unknown ill persons who may act as a source of disease in the region. Contagious disease in the region may be controlled by retraining personnel and improving diagnostic methods.

1.0-006

Specific immunoglobulins M, A and E in primary and secondary dengue infection in adults and children

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INTRODUCTION Dengue virus is a mosquito-borne flavivirus that causes a spectrum of illness ranging from an unapparent infection to mild undifferentiated fever, the classical dengue fever and the severe form, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). In this work the immune response to IgM, IgA and IgE antibodies in patient groups with differences in the epidemiological conditions, ethnicity and ages were studied.

METHODS AND MATERIALS The study groups were constituted of 127 serum samples from adult patients of the Cuban dengue three epidemic of 2001–02, 71 serum samples from children patients collected during the dengue four epidemic of El Salvador (2002) and 145 hospitalized Honduran pediatric cases from the epidemics of 2004 and 2005. All were characterized for a clinical picture of dengue fever or dengue hemorrhagic fever and with primary or secondary infection. All samples were collected within 5–7 days of fever onset and tested by capture ELISA in order to detect dengue antibodies.

RESULTS Significant differences were observed in the IgM, IgA or IgE response in the studied groups. Higher optical density ratios

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for IgA and IgE antibodies in secondary dengue cases than primary cases were found. The relationship between antibody responses and severity are discussed. The usefulness of serotype specific IgM antibody detection is also analyzed.

CONCLUSIONS The significance of this result should be considered in future studies to elucidate the role of these immunoglobulins in terms of protection, recovery from infection and immunopathogenesis, and also in the studies of dengue vaccine evaluation.

1.0-007

Updated epidemiology of cysticercosis in Madagascar

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Neurocysticercosis is the most important neurological diseases occurring in developing countries where pork is eaten and hygiene is low. Madagascar is a highly endemic country with a seroprevalence of 7–21%. However, previous studies were based on: 1/various small surveys with different epidemiologic criteria (imperfect for a cartography) 2/antibody detection ELISA test that cannot be considered as a valuable assay because of its poor sensitivity (<30%). In this study, data were updated with a combination of Antigen detection ELISA (ITM, Anvers, Belgium) and Antibody detection ELISA (CDC, Atlanta, USA). Two groups were included: Group 1: Antananarivo (all ages): Group 2: Children aged between 10 and 15 y.o. from eight different cities in four different biotopes.

RESULTS Groupe 1: Positive Negative Elisa Ag (N = 2094) 527 (25.2%) 1567 (74.8%) Elisa rt24 (N = 2048) 269 (13.1%) 1779 (86.9%) Groupe 2: Positive Negative Elisa Ag (N = 1760) 489 (27.8%) 1271 (72.2%) Elisa rt24 (N = 1799) 242 (13.4%) 1557 (86.6%)

DISCUSSION These results show high exposure of the population to the parasite and a relatively high prevalence of active infections (>25% of positive Elisa Ag tests), occurring equally in children and adults. No significant differences were found between rural and urban population ($P < 0.005$). Antibody detection may be underreported due to the poor sensitivity of this test for low cysts carriers. Antigen rate may decrease after infection except if reinfection occurs, thus no test is ideal for epidemiology study but a combination of tool may reflect the current situation. Without improving hygiene education, implementing sanitations projects and ensuring meat inspectors integrity, the pig tapeworm cycle will not stop and the disease will continue to spread over the island.

1.0-008

Epidemiological studies on ticks and tick borne parasites, in Shalatin city, red sea governorate, Egypt

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The purpose of this study was to examine tick infestation in camels, sheep and goats in Shalatin City, Red sea governorate, Egypt to determine the epizootiology of the tick species and to investigate blood borne parasites. Tick infestation was detected in 27.94% of camels, 18.22% of sheep and 23.58% of goats. Tick infestation on camels by *Hyalomma dromedarii* was predominant (90.90%). Other tick species found in smaller numbers were *Hyalomma impeltatum* (3.90%), *Hyalomma anatolicum* (2.93%) and *Amblyomma lepidum* (2.26%). Ticks were found on camels throughout the year and increased in numbers during May, June, July and August with a peak in August and a mean monthly total of 22.91–26.56 ticks per animal. On sheep and goats *Rhipcephalus* spp. and *Haemophysalus* spp. were predominant (45.14% & 38.19%) (44.23% & 38.46%) followed by *Hyalomma dromedarii* in sheep (16.66%) and *H. anatolicum* (17.30%) in goats. Ticks were found on sheep and goats during May, Jun, July and August with a peak in August and a mean monthly total of 22.91–26.56 ticks per animal. Giemsa-stained thin blood smears prepared from infested camels, sheep and goats were examined for presence of tick borne haemoparasites. Monthly and cumulative incidences are presented of Theileria camelensis (37.01%), *Babesia* sp. (9.09%) and *Anaplasma marginale* (3.90%) in camels. Where in sheep Theileria ovis (24.39%), *Babesia* sp. (12.20%) *Anaplasma marginale* (14.63%) *Eperythrozoon* sp (9.76%) *Theileria* spp (40%), and *Anaplasma marginale* (24%) in goats. Examination of the haemolymph and egg smears of the obtained engorged adults ticks revealed presence of five comma, banana, spindle, club, crescentic and rod. While of salivary gland of adult ticks *Hyalomma dromedarii* showed stages of *Theleria* sp., and *Babesia* sp.

1.0-009

Immunological determinant underlying the control of *Trypanosoma brucei gambiense* infection in humans

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Human African Trypanosomiasis or sleeping sickness is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rodhesiense* parasites that are transmitted to humans by tsetse flies. As for many infectious diseases it is now clear that a wide range of outcome may result from the infection by trypanosomes. The disease is classically characterised by an early haemolymphatic phase (stage 1) followed by a meningoencephalitic phase (stage 2) leading to neurological disorders and death if left untreated. However, in *T. b. gambiense* endemic area where mass screening of the population is routinely performed by the Card Agglutination Test for Trypanosomiasis (CATT), a high proportion of individuals displaying positive serological results are negative to direct parasitological investigations. Increasing evidence now indicate that at least part of these subjects are infected but harbour parasitaemia levels that are below the detection limit of the parasitological tests used in the field, suggesting that they are able to control infection. The nature of the immune response in these individuals has yet received poor attention. In this communication we report on the quantification of the cytokine levels (IL-12, IL-2, IL-4, IL-5, TNF-£\, INF-£^, IL-8, IL-1*f*Ò, IL-6, IL-10) measured in healthy endemic controls, stage 1 and stage 2 patients and on a cohort of seropositive subjects from Guinea that were followed up in time to assess the evolution of their parasitological status. Whereas HAT patients were characterized by elevated levels of IL-1*f*Ò and IL-10, seropositive subjects exhibited high levels of IL-6, IL-8 and TNF-*f*Ñ and low levels of IL-1b, IL-12 and IL-10. Interestingly high levels of IL-10 in seropositive subjects were also associated with an increased risk of developing the disease in this category of subjects.

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1.0-010

Acute pesticide poisoning in the region of Tadla-Azilal, Morocco, 1980–2007

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INTRODUCTION AND OBJECTIVE The cases of acute pesticide poisoning (APP) are the cause of morbidity and high mortality in the world. Developing countries are particularly affected by this scourge because of lack of regulation, and surveillance systems and inadequate access to information systems. The objective of this study was to describe the clinical and the evolutionary aspects of epidemiological patients poisoned by pesticides in the region of Tadla-Azilal in Morocco.

MATERIALS AND METHODS A retrospective descriptive study over a period of 19 years, based on the database toxicovigilance at the Moroccan poison center.

RESULTS Among cases of APP (severe acute poisoning by a pesticide) 66% were of rural origin. Patients were symptomatic in 98% of cases, and then disorders of the gastrointestinal tract were more frequent with 92.2%, followed by disorders of the central and peripheral nervous system (5.7%), disorders of the frequency and heart rate (2%). The poisonings occurred in circumstances volunteers in 62%. Organophosphates were the most often implicated products in the study area with 78.5%. Oral exposure was predominant in 74.8% of cases. The most affected age group was adults (56.4%), followed by of adolescents (25%); the average age of addicts was 13.56 ± 23.59 years. By PSS, poisoning grade 2 represented 85.8%. The outcome was favorable in 6064 cases, 28 cases had complications, with a fatality rate of 0.04%.

CONCLUSION Pesticide poisonings are a real concern in the region of Tadla-Azil, which is predominated by the agricultural sector. However, public awareness and health professionals seem to be the first necessary step to reduce the severity and extent of the problem.

KEYWORDS epidemiology, pesticides, poisoning, Tadla-Azilal

1.0-011

First molecular epidemiological study of cutaneous leishmaniasis in Libya

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Cutaneous Leishmaniasis (CL) is a major public health problem in Libya. Diagnosis was based on clinical symptoms and microscopic observation of parasites in stained skin biopsies. Disease outbreaks and parasite characterisation were not investigated sofar. In our study we screened 450 cases from all endemic areas (49 villages). The age ranges from 9 months to 87 years (median age 25 years), 54% of the cases are males and 46% females. Skin scraping spotted on glass slides were collected from 195 patients and subjected to DNA extraction using phenol-chloroform method. Ribosomal internal transcribed spacer 1 (ITS1) followed by restriction fragment length polymorphism (RFLP) analysis was performed for molecular characterization of the parasite. One hundred and forty-eigt (75.9%) of the samples have a profile identical to the WHO reference strain of *Leishmania* major and 47 (24.1%) were *Leishmania* tropica. The results were confirmed by DNA sequence analysis of the ITS1 PCR products from 10 samples (5 *L. tropica* and 5 *L. major*) which were compared by multialignment and were identical with DNA sequences from *L. tropica* and *L. major* reference strains. To our knowledge, this is the first molecular study on cutaneous Leishmaniasis in Libya. The focuses are shown to follow the epidemiological pattern of Old World CL caused by *L. tropica* and *L. major* in the Mediterranean region. These results must be considered by physician during treatment as CL caused by *L. tropica* tend to last longer and are more difficult to treat than those caused by *L. major*. Further investigation of the parasite vector and host is needed to understand the parasite life cycle in this area.

KEYWORDS Libya, Cutaneous Leishmaniasis, *Leishmania tropica*, *Leishmania major*, internal transcribed spacer 1

1.0-012

Survival of people living with HIV/AIDS in Brazzaville: before antiretrovirals, with antiretrovirals and with free antiretrovirals

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BACKGROUND Ambulatory Treatment Center (CTA) of Brazzaville, created since November 1994, is the first center to care for people living with HIV/AIDS in Congo, as such it has witnessed three stages that marked the care of HIV patients in our country, namely a symptomatic treatment initially without antiretroviral drugs from 1994 to 2001, then with antiretrovirals from 2002, these antiretroviral drugs were first paid and then became free from 2007. Our objective was to assess changes in survival of people with HIV followed CTA during these three stages.

METHODS Patients infected with the CTA consulted in Brazzaville, Congo between November 1994 and December 2009, have been divided into three groups: before the arrival of antiretroviral drugs (group 1: 1014 patients) [1994–2001], with paid-for antiretrovirals (group 2: 2390 patients) [2002–2006] and with free ART (group 3: 669 patients) [2007–2009]. The probability of survival was calculated using Kaplan–Meier, and log-rank test.

RESULTS The mean age of patients arriving at CTA was 36.68, 36.22, 35.92 years respectively in group 1, 2 and 3, and 58.9%, 66.2% and 63.4% respectively were women. BMI was starting 21.29, 21.22 and 20.83 kg/m^2 respectively in group 1, 2 and 3, average CD4 count at the finish was respectively 321.85, 232.23 and 291.69 cells/mm^3 and average rate of hemoglobin was 10, 10.51 and 10.52 g/ml. A total of 604 deaths and 2008 lost to have been recognized namely 360, 239 and five deaths, and 647, 1112 and 249 lost to respectively in group 1, 2 and 3. The probability of survival calculated by contributing to each group was 50%, 93% and 99% (P = 0.0000) at 13 months respectively in group 1, 2 and 3.

CONCLUSION Antiretroviral drugs have significantly improved the survival of our patients; the decision to make ARVs free has been salutary.

1.0-013

The hidden hunger: understanding the burden of anaemia and its determinants among pregnant and non-pregnant women in Ethiopia

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INTRODUCTION Anaemia is a global public health problem associated with increased mortality and morbidity. The highest prevalence of anaemia exists in developing world where its causes are multi-factorial.

OBJECTIVE To assess burden of anaemia and its determinants among pregnant and non-pregnant women in Ethiopia

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METHODOLOGY The 2005 DHS data of Ethiopia were used. Permission to download and analyze the data was granted from ORC Macro, in Calverton, USA. SPSS v16.0 was used to analyze the data. Both univariate and multivariate analysis were carried out. For all statistical tests significance level was set at P-value of 0.05.

RESULT Five thousand nine hundred and sixty women of child bearing age were included in the analysis. The prevalence of anaemia was 33.0% (95% CI: 28.7, 37.2%) and 27.3% (95% CI: 26.1, 28.4%) among pregnant and non-pregnant women respectively. Prevalence of anaemia was positively associated with past 5 years fertility level (chi-square for linear trend, P < 0.0001). Not possessing any toilet facilities (OR 1.85 95% CI: 1.66, 2.1), being resident of rural area (OR 2.02 95% CI: 1.75, 2.32) and not using contraceptive methods (OR 1.63 95% CI: 1.34, 1.98) were also associated anaemia. Logistic regression showed toilet possession to be the only independent predictor of anaemia among pregnant women (Adj OR 2.17 95% CI: 1.28, 3.85). Regression analysis among non-pregnant women revealed not having any toilet facilities (Adj OR 1.20 95% CI: 1.02, 1.42), lower altitude of residential areas and not using contraception methods (Adj OR 1.39 95% CI: 1.13, 1.72) to be independent predictors of anaemia.

CONCLUSION Anaemia is a moderate public health problem among women in Ethiopia but there exist significant magnitude differences by socio-economic status of women, their families and where they live. Interventions designed to address maternal anaemia should pay attention to both nutritional and non-nutritional interventions and may include environmental sanitation, deworming, provision and promotion of family planning methods.

1.0-014

Cross reaction between crude hydatid cyst fluid (HCF) antigens of human and animals origin (Mice, Sheep, Cattle) in response to human IgG class, IgG subclasses and IgM antibodies

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OBJECTIVES The use of crude HCF antigen for the diagnosis of hydatid cystic is one method which can be helpful and effective in rapid treatment of the disease. The current study aimed to evaluate the cross-reactivity of human sera against crude HCF antigens of animals in order to find the target antigens with the highest IgG class, IgG subclasses and IgE response from the human immune sera.

MATERIALS AND METHODS Human and animal crude HCF used as the source of antigen for ELISA, Western blotting and immunization the mice. Sample sera were collected from patients together with some human or animal sera with no history of hydatidosis with negative HC using ELISA and IFAT as controls. Totally 30 positive samples from each animal and human sources were used as the case together with 30 healthy sera from each as control. ELISA and SDS PAGE gel electrophoresis was carried out under reducing conditions.

RESULTS The highest mean OD of the human IgG was against antigen B (0.93) and the lowest against cattle HCF antigen (0.32) (P < 0.001). ELISA revealing that the highest mean OD value in response to human, sheep and mice HCF antigen was related to IgG4 while the lowest to IgG3. The sensitivity and specificity of ELISA test that was used for evaluating the responses of human total IgG to different HCF antigen was 100% and 95.8% respectively. Cross reaction of human IgG class and subclasses and IgE response was found almost for all antigens with the best reaction against human HCF antigen and antigen B.

CONCLUSION Human sera showed a considerable cross-reactivity against human, sheep, cattle and mice HCF antigens by ELISA test. The human HCF and antigen B were superior over other antigens in that their mean OD values and their OD ratio significantly higher than others.

1.0-015

When first-line antiretrovirals fail: delay and impact on the immunologic and virologic profile

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BACKGROUND In poor countries, the change of antiretroviral therapy after treatment failure often occurs later due to technical failures. Our objective was to evaluate impact of the delay on immuno-virological profile of patients after switching to second line.

METHODS We conducted a retrospective study. We applied the criteria for antiretroviral treatment failure as described in WHO recommendations for a new public health approach 2010 to define failure in our patients aged 18 and over followed at the Ambulatory Treatment Center in Brazzaville, Congo between October 2005 and August 2010. We noticed there was an interval of time between the effective date of the occurrence of immunological or virological failure (fall in CD4 over 50% of its peak, CD4 always <100 cells/mm^3 and viral load >5000 copies/ml on two samples) and the effective date of the switch 'delay'. The Fisher exact test was used.

RESULTS A total of 75 patients failing antiretroviral therapy first-line have been identified, 57.3% were women. The mean age was 43.24 years (range: 18.95–65.56 years). The average delay was 20.80 months (range: 1.11–48.66 months), during this period the average CD4 count decreased from 146.77 cells/ml (range: 3–931 cells/ml) to 75.56 cells/ml (range: 1–322 cells/ml), median viral load had increased from 60,000 copies/ml (range: 5000–400,0000 copies/ml) to 100,000 copies/ml (range 5000–940,000 copies/ml). Protocol ABC/DDI/LPV/RTV represents 70.7% of patients on second line, 12% of patients had new criteria for immunological and virological failure during second-line treatment. Four percent of patients died and 4% lost to. Thirty-eight patients was in the group <20 months while in the group ≥20 months the number of people was 37 patients. The delay before the switch was not associated with the onset of failure when it was 20 vs. <20 months (RR = 0.82, 95% CI:0.24–2.82 P = 1).

CONCLUSION AND RECOMMENDATION The impact of delay on immuno-virological profile of patients is obvious, but we found no association between delay and the occurrence of a second failure.

1.0-016

Convincing HIV prevalence declines in Zambia among young people (15–24 years): analysis and review of different data sources

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INTRODUCTION Prevalence studies from several sub-Saharan African (SSA) countries have shown major declines in HIV prevalence among young people (15–24 years) recently. Therefore, this study examined HIV prevalence trends among young people aged 15–

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24 years in Zambia employing different data sources, and analysis was conducted at different geographical-levels and in different population sub-groups.

METHODS AND MATERIALS We analysed ANC-data from 22 sentinel sites consistently covered in the period 1994–2008 and HIV data from the Zambia Demographic and Health Surveys (ZDHS) 2001/2 and 2007. In addition, we systematically reviewed peer-reviewed articles that reported findings on HIV prevalence or incidence in Zambia.

RESULTS Overall ANC-data showed that HIV prevalence declined by 39% (P < 0.0001) and 17% (P = 0.001), among urban and rural attendees, respectively. Provincialdeclines differed substantially, i.e. between 10% and 68% among urban women, and from stability to 86% decline among rural women. Prevalence declines were steeper in the group with highest educational attainment compared with the least educated group. The ZDHS data indicated a reduction in prevalence between the two survey rounds among women and a tendency towards increase among men. Provincial-level ZDHS changes were difficult to assess due to small sample sizes. ANC-based trend patterns were consistent with those observed in PMTCT-based data (2002–2006), and findings from population-based surveys in Ndola and Chelstone.

CONCLUSION The findings suggest overall HIV incidence declines in Zambia since early 1990s. These overall trends masked substantial differentials by place and by educational attainment, demonstrating critical limitations in current focuses on country-level trends in epidemiological reports.

1.0-017

Larval mosquitoes surveillance with emphasis in malaria, dengue and lymphatic filariasis vectors after earthquake, Haiti, 2010

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INTRODUCTION The earthquake occurred in January, 2010 caused large-scale devastation in Haiti. The Cuban Minister of Health assisted the Haitian Minister of Health by conducting a rapid field assessment of entomological-related issues. Among the actions recommended by the Cuban team was immediate vector control assistance to reinforce and expand entomological surveillance.

OBJECTIVES (i) To identify mosquito species (ii) To identify the mainly breeding sites for malaria, dengue and lymphatic filariasis vectors (iii) To know the density fluctuations for these mosquito vectors.

METHODS The survey was carried out in 26 communes in the 10 departments in Haiti. For *Anopheles albimanus* and *Culex quinquefasciatus* standard dipper for the survey was used. *Aedes aegypti* traditional index were estimated. The survey was executed from May to December, 2010.

RESULTS Eighteen mosquito species were identified. *Ae. aegypti* was found in all studied areas and during the whole survey A total of 3693 positive containers were found being the most frequent containers water-storage followed by used car tires and miscellaneous artificial containers *An. albimanus* was found in 17 (65,4%) and *Cx. quinquefasciatus* in the 26 communes surveyed. The mainly permanent breeding sites for *An. albimanus* were rice fields, lagoons, drains followed by river margin for *Cx. quinquefasciatus* were wells and artificial containers.

CONCLUSIONS The results presented in this study report data on the distribution, breeding sites and density fluctuations of *Ae. aegypti*, *An. albimanus* and *Cx. quinquefasciatus* populations in Haiti, which are vectors of dengue, malaria and lymphatic filariasis, respectively.

1.0-018

Emergent, unknown, misdiagnosed? Sporotrichosis and chromoblastomycosis in Madagascar: diagnostic algorithms

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Emergent, unknown, misdiagnosed may qualify two type of fungal infection in Madagascar: sporotrichosis and chromoblastomycosis. The aim of this study is to present algorithms summarizing the diagnostic steps for both of these diseases. Starting from the clinical and epidemiological data, samples are processed following laboratory procedures and clinical or surgical treatment will be administered to the patient. Sporotrichosis is a subcutaneous disease due to *Sporotriochosis schenkii*, an environmental dimorphic agent that typically causes lymphnode enlargement following a lymphatic pathway, ulceration may occurs and can lead to secondary bacterial infections. The disease is currently found in the Malagasy highlands: with a fairly cold and dry weather and is directly related to injure of wood splinters or plant thorns. Treatment is based on Potassium Iodine or Itraconazole. Pro and cons are listed. Chromoblastomycosis occurs mainly in the dry and hot South-East of Madagascar where deamacious fungi harboured by cactuses and dry plants thorns are said to be responsible of this chronic subcutaneous disease displaying many kind of clinical lesions (displayed) pathology is the base of its diagnosis, fungi are hard to isolate thanks to the overgrowth of other environmental moulds. Its treatment relies mostly on terbinafine, even though a combination of antifungal agents is strongly advised. Surgery may be used for limited lesions and relapses may occur due to a default of patient compliance or treatment inefficiency.

1.0-019

Transfer of newborns to neonatal care unit: a registry based study in Northern Tanzania

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BACKGROUND Reduction in neonatal mortality has been slower than anticipated in many low income countries including Tanzania. Adequate neonatal care may contribute to reduced mortality. We studied factors associated with transfer of babies to a neonatal care unit (NCU) in data from a birth registry at Kilimanjaro Christian Medical Centre (KCMC) in Tanzania.

METHODS A total of 21,206 singletons live births registered from 2000 to 2008 were included. Multivariable analysis was carried out to study neonatal transfer to NCU by socio-demographic factors, pregnancy complications and measures of the condition of the newborn baby.

RESULTS A total of 3190 (15%) newborn singletons were transferred to the NCU. As expected, neonatal transfer was strongly associated with specific conditions of the baby including birth weight above 4000 g [relative risk (RR) = 7.2; 95% confidence interval (CI) 6.5–8.0] or below 1500 g (RR = 3.0; 95% CI: 2.3–4.0), 5 minutes Apgar score 0–6 (RR = 4.0; 95% CI: 3.4–4.6), and preterm birth before 34 weeks of gestation (RR = 1.8; 95% CI: 1.5–2.1). However, pregnancy- and delivery related conditions like premature rupture of membrane (RR = 2.3; 95% CI: 1.9–2.7), preeclampsia (RR = 1.3; 95% CI: 1.1–1.5), other vaginal delivery (RR = 2.2;

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95% CI: 1.7–2.9) and caesarean section (RR = 1.9; 95% CI: 1.8–2.1) were also significantly associated with transfer. Birth to a first born child was associated with increased likelihood of transfer (RR 1.4; 95% CI: 1.2–1.5), while the likelihood was reduced (RR = 0.5; 95% CI: 0.3–0.9) when the father had no education.

CONCLUSIONS In addition to strong associations between neonatal transfer and classical neonatal risk factors for morbidity and mortality, some pregnancy-related and demographic factors were predictors of neonatal transfer. Overall, transfer was more likely for babies with signs of poor health status or a complicated pregnancy. Except for reduced use of transfer for babies of non-educated fathers and a high transfer rate for first born babies, there were no signs that transfer was based on non-medical indications.

1.0-020

Fishborne trematode larvae infection in freshwater fish cultured in wastewater in Northern Vietnam

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INTRODUCTION Fishborne trematodes are widespread in the world, especially in Asian countries. In Vietnam, fishborne trematode also is a major public health problem and widely distributed. The assessment of the prevalence of fishborne trematode infection in fresh water fish using waste water in rural and urban areas in Vietnam are needed, which will contribute to the development of control strategies of these diseases.

METHODS AND MATERIALS A total of 1000 freshwater fish were collected from fishponds fed with wastewater and examined for trematode larvae in the rural and urban areas of the plains and mountains of Northern Vietnam, 2009–2010. Identification of fishborne trematode species used morphology and molecular methods.

RESULTS The infection rate of trematodes in fish in the urban plain was 10.0%, in rural plains 32.8%; in urban mountains 3.2% and in rural mountains 16.0%. Fishborne trematode larvae were identified as *Clonorchis sinensis* in the *Opisthorchidae* family, *Haplorchis taichui*, *H. pumilio* and *Centrocestus formosanus* in the *Heterophyidae* family.

CONCLUSIONS Fish cultured in wastewater was infected with fishborne trematodes more often in rural than urban plains and moutains.

KEYWORDS Clonorchis sinensis, Haplorchis taichui, H. pumilio, Centerocestus formosanus

1.0-021

Fascioliasis infection in tumour liver patients in Hanoi hospitals 2006–2010

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INTRODUCTION Human fascioliasis is widespread in the world, particularly in Asian countries, including Vietnam, where it occurs in 47 of 64 provinces with over 6000 patients. Fascioliasis causes damage in human livers similar to that of a tumour. However, assessment of fascioliasis infection in tumour liver patients group is needed.

METHODS AND MATERIALS Two hundred and eighty-three patients diagnosed with liver tumour were examined for fascioliasis infection using clinical, ultrasound, CT scanner, MRI, blood, ELISA examination in Hanoi hospitals during 2006–2010.

RESULTS The results were showed that, the fascioliasis infection rate was 34.6% (98/283), the infection rate in women was higher than that in men (46.1% and 28.2%). In the fascioliasis group (women 48%, men 52%) the main clinical symptoms were fever in 74.5%, weight loss in 71.4%, liver pain in 70.4%, plodding in 63.3%, abdominal pain in 61.2%, digestive disorder in 22.4%, allergy in 19.4%, jaundice in 10.2% and bile-duct bleeding in 1%. The main para-clinical symptoms in fascioliasis patients were positive ELISA test for *Fasciola gigantica* antigen of 100% with titer of 1/3200 to 1/12,800; clearly prejudice in liver by ultrasound of 100%; eosinophilia of 89.8%; positive stool examination with *Fasciola* egg of 4.1%. The main clinical symptoms in tumour patients (non fascioliasis) were weight loss (49.2%) and liver pain (16.8%), other symptoms were less common.

CONCLUSIONS In a group of liver tumour patients, the fascioliasis infection rate was 34.6%. A comparison of fascioliasis and non-fascioliasis patients revealed that the symptoms of fascioliasis were most clear in fascioliasis patient group.

KEYWORDS tumour liver, fascioliasis, symptoms

1.0-022

TNF blocker, but not dexamethasone, is able to increase survival in mice acutely infected with a virulent strain of *Trypanosoma cruzi*

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Chagas disease is caused by the intracellular protozoa *Trypanosoma cruzi*, which causes myocarditis and organomegaly. The pathophysiological mechanisms associated to virulence, concretely the role of inflammation related to parasite replication, are still unclear. The aim of this work is to determine the effect of a TNF blocker, etanrecept (Enbrel®) and a corticosteroid dexamethasone (Decobel®) on parameters of the acute phase such as survival, sickness behavior and allodynia. NMRI mice (30 g) were infected intraperitoneally with 1000 tripomastigotes per gram of a virulent Venezuelan isolate of *T. cruzi*. Mice were divided into an infected untreated group (n = 21), an infected group treated with 0.8 mg/kg of etanrecept at 7 days post-infection (N = 9), an infected group treated daily at 7 days post-infection with 3 mg/kg of dexamethasone for 5 days (n = 10) and control groups (treated with etanrecept and dexamethasone, n = 10 and 8 respectively). The horizontal and vertical motility was determined during 3 min in an activity cage (Ugo Basile®) and was measured daily during the second week post-infection. The mechanical allodynia was recorded in a plantar aesthesiometer (Ugo Basile®) during the first and second week post-infection. Etanrecept increases the survival time whereas dexamethasone reduces it. In animals treated with both etanrecept and dexamethasone a reversion of the motility decrease was observed during the second week with respect to untreated group. Mechanical allodynia was increased during the first week post-infection but not during the second week in untreated infected mice and was reverted by dexamethasone and etanrecept treatment. In conclusion, sickness behavior and allodynia are associated to acute Chagas disease and these alterations can be improved both dexamethasone and etanrecept treatment, but only etanrecept is able to increase the survival time. The inflammation associated to TNF could be responsible of high virulence in Venezuelan *T. cruzi* strains.

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1.0-023

Causes of seeking medical attention among travelers with chronic diseases visiting La Habana

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BACKGROUND Many people with a variety of chronic diseases travel abroad which can result in complications or exacerbations due to factors related to travel, medication and poor diet.

METHODS A cross-sectional study was conducted from January to June 2010 on a sample of travelers (≥15 years of age) with chronic diseases assisted at the emergency service of the Cira Garcia Central Clinic of La Habana, Cuba. The goal was to ascertain the characteristics of travelers with chronic diseases, the causes of medical attention and their relationship with previous illnesses.

RESULTS Chronic diseases were present in 302 travelers (12.3%) of those assisted in the emergency department during the study period, of which 180 (56.2%) were male and 122 (43.8%) female, being most common in travelers aged 45–64 years (n = 167; 55.3%) and 65 years and over (n = 68; 22.6%). Most travelers were originally from Europe (n = 131, 43. 4%) and most were accommodated in houses (73.8%). Pre-travel health advice was sought for 159 travelers (52.6%), 82 (21.7%) were authorized by their doctors to travel, 24 (7.9%) forgot to bring the medication and 6 (1.9%) lost their medication. During the stay, 96 (31.8%) reported a poor diet and dietary transgressions and 52 (17.2%) discontinued treatment. In 204 cases (67.5%) the causes of medical consultation were related to their chronic diseases, the most common conditions being hypertension (n = 129; 42.7%), diabetes mellitus (n = 62; 20.5%) and ischemic heart disease (n = 40; 13.2%). Finally 127 (62.2%) travelers received outpatient treatment, 77 (37.8%) were hospitalized, 41 (53.2%) of them in the intensive care unit and 3 (1.4%) died.

CONCLUSIONS Chronic diseases were an important cause of medical attention and hospitalization in surveyed travelers being necessary to improve some aspects of the quality of the pre-travel preparation related to importance of diet and medication while abroad.

1.0-024

Tick-borne meningoencephalitis in Slovenia

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INTRODUCTION Tick-borne meningoencephalitis (TBE) has been known to be present in Slovenia since 1953; its notification has been obligatory since 1977. Slovenia remains one of the countries with the highest reported incidence rates of TBE. Celje region with a population of 300,000 is the third largest in Slovenia and also one of the regions where prevalence of TBE is an important health issue.

METHODS The purpose of this paper is to present the incidence of TBE in Slovenia and in the Celje region through a descriptive epidemiological method for the 1989–2010 periods.

RESULTS Within the monitored 22-year period, we have recorded 5139 cases of TBE in Slovenia (11.4/100.000). The lowest annual number of patients in the Celje region was recorded in 1989 (four cases) and the highest in 1994 (79 cases), the latter also being the year with the highest number of reported cases in Slovenia (531 cases). The reported incidence rate in the region within the monitored period was 11.4 per 100.000 inhabitants. Within the observation period, the highest number of patients in Celje region was recorded in July (212) and none in February 0.62% of the patients were male and 38% were female. Among the reported cases there were 24 children under 5 years of age, while most patients were from the 36–45 age group.

CONCLUSIONS TBE is an endemic disease in Slovenia and also a vaccine-preventable disease. For occupationally exposed or during education/training, vaccination in Slovenia is reimbursed by employer or within mandatory health insurance, while others to pay. Due to serious sequelae of the TBE, the pre-travel consultation (protection against tick bites, measures after tick-bites and possibility of vaccination against TBE) for travellers to Slovenia must be provided.

1.0-025

Re-emergence of trichinellosis in Northern Greece: an outbreak after consumption of wild boar

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Trichinosis is a zoonotic disease with worldwide distribution. The most important source of human infection in EU is the domestic pig, meats of horses and wild boars have played a significant role during outbreaks. In Greece there are only sporadic reports about *Trichinella* sp. infections among wild animals, stray dogs and rarely in pigs. The latest report about human infection was in 1982 -an outbreak in a small village in northern Greece. We report an outbreak occurred on a small family-owned pig farm in northeastern Greece. Although in total eight persons appeared to have high titers of anti-*Trichinella* antibodies only one person developed the full clinical syndrome. The patient is a 30-year-old man who was admitted because of a history of high fever with marked eosinophilia. On admission the patient was febrile up to 41°C with a maculopalular rash all over the body, facial edema and mild edema at lower extremities. He was complaining for myalgia and general muscle weakness. After thorough examination it was elucidated that he had consumed inadequately cooked wild boar meat from a farm in northern Greece. Laboratory results showed mild leucocytosis with marked eosinophilia and moderate elevation of liver enzymes and creatine phosphokinase. Muscle biopsies from gastrocnemius muscle revealed encysted parasites into degenerated muscle fiber cells, a microscopic image compatible with larvae from *Trichinella spiralis*. The serology assessment for the *Trichinella spiralis* antibodies was positive in high titers. During hospitalization the patient developed heart failure with ECG abnormalities which was treated symptomatically. The patient recovered fully after a course of albendazole and corticosteroids. In conclusion, the outbreak of human trichinellosis in an area where feral animals have been previously considered free of *Trichinella* was unexpected. This warrants the need of high clinical suspicion in order to detect and treat such patients.

1.0-026

Prevalence of chagas heart disease outside an endemic area in a population at risk in Spain

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Chagas disease is the third most important parasitic disease worldwide. It is the leading infectious cause of myocarditis. An estimated 10 million infected people in Latin America and 30% develop heart disease. There is an increasing detection of patients (p) with this disease in non-endemic areas. In Spain may have more than 60,000 in chronic illness. Several factors influence the evolution of the disease, parasitic, immunological, therapeutic,

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nutritional, psychological, socioeconomic status and length of stay in endemic areas.

OBJECTIVE To determine the prevalence of Chagas heart disease in health care settings-hospital in a risk population residing in Spain.

METHODOLOGY Diagnosis of Chagas using two different serological tests. Medical history electrocardiogram (ECG) chest radiograph echocardiography (ECO) and/or Holter was performed.

RESULTS Between 2007 and 2011, 547 persons were analyzed with mean age 37 years. Four hundred forty were asymptomatic (80%), dyspnea 35 (7%), chest pain 32 (7%), palpitations 23 (4%) and dizziness 7 (2%). The ECG was normal in 437 (79.7%), atrial fibrillation or flutter 3 (0.6%), incomplete right bundle branch block 12 (2.2%), complete right bundle branch 20 (3.6%), left anterior hemiblock 18 (3.3%), combination of both 23 (4.2%). The mean heart rate (HR) was 61 bpm and 51 p (9.3%) had <50 bpm. The ECO was conducted in 101 p (18.5%). normal in 86 (85%), ventricular dysfunction, 3 (3%), ventricular aneurysm 3 (3%) and congenital or rheumatic heart disease 6 (6%). Holter monitoring was performed in 21 cases (3.8%), it was normal in 9 (43%), sinus node dysfunction 10 (48%), flutter 2 (9.5%) and extrasystole 2 (9.5%).

CONCLUSION ECG abnormalities were detected in 11.7% (stage II) and ECO changes in 1.1% (stage III). The prevalence of cardiac involvement is apparently lower than that in endemic areas but cardiac screening and follow-up of young people and chronic longstanding patients is important.

1.0-027

Access to antioxidant rich diet and development of schistosomal periportal fibrosis (PPF): report from an Ethiopian cohort revisited after 10 years

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OBJECTIVE Morbidity and mortality associated with *Schistosomiasis mansoni* are mainly the result of Symmers' peri-portal fibrosis (PPF) of the liver. Often disparities between community prevalence of infection and levels of PPF are observed. Our previous studies suggested that dietary factors related to antioxidant deficiency and oxidative stress may contribute to the development of fibrosis in the liver, either through direct stimulation or by promoting the production of pro-fibrotic cytokines. To test these hypothesis two study cohorts were established in different parts of Ethiopia.

METHODOLOGY A study cohort comprising of 333 schoolchildren (mean age 12.6 years) with comparable levels of *S. mansoni* infection was established in two *S. mansoni* endemic communities, in Worke-Mado (171) with poor access to antioxidant rich fruits and vegetable and in Sille (162), with good access to antioxidant rich fruits and vegetables.

RESULTS In the year 2002, the prevalence of *S. mansoni* infection in study subjects of Workemado and Sille were similar; 90.6% and 95%, respectively. However, 12 subjects (7.0%) from the study subjects in Worke-Mado but only one (0.6%) in Sille had PPF. In 2011, efforts were made to evaluate 159 subjects in Worke-Mado and 161 subjects in Sille who had no PPF at initial examination but found 145 and 120 of them, respectively. The proportion of subjects with *S. mansoni* infection at this time in Worke Mado and Sille were still comparably high, 82% and 61%, respectively. Among these, 20 (13.8%) in Worke-Mado and only 3 (2.5%) in Sille developed PPF over a period of 10 years. This difference is statistically significant ($P < 0.01$).

CONCLUSION These findings support our suggestion that access to antioxidant rich dietary sources may help prevent development of schistosomal PPF. Further analysis of dietary questionnaires, serum levels of antioxidants and markers of fibrosis is currently underway in both cohort groups.

1.0-028

Seroepidemiological study of canine visceral leishmaniasis in Sarab, Iran with elisa and IFAT

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In this study 384 serum samples from dogs of Sarab district were examined by using Elisa and IFA tests. Seroprevalence rate (SPR) of CVL in studied dogs with the Elisa and IFA, was respectively 9.1% and 8.5% (CI, 95% 6.6–12.4). The highest number of infected dogs was among three year old dogs (25.7%) and the lowest number of infections in 1 year old dogs (2.9%). Three hundred and six (79.7%) were male and 78 (20.3%) female. Twenty eight (9.2%) male dogs and seven (9.0%) females were seropositive. The difference infection and gender was $P = 0.962$. A statistically significant correlation between age and infection was observed ($P = 0.332$). Nine (23.2%) dogs had at least one positive symptom, including cutaneous ulcers, hair loss, impotence, onicocriptosis, local or general lymphadenopathy, keratitis, hepatosplenomegaly and diarrhea. Four (17.4%) symptomatic dogs and 31 asymptomatic dogs (8.6%) were positive and had no symptoms of visceral Leishmaniasis. The most common clinical symptom in dogs was marked wasting and hair loss with 25.7% in nine dogs, the least common symptom, hepatosplenomegaly, was found in five (14.3%) dogs. A statistically significant correlation between visceral Leishmaniasis and wasting and hair loss dogs was observed ($P = 0.031$). No Significant correlation between visceral Leishmaniasis and hepatosplenomegaly was seen ($P = 0.065$). Between symptomatic and asymptomatic dogs with visceral Leishmaniasis significant relationship was observed ($P = 0.015$). In this study antibody titers in male dogs were greater than in female dogs ($P = 0.023$). The highest rate of infection was in dogs from Jalda Bakhan village with 9 (33.35%) dogs and lowest infection rate was in Asbfroshan village with 1 (1.4%) dogs. Mosquito control plans are necessary.

1.0-029

Systemic quality improvement – Moroccan case scaling up to improve access to care

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The SQI is an approach to improve the management of quality of care developed with assistance of German agency of cooperation (GTZ) for decentralized health systems.

OBJECTIVES Targeted by the approach SQI (Concours Qualité)are: (i) To strengthen organizational development and a better functioning of the system, (ii) To improve the quality of services management and care provided with a view to make them more adapted to expectations of the population; (iii) To promote efforts of teams by establishing a culture of recognition.

METHODS The steps of the continuous SQI cycle consist of health care and health services quality self assessment, participatory auditing with peer evaluation, identifying improvement potentials and participatory planning, and implementing improvement

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activities. It results in a benchmark using agreed quality criteria and establishes national rankings, which give information on the performance of service delivery or management organizations. Between 2007 and 2011, four national editions have been organized the number of participants registers an improvement of 223%, which indicates the positive influence on the health system structures.

RESULTS AND THE ADDED VALUE The recognition of efforts and promotion the good performances; A special dynamic recognized by the representatives of participating structures and their hierarchy; The ability to share and to amplify good practices within the health system; The creation of a culture of evaluation and accountability; The strengthening of coherence between strategic and local levels. The performance of aspects of quality evaluated by the SQI has been improved especially at district and primary health centers levels: Satisfaction (from 49.7% to 62%), Leadership: (45% to 50%), Safety: (50% to 57%).

CONCLUSION The SQI has demonstrated its impact on the dynamic of quality improvement. As a result, the approach was institutionalized by the ministry of health (with the support of EU) indicating a high level of ownership by the system.

1.0-030

Battling stigma and discrimination of leprosy in India: a double jeopardy

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Leprosy Patients in India face stigma and discrimination including significant health challenges. Social, religious and cultural contexts most often contribute to various forms of stigma and discrimination among these patients. They pose extra challenges by hindering early interventions, loss of employment, exclusion from society while causing physical and emotional distress among the affected population. India currently has about 54% of all the new leprosy cases in the world, followed by Brazil with about 17%, then Indonesia with about 7%. As of April 2008, India reported 87,000 leprosy cases with a Prevalence Rate (PR) of 0.74/10,000. A total of 134,000 new cases were detected during the year 2008–09. This paper provides a systematic literature review about the stigma and discrimination faced by this population in India. The literature review was conducted through the systematic search of several online databases including Pub Med, Medline and Google.com. The review findings indicate that although the National Leprosy Elimination Program (NLEP) recognizes disease related stigma and discrimination as a key challenge to fight against the disease, comparatively little progress has been made in systematically addressing stigma by the national government, often resulting in discrimination in intervention programs under the NLEP strategic Framework. Therefore, further research is needed in this field to understand the dynamics of this complex psychosocial, cultural and behavioral problem and to identify which strategies are most effective in reducing stigma and discrimination. More rigorous study designs are needed to evaluate intervention programs targeting stigma. We propose that the national government may consider prioritizing context-specific and culturally appropriate strategies and allocate budgets under NLEP to address stigma & discrimination faced by the affected population.

1.0-031

Knowledge and interest of family physicians in the control and treatment of diabetic muslims during Ramadan in Spain

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OBJECTIVE To assess the knowledge of Spanish family doctors (FD) about general aspects of Ramadan and management of Muslim diabetic patients and to determine what factors influence this.

METHODS AND MATERIALS A questionnaire on knowledge (two questions on Ramadan and seven on DM treatment guidelines), nine items on right attitudes on DM and a degree scale about interest on DM and Ramadan was given to FD in 17 urban health centers. Variables: age, gender, workload, knowledge on Ramadan and DM, attitudes in DM management, degree of interest in management of DM and in culturally diverse patient care. Comparison of knowledge according to different variables was done by chi-square test.

RESULTS One hundred and sixty-three FD answered the questionnaire, 67.9% women, 32.1% men; 68.3% were aged >34, 31.7% <35, workload: 69.6% visited >29 patients per day, 30.4% <30. High/low personal interest in DM: 64.2%/35.8%; high/low personal interest in culturally diverse patient care: 52.8%/47.2%. Significant difference in knowledge those that had the attitude to make the necessary changes in treatment ($P = 0.021$) and the correct answer to question relating to Ramadan in those with high interest in the care of patients from different cultures ($P < 0.03$). Physicians with high interest in the care of culturally different patients, and those who planned the process beforehand rated higher on the questionnaire with a trend to statistical significance (both $P = 0.06$). There were no significant differences by age, gender, workload and personal interest in the management of diabetes mellitus.

CONCLUSIONS FD who are more interested in caring for culturally diverse patients attain the highest score in a questionnaire on the management of diabetic patients during the Muslim month of Ramadan. This supports the fact that cultural competencies are an important aspect in the work of FD, and training in these capabilities is therefore highly recommended.

1.0-032

Influence of immigration factor on vaccination uptakes in children with chronic diseases

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INTRODUCTION There are few data about vaccination coverage in children from immigrant families but some data suggest lower levels compared with native population. In addition, certain risk groups need to be immunized against some common diseases in these populations.

OBJECTIVES To study the uptakes of scheduled vaccines in children comparing immigrant and native population. To investigate the vaccination coverage found in immigrant children belonging to risk groups.

MATERIAL AND METHODS Children attended in emergency department were surveyed using a questionnaire including demographic, health and vaccines data. Study period: March to October 2010. Descriptive analysis and comparing frequencies were done using SPSS system.

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RESULTS Four hundred and twenty-six questionnaires were completed including 42 (10%) immigrants families. There were no differences between natives and immigrants in the average age (4.32 and 3.91 respectively), percentage of risk group subjects (46.7% vs. 51.2% respectively) routine vaccine coverage (7% vs. 3.9% respectively). Vaccine uptakes were higher frequency cases of pneumococcal vaccine, varicella and rotavirus vaccine in natives than in inmigrants ($P < 0.001$, $P = 0.003$ and $P < 0.001$ respectively). When risk groups were analyzed, none of immigrant children had been immunized against rotavirus, pandemic flu and chickenpox. In the case of vaccination against seasonal influenza coverage was greater among the population risk both in the native population and the immigrant. There were no differences in vaccination coverage against pneumococcal vaccine among risk groups and healthy, both in immigrants and natives children.

CONCLUSIONS Overall, vaccines uptakes of the immigrant population are similar to natives, but in risk groups coverage is still low. Therefore, it is necessary to implement activities in populations at risk to facilitate better uptake of vaccines.

1.0-033

Congenital malaria in bobo-dioulasso (Burkina Faso): case series report

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INTRODUCTION Congenital malaria is poorly reported in recent literature from Africa. Prophylactic strategies such as intermittent preventive treatment during pregnancy (IPTp) using sulfadoxine-pyrimethamine (SP) were reported to reduce significantly maternal parasitaemia and hence the risk of congenital malaria. We investigated in a cross-sectional study in Bobo-Dioulasso the prevalence of congenital malaria.

METHODS A cross-sectional study was implemented in two primary health facilities of Bobo-Dioulasso where IPTp was the national policy for malaria prevention during pregnancy. Maternal baseline was recorded among women delivering in these two centres. Samples of maternal, placental and cord blood were stained with Giemsa and examined for malaria parasites.

RESULTS One hundred and ninety-three pregnant mothers were included in the study. Three neonates were found to harbour falciparum malaria resulting in prevalence of 1.5% (3/195) for congenital malaria. Analyses of maternal baseline revealed that the three mothers of those newborns had received IPTp during pregnancy (two doses of SP), two were primigravidae and only one mother was sleeping under ITN during pregnancy. No history of blood transfusion was reported in any of the mothers. At admission, one mother was febrile (39°C) and her baby had also fever at birth (38°C). All three mothers had both peripheral and placental falciparum parasitaemia. Among the three newborns infected by falciparum malaria, parasites densities were 200, 472 and 1120 trophozoites/µl, respectively. Birth weights were 1430, 2000 and 2500 g, respectively. The three newborns were successfully treated with intravenous quinine and all were alive after 28 days.

CONCLUSION The prevalence of congenital malaria was low in this study. Babies born from mothers with malaria should be screened for congenital malaria. Larger studies are needed for more accurate prevalence and risk factors analyses.

1.0-034

Longitudinal audit of treatment of (Pre-) cancerous cervical lesions

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While most women in industrialized countries have easy access to cervical cancer preventive services, women in the developing world generally lack this. The cost of such screening measures and the lack of awareness of the importance of screening and treatment, make it even more difficult to manage cervical abnormalities in low resource areas. Cervical cancer is the second most common form of cancer amongst South African women. An audit was applied to test the hypothesis that women in South Africa, in the rural areas, do not attend treatment for cervical disease sufficiently. This would give a backup for the Female Cancer Foundation to implement the See and Treat program through which women will be treated on the spot. The audit was done on the data of pap-smears and colposcopy attendance to test the hypothesis of lack of attendance for colposcopy; also to recognize cofactors adding to it such as HIV, age of women, months of attendance for pap-smears and colposcopy and progression of disease (CIN staging).

RESULTS A relatively small number of patients visited colposcopy clinics after an abnormal pap-smear: 501 (54%) of 928 patients. Almost half of the patients with abnormal pap-smears do not attend for treatment. HIV, month of attendance, age and staging of the lesion did not have a statistically significant influence on (non)-attendace behaviour.

CONCLUSION We saw significant lack of attendance for colposcopy and treatment after abnormal pap-smear results. The factors considered in the research however do not explain for this non-attendance behavior. Long distance travelling, hospital reputation and the level of involvement of hospital staff, could be of considerable relevance; further attention to these issues is needed.

1.0-035

Retaining health workers in rural and remote areas: a realist review

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INTRODUCTION Limited availability of qualified health workers in remote and rural areas is an important obstacle to accessing care in many countries. The aim of this paper is to identify key elements of retention interventions that might explain why interventions were effective in one setting or failing in another.

METHODS AND MATERIALS Realist review of 30 published interventions on retention in low and middle income as well as high income countries. Realist inquiry intends to answer the question:'What is it about this programme that works, for WHO and in what circumstances?' Interventions were categorised into education, regulation, financial incentives or management and social support or bundled and analysed to identify intervention components, important contextual factors and mechanisms for change.

RESULTS Successful strategies in education were selecting students with rural background and/or intending to work in rural areas combined with a rural curriculum. As for regulation: compulsory service in rural areas, without preparation to provide health services in resource-constraint setting is not likely to be successful. Studies showed a wide variety of financial incentives with differing success. These incentives appeared not successful in a situation of stark shortage. Non-financial incentives were seen as more important. Personal and professional support can reduce isolation by triggering a sense of belonging, recognition and professional

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identity. Bundled approaches showed success, in particular when involving local stakeholders and with adequate management capacity to implement schemes. Gender and age are neglected aspects in retention. Studies described methodology or intervention to a limited extent and contextual factors were often not described which limited data analysis.

CONCLUSIONS Though evidence is weak, retention in rural areas can be addressed through bundled approaches consisting of education, financial incentives and personal and professional support interventions. Management capacity, procedures and labour market are important contextual factors.

1.0-036

Identification of common dermatophytes in Yasouj by phenotypic and genotypic methods (2010–2011)

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BACKGROUND Tinea (Dermatophytosis) is caused by dermatophyte colonization in tissues containing Creatine. It initially creates an eczematous reaction in the host's body followed by allergic inflammation as well as strains and species of dermatophytes. This study aimed to identify dermatophytes isolated from patients with various forms of dermatophytosis and species by use of phenotypic and genotypic methods in Yasouj, Iran, 2010–2011.

METHODS The present study was conducted on 273 patients referring to the laboratories of Yasouj. After testing the samples directly using transparent solution (KOH or Lactophenol), they were transferred for culture on the environment Dextrose agar containing cycloheximide and choloramphenicol (SCC) cultured, Slide culture, hair perforating test, urease test (phenotypic methods) dermatophytic fungi species were determined. Then the dermatophyte fungi using the Genotype methods PCR and RFLP were again identified.

RESULTS The incidence of Tinea in patients of this study was 12.8 and the head Tinea *Epidermophyton floccosum*, *Trichophyton Mentagrophytes* in the Tinea, Tinea bodies in *Trichophyton rubrum* and *Microsporum canis* in tinea fungal nails were the most important factor. Most types of tinea have been allocated to body hair loss in men (43.9%) and tinea in women's hands (59.4%). Thirty-five dermatophyte fungi were isolated from the samples. The most isolated isolated dermatophyte fungi were *Trichophyton mentagrvphytes* (60%), *Epidermophyton floccosum* (20%), *Trichophyton rubrum* (17.1%) and *Microsporum canis* (2.9%). Between gender and age at ringworm infection, there was no significant relationship.

CONCLUSION This study demonstrated that a rapid molecular technique, stable, and easy, repeatability for detecting species of etiologic factors and results of molecular Dermatophytes were absolutely in line with the phenotypic results. *T. mentagrophytes* and *M. canis*. This should be considered important and programs to control and combat the fungi in this area should be carried out.

KEYWORDS dermatophyte, PCR-RFLP, Yasouj

1.0-037

Antimicrobial activity of extracts from *Stevia rebaudiana Bertoni* against microorganisms of importance in dental caries

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BACKGROUND From an ecological standpoint the infectious diseases in oral cavity are the result of an imbalance in the oral ecosystem leading to the predominance of a microbial flora. Dental caries is a pathological, infectious, localized process that leads to the destruction of dental hard tissue. This infectious disease is the predominant cause of tooth loss in children and young adults. The main goal of research into medicinal plants is the search for compounds having antimicrobial activity that could be used to control or prevent infectious diseases. *Stevia rebaudiana* Bertoni is a major source of high potency sweetener for the growing natural food market.

OBJECTIVE To evaluate the antimicrobial activity of extracts obtained from *S. rebaudiana* against bacterial species of importance in dental caries.

METHODS AND MATERIALS Leaves of *S. rebaudiana* were powered in a knife mill and stored. The plant material was extracted with ethanol, chloroform and hexane. The antimicrobial activity of the extracts was evaluated against *S. mutans* ATCC 25175, *S. mutans* ATCC 31989, *S. sobrinus* CIO 428, *S. salivarius* NCTC 8606, *L. acidophilus* 903 ATCC 4365, *L. plantarum* 748 and *L. casei* 475 according to the well diffusion method. After incubation, the zones of inhibition produced by the extracts were determined.

RESULTS With differences in the halos of inhibition, a concentration of 1 mg/well hexane extract presented activity against all microorganisms tested. Only from 4 mg/well the chloroform extract showed antimicrobial activity against microorganisms. Of all the three extracts tested, the ethanol extract showed the lowest inhibitory activity.

CONCLUSIONS The antibacterial activity of hexane extract of *S. rebaudiana* was higher than that of the other extracts. The hexane extract of *S. rebaudiana* may become a promising source for finding new antimicrobial agents against microorganisms of importance in dental caries.

1.0-038

Intense *Plasmodium falciparum* antifolate resistance and pfmdr1 alleles in the Southern highlands of Rwanda

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Rwanda has achieved considerable reductions in malaria in recent years, partly due to the large-scale deployment of artemether-lumefantrine (AL). However, data on *Plasmodium falciparum* pfmdr1 alleles which show some degree of association with the response to AL are not available. Also, frequent mutations in the pfdhfr and pfdhps genes have suggested intense sulfadoxine-pyrimethamine resistance in parts of Rwanda. From southern highland Rwanda, no such information is available so far. During a combined community- and facility-based survey among 749 children below 5 years of age in Huye district, southern highland Rwanda, 104 *P. falciparum* isolates were obtained. *P. falciparum* polymorphisms associated with drug sensitivity were typed including pfdhfr, pfdhps, pfmdr1, and pfcrt. Concentrations of chloroquine and pyrimethamine in plasma were measured by ELISA. 12.5% of the respondents reported treatment with AL within the preceding 2 weeks. Chloroquine in plasma was detected in 18% of the children but pyrimethamine in none. The pfmdr1 pattern revealed more than 50% of the F184 polymorphism and almost 40% of the N86-F184-D1246 (NFD) haplotype which both have been reported to be selected in infections reappearing following artemether-lumefantrine treatment. Parasites of children

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who reportedly had taken AL in the preceding two weeks in 69% revealed the pfmdr1 F184 mutation as compared to 49% of isolates from not pre-treated children (P = 0.18). For the NFD haplotype, this difference was 62% vs. 35% (P = 0.07). As for markers of antifolate resistance, pfdhfr triple (75%) and pfdhps double/triple mutants (93%) predominated. 69% of the isolates harboured pfdhfr/pfdhps quintuple or sextuple mutants associated with high-grade sulfadoxine-pyrimethamine resistance. Pfdhfr L164 was absent. The present, first-time data on pfmdr1 alleles from Rwanda reveal a pattern which is suggestive of substantial artemether-lumefantrine pressure on the local parasite population. Molecular markers demonstrate intense antifolate drug resistance of *P. falciparum* in southern Rwanda.

1.0-039

Imported malaria in travellers and immigrants: results from the Spanish network on imported infectious diseases by travellers and immigrants (+Redivi)

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INTRODUCTION Immigration from and travel to malaria endemic areas are increasing . Local series about imported malaria, often disclosed a partial view of this disease, while countrywide networks provide a more representative picture. We describe imported malaria based on al-hoc network.

METHODS +Redivi is composed of 11 medical centres, attending travellers and immigrants across Spain. We report data from patients diagnosed of malaria, (January 2009–March 2011).

RESULTS There were 127 patients diagnosed of malaria distributed as follows: Immigrants (n = 48): Median Age 27 years, females 49.2%, from E. Guinea 62.8% and Nigeria 20.8%. Main reason for consultation: febrile syndrome (64.5%), 22.9% were asymptomatic. 92% were diagnosed of *P. falciparum* malaria, 2% of *P. ovale* and *P. vivax* malaria and 4% Plasmodium sp. Malaria. VFR (Visiting friends and relatives) (n = 49): median age 35 year, females 48.9%, Arrival from E. Guinea 61.2% and from Nigeria 16.3%. Main reason for consultation: febrile syndrome (89.8%). Ninety-two percent were diagnosed with *P. falciparum* malaria, 4% *Plasmodium* sp. and 4% mixed infections. Travellers: median age 32 years, females 53.2%, arrival from Nigeria (16.7%), India (10.3%) Main reason for consultation: febrile syndrome (86.7%), 6.3% were asymptomatic. Fifty-nine percent were diagnosed with *P. falciparum* malaria, 20% with *P. vivax* malaria and 7% with *P. ovale*, *Plasmodium* sp malaria or mixed infection. Although all VFR and 86.7% of travellers were high-risk travellers, 24.5% and 50% respectively sought pre-travel medical advice. Only in one traveller chemoprophylaxis was carried out properly.

CONCLUSION Imported malaria is almost restricted to Sub-Saharan Africa. Less than half sought for pretravel advice and incorrect chemoprophylaxis was the rule. Fever is the main reason for consultation, but other symptoms may be present. The high proportion of asymptomatic patients suggests testing for malaria in high-risk travellers. The need of educational measures and awareness of this disease in travellers and VFR must be stressed.

1.0-040

Positive impact of real-time expert advice on the care of patients with cutaneous leishmaniasis in France

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Therapeutic decision in Cutaneous Leishmaniasis (CL) is complex. *Leishmania* species and Leishmanial drugs are diverse, and treatment may be associated with severe adverse events. We compared the therapeutic option proposed by physicians seeking expert advice to that of the answering expert. This formalized expert advice was provided to 105 patients with parasitologically proven Leishmaniasis. Patients had travelled to 26 endemic countries, and only 9% of them had an underlying immunodepression. The infecting *Leishmania* species was accurately suspected by the expert from clinical data and travel history in 87% of cases, as shown by post-hoc confrontation with the laboratory-identified species in 45 patients. All inaccurate species suspicions except one were in patients from the New World. For the therapeutic management of patients, simple observation was proposed by physicians and recommended by the expert in 5% and 20% of cases, respectively. Local therapy was proposed by physicians and recommended by the expert in 29% and 41% of cases, respectively. Overall, only 14% of therapeutic choices proposed by physicians, vs. 85% of those recommended by the expert were consistent with published French guidelines (P < 0.0001). Expert advice resulted in 83% reduction in the number of patients hospitalized for treatment (5 vs. 30). Evolution of disease 45–50 days after starting therapy resulted in a 72% cure rate and a 18% initial failure rate. Second- and third-line treatment after initial failure resulted in a final cure rate of 96%. In conclusion, expert advice in CL results in treatment options associated with a smaller risk of adverse events, and provided at a lower cost. Taken together, these observations suggest that the therapeutic approach recommended by WHO and French guidelines is feasible in specialized settings. Simple and safe topical or oral agents are still urgently needed for the treatment of CL in the field.

1.0-041

Diagnosis of visceral leishmaniasis in peripheral blood and bone marrow using conventional microbiologic and kDNA-based techniques

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Visceral Leishmaniasis (VL) is a neglected disease and Brazil concentrates 90% of theVL cases registered in the Americas. From 1980 to 2005, Brazil recorded 59.129 cases of VL, and the majority in the northeast region. Recently, the proportion of regions outside the northeast increased to 44% showing the spread of VL. Given all this, it is imperative to equip laboratories for diagnosis of visceral Leishmaniasis. Our laboratory has initiated studies examining diagnostic techniques aiming the implement of them in the routine.

METHODS AND MATERIAL We analyzed samples of bone marrow and peripheral blood using the following laboratory diagnostic

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techniques: microscopical examination of smears and culture in NNN/BHI, and PCR amplification of a band of 120 bp.

RESULTS Still in its preliminary stages nine paired samples were analyzed, and obtained the following results. Microscopical analysis of the smear slide, five samples were positive, both originating from bone marrow and peripheral blood. One sample was positive in culture in NNN/BHI material from bone marrow. Eight samples obtained from bone marrow were positive in PCR, while five were positive by microscopic examination of bone marrow smear slide. From nine peripheral blood samples PCR positive, five were positive by microscopic examination of peripheral blood smear. And still using the PCR, from nine samples positive in peripheral blood, eight were positive in bone marrow.

CONCLUSION The value of examination of peripheral blood, both by microscopy and PCR was similar to the examination of bone marrow sample. This preliminary finding indicates the possibility of replacing the examination of samples collected from bone marrow, which is invasive and painful, by examination of samples collected from peripheral blood. So far the culture technique has not demonstrated a diagnostic value. Our preliminary results are promising and the study will follow more samples, allowing us to provide definitive results.

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1.0-042

Diagnosis of placental *Plasmodium falciparum* malaria infection using HRP, 2 rapid diagnostic tests in an endemic setting in Uganda

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Pregnant women with little or no pre-existing immunity are at high risk of cerebral malaria, hypoglycemia, pulmonary edema, and severe hemolytic anemia which contribute 60–70% of fetal and perinatal losses. Peripheral blood smear microscopy underestimates sequestered placental infections, therefore malaria rapid diagnostic tests (RDTs) detecting Histidine Rich Protein-2 antigen (HRP-2) in peripheral blood are a potential alternative. In an endemic setting in Uganda, we compared the accuracy of HRP-2 RDTs to microscopy and placental histopathology in pregnancy. Discordant results samples were spot checked using PCR techniques. Among 434 febrile women tested, 38% had malaria. RDTs had a sensitivity of 96.8% (95% CI 92–98.8), specificity of 73.5% (95% CI 67.8–78.6), a positive predictive value (PPV) of 68.0% (95% CI 61.4–73.9), and negative predictive value (NPV) of 97.5% (95% CI 94.0–99.0) in detecting peripheral *P. falciparum* malaria during pregnancy. Mosquito net use (OR 2.1) and increasing parity (OR 2.7) were associated with lower risk for malaria. At delivery, RDTs had a 80.9% sensitivity (95% CI 57.4–93.7) and a 87.5% specificity (95%CI 80.9–92.1), PPV of 47.2 (95% CI 30.7–64.2) and NPV of 97.1(95% CI 92.2–99.1) in detecting placental *P. falciparum* infections. At delivery, 25% of peripheral infections were detected by microscopy without concurrent placental infection. Compared to placental histopathology, the combination of RDTs and microscopy improved the sensitivity to 90.5% (95% CI 68.2–98.3) for detecting placental malaria infection and the specificity to 98.4% (95% CI 93.9–99.7). Presence of malaria in pregnancy and active placental malaria infection were 38% and 12% respectively. Use of HRP-2 RDTs to detect malaria in pregnancy was accurate when performed by midwives. A combination of RDTs and microscopy provided the best means of detection placental malaria. With a high sensitivity, RDTs could be a useful tool for assessing Malaria in pregnancy, with further (cost-) effectiveness studies.

1.0-043

Lessons learned from the international response to influenza a (H1N1) – the 1st pandemic of the 21st century

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BACKGROUND The World Health Organization (WHO) has the global mandate to coordinate pandemic preparedness and response under the International Health Regulations (IHR 2005). The emergence of a novel influenza A (H1N1) virus provided the first major global stress test since the IHR came into force. On 11 June 2009, WHO announced a pandemic phase 6 when the virus had reached 74 countries of all WHO regions in <9 weeks.

MATERIALS AND METHODS The presentation reviews different stages of the first pandemic of the 21st century from April 2009 to August 2010 and its various challenges in terms of responding adequately to a global threat. We assessed internal and external reports and incorporated the subsequent recommendations of the independent IHR expert review committee.

RESULTS Pandemic preparedness planning was based using different scenario models. First, one of the lessons learned was that predictions are very difficult when related to influenza viruses due their unpredictable evolution and the complexity of factors leading to a pandemic. This uncertainty makes mid term preparedness and planning challenging. Second, the heterogeneity in the levels of performance of surveillance systems in the world, does not allow a continuous and real-time monitoring of the disease and its spread. Third, modern communications tools enable rumours and information to spread faster than ever as compared to other pandemics.

CONCLUSION The international community has responded well to the pandemic threat due to positive effect of pandemic preparedness planning. In particular, countries have been collaborative regarding the exchange of information and the global access to supplies such as vaccine and antiviral drugs. The nature of this pandemic with moderate severity and very rapid spread imposed to revise national pandemic preparedness plans during the course of the event. Risk communicationneeds to take into account public perception to better understand control measures.

1.0-044

Association of blood culture, xenodiagnosis, buffy coat and PCR results for diagnosis and monitoring of heart transplant patients at risk of reactivated Chagas disease

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INTRODUCTION Around 30% of all patients with chronic Chagas disease will develop cardiomyopathy and heart insufficiency and one third of this contingent will undergo heart transplantation . After surgery, these patients will be more prone to reactivation of Chagas disease due to immunosuppression. It is necessary to treat the patient when reactivation is present. Routine tests used for demonstration of the parasite are blood culture (BdC), xenodiagnosis(X) and buffy coat (BC) examination. Positive BC indicate episodes of parasitological reactivation. To detect this episodes, more recently it has been recommended that BdC and X should be

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performed with earlier microscopic readings. PCR is recommended too. In this study we have evaluated the ability of parasitological techniques (BC, BdC and X) and PCR to detect patients in risk to reactivate Chagas disease.

MATERIALS AND METHODS Blood samples from 46 patients treated at the Instituto do Coração do HCFMUSP were analyzed. Patients were sampled irrespective of the presence of clinical signs and symptoms suggesting reactivation. The samples were analysed by BC, BdC and X. After the results, all the positive samples and some negative samples were examined by PCR, which amplify a 144 pb from the *T. cruzi* genome.

RESULTS None was positive by BC. Eleven blood samples (11/46 or 24%) were positive by BdC and/or X. These 11 samples were also positive by PCR. However, among the other 10 negative BdC and/or X samples, PCR has found other three positives.

CONCLUSION In conclusion, the results of the present study can reinforced the role of PCR coupled to conventional laboratory tests to improve the detection of patients in risk to reactivate Chagas disease.

1.0-045

Application of molecular techniques in the diagnosis and follow up of Chagas disease patients

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INTRODUCTION Evaluation of a real-time PCR (RT-PCR) assay for Trypanosoma cruzi detection in blood samples from acute and chronic phase of Chagas disease patients.

MATERIALS AND METHODS Peripheral blood samples were collected and pre-treated with 6 M guanidine hydrochloride during 24 h. DNA extraction was carried out with silica–membrane technology (EasyMag; BioMerieux). TCZ1/TCZ2 primers and TZ3 probe from satellite sequence were selected and used in a TaqMan-based assay. The human RNase P gene was detected as internal control of amplification. Negative and positive controls were included in each RT-PCR, all samples were analyzed in duplicate.

RESULTS To evaluate our RT-PCR we used 10-fold serial dilutions of 107 ps/ml (0.7 ng/μl) purified T. cruzi epimastigotes (ps) DNA. Our detection limit was 103 ps/ml (0.0007 ng/μl). In addition, we used blood spiked with T. cruzi I (1.8×103 ps/μl) and II (2.9×103 ps/μl) epimastigotes. Detection limit was 0.56 ps/μl and 0.93 ps/μl respectively. Specificity and cross-reactivity were assessed using 55 samples, negative or positive for other pathogens including Plasmodium sp and Leishmania sp. A total of 474 RT-PCR corresponding to 446 patients was carried out. We studied: (i) Twenty-four children (24 h to 7 year), whose mothers presented a positive Chagas' serology: four of them gave a positive PCR result. (ii) Four immunosuppressed patients were monitored for reactivation risk of T. cruzi: (iii) with previous positive Chagas' serology (one leukaemia, one chronic renal failure and one single-lung transplantation), and one seronegative liver recipient from a seropositive donor. Seventeen PCRs were performed during the monitoring process and were all negative 3. We also studied 418 asymptomatic adults with positive serology: 163 (39%) presented a positive PCR.

CONCLUSIONS The RT-PCR is a high sensitivity technique for acute phase diagnosis. In addition, it allows detection of parasitemic patients in the chronic stage of Chagas disease.

1.0-046

The impact of the H1N1 virus a influenza pandemic in a secondary referal hospital in catalonia, 2009–2011

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INTRODUCTION During 2009, the first influenza pandemic of the twenty-first century, due to the swine-origin influenza A (H1N1) 2009 virus, was declared. This study aimed to describe the characteristics of the patients infected with H1N1 during a 2 years follow-up in our hospital. We determined the prevalence of the disease, as well as co-morbility and features of patients with confirmed infection with H1N1 influenza A virus.

MATERIAL AND METHODS An observational study of patients diagnosed of Influenza A virus H1N1 between April 2009 and February 2011 was performed at the hospital. We analized nasopharyngeal and throat swabs from patients with suspected influenza. Influenza A virus H1N1 infection was confirmed in all pacients through real-time PCR test. We report the clinical and epidemiological features of those patients (comorbidities, need for hospitalization, treatment and outcome) were elicited by interview; we compared data obtained both years.

RESULTS Fifty patients were diagnosed of influenza A virus H1N1 during the follow-up period (21 in 2009 and 39 in 2010). There were not differences between sex. There was higher incidence in younger patients (15–44 years old), and or age. The underlying risk conditions were respiratory disease (COPD or asthma), cardiovascular disease, obesity, diabetes and HTA. Clinical presentation included fever, dyspnea, cough, chest pain and weakness; admitted patients with influenza also were diagnosed with respiratory insufficiency, pneumonia, and gastroenteritis. The major determinant of ICU admission was shortness of breath. Five patients required admission at ICU for mechanical ventilation and two died (80% during second year). Oseltamivir and wide spectrum antibiotic was given to almost all patients.

CONCLUSION The majority of cases of H1N1 were mild and self-limiting, but some people developed complications and two died. We need more data to understand pandemic influenza viruses behaviour.

1.0-047

IgGs against the surface of *Plasmodium falciparu*m infected erythrocytes increase 1 month after delivery

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BRIEF INTRODUCTION Infections due to several microbial pathogens and autoimmune diseases begin or worsen during the early postpartum period. In particular, the risk of falciparum malaria increases not only during pregnancy, but also at early postpartum, as compared to non-pregnant women. Whereas susceptibility to malaria during pregnancy has been attributed to sequestration of *P. falciparum* in the placenta, little is known about mechanisms underlying the increased risk of infection after delivery.

METHODS AND MATERIALS To determine changes in antibody-mediated responses after pregnancy, levels of IgGs against the surface of erythrocytes infected with a chondroitin sulphate A binding line (CS2) and a paediatric isolate (MOZ2) were

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compared in 200 pairs of plasmas collected from Mozambican women at delivery and during the first 2 months postpartum.

RESULTS Levels of IgGs specific for CS2 and MOZ2 were higher at postpartum than at delivery in 115 (58%) and 120 (60%) of the 200 women, respectively. The paired analysis showed that levels of IgGs specific for MOZ2 increased at postpartum compared to levels at delivery (P = 0.001), and a similar trend was found for IgGs specific for CS2 (P = 0.054). Analysis stratified by parity, postpartum period and intervention showed that this difference was significant in multigravidae women at late postpartum (for MOZ2), and among women who received IPTp.

CONCLUSIONS Results of this study support the view of the postpartum as a period of immune reconstitution in which the level of antimalarial antibodies gradually increase after pregnancy, and suggest that recovery of immunity might take at least 1 month and might be faster in multigravidae than primigravidae. This readjustment of immunity at early postpartum might still render women susceptible to new malaria infections or allow the persistence of parasites acquired during pregnancy, which might recur after delivery.

1.0-048

Loss of leishmanin skin test reactivity in longitudinal studies of zoonotic cutaneous leishmaniasis in central Tunisia

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We report here the results of LST surveys conducted for two consecutive years in a Zoonotic cutaneous leismaniasis (ZCL) endemic community in central Tunisia for two populations: a school cohort (2001–2002) and a general population cohort (2009–2010). Our primary objective was to explore the longevity of LST reactivity and to assess the loss of LST reactivity. We observed a loss of LST reactivity in 30 (4.3%) of 699 people tested in 2001. In the 2010 population based study, we observed a loss of LST in 47 of 1730 subjects tested in 2009 (3%). In the two surveys, the loss of reactivity was significantly higher in women than in men. The mean age was significantly higher in subjects without a loss of LST reactivity. No difference was observed between villages. As is consistent with most studies, positive test results increased with age, as an indicator of time of exposure to the parasite. The observation of unexpected permanent loss of skin test reactivity in these two studies may not be related to a fluctuation in the delayed-type cutaneous hypersensitivity (DTH) response but a steady decline over time. This is not in concordance with the hypothesis of a permanence of DTH due to continued exposure to *Leishmania* antigens either through latent infection or repeated biting by *Leishmania*-infected sand flies. The loss of reactivity decreased with age. This finding could be related to the cumulative contact with the parasite. Young people are less exposed to the bite of sand fly. Gender differences were documented in several studies and males seems to react more commonly than females because they are more exposed. This would justify the higher loss of reactivity among women. Our data underscore the need for better methods of standardization and documentation of sensitivity, potency and stability of leishmanin antigen.

1.0-049

Validation of two rapid tests for diagnosis of visceral leishmaniasis in Kenya

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INTRODUCTION Accurate and simple diagnostic tests are needed to improve patients¡ access to diagnosis of visceral Leishmaniasis (VL) in Kenya. We evaluated two rapid diagnostic tests (RDT) for VL in two treatment centres in Western Kenya.

METHODS AND MATERIALS VL clinical suspects (fever for ≥14 days and splenomegaly) presenting at Kimalel Health Centre in Baringo district or Kacheliba Kala-azar Treatment Centre in Pokot North district were enrolled prospectively after consenting to participate in the study. In the absence of clinical (e.g. jaundice) or biological (e.g. thrombopenia) contra-indication(s), splenic aspiration was done. Two serological RDTs, the rK39 antigen-based Diamed-IT LEISHTM (Diamed AG, Switzerland) and the rKE16 antigen-based Signal KATM (Span Diagnostics Ltd, India), were performed on patients' serum by a laboratory technician who was blinded to the results of the spleen aspirate. The sensitivity and specificity of the two RDT were assessed using splenic aspiration smear results as the reference (gold) standard.

RESULTS A total of 187 patients were enrolled by May 31, 2011 and we show here the results of a preliminary analysis. Spleen aspiration confirmed VL in 103 patients and was negative in 84 patients. The Diamed-IT LEISH was 91.3% sensitive and 85.7% specific, and the Signal KA test was 82.5% sensitive and 91.7% specific.

CONCLUSIONS The final results will be presented at the congress. The implications of the RDT diagnostic performance and their potential to be integrated into diagnostic algorithms for primary diagnosis of VL in Kenya will be discussed.

1.0-050

Documentation of clinical data and its transference from Ebola and Marburg wards: health care workers' experiences and preferences

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BACKGROUND The understanding of clinical manifestations of filoviral hemorrhagic fevers (FHF) and the evaluation of treatment strategies requires the collection of clinical data in outbreak situations. However, to date, clinical documentation inside isolation wards during FHF outbreaks has been limited and data were lost because clinical records were considered contaminated and destroyed. There is no consensus on the best way for documenting clinical FHF data and their transfer from the Ebola/Marburg ward to the outside world.

MATERIALS AND METHODS Semi-structured interviews were conducted with health care workers involved in FHF outbreaks in Africa, and with persons experienced in clinical documentation and transfer from high biosecurity areas in Europe. Data transfer methods were categorised depending on need of electricity and ranked by interviewees.

RESULTS Lack of interest in data, other work-related priorities, difficult working conditions during outbreaks, and lack of standardized data collection forms were the main reasons for limited documentation of clinical data. Interviewees confirmed that clinical records were often destroyed, identified a range of data transferring methods, and proposed two most appropriate methods: (i) Dictating data over the fence when no electricity is available and (ii) Use a Personal Digital Assistant (PDA) otherwise.

CONCLUSION Recommendations for improved data collection in FHF wards in future outbreaks were developed: (i) Using standardized forms for clinical documentation at outbreaks, and (ii) Documentation by a second health worker following the one working clinically. For data transfer, high technology solutions like PDAs, although popular among interviewees, should be regarded with scepticism due to the usual lack of electricity. A health worker entering a ward and manually copying or

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photographing clinical data without touching anything else could become a standard procedure. Usage of a scanner where electricity is available should be evaluated. Further research on safety of disinfecting paperwork by sunlight is warranted.

1.0-051

Clinical profile of concurrent dengue fever and *Plasmodium vivax* malaria in the Brazilian Amazon: case series of 13 hospitalized patients

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BACKGROUND Malaria and dengue fever are the most prevalent vector-borne diseases worldwide. The present study aims to describe the clinical profile of patients with molecular diagnosis of concurrent malaria and dengue fever, in an endemic area where these diseases show incidence peaks on distinct seasons.

METHODS This case series is part of a larger prospective study assessing admitted *P. vivax* cases, in which extensive comorbidity evaluation was performed. A total of 311 inpatients with PCR confirmed *P. vivax* monoinfection were also investigated for dengue, 13 of which being confirmed by RT-PCR and/or NS1. Data were collected prospectively in a standardized questionnaire and *P. vivax* monoinfection was also confirmed by real-time PCR.

RESULTS Thirteen patients with confirmed concurrent dengue and *P. vivax* infection were detected among 311 inpatients. Similar frequencies of DENV-2, DENV-3 and DENV-4 were found. In nine patients, World Health Organization's criteria for severe malaria could be fulfilled, and only one pregnant patient had severe dengue, but warning signs were present in 12.

CONCLUSIONS Delayed diagnosis and inappropriate treatment of both diseases can result in enhanced hospitalization and severity. Syndromic surveillance systems must be ready to identify this peculiar condition and to avoid misinterpretation of severity attributed to a single disease, such as *P. vivax* infection, which is also frequent in this region. Further studies are needed to estimate the real burden of this coinfection, its repercussions, and if it actually leads to a distinct clinical presentation.

1.0-052

Resistance of *Aedes aegypti* to insecticides in Martinique and implications for dengue vector control

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Dengue virus, transmitted by *Aedes aegypti*, is reemerging dramatically in Martinique Island (French West Indies). One of the principal recourses to reduce the transmission remains the fight against the vector by the use of insecticides. Unfortunately, insecticide resistance (metabolic and target site mutation mechanisms) to conventional insecticides (pyrethroid and organophosphate) is strong and widespread among local mosquito populations. The present study was designed to measure and understand the phenotypic impact of resistance on the efficacy of adulticide and larvicide treatments at an operational scale. To assess the impact of pyrethroid resistance on the efficacy of treatments, three rounds of applications of deltamethrin and natural pyrethrins were performed with vehicle-mounted thermal foggers in nine localities of Martinique. Efficacy was assessed by monitoring mortality rates of naturally resistant and laboratory susceptible female mosquitoes placed in sentinel cages. Results showed high mortality rates of susceptible sentinel mosquitoes treated with deltamethrin while resistant mosquitoes exhibited very low mortality. There was no reduction of either larval or adult *Ae. aegypti* population densities after treatments. This suggested a limited efficacy of pyrethroid treatments for reducing the virus transmission during epidemics. Conversely, we showed the potential of using alternative larvicides (spinosad, pyriproxyfen and diflubenzuron) for the control of organophosphate resistant *Ae. aegypti* larvae. Spinosad (naturalyte) and pyriproxyfen (growth regulator) were also used in mixture to measure the residual efficacy of the combination of their different modes of action. Under field conditions, pyriproxyfen and Bti failed to curtail *Ae. aegypti* populations after 4 weeks. Conversely, diflubenzuron and spinosad showed a residual efficacy of 16 weeks suggesting that these chemicals may be promising alternatives to Bti and temephos for controlling insecticide-resistant mosquitoes in Martinique. The mixture remained effective for 18 weeks, showing that the combination of the two larvicides acted to increase the residual activity of the treatment. The mixture could preserve the utility of both insecticides in public health programs. This study emphasizes the urgency in the need for further research to provide new tools and innovative strategies to manage insecticide resistance in dengue vectors.

1.0-053

Relationship between climate change and zoonotic cutaneous leishmaniasis in Tunisia

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Tunisia is one of the most exposed countries to Climate Change (CC) phenomena. Between 1976 and 2004, temperatures rose and reached an average 1°C more and more dryness and floods were recorded. The Meteorological models applied to the Tunisian Data predicted that, by 2050, the average temperature will have increased by 2.1°C. Since 1982 zoonotic cutaneous Leishmaniasis (ZCL) due to *Leishmania major* has expanded from the centre of the country to neighboring areas. Now ZCL is the most frequent vector born disease in the whole country and covers 2/3 of the territory. In order to analyze the relationship between the emerging of ZCL and CC we used a time series of meteorological data (1963–2010) recorded in the Sidi Bouzid meteorological station and compared it to ZCl occurrence (statistics of Health facilities related to ZCL cases) in Sidi Bouzid governorate, which is the most affected governorate in the country. Emerging ZCL recorded in Tunisia since the beginning of the 1980s seems to be strongly related to the increasing temperature and intense heat waves recorded in the same period. In addition to the increase of temperature, we recorded a decrease of the quantity of rain.

1.0-054

Perception of the climatic changes inquire near the population of Sidi Bouzid

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Sidi Bouzid is a governorate in the center of Tunisia where zoonotic cutaneous leishmaniose by *Leishmania major*, a disease

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with vectorial transmission very sensitive to the climatic changes (DC), is endemic-epidemic. Our study is within the framework of a research project which seeks to analyze the methods of adaptation to the effects on health of the climatic changes in the case of Zoonotic cutaneous Leishmaniasis. It involved 24 health professionals in the area whose knowledge of DC, its major causes, how they occur, which are their effects on health and which are the measures to be taken with respect to the DC was assessed by questionnaire. Twenty-five percent gave an incorrect definition of the DC. Only 9% evoked greenhouse gases (GES) as causes of DC and very few (8%) stressed that DC has an impact on health. Total ignorance of measurements of control of DC (Attenuation and adaptation) was noted.

1.0-055

Risk of exposure to the vector of zoonotic cutaneous leishmaniasis in Tunisia

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Zoonotic Cutaneous Leishmaniasis (ZCL) due to *Leishmania* major is a vector borne disease transmitted by *Phlebotomus papatasi* from a *Psammomys rodent* (parasite host) witch is pledged to the salted wetlands (Garâa). Irrigated perimeters (IP) around such zones would expose farmers and population to the risk of ZCL transmission. We aimed to assess the risk of exposure of farmers to the vector of ZCL the through analyzing their behaviors during irrigation process and carried out an entomological investigation in order to study spatial distribution of *P. papatasi* in various biotopes and time activity of the vector. The method used to study farmer's behaviors is based on recording hourly duration of irrigation session and CDC luminous traps allowing the segregation of the time collections of phlebotomus were used to capture vectors. On the whole 616 phlebotomi were captured. *P. papatasi* accounts for 72.9% (449). They are distributed as follows: 48% (222) in Garâa, 32% (144) in IP and 20% (90) in the village. Proportion of females is more significant in IP (75%). The study of the time activity of the vector showed that *P. papatasi* is active from 23 pm to 4–5 am with a maximum of activity towards 0–1 AM. Irrigation was continuous almost all day. Farmers are exposed to the risk of contact with the vector of ZCL.

1.0-056

Monitoring influenza in Tunisia

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During the H1N1 pandemic in 2009/2010, Tunisia reinforced its existing monitoring activities. These were based on a sentinel network of 200 health centers (10% of all centers), which ensured active monitoring from the first week of October to the last week of April. Two weekly indicators of follow-up were used: proportion of consultations for flu-like symptoms and proportion of consultations for ARI. We report here the results covering five transmission seasons, from 2004/2005 until 2008/2009. The shape of the epidemic curves was the same for each: the peak occurred between the fourth and the eighth week with knowing of the end of January until the end of February. Its values varied from year to year between 12% and 19%, being highest in 2004–2005.

1.0-057

Detection and quantification of human herpes virus 6 in samples from patients with AIDS and progressive multifocal leukoencephalopathy (PML)

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BACKGROUND Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS). PML usually occurs in immunocompromised patients and mainly in aids. There are few reports suggesting the association between JCV and HHV6 in PML and other demyelinating diseases. The aim of this study was to investigate the presence and quantification of HHV6 DNA in samples of cerebrospinal fluid from patients with PML and other neurological diseases, by Sybr Green real time PCR.

METHODS Cerebrospinal fluid samples from patients with aids with suspected PML were tested for HHV6. DNA was extracted by QIAamp® DNA Mini Kit according to the manufacture's instructions. Five microliters of DNA were analyzed for the presence of HHV6 by Sybr Green Real Time PCR with primers which codify the main viral capsid protein (MCP) – HHV3: 5′ TTG TGC GGG TCC GTT CCC ATC ATA 3′; HHV4: 5′ TCG GGA TAG AAA AAC CTA ATC CCT, amplicon size 214 bp.

RESULTS Among the 82 patients investigated, HHV6 DNA was found in 4 (4.87%). One of them had a PML diagnosis confirmed by clinical, neuroimaging and laboratorial findings. JCV DNA was not detected in the other three positive samples.

CONCLUSION Our data show that, despite being a rare occurrence, the presence of HHV6 in cerebrospinal fluid samples from patients with aids and PML can be detected.

1.0-058

The study of the amount of contamination of meats of sheep, cattle and meat products to Sarcocystis in Ahwaz in the year 2010 through protein digestion method

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BACKGROUND The existence of parasitology cyst in the meat have been provide in some extensive studies .some parasites Coccidia like Sarcocystis can infect human beings through this way .The Sarcocystis is a part of Coccidia through which human beings can be as a main or intermediate host in some species. Since meat and meat products in transmission of this sickness play an important role, in this study we try the amount of tissue contamination in meat Coccidia will be determined.

MATERIALS AND METHODS Fifty five samples of cattle meat and 50 samples of sheep meat and 25 samples hotdogs and sausages and hamburgers (80–100 gr) prepared and after LABELING and registrating the information, their proteins were digested (1% HCL and Pepsin) afterwards centrifuged sediment residuals were stained with Giemsa.

RESULTS In this study 100% of sheep and cattle samples, 20% of hotdog, 8.6% of sausage and 56% of hamburgers were contaminated by Sarcocystis.

CONCLUSION The result of this study has a full agreement with other researches .It remined the people the complete cooking of meats and meat products .It is recommended that in next studies parasite species are identified through molecular method.

KEYWORDS Sarcocystis, digestion method, meat

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1.0-059

Recreational waters of Chaharmahal va Bakhtiyari province of Iran as an important potential source of human cryptosporidiosis

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INTRODUCTION Cryptosporidiosis is a prevalent infection in developing countries, due to the high intensity of environmental contamination and poor hygiene conditions. Cryptosporidium oocysts from infected human and non-human hosts, livestock and agricultural practices, and infected feral and transport hosts are frequent contaminants of waters, including recreational waters.

BACKGROUND The aim of this study was to detect and characterize Cryptosporidium spp. in water samples collected from the main recreational ponds of Chaharmahal va Bakhtiyari Province, Southwest of Iran.

METHODS From the November 2009 to May 2010, thirty water samples (each contained 10 l) were collected from the main recreational waters of the area. The samples were then filtered through Millipore filters, pore size 1.2 m. The pellets introduced into microtubes containing 2.5% potassium dichromate were refrigerated at + 4 °C. After washing the pellets, DNA extraction of the products was accomplished using the QIAamp DNA Mini Kit, Germany. Finally the SSU rRNA-based PCR-RFLP technique was used to detect and characterize Cryptosporidium spp. in the samples.

RESULTS Out of thirty samples examined, 6 (20%) were positive for different Cryptosporidium spp. Restriction pattern analysis showed that *C. parvum* has been the most prevalent genotype,- followed by *C. hominis* and *C. canis*, respectively. The higher prevalence of *C. parvum* compared with other genotypes is consistent with the distribution of cattle in this area.

CONCLUSION Farm animals, particularly cattle may be considered as the main source of cryptosporidial contamination for these water sources and the occurrence of human cryptosporidiosis, particularly in tourist and travelers could be expected after the exposure with such water sources.

1.0-060

Prevalence intestinal parasites of North Khorasan in Iran 2010–2011

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BACKGROUND New efforts are being made to improve understanding of the epidemiology of intestinal parasites of north Khorasan in Iran.

METHODS AND METHODS Stool samples were taken from 3000 patient and preserved in SAF (sodium acetate-acetic acid-formalin). The samples were analyzed by formalin-ether concentration direct smear.

RESULTES AND CONCLUSION Prevalence of *Giardia lamblia*, *Hymenolepis nana*, *Entamoeba coli* and *Enterobius vermicularis* were, respectively, 3%, 0.3%, 1.1% and 0.1%. These four species of pathogenic parasites are present with substantial prevalence in this area of north Khorassan in Iran Although their spatial distribution is not focused in any one place, determination of the population segments with the level of infection will help to target the chemotherapeutic fight. To reinforce treatment with chemotherapeutic agents, tap water should be made available in all the localities of this are

Track 2: Women's and Children's Health

2.1 Maternal, Sexual and Reproductive Health

2.1-001

Effect of mineral and/or vitamin supplements usually consumed on iron, copper and zinc status of lactating women at a Brazilian human milk bank

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INTRODUCTION This study evaluated the effect of mineral and/or vitaminic supplements, usually consumed during pregnancy or breastfeed, on the iron, copper and zinc (blood and mature breast milk) nutritional status of 134 lactating women at a Brazilian Human Milk Bank.

METHODS AND MATERIALS Donors completed a questionnaire to obtain data on use of mineral and/or vitaminic supplements and if this was to treat or prevent anemia. Blood samples were collected for analysis of hemoglobin, iron, transferrin, ferritin, ceruloplasmin, copper and zinc. Mature breast milk samples were collected for analysis of iron, copper and zinc. First, mothers were divided in three groups: 1- took supplements to treat anemia; 2- took supplements to prevent anemia; 3- did not consumed supplement (not anemic).

RESULTS Variance analysis and Tukey test were applied, and showed that hemoglobin means were lower in anemic donors ($P = 0.0002$), but above the limit for anemia – 11.9 g/dl. No differences were observed in iron, copper and zinc from milk between groups. Thereafter, lactating women were redistributed in: group 4- lactating women who were consuming mineral/vitaminic supplements during breastfeeding; group 5- lactating women who were not consuming mineral/vitaminic supplements during breastfeeding. This redistribution showed an association between supplement consumption and nutritional status of copper, evaluated through serum ceruloplasmin and copper. Both indicators were lower in donors who were consuming supplements during breastfeed.

CONCLUSIONS Iron supplements usually indicated to treat anemia were efficient in anemic donors who mainly had to compensate for losses expected from iron to produce breast milk. Consumption of supplements to prevent anemia was positive, considering that this group presented higher levels of hemoglobin. We advise further epidemiological studies to confirm these associations.

2.1-002

Using mobile phones to promote sexual and reproductive health: Kenyan case study

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BRIEF INTRODUCTION This paper explores the use of mobile phones, specifically text messaging, to promote sexual and reproductive health.

METHODS AND MATERIALS Exploratory case study of a reproductive health project (m4RH), in Nairobi, Kenya to explore young people's experiences, knowledge and attitudes with mobile phones for a reproductive health project. We used in-depth telephone interviews to elicit stakeholders' experiences in developing and implementing mobile phones.

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RESULTS AND CONCLUSIONS So far, young people have too often been seen as a burden rather than an asset, a group to be taught but not to teach, and to receive but not to give. Youth need to be engaged in decision-making processes related to the information society – as students, and as citizens with an affinity for technology, they are informed stakeholders in the evolution of education and innovation.

2.1-003

A study of Moslem married women's practices and perceptions of termination of (Suspected) pregnancy, in Jakarta, Indonesia

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In Indonesia, abortion is prohibited, although Islamic teachings do not explicitly forbid it: most Islamic scholars allow abortion in certain circumstances. The Qur'an's Al-Hajj verse indicates that the fetus has to pass through various phases before becoming a human being with a soul. Therefore, the length of pregnancy at which abortion is still allowed is debatable. After 120 days, abortion is forbidden, except to save the mother's life. On May 2005, Majelis Ulama Indonesia (MUI, the Indonesian Moslem Assembly of Religious Leaders) declared that abortion is allowed before 40 days when certain requirements are met. This research is an exploratory-qualitative study in order to describe Muslim married women's perceptions and practices of termination of (suspected) pregnancy, or TSP. The research was carried out during 6 weeks, from May to June 2006, at Kampong Meruya, west of Jakarta. Ppurposive sampling was used and four indepth interviews of married women, jamu sellers (traditional herbs' seller) and 10 community women in focus group discussion. In summary, Muslim women did practice induced abortion, which was considered menstrual regulation from their point of view. Various methods used, such as drinking *jamu telat bulan* or *jamu peluntur*, concoctions, western medicines, and massage, revealed serious efforts to get rid of unwanted pregnancies. Women argued rationally that what they had done served the future of their families. They described themselves as mothers who had taken the responsibility for their families, in agreement with how the ideology wants them to be. Despite acting against the community's perception of abortion, they created an agency to make sure that their messages would be received successfully in the community.

KEYWORDS terminated suspected pregnancy, abortion, contraception, reproductive health

2.1-004

Peer education: the effects on knowledge, and preventive practice of pregnancy related malaria in women of reproductive age in Edo-state, Nigeria

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BACKGROUND In Nigeria, pregnant woman take limited measures to prevent malaria. Often this is because women of child-bearing age have limited knowledge about the health impact of malaria on the foetus (and mother) during pregnancy. Peer education is a strategy to improve community awareness of malaria in pregnancy. This study evaluated whether peer education is an effective tool in improving the uptake of preventative measures.

METHODS Women of child-bearing age were interviewed in their households using a structured questionnaire about their knowledge of malaria, malaria in pregnancy, and the various preventive measures available. A peer education campaign was subsequently launched in order to raise the level of knowledge amongst the community. Interviews were repeated after the campaign and the answers between the pre- and post- intervention were compared.

RESULTS Pre-assessment revealed that knowledge on malaria was high in the studied population but that knowledge on the specific health risks of malaria during pregnancy and possible preventive measures were limited. The peer education campaign had a significant impact in raising the level of knowledge of women of child bearing age; they scored better in questionnaires and improved their knowledge about malaria prevention.

CONCLUSION Peer education can lead to a significant increase in knowledge on disease transmission and prevention. However, increase in knowledge does not necessarily translate in increased preventive practice. Therefore, health interventions should also focus on addressing other problems influencing preventive practice, like structural barriers e.g. lack of availability of preventive tools (bed nets) and poor access to health services (nearby health facilities).

2.1-005

Tele safe motherhood pilot project in Rolpa district of Nepal

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BACKGROUND Rolpa district is one of the remote districts of Nepal, with a high maternal mortality rate (MMR) of 352 per 100,000 livebirths (The national MMR being 240 per 100,000 livebirths). Although 54% of pregnant women attend hospitals for the first antenatal check-up, only 19% of the total number of pregnancies, complete the fourth antenatal check-up. Institutional delivery is only 5%. Low antenatal care (ANC) during third triemester and low institutional delivery are responsible for high maternal mortality in the district. As it is with other remote districts, the main reason behind the low institutional deliveries in Rolpa seems to be remoteness of health facilities and lack of health education among women.

OBJECTIVE To reduce maternal mortality by providing pregnant women obstetric care using mobile technology.

METHODS Female community health volunteers (FCHVs) use their cellphone to send information about pregnant women to the district hospital and use templates written in their own language to send information. The district hospital collects data and encourages pregnant women to have the last four ANC visits and institutional delivery. If the mother is not willing to come to hospital, a health worker is sent antenatal check-up. The hospital divides pregnancies into normal and high-risk pregnancies. High-risk pregnancies are followed accordingly and, if necessary, are referred to a maternity-care centre for better management.

RESULTS At least 90% of pregnant women will get four ANC visits. Institutional delivery will increase to 25% for adoption of the mobile-phone technology.

CONCLUSION Incorporation of modern technology into health service-delivery system is highly recommended, especially in remote areas, to achieve the universal goal 'Healthcare for All'.

KEYWORDS antenatal care, maternal mortality, mobile phone technology, safe motherhood, Nepal

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2.1-006

Developing more effective approaches to prevent morbidity and mortality rate among women of reproductive age in Nigeria

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INTRODUCTION In Nigeria, health institutions often provide poor services to patients and increase maternal morbidity and mortality. This study focuses on women of child-bearing age who attend Jericho Specialist Hospital, Ibadan. The objective was to cultivate effective approaches to lower the high morbidity and mortality rate.

METHODS AND MATERIALS The target populations were women of child-bearing age attending Jericho Specialist Hospital. The research adopted a non-experimental design and a convenient sampling technique in which 50 respondents were chosen from the hospital as a participant for the study. Data was collected through a self questionnaire.

RESULTS The findings revealed contributing factors were poverty (40%), poor antenatal services (50%) and a lack of skilled health and medical services (10%). Respondents were asked whether provision of free health care would reduce death: 45 (90%) agreed while 5 (10%) disagreed. Forty-one (82%) of the respondents believed that inadequate essential tools and equipment in clinics contributes to maternal death while only 8 (16%) disagreed to the question and only 1 (2%) had no response to the question. Forty-three (86%) agreed that family-planning as a tool of contraception would improve women's health, while 7 (14%) disagreed.

CONCLUSIONS Women of child-bearing age had an awareness of morbidity and mortality issues but a lack of quality antenatal care and emergency obstetric care increased the risk.

2.1-007

The cause and effect of domestic violence during pregnancy: Nigerian experience

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INTRODUCTION Domestic violence is an international problem. This study examines the cause and effect of domestic violence during pregnancy.

METHODS AND MATERIALS A total of 100 questionnaires were completed by pregnant women who attended the antenatal clinic of Jericho Nursing Home, Ibadan.

RESULTS AND CONCLUSION Thirty percent of respondents were expecting their first child while 55% had been pregnant one to two times before the study, 14% 3–4 times and 1% 5–6 times. Eighty-six percent had heard about domestic violence before, the remaining 14% perceived no knowledge or understanding. Forty-nine percent of the respondent accept that behavioral changes in pregnancy could cause domestic violence in pregnancy while 51% did not accept this. Seventy-three percent also accept that unplanned pregnancy could cause domestic violence, while 27% of the respondents did not. Ninety percent of the respondents said domestic violence could cause miscarriage or abortion while 10% disagreed. Futhermore, 76% said domestic violence could causes death while 24 24% of the respondent disagreed. The fact that violence still occurs even against pregnant women calls for attention. Preconception health educations for couples, routine screening for potential victims during antenatal visits by health care-givers could be useful in combating this form of violence.

2.1-008

Chagas screening in pregnant women from Latin America: experience in western Almeria

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INTRODUCTION Chagas disease is an illness that can be transmitted from birth and is endemic in most Latin American countries. The Hospital de Poniente has been one of the first that has implemented the screening of this disease in Latin American pregnant women in Andalusia (southern Spain).

MATERIAL AND METHODS Screening of pregnant women from Latin America, captured in Obstetrics and queries ward puerperal (pregnancy was not monitored) from April 2007 to April 2011. Protocol: two serological test (ELISA and IFA). (i) Doubtful cases (one positive and one negative test): ask for a third confirmatory test. (ii) Confirmed cases (two positive tests) 2.1.Studying the newborn (vertical transmission) 2.2. The mother is referred to Tropical Medicine Unit for treatment and monitoring.

RESULTS A total of 288 pregnant women were included in this protocol. Country Frequency percentage: Argentina 62 21.5 Bolivia 17 5.9 Colombia 48 16.7 Ecuador 74 25.7 Perú 46 16.0 Brasil 17 5.9 Chile 8 2.8 Uruguay 1.3 Paraguay 1.3 Venezuela 7 2.4 Panamá 1.3 Guatemala 1.3 México 3 1.0 Honduras 2.7. Total 288 100.0 three women with a confirmed diagnosis (two Bolivian and one from Argentina): The Argentina patient was lost to follow-up. Two Bolivian mothers were treated with Benznidazol. One of them showed grade 1 esophageal disease. In all cases PCR was negative and there was no vertical transmission to newborns.

CONCLUSION In Western Almeria there is a low prevalence of Chagas disease among pregnant women from Latin America. This is probably due to the small number of Bolivian women who live in the area.

2.1-009

Measuring equity in access to emergency obstetric care at Wolisso hospital in Oromiya, Ethiopia

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INTRODUCTION There is a growing need to monitor access to health care through routine health data. Such data, however, does not allow for socioeconomic stratification of service users. This study developed a simple tool to measure equity in access to EmOC at Wolisso Hospital, Ethiopia, and to compare the wealth status of EmOC users with that of general population women.

METHODS A dataset of women with previous delivery and with usual residence in Oromiya (n = 1531) was created from the Ethiopia 2005 demographic and household survey (DHS) datasets. All proxy wealth variables were cross-tabulated against wealth quintiles in the DHS dataset and five variables, differentiated well across the quintiles, were selected. Response options for each variable were assigned weighted scores. Factor scores for all wealth variables in the Oromiya DHS dataset generated a 'gold standard'. Validity and reliability of the weighted scores of five selected variables were established by correlation and kappa analysis respectively with the 'gold standard'. A short questionnaire containing the five variables was prepared and used to collect data from 760 women at discharge from the maternity ward of Wolisso Hospital from January to August 2010. Collected data

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were compared with the DHS data both graphically and statistically.

RESULTS There was a strong positive correlation ($R = 0.876$), and fair to good agreement (κ 0.464; 95% CI 0.435–0.493, $P < 0.001$) between the 'gold standard' and weighted scores of five selected variables. Women using EmOC were wealthier, and were more likely to be urban than rural dwellers compared to general population women.

CONCLUSIONS Measuring equity in access to health services at the hospital was feasible. Approaches to redress inequity in access to EmOC at the hospital are needed.

2.1-010

Audit of the cervical cancer screening programme at Eshowe hospital, Kwazulu-Natal, South Africa

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The female population in Kwazulu-Natal (KZN), South Africa, has a very high rate of advanced cervical cancer. The services available to comprehensively treat them (surgical and radiotherapy) are in tertiary centres where first appointment clinic waiting times are up to 6 months. The high HIV prevalence (31%) in our catchment area made our target population increasingly vulnerable to cervical cancer. A cervical screening protocol has been put in place by the South African government. Our study aims to assess whether the screening protocol was being followed at Eshowe hospital (district general hospital). There is a separate protocol to follow for HIV positive patients who require annual smears. We looked at both protocols. We interviewed 100 women using a questionnaire format. We assessed HIV status, age, cervical screening status, and knowledge of the screening tool. Our results showed that 25.7% of our total population had received a cervical smear; of the HIV positive sub-group 28% had received a smear. In the government HIV negative target group (over 30 years) 44% had received a cervical smear. These are below national targets for screening uptake. Only 50% of the women had any basic knowledge of the test. The results show that there needs to be an increase in cervical screening, especially targeting HIV positive women. To increase screening rates a two-pronged approach should be put in place. Firstly improving the health education of the target population, Secondly a more rigid protocol should be in place at the clinics to ensure women attending for any complaint are offered cervical screening. An effective cervical screening programme would have a large impact on health in this area.

2.1-011

Care-seeking practices and quality of care in rural Bangladesh: baseline survey for health monitoring and advocacy project on safe motherhood

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BACKGROUND A safe motherhood project was designed to strengthen the Family Welfare Center through improving governance of local government in five rural areas of Bangladesh. Prior to implementation, a baseline survey was conducted to assess care seeking practices of pregnant women, quality of care and existence of supportive initiatives by local government in maternal complications.

METHODS AND MATERIALS Two thousand women, who had their delivery during the last year prior to the survey, were interviewed. In depth interviews were conducted among the local government. Composite scores were used to measure quality of care responses. Quality of care response was based on standard services and counseling items that are mandatory to be provided during ANC and PNC. Transcriptions were prepared and coded for in-depth interviews.

RESULTS Multiple sources were used by mothers for seeking care during pregnancy, delivery and postnatal period. Mothers commonly preferred traditional birth attendants during delivery and village and private doctors for complication management. Mean score for service items during ANC, out of maximum score 10, was around 5. Counseling during ANC was especially poor on danger signs, facility care for complications, arranging transport, money and blood donor. Overall, mean score for service items during PNC was two out of maximum 6. Among 40% mothers who did not receive PNC, 70% felt that it was not necessary. Local government was neither aware about safe motherhood policy nor about their role in safe motherhood initiative.

CONCLUSION Key recommendations are: to improve quality of care at all stages of pregnancy and increase community awareness in the use of skilled health services. The qualitative results failed to explore on local government's role in safe motherhood initiatives. Thus, to improve maternal health, there is a need to empower the communities to make health service providers, as well as local governments, demand-responsive.

2.1-012

Serological diagnosis of *Toxoplasma gondii* infection in women: comparative study using ELISA and IFA

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Toxoplasma gondii is an obligate apicomplexan parasite of warm-blooded animals in addition to humans. Although toxoplasmosis is usually asymptomatic in immunocompetent hosts, it can make a fatal visual impairment result or neurological manifestation in newborns with congenital infection and immunocompromised patients. This study was performed to establish the ELISA assay for serodiagnosis of *Toxoplasma gondii* with home-made antigen in comparison with IFA.

METHODS One hundred referred serum samples in serology laboratory of School of Public Health, Tehran University of Medical Sciences from November 2008 to July 2009, were collected for execution of assays. Antigens were prepared from tachyzoites of *T. gondii*, RH strain then tests were performed.

RESULTS This study was performed to compare the ELISA assay by IFA (the later test as a gold standard method), with the aim of serodiagnosis of *T. gondii*. The research showed that these tests have an agreement rate about 91.4% in detection of *T. gondii* infection. No statistically significant differences were observed between ELISA and IFA techniques ($P = 0.48$) by chi-square analysis. The results revealed that 23 of the cases were positive and 71 were negative for IgG antibodies against *Toxoplasma gondii* with both methods. Five seropositive samples were identified for IgG antibodies with ELISA, which were negative by IFA method. On the other hand, a detected positive serum with IFA had negative result by ELISA

CONCLUSION These assays had high agreement rate (91.4%) in detection of *T. gondii* infection. No statistically significant differences were observed between ELISA and IFA techniques ($P = 0.48$) by chi-square analysis.

KEYWORDS Toxoplasma gondii, IFA, ELISA

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2.1-013

Syphilis in pregnancy in Florence: immigration and unfaithfulness

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Introduction Syphilis in pregnancy is a reemerging problem in Italy, ascribed to immigration and changes in sexual behavior. Early antenatal screening and adequate treatment are lacking, and recent syphilis and elevated nontreponemal tests are the major risk factors identified by literature for the development of adverse events in pregnancy, such as abortion or fetal death, low weight at birth, preterm delivery and congenital syphilis.

Methods and Materials We retrospectively reviewed anagraphic, ethnical, socio-economic and clinical features of 186 syphilitic pregnant women presenting to the Tuscan Referral Center for Infectious Diseases in Pregnancy from 2000 to 2010 in order to investigate whether certain subgroups have more identified risk factors for developing adverse outcome. We followed 142 women until the end of pregnancy. Their outcomes were recorded in order to identify new risk factors for adverse events in our setting.

Results The number of pregnant women evaluated increased from two cases in 2000 to 44 in 2010. A significant association was detected between: late screening test (>16th gestational week) and unmarried status (P = 0.035); lack or inadequate treatment and both Asiatic origin (P = 0.041) and illegal migrant status (P = 0.049); early syphilis and both Italian origin (P = 0.023) and positive partner's test (P = 0.021); elevated RPR title (¡Ý1:8) and positive partner's test (P = 0.006). Furthermore in our setting we found that the diagnosis of confirmed or probable congenital syphilis was associated with Asiatic origin (P = 0.014), illegal status (P = 0.019) and positive partner's test (P = 0.040). Abortion or fetal death was associated with positive partner's test (P = 0.036).

Conclusions In our region Asiatic and illegal migrant women with syphilis are the subgroups with greater difficulty in accessing prenatal care and are at high risk of developing adverse outcome. Another group with increased risk is women who acquire a recent infection from their partner.

2.1-014

Why some women die and others survive maternal complications: findings from the qualitative assessment of Bangladesh maternal mortality survey (BMMS), 2010

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A nationwide survey shows an impressive reduction in maternal mortality in Bangladesh: a decline from 322 to 194 in 9 years, with hemorrhage and eclampsia the leading causes of death. Little is known about the circumstances that prevent or encourage women with complications to use emergency obstetric care. As part of the maternal mortality survey, qualitative research was carried out between March 2010 and January 2011 with maternal deaths (15) and near-miss (16) having experienced hemorrhage and eclampsia within the past 18 months. Maternal deaths were sampled from the survey while near-miss were identified in health facilities near to where the deaths occurred. Methods included in depth interviews with people most familiar with the death or near-miss event, including family members, health providers, and in the case of near-miss, the women themselves. Informants from both groups demonstrated limited knowledge of delivery-related complications and where to seek treatment. Maternal deaths were more likely to obtain initial treatment with informal providers, delaying care seeking to facilities, while near-miss women generally first sought facility care. Additional household level delays to care seeking faced by women who died included older family members' opposition to facility care, the fact that it was night-time, and that money was not available. Once care was sought, maternal deaths were more likely to go to a facility that was unable to provide care, either because of the unavailability of doctors or required services, forcing women to visit multiple facilities before obtaining treatment. Data showed that eclampsia was easier to recognize, signaling the need for formal care, while hemorrhage was difficult to identify, with the majority of deaths never accessing facility services. Prior to childbirth, women and family members should be informed about pregnancy-related complications and where to seek appropriate care. Health officials must ensure that EmOC services are functioning and rapid referral systems are in place.

2.1-015

NGO CSBAs: a big step in public-private partnership (PPP) in improving mother and child health in rural Bangladesh

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Introduction Low skilled birth attendant (SBA) at delivery is a concern for achieving MDG 5 in Bangladesh. The rate of SBA at delivery increased to 26%, but far from the desired 50% by 2010. Almost two in three births are assisted by untrained traditional birth attendants in the villages and one in 11 deliveries are assisted by relatives or friends. One in 10 births takes place in a facility. The government of Bangladesh initiated the community skilled birth attendant (CSBAs) training program in 2003. Nearly 5000 CSBAs were trained by June 2009, which is much less compared to the total requirements.

Objective Deploy 32 NGO health workers as CSBAs, required for the Shahjadpur sub-district to increase the SBA at delivery from 18% to 50% and to decrease neonatal mortality from 37/1000 to 20/1000 livebirths by end 2011.

Methods and Materials A pre- and post- design with a midline evaluation to observe the changes in maternal and neonatal health (MNH) status. Structured questionnaire used to interview mothers who delivered 6 months prior to interview date. The study area with a population of approximately 600,000 people required 62 CSBAs in total. It had 30 CSBAs and the additional 32 were selected from the NGO health workers and were trained and deployed.

Results The baseline in early 2009 and midline in late 2010 showed increase in skilled delivery from18% to 42%. CSBAs contributed to 18% of the total skilled delivery at midline, three times the baseline. Neonatal mortality was reduced to 22 from 32/1000 livebirths.

Conclusion Placement of CSBAs according to the government policy (1 CSBA/8000–10,000 population) improves the MNH status thereby decreasing morbidity and mortality. Home delivery is still >70% and social, economic and other cultural factors are impediments to facility delivery in Bangladesh.

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2.1-016

Husbands' knowledge on maternal health care in rural Bangladesh: an untapped resource?

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INTRODUCTION Poor utilization of care is hindering the improvement of maternal health in Bangladesh. Multi-factorial unfavorable conditions including poverty, illiteracy, and lack of knowledge regarding availability of maternal health services/providers indirectly contribute to maternal deaths and disability. Husbands can play a key role in this regard by taking care of their spouses with proper planning, arrangement of resources, and support during the entire pregnancy period and beyond; these efforts could have an impact in reducing maternal deaths. ICDDR, B, in collaboration with consortium-partners in Bangladesh and Netherlands, has initiated a project to address this. The main objective is to assess men's awareness of their wife's maternal health care needs.

METHODS AND MATERIALS A baseline survey using a structured questionnaire was conducted during June–August 2010 among 390 women who delivered in the last year prior to the interview date and their respective husbands (n = 359) residing in rural areas of several sub-districts of Bangladesh.

RESULTS Knowledge about pregnancy-related advice to diet and nutrition, avoiding heavy work, taking rest, and intake of iron tablet was significantly lower (>30%) among the husbands than their spouses. No husbands knew about arrangement of transport and blood-donor, and emergency saving-scheme for their wives' pregnancy-complication period. Awareness of the husbands about health service facilities for maternal complications during pregnancy, delivery and postnatal periods were 12%, 21%, and 11%, respectively, substantially lower than their wives.

CONCLUSION There is a huge gap in knowledge on maternal health care among husbands in rural Bangladesh. Husbands can be essential in improving maternal health by encouraging their wives to have adequate nutrition and providing financial support to do so, and facilitating visits to a health facility during any maternal complications. Therefore, efforts are required to develop interventions that enhance maternal health care knowledge amongst fathers-to-be.

2.1-017

Community support groups: expectant contributors in improving maternal health in Bangladesh

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INTRODUCTION Maternal health status remains a significant challenge in Bangladesh. Lack of awareness combined with socio-cultural and religious taboos contribute to the high maternal mortality (194/100,000 livebirths) in Bangladesh. Social mobilization through community support groups (CSGs) could be an evidence-based intervention in increasing awareness on the causes of maternal mortality, and to encourage advocacy actions that will improve maternal health outcomes. As a part of demand-side intervention, ICDDR, B have taken initiative to form CSGs by involving community members in Shahjadpur sub-district. The CSGs' objectives are to identify pregnant women, sensitize the women and other members of her family about need of using skilled care for maternal health, provide support to the poor and arranging transportations for maternal complications for transfer to facility, and establish linkage between community and facility.

METHODS AND MATERIALS Sixty-four CSGs were formed covering almost all the unions of Shahjadpur sub-district where each CSG was comprised of three different tiers including advisory, executive, and volunteer committees. The effectiveness of CSGs was assessed at community-level through a baseline (n = 3158) and a follow-up (n = 2725) survey during November 8–March 9 and October 10–November 10 among the mothers who delivered 6 months prior to the interview date using a structured questionnaire.

RESULTS After the baseline survey, 640 courtyard-sessions covering 6500 mothers were conducted by the CSGs' volunteer. After formation of CSGs, 84.0% of mothers received antenatal care (ANC) in the follow-up survey, while that figure was 69.6% during the baseline survey. Skilled attendant at delivery was increased from 26.4% to 41.7% during this period. The mothers who attended the courtyard-sessions conducted by CSGs received substantially more ANC (88% vs. 83%), and skilled delivery care (46% vs. 41%) than those mothers who did not attend.

CONCLUSION Participatory approaches through courtyard-sessions by CSGs could make a significant contribution to transfer knowledge to pregnant women in improving their health-seeking behavior in rural Bangladesh.

2.1-018

The reasons for use and non-use of urban maternal and child health services in Bangladesh: a qualitative assessment from the client's perspective

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INTRODUCTION Eighty-percent of the world's biggest cities are located in developing countries and 60% of the inhabitants are slum dwellers. In Bangladesh nearly 33% of the urban population live in urban slums. Despite being located close to skilled care facilities, 80% of deliveries in slums are conducted by neighbors or relatives at home and only 13% of births are assisted by skilled attendants. This paper explores the reasons behind use and non-use of maternal and child health services in urban slums where a Maternal Newborn and Child Health program (Manoshi) is in operation.

METHODOLOGY A qualitative approach was applied for conducting this study during June 2009 and August 2009. Thirty in depth interviews were conducted among the user and non-user of MNCH services.

RESULTS AND CONCLUSIONS From the client's perspective the reasons for using MNCH services were: counseling skills of the providers, provision of free care, proximity, unavailability of accompanying family members, privacy due to absence of males, availability of trained birth attendant, referral linkage, provision of referral cost and home service. The stated reasons for not using the services were: unavailability of providers during emergency; inadequate information about services; perceived lack of use of essential drugs such as oxytocin; lack of facility for c-section and immunization; not allowing family members; discouragement of the local TBA and neighbors; shyness; and desire for privacy as there were no obvious complications. MNCH programs such as Manoshi should figure out ways to address the barriers to accessing health services for future sustainability.

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2.1-019

Mobile phone network to access specialist care at community level in maternal emergencies

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INTRODUCTION Maternal mortality remains unacceptably high in Bangladesh at 194 per 1000 live births. More than 80% of births are occurring at home; over 70% attended by unskilled providers, more than half (53%) pregnancies or deliveries have complications such as excessive bleeding (9%), convulsions (6%) and prolonged labour (12%). Provision of skilled services, including access and utilization of them at community level, is scarce. A mobile phone network that connects mothers directly with the community-based skilled birth attendants (CSBA) can improve the situation by addressing complications at the onset and reducing response time.

METHODS AND MATERIALS A mobile phone based network was made available in a rural sub-district in Bangladesh along with a package of services. Mothers and family members communicated toll free with CSBAs. CSBAs contacted specialists (Doctors) through the other loop of network to get instructions to deliver services. The trial is being monitored through interviews, surveys and evaluation.

RESULTS AND CONCLUSION About 53% women who delivered at home in the intervention area had any complication at delivery. These included severe bleeding (5.5%), convulsion (1.4%) and difficult or prolonged labour (30%). Sixteen percent sought care from CSBAs and initial communication happened through mobile phone (60%) loop. CSBAs visited the mothers and in most cases sought advice from specialists at the district level by mobile phone. This consultation facilitated provision further management at community by drugs, maneuvers and in appropriate referral. This small mobile phone network established a direct communication with the specialists in maternal emergencies and facilitated appropriate care at the community level. This communication saved money, time and lives.

2.1-020

Maternal health care seeking behavior at Haor area in Bangladesh

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BACKGROUND Despite all measures and initiatives, utilization of Maternal Health Care (MHC) services is still falls below acceptable standard. This study was conducted in the haor area of Bangladesh, where plains are submerged under water for more than 6 months a year. Large area in the eastern part of Bangladesh are classified as haor.

OBJECTIVES This study examines mothers' knowledge about maternal health care and their practices in seeking MHC services. It also establishes factors affecting the utilization of MHC services in the haor area.

METHODOLOGY A cross sectional study was conducted in two unions of Hobigong district, eastern part of Bangladesh. Data were collected from randomly selected 400 women aged 15–49 years who gave birth in 5 years preceding the survey through a semi-structured interview schedule. Information on socio-economic and demographic of the respondents, knowledge about MHC facilities, ANC situation, delivery care and PNC have been collected.

RESULTS Sixty-one percent respondents did not know about MHC service available in the study area. Only 36% (n = 144) women received ANC. Irrespective of complications and danger signs 95% of deliveries occurred in home and 92.5% deliveries attended by untrained birth attendant. Only 13.8% of women and 12.3% of infant received postnatal care respectively. Communication, low knowledge about MHC services, low income, decision, and lack of companion to go to health services are the important factors influenced the lower utilization of MHC services.

DISCUSSION MHC service knowledge among hoar women is very low. ANC and PNC coverage is also very low. Immediate and special initiatives and program are needed to increase the MHC knowledge among women and to improve ANC, delivery and PNC utilization for the overall improvement of MHC services.

2.1-021

Ultrasonographical thyroid features in pregnant women in Lubumbashi, DR Congo

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BACKGROUND The thyroid gland is prone to several modifications during pregnancy such as change in size and malignant transformation of benign nodules.

OBJECTIVE To describe morphological changes of thyroid gland in pregnant women living in Lubumbashi, an area with known iodine deficiency.

METHOD A cross sectional study was carried out in three hospitals of Lubumbashi (University clinics, Katuba general hospital and Bongonda maternity) in DR Congo from March 15 to July 15 2009. One hundred and forty-seven pregnant women without evidence of a visible goiter were enrolled, mean age 27 ± 6 years (range: 16–43 years). Ultrasonographical characteristics of the thyroid were analyzed according to maternal or gestational age. The volume of the gland was expressed as geometric mean (SD) because of a logarithmic transformation for analyses.

RESULTS The mean thyroid volume was 11.5 (1.5) ml, 10.4 (1.4) ml, and 9.8 (1.5) at first, second, and third trimester of pregnancy, respectively. The increase in volume was correlated with maternal age and with parity. Presence of nodules was detected in 8% of pregnant women. A thyroid volume ≥18 ml was registered in 5.4% of women, suggesting infraclinical goiter.

CONCLUSION Thyroid modifications have been observed during pregnancy. To allow a thorough understanding of the link between pregnancy and the gland modifications in an area with iodine deficiency, additional studies are needed on larger samples of pregnant women with functional explorations.

2.1-022

Iodine content of household salt in Lubumbashi, DR Congo

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BACKGROUND To reduce disorders caused by iodine deficiency, DR Congo has complied with universal salt iodization since 1994. However, there is no routine monitoring of iodine content (IC) at any level of its distribution chain.

OBJECTIVE Our study aim was to evaluate IC of salt eaten in households from Lubumbashi.

METHODS We conducted a survey in households from 375 women randomly selected among women consulting in three maternities of Lubumbashi: Cliniques universitaires de Lubumbashi (CUL), general reference hospital from La Katuba (GHK), and maternity of Bongonga dispensary (MBD). Interviews were conducted targeting consumer behavior towards the kitchen salt (purchase place, packaging, and storage). We collected salt samples used at

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home to determine their CI. Iodine measurement assay was a volumetric method. CI is reported as median, Q1–Q3, and range (ppm). Recommended minimum- and maximum thresholds are 20 and 40 ppm, respectively.

RESULTS Most women (81.5%) bought their salt on markets, and sometimes at stores (8.5%) or supermarkets (6.4%). Furthermore, 93.6% were buying in bulks, 5.1% in bags, and 1.3% in boxes. Five types of containers – plastic box, metal box, bottle glass, porcelain plates, and cellulose paper – were used to store the salt in the kitchen, plastic box container being the most frequent (80.0% of households). CI were 27.5 (16.9–38.1, 0–86.8), 20.1 (5.3–31.7, 0–99.5), and 19.0 (7.4–31.7, 0–100.5) for women recruited at CUL, GHK, and MBD, respectively. Adequate salt iodization was observed in only 37.9% of households: CI was not detectable, <20, and >40 in 13.9%, 30.4%, and 17.9%, respectively.

CONCLUSION Because of inadequate salt iodization, Lubumbashi inhabitants remain at risk of hypothyroidism and hyperthyroidism. There is a need for an effective and regular control system of salt IC to prevent human iodine deficiency in DR Congo.

2.1-023

Overcoming the challenges in inequity to improve maternal and neonatal health in rural Bangladesh

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INTRODUCTION Bangladesh is committed to achieving Millennium Development Goal (MDG) 5 to reduce maternal mortality to 143 per 100,000 livebirths by 2015. Deliveries take place at home (>90%) mostly by Traditional Birth Attendants and family members. The rate of skilled birth attendant (SBA) at delivery is 26%, far from the desired 50% goal by 2010. SBA at delivery is vital for preventing maternal morbidity and mortality. There is much inequity in the availability of SBA between the urban and rural setup. This inequity is even more in the lowest local government tier, the unions, giving rise to difference in health indicators.

OBJECTIVES To deploy community skilled birth attendants (CSBAs) to address community provider inequity on PPP basis, follow up for regular holding of satellite clinics, formation of community support groups(CSGs)for creating community demand to access health: to improve maternal and neonatal health.

METHODS AND MATERIALS A pre- and post- design with a midline evaluation. Structured questionnaire used to interview mothers delivering 6 months prior to interview date. Shahjadpur subdistrict with a population of approximately 600,000 required 62 CSBAs. It had 30 CSBAs and the additional 32 were selected from the NGO and deployed. Satellite clinics were followed up and 80 CSGs were formed till May-2011.

RESULTS The baseline in early 2009 and midline in late 2010 showed increase in skilled delivery 18–42%, ANC 70–84% in the subdistrict. In the remote union Sonatoni the SBA increased from 3.7% to 36.4% after deployment of three CSBAs. The Neonatal mortality was reduced to 22 from 32/1000 livebirths. CSGs helped the community to access skilled care which was significant.

CONCLUSION Just distribution of CSBAs, formation of CSGs improves the MNH status, decreasing morbidity and mortality. This finding needs considerations as home delivery is very high in Bangladesh and social, economic and other cultural factors are impediments to facility delivery.

2.1-024

Historical and current influences on U.S. international reproductive health policy

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The United States has been a global leader in the international family planning and reproductive health arena for some 50 years, but the domestic policy debate around these issues has been increasingly characterized by politicization and partisanship. My study explores the strategies and discourse that have been designed and employed to influence the direction and scope of U.S. international family planning policy over time, including those used by interest groups and members of the United States Congress themselves. Through historical documentation, qualitative interviews and a content analysis of congressional debates, I assessed: (i) How interest groups and the messages and strategies they utilize have influenced United States international family planning policy over time; (ii) How the gender and political party affiliation of members of Congress have influenced their support for international family planning programs; and (iii) The tone of discourse employed by male and female members of Congress when speaking and voting on international reproductive health and rights issues. I find that the Religious Right has successfully managed to alter the debate about international family planning and reproductive health, including by deliberately conflating family planning with abortion. I find evidence of a growing partisan divide around these issues in the U.S. Congress, in both voting and speaking patterns. Finally, I conclude that female members of Congress engage more frequently and more positively on international family planning than do their male counterparts. These findings may provide useful guidance for supporters of international family planning who are working to restore the United States to a position of global leadership in the areas of international family planning and reproductive health.

2.1-025

Capturing the understanding of prolonged labor: voices from an urban slum

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INTRODUCTION Prolonged labor is one of the major causes of maternal death in developing countries. Many women die due to this common labor complication in urban areas of Bangladesh. Care sought for prolonged labor is associated with indirect non medical causes e.g. social norms, cultural beliefs and local practices and these causes are reflected the care seeking behavior in urban slums. BRAC conducted a multi-site study in partnership with Emory University, ICDDR,B, and LAMB Hospital (funded by Emory Global Health Institute, USA) to examine how 'cultural beliefs', social norms are related to care seeking behaviors during prolonged labor among slums dwellers of Dhaka city.

METHODS Data was collected using group interview technique with seven mothers who experienced prolonged labor and their caregivers and 80 successive free listing techniques comprising of semi-structured interviews of 20 reproductive age women, 20 elderly women, 20 traditional birth attendants and 20 informal doctors.

RESULT The result shows that, most of the respondents do not have a clear idea about the signs and symptoms of true labor pain in biomedical point of view. Regarding the length of labor, some respondents considered 2–7 days continuous pain as life threatening. In reality, the mothers residing in slum practice early

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pushing during delivery. Some harmful care practices were also found in the selected urban slums. The community strongly believes in pushing 'injection' (Oxytocin) for speeding up labor pain and spiritual care practice as a treatment of prolonged labor at home. Moreover, family beliefs, cultures and the different types of economical contextual factors incorporated in care seeking behavior and influence them to stay at home during prolonged labor.

CONCLUSION These understandings by the local women would help health programmes in setting up the appropriate community-based strategies and interventions; sensitize urban community in identifying complications and danger signs of labor pain.

2.1-026

Factors affecting the activities of volunteers in community support groups in rural Bangladesh

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INTRODUCTION In developing countries inequalities in maternal health are tremendously common. In Bangladesh, current MMR 194/100,000 live births which is unlikely to attain the MDG 5 target. Deficiency of cognizance and socio-cultural norms, religious superstition contributed a high maternal mortality in Bangladesh. As part of the demand-side intervention, the project has initiated to form community support groups (CSGs) by involving community people. And, the objective is to describe the hindering factors that affect the activities of root-level tiers of a CSG.

METHODS AND MATERIALS The project has to plan for one CSG having with 3000–3500 population. The CSG has comprised with advisory, executive, and volunteer committee. Formation of CSGs formally started in July 2009 by organizing the first advocacy meeting of the CSGs at community level under the respective union. As of May 2011, already 81 CSGs have been formed covering 8000 mothers. The volunteers committee comprises female members only and consists of 7–10 volunteers in each CSG (One volunteer per 100 households).

RESULTS The factors that affect the activities of the volunteers group in a CSG were: inadequate communication between advisory and executive committee, no incentive facility which made discomfort themselves, difficulties to manage time due to domestic home works, inadequate training and logistics supply, non-cooperation by service providers.

CONCLUSION Community support groups can play a vital role for mobilization of community people for increasing awareness regarding maternal health and contribute to decrease maternal mortality. Therefore, efforts need to develop a close monitoring system and provide some incentive giving mechanism to solve the volunteer group problems so that they can run their volunteer activities smoothly.

2.1-027

Imported malaria in pregnancy in Madrid

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BACKGROUND Malaria in pregnancy is associated with maternal and fetal morbidity and mortality in endemic areas. But information on imported cases to non-endemic areas is scarce. Our aim was to examine the incidence and the clinical and epidemiological characteristics of malaria in pregnancy in two general hospitals in Madrid, Spain.

MATERIALS AND METHODS Retrospective study of laboratory confirmed malaria in pregnant women at the Hospital de Fuenlabrada and Hospital Príncipe de Asturias over a 5 and 10 year period, respectively. Relevant epidemiological, clinical and laboratory data was obtained from medical records.

RESULTS Eighteen pregnant women among 345 malaria cases (5.2%); All sub-Saharan immigrants. Average age 28 years. Gestational age (trimester): 55% 3rd, 27% 1st, 16% 2nd All but one multigravidae. Sixteen (88%) had visited friends and relatives. None had taken prophylaxis nor seeked pre-travel advice. Two were recently arrived immigrants. Three were HIV positive. All Plasmodium falciparum malaria. Presentation: 15 symptomatic (fever 13, asthenia 2), three asymptomatic, one complicated malaria. Median delay in diagnosis: 7.5 days. Laboratory tests: anemia (cut off Hb level 11 g/dl) 83.3% (mild 33.3%, moderate 33.3%, severe 16.7%) thrombopenia 72%, hypoglycemia 11%. Quinine + clindamycin prescribed in 83%. Outcomes: no severe maternal complications or deaths, two stillbirths, 15 term pregnancies, no low birth weight newborns.

CONCLUSIONS Though incidence is low in our setting, a most at risk group is clearly defined: young Sub-Saharan mothers visiting friends and relatives without pre-travel counselling and recently arrived immigrants. The most common adverse maternal and fetal effects were anemia and stillbirth. Given that presentation can be asymptomatic, screening of vulnerable groups could be recommended. Malaria should be considered in patients with unexplained anemia and epidemiological risk. These findings could help Maternal Health programme planners and implementers to target preventive interventions in the immigrant population and should create awareness among clinicians.

2.1-028

Factors affecting formal antenatal care attendance: results from a qualitative study in Madang, Papua New Guinea

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BACKGROUND Attendance at formal antenatal care (ANC) is important for the provision of certain health interventions such as intermittent preventive treatment of malaria (IPTp), HIV and STI testing, screening of hemoglobin levels and health education. However, in Papua New Guinea (PNG) only 33% of women attend formal ANC during pregnancy. This paper explores factors that influence ANC attendance in the Madang region of PNG.

METHODS Data were collected in Madang, PNG (three sites) utilizing qualitative methods including in-depth interviews, group discussions, long-term case studies and observations during formal ANC visits. Respondents included pregnant women, their relatives, biomedical and traditional health providers, opinion leaders and community members.

RESULTS Women's main motivation for attending formal ANC was to ensure an easier delivery. However, a number of factors affected attendance. Accessibility of services (cost and distance) played a role to such an extent that some women stay with a relative who lives closer to a clinic during their pregnancy. Other reasons underpinning non-attendance, even if services were near and affordable, included negative interactions with healthcare workers (such as being scolded), long waiting times, not wanting to take medicine, fear of stigmatization (if unmarried or in case of short birth spacing), and out of spite or depression due to unwanted

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pregnancies or in response to arguments with relatives. Women delayed their first formal ANC visit for three principal reasons: waiting for the unborn child to be 'strong', avoiding repeat visits and already having children. In contrast, experience of previous pregnancy complications prompted earlier attendance.

CONCLUSIONS Studies on ANC attendance often focus on cost and distance as major determinants. This in depth, long-term study, the first of its kind in PNG, shows that other socio-cultural factors are also important which should be addressed in efforts to increase formal ANC attendance and encourage earlier first visits.

2.1-029

Promotion of normal delivery and childbirth: an alternative way for maternal and neonatal health

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BACKGROUND Quality care with an emergency service is central in reducing maternal and neonatal mortalities, where appropriate and timely intervention can be accomplished by careful observation. However, patients frequently experience verbal and physical abuse from health care providers. In addition, harmful unnecessary interventions during delivery have been observed.

METHODS Bilateral technical cooperation project between Madagascar and Japan focused on improvement of care in normal birth. It provided a systematic technical session on evidence-based care combined with human-relationship training to those who work in maternity at the first-line health facility in region Boeny from 2007 to 2010. A structured questionnaire was distributed to pregnant women in the target facilities to gauge experiences and confirm what intervention they received. We also measured practices during delivery both in the target and control facilities by self-administered questionnaire to the providers.

RESULTS The proportion women with negative opinions (i.e. experience of abuses) to the providers decreases from 23% to 9% after the training ($P < 0.01$). Utilisation rate of any injection or perfusion decreased from 27% to 13% ($P < 0.01$). The proportion of women accompanied by family during delivery increased from 88% to 99% ($P < 0.01$).

Certain differences in harmful or unnecessary practices were observed between the intervened and control facilities: routine use of lithotomy position (27% vs. 92%, $P < 0.01$); routine uterine revision (21% vs. 62%, $P < 0.05$); hourly vaginal examination (47% vs. 88%, $P < 0.05$).

CONCLUSION There must be enough reasons when one intends to alter physiological process of birth. We have confirmed unnecessary practices are common in Madagascar, although introduction of evidence-based medicine with an introspective reflexion can decrease it. Promotion of care in normal birth should be emphasized as well as the emergency care to control iatrogenic complications for woman and baby.

2.1-030

Knowledge and practice of PAP smear in women from Sucre, Bolivia

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OBJECTIVE To describe and analyze the knowledge and practice regarding PAP smear testing in women from Sucre, Bolivia, in 2010

METHODS Cross sectional study of randomly selected women aged 20–59 residents in Sucre's districts. Women were invited to answer a questionnaire which inquired about socio-demographic variables and on knowledge and practice regarding PAP smear. We considered 'adequate knowledge' if the women answered correctly the following: 'What is PAP smear for?', 'Where are PAP smears obtained from?', 'How often should you have your PAP smear done?' 'Adequate practice' was assessed by asking 'Have you ever had a PAP smear done?'. Descriptive analyses were performed and multiple logistic regressions used to study associations with the outcomes.

RESULTS Of 1563 women, 'inadequate knowledge' was present in 59% (95%CI 56.6–61.6) and 'inadequate practice' in 33% (95% CI 30.7–35.4). Factors associated with 'inadequate knowledge' were educational level [OR 2.07 (95% CI 1.58–2.71) for low educational level compared to high] and a negative perception of the need for PAP smear [OR 2.94 (95%CI 1.88–4.59)]. Factors associated with 'inadequate practice' were age [20–29 vs. 30–59 OR 4.96 (95% CI 3.84–6.41)], 'inadequate knowledge' of PAP smear [OR 1.37 (95% CI 1.06–1.77)], low educational level [OR 2.45 (95% CI 1.31–4.59) compared to high level], negative perception of the need of PAP smear [OR 2.81 (95% CI 1.82–4.34)], previous sexual transmitted infections [OR 0.39 (95% CI 0.25–0.62)], information of PAP smear gained through health service [OR = 0.35 (95% CI 0.27–0.48)] compared to that obtained from radio/TV.

CONCLUSION The proportions of women with poor knowledge regarding PAP smear and who have never had a smear done are very high. Future interventions need to improve the knowledge on PAP smear and raise awareness in order to increase uptake of PAP smear, especially in young women of low educational level. These actions should be led by the healthcare service.

2.1-032

Predominance of Toxoplasma gondii RFLP-genotype II and high frequency of atypical genotypes in 74 amniotic fluid samples of Brazilian women

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Among many factors involved in the pathogenesis of toxoplasmosis, the parasite genotype seems to play a pivotal role. The aim of this study was to determine the frequency of T. gondii genotypes in amniotic fluid samples of Brazilian women who acquired toxoplasmosis during pregnancy and transmitted the infection to

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their fetuses. In Brazil, significant linkage disequilibrium was reported in cases of ocular toxoplasmosis and Toxoplasma encephalitis of HIV patients, suggesting that the role of sexual recombination in the population structure of T. gondii is more central than in other continents. Seventy-four amniotic fluid samples were analyzed by means of a multi-loci nested-PCR-RFLP of four markers: 3′-SAG2, 5′-SAG2, SAG3 and GRA6 resulting in one sample (1.3%) with genotype I; 54 (73.0%) with genotype II; five (6.7%) with genotype III, and 14 (19.0%) submitted to DNA sequencing due to RFLP-inconclusive results. Among these 14 samples, two were genotype II and two were genotype III presenting with 100% of homology with the reference strains, while the remaining 10 samples contained a mixture of the three archetypal genotypes. The final distribution was: 1 type I (1.35%); 56 type II (75.7%), 7 type III (9.46%) and 10 atypical (13.5%). Albeit the predominance of RFLP-genotype II, atypical genotypes were much more represented in comparison to European and USA studies. Nevertheless, our data is in agreement with the findings of Brazilian ocular and cerebral toxoplasmosis. In conclusion, we have evidenced, for the first time in South America, the predominance of RFLP-genotype II and a high frequency of atypical parasites in congenital toxoplasmosis. Considering that the RFLP classification is based on a single cleavage site, it is possible that recombination has occurred in other nucleotide positions, so that it would be more reliable to adopt the amplification products sequencing as the gold standard of T. gondii genotyping.

2.1-033

Prevalence of anemia and related factors among child-bearing women receiving maternal and child health services at public health institutions of Harari region, Ethiopia

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INTRODUCTION Anemia remains one of the public health problems worldwide. It refers to a condition when the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, heavy blood loss, parasitic infections acute and chronic infection and others. About 56% of pregnant and 43% of non-pregnant women in developing countries are affected with anemia. With regional variations, the Ethiopian national survey indicated anemia prevalence among women was about 30.4% that necessitate the knowledge of specific region. The aim of the study was to determine the prevalence of anemia and related factors among women in child bearing age receiving maternal and child health (MCH) services in Harari region.

METHODS A cross-sectional study was conducted in six health centers and two hospitals from May to June 2010. Hemoglobin level was determined by Haemocue and different anthropometric measurement was done by their respective measuring tools. Data was entered to Epideta (v3.1) and different analyses were made with SPSS (v16) software.

RESULTS A total of 843(271 pregnant, 274 lactating and the 298 non- lactating) were studied. The prevalence of anemia was found to be 17.9% with mean hemoglobin level of 12.92+1.52(SD). Two percent of the women have had severe anemia (<7.0 g/dl). Women from the rural were about two times more likely affected by anemia (AOR = 1.833 (1.16, 2.89) than their counter parts, urban women.

CONCLUSIONS Anemia was found to be moderate public health problem. Rural resident women were more affected by anemia than urban once. Interventions encompassing dietary education and iron supplementation have to be programmed. Women from rural area require special emphasis. Intervention can be done through community health extension workers and at health institutions when women come for routine maternal and child health care services.

2.1-034

The use of mobile phones (mHealth) as a tool for reproductive health in Sub-Saharan Africa

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Since the 1994 International Conference on Population and Development in Cairo there has been an international push to improve reproductive health throughout the world, especially in Sub-Saharan Africa where some of the highest rates of reproductive mortality and morbidity are found. While the last two decades have been marked by an increase in attention on reproductive health, they have also been greatly affected by the global technological boom. To that end there has been a recent movement toward the use of technological solutions for the improvement of reproductive health, in particular the use of mobile phones. This use of 'mobile communications for health services and communications' is referred to as *mHealth*, and has earned the attention of international organizations such as UN Foundation and the World Health Organization. Its applications include 'Education and awareness, Remote data collection, remote monitoring, Communication and training for healthcare workers, Disease and epidemic outbreak tracking and Diagnostic and treatment support'. *mHealth* programs may be of particular value in Africa, where mobile phone use has spread at unprecedented rates- use increased by an estimated 550% between the years 2003 and 2008. From Uganda to Senegal to South Africa, *mHealth* is being employed for patient education and contraception reminders, to remote diagnosis support for birth attendants, to simply using the mobile to call an ambulance in an emergency. The purpose of our work is to address the various ways that mobile phones are being used to improve the reproductive health in Sub-Saharan Africa. This paper presents an active discussion or work in progress as *mHealth* is in early stages of use. We will discuss the uses of *mHealth* in the areas of: family planning, public education, service provision and emergency care, providing descriptive information about *mHealth* programs and providing data obtained through academic articles and grey literature.

2.1-035

Perceptions and care seeking for obstructed labor: findings from the qualitative assessment of the Bangladesh maternal mortality survey, 2010

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BRIEF INTRODUCTION A nationwide survey shows that 7% of pregnancy-related deaths are due to obstructed labor in Bangladesh. However, little is known about community perceptions towards obstructed labor and associated care seeking behaviors when women experience the condition.

METHODS AND MATERIALS As part of a nationwide maternal mortality study, qualitative research was conducted between March 2010 and February 2011 with maternal deaths and near-miss who experienced obstructed labour. Deaths were sampled from the survey, while near-miss were identified in health facilities located in the same area. In-depth interviews included family members of women who died (2) or survived (4) obstructed labor, and in case of near-miss, the women themselves.

RESULTS Women who died experienced labour pain for 2–3 days before they sought care outside home, while near-miss cases

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typically sought treatment within a few hours after the onset of contractions. Maternal deaths suffered from severe delivery pain which was not recognized as life-threatening by family members and formal and informal health care providers. In contrast, near-miss women experienced breech position, the baby's head was stuck in the birth canal, or a hand came out first, which both family members and TBAs identified as danger signs. TBAs referred these women promptly to health facilities. However, once reaching the facility, near-miss women faced many delays obtaining appropriate care. Overall, five women eventually had c-sections, which family members initially objected to due to the costs involved. With the exception of one maternal near-miss, all women had stillbirths.

CONCLUSION Prolonged labour is more difficult for family members and health providers to identify than other signs of obstructed labour. Mothers can be saved from obstructed labour if timely care is sought. Raising awareness about the definition of obstructed labor and appropriate treatment and establishing proper referral mechanisms is important in reducing maternal mortality and preventing stillbirths.

2.1-036

Point-of-care diagnostics at the periphery: early findings from an exploratory study in Bangladesh

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INTRODUCTION Maternal, perinatal, and neonatal infections and their diagnosis are under-researched and poorly controlled contributors in mortality in developing world. A myriad of systems and community level factors affect uptake of diagnostics for early detection of infections. The current study seeks to understand these factors at the lower levels of the health system. The current analysis however focuses only on the community level factors.

METHODS AND MATERIALS This is an exploratory study that employs a gamut of Methods: transect walks (12), Observation of healthcare worker (24) and laboratory technician (24), interview of healthcare workers (24) and medical representatives (12), FGDs (24), and diagnostic product concept evaluations (24). The study is being conducted in two phases. Phase I is exploratory, with field-testing of tools in a peri-urban location, followed by Phase II which will sample three sites in rural Bangladesh to diversify the findings.

RESULTS Early findings suggest that skilled and trained workers must administer the tests. Preference of location indicates that home is preferred over facility. Postnatal care seems unfamiliar to women. Also, there are restrictions on their movements during first 40 days after delivery. Importance of diagnostic tests across the continuum of care is not well understood in these communities. Community members (i.e. pregnant women and their key influencers) showed reservations in allowing health-workers to draw blood from neonates and obtaining vaginal swabs from a pregnant/parturient woman. Men and women suggested follow-on activities like medicines after the test, but were disinterested about referral.

2.1-037

Individual and institutional predictors for caesarean delivery and maternal mortality in referral hospitals in Senegal and Mali: a cross-sectional epidemiological survey

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OBJECTIVES In the context of recent fee exemption for caesarean delivery (CD) in Senegal and Mali, we assessed the individual and institutional factors Montréal, Montréa associated with CD likelihood and maternal mortality in referral hospitals. In particular, we focused on the impact of the qualification of the medical staff.

METHOD We used data collected during an ongoing trial on the efficacy of a multifaceted intervention to improve emergency obstetric care (QUARITE trial-ISRCTN46950658). From October 2007 to October 2008 – while the intervention was not yet implemented – 87,916 deliveries were reported in 41 hospitals. For each delivery, maternal and delivery characteristics and the vital status of both mother and child at discharge from hospital were recorded. At the institutional level, available equipment and human resources were assessed twice during the study period. Hierarchical mixed logistic multivariable regression models were used to determine the individual and institutional factors associated with (i) CD – categorized as emergency (before labour), intrapartum and elective – and, (ii) maternal mortality.

FINDINGS Twenty percent (17,058/86,602) of women had a CD, with highly variable rates between hospitals (8–46%). Intrapartum CD- which accounted for 73% – were mainly determined by well known maternal risk factors, but a large part of the between-hospital variability (45%) remained unexplained. Elective CD- which accounted for 11% -were mainly influenced by institutional factors (69% of the between-hospital variability). In particular, the presence of obstetrics specialists and medical-anesthetists was associated with a 5–10-fold increased probability of elective CD. A total of 804 (0.9%) women died. Independently of maternal risk factors, intrapartum CD [AOR: 3.0 (2.34–3.90)] and delivery in facilities where the staff was unspecialized [AOR: 3.0 (2.02–4.46)] were associated with a higher maternal mortality.

CONCLUSION To improve the quality of the obstetrical referral services, a better training in clinical decision-making for CD and mentoring of health professionals should be promoted.

2.1-038

Number and timing of antenatal HIV testing: evidence from a community-based study in northern Vietnam

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INTRODUCTION HIV testing for pregnant women is important for the success of prevention of mother-to-child transmission of HIV (PMTCT). A lack of antenatal HIV testing Results in loss of

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benefits for HIV-infected mothers and their children. However, the provision of unnecessary repeat tests at a very late stage of pregnancy will reduce the beneficial effects of PMTCT and impose unnecessary costs. This study aims to assess the number and timing of antenatal HIV testing in a low-income setting where PMTCT programmes have been scaled up to reach first level health facilities.

METHODS A cross-sectional community-based study was conducted among 1108 recently delivered mothers through face-to-face interviews by a structured questionnaire.

RESULTS The prevalence of women who lacked HIV testing among the study group was 10% while more than half of the women tested had had more than two tests during pregnancy. The following factors were associated with the lack of antenatal HIV test: having two children (aOR 2.1, 95% CI 1.3–3.4), living in a remote rural area (aOR 7.8, 95% CI 3.4–17.8), late antenatal care attendance (aOR 3.6, 95% CI 1.3–10.1) and not being informed about PMTCT at their first antenatal care visits (aOR 7.4, 95% CI 2.6–21.1). Among women who had multiple tests, 80% had the second test after 36 weeks of gestation. Women who had first ANC and first HIV testing at health facilities at primary level were more likely to be tested multiple times (OR 2.9 95% CI 1.9–4.3 and OR = 4.7 95% CI 3.5–6.4), respectively.

CONCLUSIONS Not having an HIV test during pregnancy was associated with poor socio-economic characteristics among the women and with not receiving information about PMTCT at the first ANC visit. Multiple testing during pregnancy prevailed; the second tests were often provided at a late stage of gestation.

2.1-039

Prevention of mother-to-child transmission – precarious hopes and childbearing choices among HIV-infected women in a northern province of Vietnam

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INTRODUCTION The world over, increased access to treatment brings reproductive hope to women infected with HIV. Yet despite the expanding availability of programs for prevention of mother-to-child transmission (PMTCT), HIV positive women continue to face numerous problems and uncertainties in the realm of reproduction. This study explores the hopes that HIV positive women invested in PMTCT and examining how this new technology enhanced the women's faith in their futures and childbearing capacities.

METHODS The research approach by anthropological research conducted in a Northern Province of Vietnam in 2007 by In-depth interviewed 32 HIV positive women, who had either had an induced abortion or had carried a pregnancy to term after being diagnosed as HIV positive with main contents: about their motives and concerns in relation to their pregnancy decision, about their experiences with the PMTCT services and their thoughts about their children's future.

RESULTS Women who were found to be HIV-infected during their pregnancy faced with major distress and were challenged with numerous problems and uncertainties as well as the difficult decision of whether to carry the pregnancy to term or to have an induced abortion. PMTCT, however, played an important role in providing treatment and care for HIV-infected pregnant women, in supporting the women in their reproductive decision making and in helping them to feel hopeful. On the basis of our findings we discuss the new forms of gendered uncertainty that arise in the era of HIV/AIDS in Vietnam.

CONCLUSIONS PMTCT is not only a technology in medical prevention but also a tool that can increase optimism among HIV-infected women. Health staff played an important role in helping women to fulfill their reproductive desires and in building and strengthening reproductive hopes for women living with HIV.

2.1-040

Maternal mortality in Tunisia: results of the 2010 national survey

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We conducted a household survey using verbal autopsy instrument to identify and investigate the real number of maternal mortality and to assess the causes of all deaths of women of reproductive age in a Tunisia by using multiple sources of data (e.g. interviews of family members, vital registrations, health facility records, burial records, traditional birth attendants). All women death in age of reproduction (15–49 years), occurred during the year 2008, have been listed and investigated by verbal Autopsy so as to determine causes of death. Maternal death made of a specific study. Eighty-two deaths reply to the maternal death definition, the rate of mortality is estimate at 44.8 for 100,000 live births at the national level, with a regional heterogeneity and an excess of mortality in the region of Tunis. The main causes of mortality was hemorrhage (47.3%) followed by toxemia (16.4%) and infection (14.5%). The reduction of 35% of the maternal death in two decades. Our next challenge is to reduce the maternal mortality rate to 30 for 100,000 live births with the improving of the quality of cares and the intersectorial partnership.

2.1-041

Migrant women and maternal health services: utilization and determinants

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INTRODUCTION There is limited knowledge about the health status of migrant women in reproductive age. The present study tries to assess the determinants of utilization of the selected maternal factors such as Antenatal care and Natal (Place of Delivery) factors among migrants at urban slums in India.

METHODS Cross sectional study was conducted in five slums of Jaipur city of India among 196 recently migrated women with at least one child <2 years of age. Migration, Socio-demographic, health Service and knowledge factors were studied. Data were presented using descriptive statistics, chi-square tests and odds ratio (OR).

RESULT Six percent of the participants received complete ANC services and 69% had institutional delivery during their most recent delivery. Standard of living is a significant predictor for utilization of ante natal services. Mother's education, Caste and Information on JSY (scheme whereby cash incentive is paid for institutional delivery) are important predictors for seeking institutional delivery. Women migrating for marriages are less likely to use institutional delivery than that of women migrating for other reasons(OR = 0.30, P = 0.01)

CONCLUSION Social development is a prerequisite for service utilization. Campaigns to promote institutional delivery should pay special attention to education and a special targeting of lower caste women for institutional delivery is needed. Similarly migrant women labourers could be targeted upon. JSY should emphasize on complete package of ANC besides institutional delivery.

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2.1-042

Pregnant women's experiences of routine counselling and testing for HIV in eastern Uganda: lessons for improving the prevention of mother-to child transmission of HIV programme

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BACKGROUND Routine HIV counselling and testing as part of antenatal care has been institutionalized in Uganda as an entry point for pregnant women into the Programme for the Prevention of Mother to Child Transmission of HIV (PMTCT). However, utilization of PMTCT services remains low. We explored pregnant women's experiences of routine counselling and testing and formulated suggestions for improving the PMTCT programme in Mbale District Eastern Uganda.

METHODS This was a qualitative study conducted at Mbale regional hospital in eastern Uganda between January and May 2010. Data were collected using in-depth interviews with 30 pregnant women (15 HIV positive and 15 HIV negative) attending an antenatal clinic. In addition, key informant interviews (6) were conducted with health workers involved in antenatal care for pregnant women. Data management was done using Nvivo version 9, and the content thematic approach was used for analysis.

RESULTS Most women knew that the hospital provided HIV counselling and testing services as part of ANC prior to going to hospital. Most women felt that HIV testing was compulsory but recognized its benefits for themselves and their unborn babies. HIV positive women identified a need to receive HIV treatment within the same setting, follow up counselling after the test to address fears of living with HIV and infecting their babies. Most HIV negative women mentioned that they were given inadequate time during post test counselling. This left them with unanswered questions and with some doubts about the negative test Results.

CONCLUSIONS Routine counselling and testing services are known and acceptable to most mothers. There is need to strengthen post test and follow up counselling for both HIV positive and negative women to maximize opportunities for primary and post exposure HIV prevention. Integration and linkages with community structures such as PHA networks and Village health teams should be prioritized.

2.1-043

Investigating maternal mortality and health service factors in Sierra Leone

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BACKGROUND Many challenges exist in achieving a 75% reduction in national maternal mortality ratios (MMRs) by 2015, as proposed by the MDGs. In most developing countries, deaths are unregistered and measuring population-based outcomes, such as MMR, is resource-intensive, impractical and fails to highlight priorities at a sub-national level. The use of process indicators such as case-fatality rates (CFR) has been advocated as a potential solution. This study describes health services and maternal mortality in Sierra Leone, with the aim of using process and outcome indicators at district-level to identify local public health priorities.

METHODS Administrative, policy and strategy documents were scrutinized to describe health services in Sierra Leone, including structure of the system, workforce available, and distribution of health facilities and obstetric care. Routine health facility data from 13 Government District Hospitals (GDHs) were extracted, linked to geographic and demographic data and analysed at district-level.

FINDINGS Primary health care facilities all lacked basic EmOC and only four of 13 GDHs had comprehensive EmOC facilities. Routine hospital data found the MMR in GDHs ranged from 2051 to 10,667 deaths per 100,000 live births and CFR ranged from 1.9% to 7.9%. With the exception of one hospital, the Northern Province had the lowest mortality in hospitals and the greatest availability of EmOC. Nationwide, obstructed labour was responsible for the greatest number of deaths (40%), followed by haemorrhage, eclampsia and abortion (22%, 15% and 7%, respectively).

INTERPRETATION This study has shown that the hospital-based MMR in Sierra Leone is substantially higher than population-based estimates with great variation between districts. Health services in Sierra Leone are characterised by the unavailability, inaccessibility and inequitable distribution of emergency obstetric care (EmOC) and skilled health workers, with non-poor districts receiving more resources. Further efforts to improve maternal mortality and health services must explicitly consider issues of equity.

2.1-044

Controlled lives: impact of social factors on reproductive rights of women in urban South India

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INTRODUCTION Despite several policy initiatives on meeting Millennium Development Goal 5, ensuring maternal health as a right has not been fully realised in India. The paper in this context highlights some important social barriers for women to realise their reproductive rights. It draws evidence from KG Halli, a poor urban neighbourhood in Bangalore, locus of a health systems research conducted by the Institute of Public Health.

METHODS We gathered the pregnancy and childbearing experiences of five women using data generated through in-depth interviews as well as information from their family members/neighbours. Community health workers had identified these women as being at 'high risk' of adverse maternity outcomes during routine home visits aimed at following pregnant women in the community. We discussed these case studies in the research team to validate observations and identify emerging themes. We also draw insights from observational field-notes.

RESULTS In four of the five case studies mothers were expected to produce a male child, even though they already had 5–6 living children, highlighting strong male child preference. In the fifth case, the husband used his power as a male to repeatedly induce abortions (using oral medication) arguing that he was not yet 'ready' to have a child. In other instance, the husband's perception of contraception/sterilization as antithetical to social norms had deprived his wife from adopting contraception despite feeling 'ashamed' to undergo further pregnancy.

CONCLUSION Our study reveals impact of certain social factors on women's reproductive rights in urban poor neighbourhood in India. Health workers should be attentive to these factors; society as a whole should address it.

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2.1-045

Young domestic staff represent a vulnerable population in relation to reproductive health and HIV prevention in Bujumbura, Burundi

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In Burundi, pushed by socio-economic circumstances, youngsters migrate to Bujumbura. Many work in the 'informal' economy as domestic staff. Due to their age, lack of professional training they represent a vulnerable population. We conducted a randomized cross sectional survey in the urban commune of Bwiza, Bujumbura. The main objective was to evaluate knowledge, attitude and practices of domestic staff, aged 10–24 years, in relation to reproductive health and HIV prevention. In October 2009, we interviewed 306 participants. Mean age was 16.5 years and 57.2% were girls. Risk factors for vulnerability: 24.8% were orphans, 53% came from big family (¡Ý6 children) and 93.5% were single. 98.7% told to be informed about reproductive health. Main sources of information were radio (90.2%), friends (50%) and television (37.2%). Contraceptive methods stipulated by 97.7% of the participants were: sexual abstinence during fertile period (83.9%), use of male condom (76.5%), oral (46.5%) and injectable (31.1%) anticonceptives. HIV was known by 97.4% participants and 88.9% were frightened by HIV. Other known Sexual Transmissible Infections (STI) were syphilis (40.5%), gonorrhoea (32%), weak chancre (23.9%) and hepatitis B (21.2%). HIV/STI preventive methods known were sexual abstinence (88.9%), monogamy (45.8%), male condom use (71.9%) and avoid contact with seropositive blood and skin cutting objects (55.9%). 25.8% youngsters already had sexual contacts and 39.2% had more than one partner. First contact was on average at 15.1 year and 51.9% were in Bujumbura and 29.1% on the countryside. 20.3% were with condom use. Of the sexual actives, 12% had suffered from an STI. Thirty-one percent of the girls and 24.3% of the boys had an unwanted pregnancy: 31.9% of them were voluntarily interrupted. Young domestic staff are confirmed to be a vulnerable population who should receive, based on our findings, specific attention in relation to reproductive health and STI/HIV prevention.

2.1-046

Knowledge, attitudes and practices related to HIV and vulnerability factors in pregnant women treated in public hospitals in the province of Buenos Aires, Argentina

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INTRODUCTION A comprehensive prevention program is developing in Great Buenos Aires suburbs. Before training health providers a baseline study was carried out to identify risk factors and gaps related to MTCT of AIDS and Syphilis.

METHODS AND MATERIALS During May 2011, a personal survey was applied among reproductive aged women who were pregnant in the last 2 years (n = 100).

RESULTS Half of the sample had their first pregnancy before the age of 19. Fifty-six percent had one or two pregnancies. Nine percent of the pregnancies ended in abortion. At their last pregnancy, 86% sought healthcare during the first trimester. One hundred percent carried out at least one HIV test and 96.8% look for the result, 54.8% didn't receive pre-test counseling and 78.9% post-test counseling. Only 39% performed a syphilis test (VDRL) and 73% said that it was not offered. Ninety-one percent have a steady partner during pregnancy. Seventy-four percent of partners attended health consultations but only 14% received the offer of testing for HIV and 3.3% for VDRL. Most women recognize sexual relations as a mode of HIV transmission (88% vaginal, oral 34%, 30% anal) and only 2% vertical transmission. Fifty-five percent never used condom with their steady partner and only 17% used it always; for the 35.2% condom use was a matter of disagreement or tension with the couple. 26.4% suffered once verbal aggression and 17.6% physical aggression by their partner and 5% was forced to have sex, 53.8% feels dependent or vulnerable on their partners. Thirty-one percent suffered abuse once in their lives, 22% abandonment and 46% drop out of school to work.

CONCLUSIONS Even though these women attend consultation during pregnancy there are serious shortcomings in preventive information and adoption of preventive methods. Health workers must acquire tools for HIV and syphilis early detection, couple testing and intervention on risk factors such as situations of violence and other vulnerability factors.

2.1-047

Childbirth planning and preparation: findings from the qualitative assessment of the Bangladesh maternal mortality survey (BMMS), 2010

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INTRODUCTION Planning is needed to ensure that women prepare adequately for childbirth. Birth planning may be made with family members, and antenatal care (ANC) provides an opportunity for women to learn about essential birth preparations. To increase use of skilled attendants and improve birth outcomes, it is important to understand how women view birth preparedness and plan prior to delivery.

METHODS AND MATERIALS As a sub-component of a nationwide maternal mortality survey, indepth interviews were carried out between March 2010 and February 2011 with 20 women in their third trimester of pregnancy to assess what preparations they had made for childbirth. When available, the pregnant woman's husband, mother and mother-in-law were also questioned.

RESULTS While women viewed delivery as risky, most failed to discuss childbirth and make formal birth plans with household decision-makers due to shame, the pregnancy was unwanted, or they relied on family members to make necessary arrangements. The majority of respondents expected to deliver at home with a traditional birth attendant (TBA) because they assumed the delivery would be normal, had confidence in the TBA, and wanted to avoid a facility delivery. Women attended on average two antenatal visits, with some never receiving ANC. ANC health workers consistently failed to give information on birth planning or pregnancy-related complications. Women expressed reservations about delivering in a facility mainly due to concerns about costs and exposing private body parts to male health workers. While most women stated they would go to a health facility if complications occurred, virtually no preparations were made for transport and payment.

CONCLUSION Although childbirth is considered a dangerous time, minimal preparations are made prior to delivery. Household members and ANC health workers often fail to share information necessary to prepare for childbirth. Efforts are needed to improve ANC so that families are better prepared to respond to delivery complications.

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2.1-048

Obstetric fistulae is still a major and stigmatizing public health problem in rural Burundi

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Obstetric fistula (or vaginal fistula) is a severe medical condition after severe or failed childbirth, when adequate medical care is not available. Following former studies, we expect about 1000 new cases annually in Burundi. In the framework of a capacity building project we wanted to mobilize and perform corrective surgery for women suffering from fistulae in two provinces in Burundi, Ngozi and Makamba province. After a public awareness campaign through i.e. radio messages and via church announcements women were invited to present themselves with stigmatization. Of the 230 women that voluntarily presented themselves, 185 (80.4%) were diagnosed with fistulae. Their average age was 34.5 years (16–63 years) and 45% developed the fistulas as primi- and secundigravidae. Four percent had fistulae for 20 years and 34% were rejected by their husbands. Thirty-eight percent of the cases had delivered through a caesarian section during the pregnancy causing the fistulae. We operated 51 fistulae of 45 women at the University teaching Hospital in Bujumbura: 12 simple fistulae (23.5%), 30 complex fistulae (58.5%), six severe fistulae (11.8%) and three rectovaginal fistulae (5.9%). Among these women, 58.1% had been in labour for more than 3 days, 51% were transported with stretcher, 62% had visited two or three health centers before assistance was given and 58.8% had a stillbirth. After the surgical intervention, 5 (11.1%) had leakage and two were still incontinent. Overall, the operation was successful in 38 (84.5%) women. Adequate obstetric care but certainly primary obstetric care level is a major problem in rural Burundi, which represents 90% of the population. Obstetric fistulae are a well known stigmatizing consequence. Therefore, unfortunately, even after intensive mobilization, only few women presented themselves for assistance. Even in case of long term sequels, surgical intervention is successful.

2.1-049

'I wanted to deliver at the hospital'. Narratives of Ugandan women who almost died during childbirth

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INTRODUCTION Maternal mortality and morbidity remain challenges to reaching the Millennium Development Goals in low-income countries. The experiences of women who survive severe complications during childbirth are important to understand the processes leading to severe maternal disease and maternal death. The aim of the study was to explore underlying factors and delays that are threatening maternal health in Uganda.

METHODS AND MATERIALS A qualitative design was adopted, implying flexible and participatory research strategies during 12 weeks of fieldwork in a rural hospital and attached health centres in Kasese district, Western Uganda. Thirty women affected by obstetric fistula or who had survived other severe maternal morbidity were the main informants. Data collection included 13 in-depth interviews, two focus group discussions and participant observation in the hospital. Thematic content analysis was performed. Ethical approval was given by the Uganda National Council for Science and Technology.

RESULTS Almost all women had planned and opted for a facility-based delivery. Women described neglect and poor treatment at hospitals and dispensaries. They experienced disrespect of health workers during labour and after delivery. Among more than half of the interviewed women low quality of care and delays at the health facility appeared to be the main causes of morbidity. Delays in seeking care as a consequence of lacking decision-making power and non-supportive husbands were also mentioned, while cultural beliefs did not seem to delay care seeking.

CONCLUSIONS Substandard care and delays at the health facility seemed to be of more relevance for severe maternal disease than cultural barriers to seek care. Giving a voice to the women who survived severe obstetric complications and awareness raising and training of health personnel is crucial to improve reproductive health services.

2.1-050

Clients' and providers' perspectives on caesarean sections: an operational study into the high caesarean section rate in Georgia

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INTRODUCTION Over the past decade Georgia has seen a rapid increase in the number of Caesarean Sections (CSs). The operational study on CSs aimed to determine trends and factors associated with current practice, in particular demand and supply factors associated with CSs without medical indication. In addition the study strengthened midwives' capacity in their analytical skills by involving them in all stages of the research.

METHODS AND MATERIALS The study followed a cross-sectional comparative and descriptive design. Participants were found within 19 maternity houses spread over Georgia, which in total accounted for almost 50% of all institutional deliveries and 43% of CSs in 2010. Semi-open questionnaires were administered to 171 pregnant women, to 119 women who underwent a CS and to 175 women who delivered vaginally. Data from delivery logs were analyzed applying a checklist considering the 'Robson classification system'. Semi-open questionnaires were applied to 83 midwives and 109 obstetricians/gynaecologists.

RESULTS AND CONCLUSIONS Study findings confirm national data on high rate of CSs, with an average rate of 32%. This figure varied greatly among the studied health facilities, ranging from 6% to 79%. Findings indicate lack of information and insufficient birth preparedness as determinants of an increasing CS rate. Thirty-one percent of the women who underwent a Caesarean section could not state the reason for the surgery. A majority of study participants were not insured, facing additional costs associated with the delivery. National guidelines and medical protocols are absent, and common (evidence-based) standards are not well known among providers. Analysis of delivery logbooks show a low practice of assisted vaginal deliveries, exemplifying a lack of information and practical skills to perform vacuum and forceps among providers. In order to curtail a further increase in CSs in Georgia, improvements are required in perinatal care, including enhancing birth preparedness and improved pain management during delivery.

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2.2 *Neonatal Health*

2.2-001

Neonatal mortality in Timor-Leste: a need to reappraise determinants

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INTRODUCTION Timor-Leste is a post-conflict and fragile state; ranked 120 of 168 in the 2010 human development index. Its health and development indicators are the poorest in this region. The 2009–2010 Demographic and Health Survey (DHS) shows a high maternal mortality ratio (557/100,000 live births); and under-5 children, infant, and neonatal mortality rates, at 64, 44, and, 22 per 1000 live births respectively. In Timor-Leste 34% of child deaths occur in the neonatal period. This is lower than the global estimate of 38% and of other nearby countries such as Indonesia (43%), and Bangladesh (57%).

METHODS AND MATERIALS Systematic reviews of DHS of different countries, different published and unpublished studies of Timor-Leste, national data bases, and Lancet publications on newborn survival are used to help inform these findings.

RESULTS Under-5 mortality has been reduced substantially in recent decades; however, insignificant progress has been seen in neonatal mortality rates (NMR). Although progress in NMR in Timor-Leste is remarkable, it hardly correlates with the established positively influential factors, such as appropriate care-seeking behavior, equity in socio-cultural status, or maternal and child health services. The health system in Timor-Leste is weak, including in human and financial resources. The quality of neonatal care is yet to be standardized. Health care is not accessible due to distance, rugged terrain, poor transport and a weak communication system. Local beliefs and practices are diverse. Only 29.6% of deliveries are conducted by skilled birth attendants. Only 22% are at health facility. The total fertility rate is 5.9. Twenty-eight percent of pregnant women are anemic.

CONCLUSION In spite of the weak health system and health status, NMR is relatively low without having obvious correlates in known determinants. It requires further in-depth investigation; including the exploration of the role of positive indigenous knowledge and practices.

2.2-002

Cause specific neonatal mortality in a neonatal care unit in northern Tanzania: a registry based cohort study

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OBJECTIVE The current decline in under-five mortality shows an increase in share of neonatal deaths. In order to address neonatal mortality and possibly identify areas of prevention and intervention aiming at increased survival, we studied causes of admission and causes of neonatal deaths in a neonatal care unit at Kilimanjaro Christian Medical Centre (KCMC) in Tanzania using a medical birth registry and neonatal registry data.

METHODS A total of 5033 neonates admitted to a neonatal care unit (NCU) from 2000 to 2010 were studied. Clinical diagnosis, gestational age, birth weight, Apgar score and age at discharge were registered. Causes of neonatal deaths were classified by modified Wigglesworth and NICE classifications. Statistical analysis was performed in SPSS 18.0.

RESULTS Leading causes of admission were birth asphyxia (26.8%), prematurity (18.4%), and risk of infection (16.9%), neonatal infection (15.4%), and birth weight above 4000 g (10.7%). Overall case fatality was 10.1% (536 deaths). Leading single causes of death were birth asphyxia (n = 245, 45.7%), prematurity (n = 188, 35%), congenital malformations (n = 49, 9.1%), and infections (n = 46, 8.6%). Babies with birth weight below 2500 g constituted 29% of all admissions and 53% of all deaths. Birth asphyxia was the most frequent cause of death in normal birth weight babies (n = 179, 73.1%) and prematurity in low birth weight babies (n = 178, 94.7%). The majority of deaths (n = 304, 56.7%) occurred within 24 h, and 490 (91.4%) within the first week. Deaths after the first week were dominated by neonates with infections.

CONCLUSIONS Birth asphyxia in normal birth weight babies and prematurity in low birth weight babies each accounted for one third of all deaths in this population. Possible preventive measures to reduce mortality are proper antenatal care screening; timely referral and interventions for mothers at risk, monitoring signs of fetal distress during labour, proper resuscitation skills, prevention of hypothermia, hypoglycaemia, nutritional and feeding support.

2.2-003

The effect of integrated mother-and-child health services on the follow-up of HIV exposed infants in Mozambique

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INTRODUCTION The follow-up of HIV-exposed infants remains challenging in Sub-Saharan Africa. This study evaluated the feasibility of the integration of mother-and-child health (MCH) care into a one-stop consultation and its effect on the follow-up of HIV exposed infants, in the public health system of Tete Province in Mozambique.

METHODS During 2009–2010, an intervention-control study was implemented in three matched pairs of public health clinics, randomly assigned to the intervention or control group. The intervention comprised the reorganisation of MCH services, to deliver integrated, one-stop, consultations for mothers and under-five children. Routine monthly MCH clinic data were collected 6 months before, and 13 months after the intervention, and analysed for differences between the pre- and post-intervention period, and between intervention and control group. Semi-structured interviews were held with clinic staff on experiences with MCH service delivery, at the start, 6 months, and end of the study, and were manually analysed for themes.

RESULTS The delivery of one-stop MCH consultations was feasible and appreciated by the personnel. There was no impact of the integration on the follow-up of HIV-exposed infants or other MCH components. In the post-intervention period children started follow-up earlier and attended the services more frequently, but without significant differences between intervention and control clinics. Staff absences and an irregular supply of consumables and drugs were more important performance determinants.

DISCUSSION One-stop MCH consultations are feasible and acceptable to the personnel of public health facilities in Tete Province, Mozambique. Although the follow-up of HIV-exposed infants overall improved, it could not be attributed to the integration. Structural health system limitations, such as staff absences and irregular supply systems, appear stronger determinants. Regular supervision and improvement of working conditions form valuable

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motivators and are of critical importance for the improvement of the performance of MCH care in peripheral public health facilities.

2.2-004

Understanding the perceived cause and care practice of birth asphyxia among urban slum dwellers: BRAC experience

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INTRODUCTION Beliefs and culture control the community to acquire a particular perception of a specific health problem and set up people's mind to take necessary steps to solve the particular problems. A multi-site study was carried out by BRAC in partnership with Emory University, ICDDR, B, and LAMB Hospital to understand the perception about causes and local care practices of birth asphyxia (BA) among urban slum-dwellers in Dhaka.

METHODS AND MATERIALS Data was used from five group interviews (GI) with mothers whose babies had BA during birth and their caregivers and 80 semi-structured interviews of 20 reproductive age women, 20 elderly women, 20 traditional birth attendants and 20 informal doctors.

RESULT Interestingly, most of the respondents mentioned that 'prolonged labor' (baby stuck in birthing canal for long time/big baby or fatty baby/head stuck in birth passage) is the cause of birth asphyxia (BA). Nevertheless, others believe that 'taking inadequate food', 'if mother falls down/has abdominal pain', 'effect of evil spirits', 'mother gets cold' during pregnancy period, are the causes of BA. Regarding care for birth asphyxia, the caregiver takes one action after the other until the baby breathes. Alarmingly, instead of giving the immediate appropriate care for BA, many potentially harmful practices are found to take place in the study area, e.g. 'delayed cord cutting', 'oil massage on baby's body', 'slap or give strike on baby's back', 'pour/spread water on the baby', 'give spiritual treatment', 'blow in to baby's ear or head' etc.

CONCLUSION BRAC Delivery Centers are replacing these harmful traditional practices through Manoshi project. Study findings will help BRAC Manoshi further tailor behavioral change in a local context and implement essential newborn care package significantly.

2.2-005

External birth defects in south of Vietnam: a population-based study

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INTRODUCTION At present, no data on birth defects from population-based studies exist in Vietnam. Our study aimed to establish a network of health professionals (HP) to perform a systematic visual examination of newborns for external birth defects (EBDs) and to assess EBDs prevalence among live newborns in Binh Thuan Province, Vietnam.

METHODS Four hundred and fifty-two HPs were trained in delivery care over 2 months in 116 Commune Health Stations (CHS) and in 28 provincial or district hospitals (DH). After a successful 6-months pilot study, a 1-year registry of EBDs was established in 2008. All live-borns were examined to detect for EBDs within 24 h after birth in all DH obstetric department and in all CHS. Trained local HP collected information in predesigned forms and photographs of affected newborns. A geneticist checked final diagnoses. EBDs were coded using the International Classification of Diseases system-10, Clinical Modification (ICD10-CM). The study was repeated in 2010.

RESULTS In 2008, we observed 90 EBDs among 16,720 live births giving a prevalence of 54 per 10,000 live births. In 2010, EBDs prevalence was 75 10^{-5} and multiple EBDs prevalence was 8.0 10^{-5} (10.7% of cases). The most common groups of EBDs were: musculoskeletal (34.6), orofacial clefts (17.3), neural tube defect (93.0) and external genital system (67.0).

CONCLUSION Although the collection and reporting techniques are simple, this study requires efforts co-ordination at the local level. Data on EBDs from this study in the Northeast South of Vietnam may be useful to setup a population-based registry of birth defects in Vietnam.

2.2-006

Comparison of newborn care practices in BRAC delivery centers vs. home deliveries in an urban slum of Dhaka, Bangladesh: a descriptive cross-sectional study

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BACKGROUND Neonatal mortality in urban slums of Dhaka is twice than in urban non-slums areas. BRAC, a Bangladeshi NGO, initiated a community based Manoshi program and established delivery centers in urban slums to improve maternal, newborn, and child health. This study was undertaken to compare newborn care practices (i.e. cord care, thermal care, bathing and initiation of breastfeeding) in delivery centers and at homes, in an urban slum of Dhaka.

METHODS A cross-sectional study included 130 mothers; 99 of whom delivered at centers and 31 at homes between January and November 2010. Six in depth interviews were also conducted to explore newborn care practices.

RESULTS Ninety-nine percent center-delivered and 93.5% home-delivered mothers received the ANC during their last pregnancy. All centered deliveries were conducted by the BRAC trained urban birth attendants (UBAs) whereas 77% home deliveries were conducted by traditional birth attendants (TBAs). In center deliveries, 96% used new blade and all used new thread to cut the cord. Of those, 70% boiled thread and 28% boiled blade. In home deliveries, all used new blade and thread to cut and tie the umbilical cord. Of those, 84% boiled blade and 39% boiled thread. Overall, 91% newborns were dried and 96% wrapped immediately after birth in new/clean clothes. Of those, 76% were dried before and 70% wrapped after the delivery of placenta. In center-deliveries, 96% newborns were placed on mother's abdomen and 92% given bath on/or after third day. About 98% mothers initiated breastfeeding within 24-h and all gave colostrum to their babies. In home-deliveries, 26% newborns were placed onto surface and 62% not given bath on/or after third day. About 23% mothers discarded colostrum.

CONCLUSION Newborn care practices were comparatively better in center deliveries but still remain a great cause of concern among home deliveries that may hamper the health of newborns.

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2.2-007

Consequences of malaria during pregnancy on neonatal antigen presenting cell activation and on responses to toll-like receptors and *P. falciparum* antigens in Benin

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Pregnancy-associated malaria is a public health problem. During pregnancy and early childhood, there is an increased susceptibility to malaria due to parasite-induced modulation of pro-inflammatory responses. The development of a protective immune response requires correct function of Toll-like receptors (TLR) that are expressed by antigen-presenting cells (APC). TLR activation induces cytokine production and the expression of co-stimulatory molecules to trigger antigen presentation to T cells. In newborns, stimulation of APC via their TLR is involved in the progressive development of immune responses during the first months of life and our hypothesis is that pregnancy-associated *P. falciparum*-malaria would adversely affect this development. The study is part of the STOPPAM project conducted in Benin on a cohort of 200 pregnant women and their children followed from birth to 12 months. To assess the impact of malaria on neonatal immunity, clinical and parasitological data were collected both from the mother during pregnancy and from the child. Peripheral whole blood of children (0, 3, 6 and 12 months) was stimulated either by TLR ligands (polyI:C, LPS, R848, CpGODN) that have distinct effects on different APC subsets or by *P. falciparum*–infected red blood cells. Concentrations of pro-inflammatory and anti-inflammatory cytokines were then evaluated in culture supernatants to investigate activation levels of APC. We performed the stimulations and cytokine assays on blood from 137 newborns from mothers with different malaria histories during pregnancy in order to evaluate the impact of an in utero contact with *P. falciparum* antigen on the newborn immune system. Statistical analysis is ongoing and results will be presented.

KEYWORDS toll-like receptors, *P. falciparum*, antigen-presenting cells, cytokines, newborns

2.2-008

Knowledge, practice and care seeking behavior on neonatal health in Matlab, Bangladesh

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BACKGROUND Improving neonatal health is a challenge in many developing countries including Bangladesh. Knowledge and awareness on essential newborn care and health seeking behavior among mothers is vital to address health needs and formulate preventive heath care.

OBJECTIVES The objectives of this study were to determine the knowledge, practice and health seeking behavior of mothers on neonatal health and to identify the high-risk practices for neonatal health.

METHODS This study was based on two cross sectional surveys conducted during 2007, baseline (B) and 2010, end line (E) in Matlab, Bangladesh where international centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) has been maintaining a health and demographic surveillance system (HDSS) comprising the area into two. Both surveys were conducted in two areas of HDSS. Study participants were women who delivered live birth babies from 2006 for baseline and 2009 for end line. In total 4704 women were interviewed through a semi-structure questionnaire based on knowledge, practice and health seeking behavior of neonatal health.

RESULTS The knowledge on essential newborn care (drying with wrapping and breast feeding) was quite good in ICDDR, B area (IA) but interestingly it went down in GOB area (GA). Dry and wrapping:86% (B)-92% (E), P = 0.001 and 76% (B)-65% (E), P = 0.001 in IA and GA respectively. Breast feeding practices were 72% (B)-76% (E), P = 0.04 and 75% (B)-38% (E), P = 0.001 in IA and GA respectively. However these knowledge were hardly practiced (Dry with wrapping: 8% (B)-34%(E), and 04% (B)-19% (E), and breast feeding: 0.5% (B)-0.8% (E), 0.4% (B)-1.1% (E), P = 0.001 in IA and GA respectively. Bathing practice of newborn babies within first 3 days of life has substantially decreased in both the areas 70% (B)-29% (E), P = 0.001 and 68.3% (B)-54.0%(E), P = 0.001 IA and GA respectively. Sought care for newborn first month problems from unskilled providers were also decreased about 7%-10% in both the areas [IA: 26%(B)-19% (E), P = 0.001 and GA:47% (B)-37% (E), P = 0.001].

CONCLUSION Application of knowledge on essential newborn care especially immediate breast-feeding just after birth is poor and harmful practices are still prevalent in the study areas. These should be addressed to improve neonatal health.

2.2-009

Neonatology in reference hospital level III in LIC: an experience of integrated support to a national existing structure – neonatal inse, Donka hospital, Conakry, Guinea

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INTRODUCTION Neonatal mortality is the first cause of death (41%) among children <5 years in LIC and most of the health structures have not the capacity to cope with the needs and demands. MSF Switzerland is supporting the neonatal unit in INSE in Conakry since May 2009 (55 cradles and 20 beds) and this support is a multi-sector based integrative rather than substitutive approach.

METHODS AND MATERIALS Data are collected weekly and entered on Excel for statistical analysis on Qlik View. This work is a retrospective analysis of admission and mortality trends from 2009 to April 2011.

RESULTS Admissions were rapidly increasing from <80 monthly before MSF intervention to 150–250 mid 2010 and then, above 250 till April 2011. Bed occupation rates were permanently above 150%. Concurrently, mortality rates declined from above 30% before MSF intervention to 25–30% until January 2010 and 14–18% from November 2010 to April 2011. Major proportional morbidity are asphyxia (26%), infections (31%) and Low Birth Weight (34%), including premature babies.

CONCLUSION Despite improvement, the major difficulty faced by MSF in Guinea is its absence of implication in the maternity level III and communities regarding neonatal care. Definitively basic (Kangaroo Mother Care) and/or comprehensive neonatal care (Neonatal Intensive Care/PMTCT) should systematically be developed jointly with emergency obstetrical care since the main causes of neonatal deaths are directly dependent of maternity's activities. In order to improve permanently and significantly neonatal care in LIC, a multi-sector approach targeting maternal and neonatal care jointly and including logistics, supplies, equipments, guidelines and training of health staff is necessary. Collaboration with community health workers should be rein-

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forced to deliver basic neonatal care, key messages at home and, to refer babies on time to the adequate health structures.

2.2-010

Barriers to skilled attendance for childbirth

A survey among mothers in rural Gambia

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OBJECTIVES: Millennium Development Goal (MDG) five addresses maternal health. The international community has agreed to reduce maternal mortality worldwide by three quarters between the year 1990 and 2015. Maternal mortality ratio and the percentage of births attended by skilled health workers are key indicators for measuring progress. Underutilisation of maternal health services is widespread, especially in rural and poor areas. Barriers to accessing health care are keeping women away from lifesaving interventions. Our objective is to assess the most important barriers for seeking skilled care in rural Gambia.

METHODS: This is a cross-sectional study. Convenience sampling was used to recruit the participants. Four hundred and thirty-two Women who recently gave birth outside a health facility was interviewed and asked to state the barriers they perceived as the most important for not giving birth in a health facility.

RESULTS: Seventy percent of the participants gave birth attended by a traditional birth attendant. The most important barriers for giving birth in a health facility were shortage of time and lack of transport. The majority of the women stated that complications can arise, and that health workers can provide sufficient assistance if needed. These were the main reasons given for the preference of being attended by a health worker.

CONCLUSION: If the goal of The Gambia is to increase use of skilled attendants for childbirth, there is a great potential to reach the target of the MDG 5. Our findings suggest that the participants hold knowledge and motivation, which is important if practice is to be changed from giving birth at home to seek skilled care for childbirth. However, barriers need to be reduced, and access barriers stand out as the most important.

2.3 Children's Health

2.3-001

The emergence of the HIV infection in the child in Algeria and the interest of the prevention of contamination mother to child

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INTRODUCTION In Algeria the prevalence of HIV in the general population is 0.1%; 150 children are infected and half of this number are in Oran.

OBJECTIVE To highlight the reality of the problem in Oran by describing the principal epidemiologic aspects and considering prevention methods.

MATERIALS AND METHODS Retrospective study of 73 children infected by HIV in 1997–2008.

RESULTS 78.43% <5 years (mean: 26 months 2 months), and sex ratio: 1.51. The vertical transmission was 94.5%, the breast-feeding was noted at 63 cases (86.3%). The chronic diarrhoea 41 (56.1%), tuberculosis 17 (23.28%), lost of weight 29 (39.72%), adenopathy 47 (64.38%), molluscum contagiosum 19 (26%), oral candidosis 42 (57.5%), bad teeth 26 (35.6%), unexplained fever 18 (24.6%), lymphoid pneumonia interstitial 14 (19.1%), chronic parotitis 9 (12.3%), otitis 26 (35.6%), neurological signs 7 (9.5%). The HAART was found in 34 children (46.5%); 27 (37.6%) died and 19 (26%) were lost to follow up.

CONCLUSION Virus transmission was vertical; children become symptomatic in an age 26 months. Weight loss, generalized lymphadenopathy, respiratory issues, chronic ORL and digestive problems were the most frequent signs. Chronic parotidis, molluscum contagiosum and multiple-early dental decays were signs that characterized these children. When chronic symptoms are noted, ways of tracking their outcomes need to be employed.

2.3-002

Child survival in Nicaragua looking for evidence of progresses and challenges

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INTRODUCTION Nicaragua has demonstrated impressive advances towards the improvement of the health of the children, in spite of limited resources. The key success has been a combination of improvements of access to health services mainly to those populations with more needs. This study analyse the under-five mortality (U5MR) of Nicaragua, as well as indicate new challenges to improve the child health.

METHODS AND MATERIAL The findings of this study were obtained from two communities of Nicaragua. One of them, a mix of rural-urban called León and the other, a pure rural community called Cuatro Santos. In both setting a series of reproductive surveys were implemented in women of reproductive age (15–49 years) collecting information on full birth history, as well as background data of the mother with the U5MR as outcome. The trend and social determinants were analyzed. Ethical principles were followed during the study.

RESULTS The U5MR reduction in León (1970–2005) and Cuatro Santos (1990–2009) was 74% and 73%, respectively. In León U5MR was higher in rural than in urban areas. Neonatal mortality in León was 85% of U5MR in 2005 compared to 46% in 1970. In León, social inequalities trend showed a gap reduction, mainly during the 1990s, however during 1994–2005 this inequality gap seems to increase. In Cuatro Santos social inequalities were not found significant, but geographically, there were regions with an increased level of mortality.

CONCLUSIONS Both communities are examples of learning for the international perspective of child survival. A poor rural community reached the Milleium Development Goal 4 early, most probable through a social development. But, progress can stagnate if low-income countries as Nicaragua do not make investments to improve the neonatal health services as well as a reduction of the social inequalities that allow scaled-up interventions to those children with more needs.

2.3-003

A cluster-randomized evaluation of a responsive feeding and stimulation intervention on nutrition and development outcomes in rural Bangladesh

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INTRODUCTION It is well known that malnutrition in children under 5 years causes serious long-term health and growth problems. Furthermore, mental development and learning potential are also challenged. Parenting education for feeding and stimulation of infants and young children is needed, particularly in South Asia where 45% of children are malnourished and many do not achieve

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their learning potential. The hypothesis was that mothers in the intervention would exhibit better parenting skills and children would exhibit better developmental and nutritional outcomes than controls.

METHODS AND MATERIALS A cluster-randomized field trial was conducted with 302 randomly assigned children aged 8–20 months and their mothers in rural Bangladesh. The control mothers received 12 sessions on health and nutrition. The intervention groups received additional six sessions delivered by peer educators who demonstrated modeling and practice in self-feeding and verbal responsiveness with the child during play. Second intervention group received, along with the sessions, 6 months of food powder fortified with minerals and vitamins. Developmental outcomes included the Home Observation for Measurement of the Environment (HOME) Inventory, mother-child responsive talk, and language development. Nutritional outcomes included weight, height, self-feeding, and mouthfuls eaten. We used analysis of covariance to compare the three groups at the posttest and follow-up, covarying the pretest levels and confounders.

RESULTS At follow-up, responsive stimulation-feeding groups had better HOME inventory scores, responsive talking, language, mouthfuls eaten, and hand-washing. Micronutrient fortification resulted in more weight gain.

CONCLUSIONS A brief behavior-change program that focused on modeling and practice in stimulation and feeding was found to benefit children's nutrition and language development. Micronutrients benefited children's weight but not length.

2.3-004

Soil-transmitted helminth infections in schoolchildren on the Caribbean Coast of Nicaragua

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A survey was carried out to determine the prevalence and intensity of infection of soil-transmitted helminths in schoolchildren living in a rural community on the Caribbean coast of Nicaragua (RAAS). Prevalences and intensities were analysed through coprological studies of a total of 109 schoolchildren (64 boys and 45 girls), 1–15 years old (age-groups considered: 1–5; 6–11; 12–15). Each stool sample was examined using formol-ether concentration and the Kato-Katz techniques to determine helminth prevalences and intensities. Statistical comparison of categorical variables was carried out with? 2) *Ascaris lumbricoides* and *Trichuris trichiura* were the only geohelminths detected, with overall prevalences of 21.1% and 80.7%. No significant differences in prevalence rates with respect to age or sex were detected in either helminth found. The analysis also revealed that all children infected with *A. lumbricoides* were also likely to be infected with *T. trichiura*. Global intensity ranged from 24 to 38,376 eggs per gram of faeces (epg) and from 24 to 28,560 epg for ascariosis and trichuriosis. Although without statistical differences, intensity peaked in the 1–5-year-old group both in *A. lumbricoides* and *T. trichiura* infections, and higher intensities of *A. lumbricoides* were noted in boys, while *T. trichiura* present higher intensities in girls. Among the subjects positive for ascariosis and trichuriosis, the vast majority detected (65.2% and 61.4%) can be classified with statistical differences as light-intensity infections. The percentage of light and moderate infections was very similar among the different sex and age-groups considered. The proportion of heavy infections for *T. trichiura* was 4.5%. No heavy infection for *A. lumbricoides* was detected. The highest percentage of *T. trichiura* heavy infections was detected in girls (5.9%) and in the youngest age-group (7.7%). Prevalences and intensities detected are high enough to recommend the immediate establishment of therapeutic and control measurements of these soil-transmitted helminth infections.

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2.3-005

Iodine deficiency disorders in Cuba

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INTRODUCTION The eradication of iodine deficiency disorders (IDD) is one of the most important social and health-related goals for this century. Concern over this deficiency in Cuba has extended to different areas of medical care involved in treatment, but above all, prevention is key.

OBJECTIVES To assess the effectiveness of actions aimed at the sustainable elimination of DDY.

METHODS Stratified multistage cluster sampling in three different strata: lowlands, foothilds and mountainous areas. Setting: Field study in 67 municipalities in all country. Subjects: 2101 school-children of either sex and 6–11 years from 87 primary schools. IDD was characterized through determining iodine levels in urine samples and goiter prevalence by performing an inspection and physical exam.

RESULTS The median urinary iodine excretion was 246.9 μg/l, with only 1.1% of samples under 50 μg/l and 29.8% above 300 μg/l was found association with iodine urinary levels and geografical stratum (OR = 2.60, LI 2.52–LS2.67, P < 0.0004).The age was considered as protector factor (OR = 0.68, LI 0.67 LS 0.68, P < 0.0001). After analyzing the goiter rates by grouped provinces and as a country, Cuba classifies as moderate endemic with a rate of 27, 3%. The high prevalence was found in the mountain (OR = 1.72, LI 1.69–LS 1.76, P < 0.001).

CONCLUSIONS Cuba has been declared a country with sustainable eradication of DDY, and the political will of the State is committed to achieving this goal. The findings show the impact of iodized salt evaluated through urine samples as an indicator of recent salt intake. Endemic goiter continued being a nutrition problem in the population. Thorough studies are required in order to identify possible relations.

2.3-006

Measuring fitness of Kenyan children with polyparasitic infections using the 20-m shuttle run test as a morbidity metric

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To date, there has been no standardized approach to the assessment of aerobic fitness among children who harbor parasites. In quantifying the disability associated with individual or multiple chronic infections, accurate measures of physical fitness are important metrics. This is because exercise intolerance, as seen with anemia and many other chronic disorders, reflects the body's inability to maintain adequate oxygen supply (VO2 max) to the motor tissues, which is frequently linked to reduced quality-of-life in terms of physical and job performance. The objective of our

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study was to examine the associations between polyparasitism, anemia, and reduced fitness in a high risk Kenyan population using novel implementation of the 20-m shuttle run test (20mSRT), a well-standardized, low-technology physical fitness test. The 20mSRT proved easy to perform, requiring only minimal staff training. Parasitology revealed high prevalence of single and multiple parasitic infections in all villages, with Schistosoma haematobium being the most common (25–62%). Anemia prevalence was 45–58%. Using multiply-adjusted linear modeling that accounted for household clustering, decreased aerobic capacity was significantly associated with anemia, stunting and wasting, with some gender differences. The 20mSRT, which has excellent correlation with VO2, is a highly feasible fitness test for low-resource settings. Our results indicate impaired fitness is common in areas endemic for parasites, where, at least in part, low fitness scores are likely to result from anemia and stunting associated with chronic infection. The 20mSRT should be used as a common metric to quantify physical fitness and compare subclinical disability across many different disorders and community settings.

2.3-007

Cuban nutritional intervention program to prevent and control iron deficiency anemia in children

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INTRODUCTION Within the Cuban National Plan for Prevention and Control of anaemia a program was implemented to fortificate the infant fruit purees, aimed to children from 6 to 35 months.

OBJECTIVE To evaluate the effectiveness of the program through the determination of hemoglobin, as an indicator of impact and associated epidemiological variables.

METHOD The national sample (1788 children) consisted of the total samples of the strata (urban, rural and mountain). Hb was determined with the use of Hemoglobin Hemocue System. As anemia is considered a Hb < 110 g/l. An epidemiological survey was conducted on a history of maternal anaemia during the pregnancy, breastfeeding practices and supplement use. A survey of habits and frequency of consumption of foods rich in iron was implemented. To evaluate the strength of association between variables was calculated OR with confidence intervals (CI).

RESULTS Prevalence of anaemia of 37.5% (CI from 32.68–42.76) and a reduction of 25% compared with baseline. 22.2% (CI 18.1–25.4) and 15, 3% (CI 12.8–17.9) had anaemia, as mild and moderate, respectively. There were no hemoglobin values indicative of severe anaemia. The highest rates of anaemia were found in children from 6 to 11 months and from 12 to 23, respectively (43.7% and 39.3%) (P 0.01). The association was stronger in children from 6 to 11 months (OR = 2.39, CI 1.84–3.09) and for children from 12 to 23 (OR = 1.84, CI = 1.45–2.35). Most likely to suffer from anaemia was found in rural and mountainous areas.

CONCLUSION Effectiveness of actions contained within the National Plan were demonstrated. The greatest strengths have been the political will, intersectoral, multidisciplinary and comprehensive action.

2.3-008

Nutritional assessment in an elementary school in Kenya

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OBJECTIVE Analyze the usefulness of children's examinations in schools in depressed areas of Africa. To estimate the nutritional characteristics of the 215 primary school children Chazon, Children Centre (Molo, Kenya)

DESIGN AND METHODS Health surveys were conducted at an elementary school from November 2010 to January 2011. This descriptive study of sociodemographic characteristics analysed sex, weight, height, personal history and BMI in 215 children.

RESULTS The population is homogeneous in terms of sex (54.5% of girls and boys 45.6), the average age is 10.30 years (CI 9.93–10.67) and a standard deviation 2.79. In relation to BMI, 25.6% of the population were malnourished: 2.8% moderate and 0.9% severe.

CONCLUSIONS It is important to carry out school surveys in a systematic way especially in impoverished areas with high malnutrition rates. The detected cases of severe and moderate malnutrition indicate that early detection significantly improves the prognosis.

2.3-009

Prevalence of intestinal parasitic infections in primary school students in Gorgan, Iran

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OBJECTIVES Intestinal parasitic infections are among the most widespread human infections in developing countries, and children are the most vulnerable. Surveys on the prevalence of various intestinal parasitic infections in different geographic regions are prerequisite for developing appropriate control strategies. The aim of this study was to determine the prevalence of protozoa and intestinal helminthes and also associated sociodemographic factors, as well as the risk factors of intestinal parasites for students in various schools of Gorgan city, Iran.

METHODS The study was conducted in 7–12 year old primary school students from October 2010 to March 2011. Participants provided fecal samples and answered a questionnaire about their demographics and hygiene habits. The collection of stool samples was performed over 2 days (j1, j3). The samples were examined by direct smear and formalin-ether concentration in the Laboratory of Parasitology, Golestan University of Medical Sciences.

RESULTS A total of 801 students were assessed, average age 9.5 years. Overall 317 children (39.6%) were infected with trophozoites and/or cysts of protozoa and/or eggs of helminth parasites. One hundred and three (12.8%) students were polyparasitized. The rates of infections with *Entamoeba histolytica/ Entamoeba dispar*, *Giardia intestinalis*, *Blastocystis hominis*, *Entamoeba coli*, *Endolimax nana*, *Entrobius vermicularis*, *Hymenolepis nana*, hook worms and *Ascaris lumbricoides* were ½%, 33/7%, 31/2%, 56/5%, 26%, 2/8%, 1/8%, 0/5% and 1.3% respectively. There was no significant association between sex and rates of infection (P-value < 0.05). However, significant correlations were obtained among parasite infections with parents' education, place of residence, washing hands habits (P-value < 0.05).

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Conclusions This work shows that the prevalence of intestinal parasitism is high among primary school students in Gorgan city and suggests an imperative for the implementation of control measures.

2.3-010

Participation in a child growth monitoring program in a low-income community in the Dominican Republic

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Introduction Growth monitoring programs (GMP) may aid in the prevention of child malnutrition. Extent of exposure to a GMP may be an important variable influencing impact, however, participation patterns are rarely reported, nor factors influencing participation.

Methods and Materials Extent of participation in a new GMP in a Haitian-Batey community in the Dominican Republic was determined by extracting attendance information from program records from the first 2 years of operation and linking these with community census information. Hypothesized predictors of attendance were drawn from associated health records and linked questionnaires. Finally, factors perceived as influencing attendance were derived from exploratory qualitative interviews with participants and key informants.

Results An estimated 91.7% of the eligible community population participated in the GMP on at least one occasion. Of those who registered for the program, (n = 227), mean percentage of attendance of eligible sessions was 75.5% (SD 32.3). Children of caregivers reporting their first language as Haitian Creole had higher levels of participation than those reporting Spanish as their first language. Child age, gender, initial weight-for-height Z score and initial caregiver's perceived thinness of their child were not related to level of participation. Interest in the program and the lack of charge for the services were perceived as factors positively influencing attendance. Confusion about the program, lack of adequate reminders, lack of interest and interference with work and household chores were cited as factors negatively impacting attendance.

Conclusions The lack of reported data from similar programs does not allow comparison of these utilization patterns, however, there is room for attendance improvement in this program. Further research on participation in GMP should examine the extent to which identified influencing factors impact attendance and to what extent program modifications can enhance attendance.

2.3-011

Management of childhood illnesses: development of a new algorithm (ALMANACH) to promote evidence-based medicine and rational use of drugs

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Introduction The Integrated Management of Childhood Illness (IMCI) has proved to be a useful tool to decrease child mortality. New evidence from randomized controlled and etiology of fever studies, the availability of reliable RDT for malaria, and novel technologies call for a revision of the IMCI algorithm with a focus on appropriate care and rational use of drugs.

Methods and Materials The IMCI decision tree was used as a basis. We did a systematic review of the branches that recommended the use of antibiotics to evaluate the current evidence behind the recommendation. We also used the results of an exhaustive clinical and microbiological investigation of 1005 Tanzanian children aged <5 years with fever to estimate pre- and post-test diseases probabilities and outcomes depending on predefined management procedures. We finally took IMCI expert opinions around the world.

Results Major changes of ALMANACH algorithm when compared to IMCI are the following (some procedures are applied on selected patients): structure separates children into febrile and non-febrile; non-febrile children without danger signs never get antibiotics; respiratory rate threshold increased from 40 to 50 (assessed twice) for children 12–59 months to define pneumonia; inclusion of malaria RDT for all febrile children, antimalarial treatment based upon result; abdominal tenderness to suspect bacterial sepsis in febrile children (>2 years); inclusion of urine dipstick for children (<2 years) with no identified cause of fever.

Conclusion This algorithm based on new evidence and reliable diagnostic tests should improve the quality of care of young children and lead to more rational use of antimalarials and antibiotics. The ALMANACH has now been integrated into a smart phone support and is currently tested for its safety in Tanzanian children; results are reported in a companion abstract.

2.3-012

Timeliness of child vaccinations in Kampala, Uganda

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Timely vaccination is important to ensure optimal response to vaccines, yet data is sparse. We examined delay in receiving recommended vaccines and its influencing factors among respondents with children <2 years in Kampala. This household survey used cluster sampling with 825 respondents. Respondents answered a questionnaire with three components: attitudinal factors such as perceived benefits from vaccinations; social factors such as support from significant others; and self-efficacy factors like being able to cope with poor communication with the spouse. Mobile telephones were used to collect data. Multinomial logistic regression was used to identify influences on delayed vaccination. Delayed vaccination was considered for each dose: DPT-Hib-HEB1 (>2 months), DPT-Hib-HEB3 (>6 months), and measles (>12 months). Vaccination was delayed for DPT-Hib-HEB1 in 25.5% of the children with a median delay of 49 days, in 15.4% of children for DPT-Hib-HEB3 with a median delay of 121 days, in 24.6% of children for measles with a median delay of 413 days. Delayed vaccination for DPT-Hib-HEB1 was reduced by the father's involvement in decision making for childhood vaccination (OR = 0.69, 95% CI = 0.48–0.99) and if respondents reported that they had less work (OR = 0.64, 95% CI = 0.40–0.98). Those that said they found it difficult to discuss vaccination issues with their partners tended to be delayed for both DPT-Hib-HEB3 (OR = 0.30, 95% CI = 0.12–0.82) and measles (OR = 0.59, 95% CI = 0.37–0.94). For measles, respondents that said the father had visited the vaccination post were less delayed than those that did not report this (OR = 0.51, 95% CI = 0.28–0.90), and respondents that said they would not immunize a child with fever were more delayed than those that said they would immunize a child with fever (OR = 1.64, 95% CI = 1.04–2.57). Vaccination programs should increase male involvement to improve timeliness of child vaccinations.

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2.3-013

Front line health care providers in a rural community of Bangladesh: how they perceive infant and young child feeding practices?

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INTRODUCTION Introduction of breast milk substitutes to infants during first 6 months and early initiation of complementary foods with other inappropriate infant feeding is widely practiced by mothers in Bangladesh. To change these feeding practices, BRAC initiated an intervention utilizing frontline community health workers. These workers have access to the household and influence on feeding practices. Therefore it is crucial beforehand to know their perception on feeding practices, to identify how and why these perceptions concord or mismatch with the WHO recommended infant and young child feeding practices. This is important for designing the intervention as well as providing relevant training to the health workers. This study aims to explore the perceptions of frontline workers on infant and young child feeding in rural Bangladesh.

METHODS An exploratory qualitative research was conducted on frontline workers in a rural community. A total of 69 purposively selected frontline providers participated in the study. Semi-structured interviews, focus group discussions, and key informant interviews were used as data collection tools. Content analysis was done to analyze data.

FINDINGS The majority of frontline worker's perceptions do not concord with the WHO recommended infant and young child feeding practices: they reported suggesting breast milk substitutes within 6 months and early initiation of complementary food when mothers complained of producing insufficient milk. They also perceived that colostrum alone is not sufficient to satisfy a baby's hunger. Such misconceptions could have serious implications for child health.

CONCLUSIONS These findings highlight the urgent need to train frontline health care workers based on the WHO recommended infant and young child feeding practices.

2.3-014

Under nutrition prevalence and associated factors among school age children in Amhara state, Ethiopia

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BACKGROUND Child under nutrition is a leading cause of mortality in Libokemkem and Fogera, two provinces of Amhara, Ethiopia. In order to propose effective nutrition and public health strategies it is important to have reliable information on the nutritional status of the population and the underlying factors affecting it. The aim of this study was to estimate the prevalence of under nutrition in school age children, and to identify the risk factors associated with it.

METHODS A cross-sectional survey using a multi staged stratified cluster sampling was conducted. Four hundred and fifty-eight children aged 4–15 years were surveyed. The survey included a socio-demographic, health and dietary questionnaire and anthropometric measurements, from which nutritional indicators based on the WHO references, were calculated. In order to define under nutrition two indicators were used: Body Mass Index for Age *Z*-score <-2 for wasting and Height for Age *Z*-score <-2 for stunting. Logistic regression models were used to identify associated risk factors.

RESULTS Overall prevalence of wasting and stunting was 23% and 35.7%, respectively. In the multivariate analysis, the risk factors associated with wasting were child's increasing age (OR = 1.3 per year; P < 0.001), being male (OR = 2.5; P = 0.005) and not consuming products of their own cattle in the household (OR = 2; P = 0.038). The factors associated with stunting were child's increasing age (OR = 1.2 per year; P < 0.001), living in a house with no bed nets (OR = 2; P = 0.003) and with poorly conditioned walls (OR = 2.4; P < 0.001).

CONCLUSIONS There was a high prevalence of under nutrition in the school age children of this area of Ethiopia. Results suggest that living conditions and health related behaviour are associated with stunting while the factors associated with wasting are related to gender and household dietary habits. This project was financially granted by the UBS Optimus Foundation and supported by the Red de Investigación Cooperativa en Enfermedades Tropicales (RICET).

2.3-015

Coexistence of maternal obesity and child stunting in rural communities

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INTRODUCTION In Mexico the National Nutrition Health Survey 2006 reported that the prevalence of child stunting in <5 year is more prevalent in rural communities: 19.9% compared with 10.1% in urban communities. Prevalence of obesity in women >20 year is 67.9% in rural communities. Therefore, it is suggested the coexistence of maternal obesity and child stunting in rural communities.

OBJECTIVE To assess the coexistence of maternal obesity and child stunting in rural communities.

MATERIAL AND METHODS A sample of 11,326 mother/child pairs were obtained in six different states in Mexico. The pairs are beneficiaries of the Mexican Social Programme 'Un Kilo de Ayuda'. In children anthropometric *Z*-scores for height-for-age (HAZ) were calculated using the WHO 2006 reference standards. Children with a HAZ score value lower than -2 were classified as stunted. In the mother's body mass index were determined based on the standars of the WHO. Localities lower than 2500 habitants were classified as rural community.

RESULTS The 32.7% and 22.2% of the women were overweight and obesity respectively. The 59.22% of the children were stunting. The coexistence of maternal obesity with child stunting was 11.4%. However, in overweight women the phenomenon was more prevalent 19.0%. Therefore, it is suggested that stunted children, three of each 10, have overweight/obesity mother. Only the 16.51% of the mothers with normal weight haven not stunting child.

CONCLUSION In the rural communities there is a coexistence of maternal obesity and child stunting. It is necessary identify the different environmental, behavioral and individual risk factors. These risk factors must be considered for public health programs that are able to address child and maternal weight issues simultaneously.

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2.3-016

Comparing nutritional status: <2 year-olds of mothers working in the ready made garment industry and of housewives in Bangladesh

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BACKGROUND Under-5 nutrition status has not been improving in Bangladesh in the past decade, primarily due to the improper IYCF (infant and young child feeding) practices. A burgeoning female workforce of the readymade garments (RMG) industry now poses a new threat to good IYCF practices as they are compelled to forgo proper breastfeeding practices for the sake of their employment.

OBJECTIVE To compare nutritional status between under-2 children of housewives and garments working mothers attending a semi-urban health centre in Bangladesh.

METHODOLOGY This cross-sectional study was conducted from December 2010 to January 2011 in the paediatric outpatient and immunization departments of the Centre for Woman and Child Health (CWCH). One hundred and four mothers and their under-2 children (half were healthy) pairs, either housewives or female garments workers were recruited to collect information using a semi-structured questionnaire. Children's anthropometric information was also collected. Chi-square tests were undertaken to test for the differences in the nutritional status and feeding practices between the two groups.

RESULTS The two groups were similar in terms of family size, parental educational status and occupation, maternal age and mean BMI. Wasting (WHZ < -2.00) was more than twice as prevalent in the garments workers' children (19.4% vs. 7.8%; $P < 0.05$). There were no statistically significant differences between the groups regarding underweight (WAZ < -2.00), stunting (HAZ < -2.00), any breastfeeding rate at all age groups or the introduction of soft, semi-solid foods at 6–8 months. Considering only healthy children, stunting was significantly more common in the garments worker group (23.5% vs. 15.5%; $P < 0.05$). In children aged <6 months, exclusive breastfeeding was 10 times less common in the garments worker compared to the housewife group (5.8% vs. 58.2%; $P < 0.00$).

CONCLUSION These study findings point to the urgent need of support and counselling for garments working mothers for proper IYCF practices.

2.3-017

Determinants of 1st hour initiation of breastfeeding in infants attending the paediatric outpatient department of a semi-urban hospital in Bangladesh

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BACKGROUND It is estimated that universal initiation of breastfeeding within 1 h of birth would save 37,000 newborns' lives and 52,000 under five lives every year which would reduce under five mortality rate by over 20%. The first hour initiation of breastfeeding was 43% in Bangladesh in 2007. It is important to study the determinants of this life-saving behaviour so that interventions can be designed to increase its prevalence.

OBJECTIVE To describe the determinants of breastfeeding initiation within 1 h of birth in infants attending the paediatric outpatient department of a semi-urban health centre in Bangladesh.

METHODOLOGY In this cross-sectional study, 150 randomly selected mothers of infants from the outpatient department of the Centre for Woman and Child Health (CWCH) were interviewed in October 2008 to collect information on socio-demographic factors, breastfeeding practices and factors affecting breastfeeding practices using a structured questionnaire. Chi-square tests were undertaken to compare children who had initiation of breastfeeding within 1 h of birth with those who had not.

RESULTS Seven percent of fathers were illiterate and 69% were garment workers whilst 7% mothers were illiterate and 89% were housewives. Most of the families (75%) were nuclear, average family size was 5 and 73% of families were living in rented houses. Mean maternal age was 23 years, age at marriage was 17.5 years and age at first child-birth was 20 years. Mean age of the children was 3.7 months (SD 2.7), 56% of babies were male and mean birth order was 1.45. Initiation of breastfeeding within 1st hour was 43% and was significantly associated with normal delivery (88% vs. 67%, $P < 0.005$), delivery conducted by trained birth attendants (48% vs. 25%, $P < 0.005$) and breast milk being the 1st feed (72% vs. 42%, $P < 0.00$). Maternal sickness (65%) was the major cause of delayed breastfeeding initiation beyond the 1st hour.

CONCLUSION These study findings indicate a need for breastfeeding promotion and counselling among all mothers and birth attendants to ensure breastfeeding within the 1st hour of life.

2.3-018

Nutritional status of schoolchildren and influencing factors in TUnja, Boyacá, Colombia

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INTRODUCTION Malnutrition is a relevant Public Health problem in Colombia. In rural areas chronic malnourished children are reached with difficulty by government nutrition programs. Boyacá is mainly a rural department. We conducted a nutritional survey in its main municipality, Tunja, in order to assess the magnitude of the problem and to serve as a baseline for future evaluations.

METHODS We randomly selected schoolchildren stratified by grade among all private and public schools in Tunja. Information on health status of the children and socioeconomic and demographic characteristics of their families was collected using questionnaires. In them we also included the Colombian Household Food Security Scale (CHFFS) to assess food insecurity. Anthropometric measurements of 1177 schoolchildren aged 5–19 years old were obtained, analyzed with WHO AnthroPlus, and related with the other variables through further statistic analysis.

RESULTS The overall prevalence of underweight, stunting and thinness were 4.4%, 11.3% and 1.7%, respectively. In rural areas 23% of the children were stunted. Higher prevalences were also found in poor households, children attending public schools, and in female-headed households. Stunted children were more likely to be diseased and to live in households with more food insecurity. 48.6% of the households were classified as food insecure, with higher rates among poor and rural households, and children from public schools. Overweight was present in a 17.6% of the children in the sample, reaching apercentage of 26.9% in children in private schools.

CONCLUSIONS Periodic controls are needed to evaluate the impact of the programs in the overall nutritional status of the children in Tunja. The situation in other rural areas of Boyacá should be deeply assessed. A standardized food security scale, as the CHFFS, could be used complementarily for the better planning of the programs. Obesity should also be addressed mainly in private schools.

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2.3-019

Risk factors contributing to child's progression to severe disease in rural Tanzania

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INTRODUCTION Mild cases of malaria, pneumonia and diarrhea are treatable with complete recovery, however mortality is significantly high when children presents to the hospital with severe disease. We assessed at how care seeking behaviours and other factors contributed to severity of disease among children presenting to hospitals in rural Tanzania.

METHODS AND MATERIALS We used an interviewer administered questionnaire among care-takers seeking care for their underfive children between July 2009 and January 2010 at Korogwe and Muheza district hospitals in north-eastern Tanzania.

RESULTS A total of 293 children with severe and 190 with non-severe disease were studied. We found persistant associations between severity of disease and caretaker's no formal education [OR 3.0 (1.2–7.6)] compared to those with post-primary education), low socio-economic status [OR 2.1 (1.2–3.5)], having four or more children [OR 2.2 (1.2–4.1)] compared to having one child, having utilized a nearer primary care facility [OR 7.6 (4.0–14.6)] and having first treatment purchased from local or drug shops [OD 4.1 (2.1–8.0)] compared to when obtained from public hospitals. The old first line anti-malaria drug SP was reported to have been given to 19 children as a single treatment for malaria. Nine were confirmed at the district hospital as malaria cases of which eight presented with severe malaria (two of them died). SP use for malaria treatment was significantly associated with child's progression to severe malaria [OR 11.2 (3.2–92.6)].

CONCLUSIONS Our study revealed several further areas and target groups for further interventions in the fight to reduce child mortality from treatable illnesses. Quality of the available primary care facilities need to be careful assessed while strict regulations and close monitoring of drugs that have been phased out need to be put in place.

2.3-020

Prevalence and clinical implications of *Helicobacter pylori* infection in children seeking medical care in rural Ghana

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BRIEF INTRODUCTION Helicobacter pylori infection is highly prevalent in developing countries, and has possible implications for childhood morbidity. We report data on the prevalence of *H. pylori* in children seeking medical care in Ghana, and on the association with clinical parameters and selected parasitic infections.

METHODS AND MATERIALS Clinical, biometric and laboratory data of children presenting to the paediatric outpatient department of the Agogo Presbyterian Hospital in Ghana were recorded. Stool and blood samples were analysed for parasites. A commercial *H. pylori* stool antigen test (Premier Platinum HpSA Plus®; Meridian Bioscience Inc., Cincinnati, OH, USA) was used to identify children with *H. pylori* infection.

RESULTS A total of 1903 children were recruited for the study. Mean age was 51.3 ± 42.2 months, 46.9% were female. Overall prevalence of *H. pylori* was 32.5%, increasing with age (<16 months 9.2%, 16–33 months 19.7%, 34–73 months: 40.4%, 74 months: 52.3%). 27.2% of children were diagnosed with malaria, and 37.7% with *G. lamblia* (stool PCR). Sixteen percent of children were malnourished (*z*-score weight-for-age -2). Children with *H. pylori* infection were more likely to have malaria [Odds ratio (OR) 1.53, 95% confidence interval (CI) 1.2–1.9, P < 0.001] and *G. lamblia* infection (OR 1.3, 95% CI 1.07–1.6, P = 0.01), but were less likely to be malnourished (OR 0.7, 95% CI 0.51–0.97, P = 0.03). No significant association was found between *H. pylori* infection and the presence of diarrhoea and anaemia.

CONCLUSION *H. pylori* infection was highly prevalent and associated with malaria and *G. lamblia* infection. Children with *H. pylori* infection were less likely to be malnourished; there was no association with anaemia and diarrhoea in this study. No data on the association of *H. pylori* and malaria has been previously reported to our knowledge. The interplay between *H. pylori* and parasitic co-infections could be relevant in highly endemic settings and warrants further investigation.

2.3-021

Factors associated with nutrition status among breastfed infants in communities in Mangochi district, Malawi

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Child malnutrition underlies more than a third of all deaths in children under 5 years. It remains to be a public health challenge in many low income countries including Malawi. We investigated the rates of malnutrition and its correlates among breastfed infants in a rural and semi-urban community in Mangochi district, Malawi, basing on both the 1978 NCHS references and 2006 WHO standards. Cross-sectional data were collected from 349 mother-infant pairs. The rate of stunting was 16.9%, being underweight was 17.8%, and wasting was 13.3% basing on the 2006 WHO growth standards. Thirty-four percent and 41% infants were additionally classified as stunted and underweight with the use of the 2006 standards instead of the 1978 NCHS references. Whilst as an additional 50% were classified as wasted with the use of the same standards. The use of either the standards or references yielded a significant association between being stunted and food security in logistic regression analyses; when breastfeeding patterns were adjusted for. In this regard the odds ratios (95%CI) were 2 (1.0–4.6) and 3 (1.2–7.4) using the 1978 references and 2006 standards respectively. Being between 6 and 8 months old and having a mother who has a petty occupation like subsistence farming were independently associated with being underweight when WHO standards were used. The significance of the associations was lost when NCHS references were used. In conclusion the findings underline that food shortage negatively influences the nutritional status of infants, even when they are well breastfed. Breastfed infants are still prone to malnutrition especially in the period which coincides with prominent complementary feeding. The use of the 2006 WHO standards may not pose a challenge in mapping out the factors associated with malnutrition.

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2.3-022

Prevalence of malnutrition in children attending at Manhiça district hospital Southern Mozambique

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INTRODUCTION Malnutrition is associated with at least four times increased risk of death in childhood in developing countries and almost half of these deaths occur in sub-Saharan Africa. The main objective of this study was to determine the prevalence of malnutrition and describe its clinical features at admission to hospital in children <5 years.

MATERIAL AND METHODS The study was carried out at Manhiça District hospital (MDH), Manhiça in southern Mozambique. Retrospective analysis of data collected through the morbidity surveillance system in place in the hospital, between January 2000 and December 2006 were performed using STATA statistical software.

RESULTS 76.033 children under 5 years of age attended the outpatient service of the MDH. The prevalence of severe malnutrition was 7% (5.146/76.033); however 50% (38.021/76.033) of children presented with any form of malnutrition. Mortality in hospitalized children with severe malnutrition was 7% (234/3359). Most clinical symptoms and signs were statistically significantly higher among hospitalized severe malnourished children compared to non-hospitalized children. Hypoglycaemia (OR = 4.58 95% CI, 2.44–8.62), and dehydration (OR = 2.63 95% CI, 1.58–4.37) were associated with hospital mortality in severe malnourished children. On the other hand, malaria parasitemia (OR = 0.48 95% CI, 0.29–0.78) was lower among severe malnourished and associated with reduced mortality.

DISCUSSION Hospital prevalence and mortality associated with severe malnutrition in children was high in this area of Mozambique. Hypoglycaemia and dehydration were poor prognosis signs in severe malnourished children. In contrast, malaria parasitemia was associated with reduced risk of mortality. More studies on the pathophysiology associated with severe malnutrition in children is are needed to improve clinical management and reduce mortality.

2.3-023

Congenital syphilis in immigrant and adopted children arriving in Spain

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INTRODUCTION Internationally adopted and immigrant children population has increased in Europe during the last decade. Many of these children were born in countries where pregnancy control is poor or non-existent. A consequence of this poor pregnancy control is congenital syphilis (CS). The objective of the present study is to assess the incidence of CS in the immigrant and internationally adopted children attended at a pediatric hospital in Barcelona.

PATIENTS AND METHODS Medical records and serological testing for syphilis were retrospectively reviewed from all patients who were routinely evaluated after their arrival in the International Health Department of Hospital St Joan de Déu (Barcelona) between 2000 and 2010. Nontreponemal (RPR) and treponemal (FTA-ABS) tests were assessed in all patients. In those with reactive treponemal test, lumbar puncture and VDRL determination in cerebrospinal fluid, long-bone radiographs and hearing test were additionally performed.

RESULTS During the period studied 4086 children were evaluated. The most represented regions of origin include Eastern Europe (26%), China (23.5%), Sub-Saharan Africa (12%), South America (8.3%), and India and Nepal (6.5%). Eight cases (0.2%) of CS were observed. Clinical characteristics included neurosyphilis in one case, radiographic long-bone abnormalities in another patient and one case of sensorineural hearing loss. The remaining five patients were asymptomatic. Four patients, including one of the symptomatic ones, had been previously treated in their country of origin and the remainder were diagnosed and treated at their arrival.

CONCLUSIONS CS remains one of the most severe, preventable adverse pregnancy outcomes worldwide. CS can be prevented if infected pregnant women are treated with penicillin, but in areas were coverage of prenatal care is low, women do not receive routine syphilis testing during pregnancy. Thus, it is important to test for syphilis all those children with non-existent or unknown prenatal care, considering the availability of curative treatment.

2.3-024

Childhood disability in rural Zambia: a qualitative study on the use of health care services

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There are 650 million people living with disabilities worldwide, an estimated 200 million are children. A majority of disabled children live in poverty and lack access to basic health services and rehabilitation opportunities. As such, they are highly susceptible to the risk of missing out on essential developmental opportunities. In Zambia, poverty levels are high and a national policy about disability is yet to be implemented, thus the care for disabled children falls on the families. This study explores the use of health care services for children with disabilities and their families. In this qualitative study, observations and interviews with 16 parents of disabled children and 13 health workers in the Kazungula District, Zambia were carried out. Facilitating factors and barriers to health care were explored, formal and informal health services identified and reasons for the choice of services examined. Systematic Text Condensation was used to analyze the material. The primary caregivers of disabled children use the rural health centers, but rarely for an assessment of their disability. Family members attend rural health centers without bringing the disabled child, thus further management of the child is based solely on information from the relatives. The main barriers to health care are long distances, lack of available transport and shortage of staff, equipment and skills at the rural health centers to manage childhood disability. Referral to higher-level health facilities is done extensively, but is difficult for families to make use of. Parents become tired of trying to respond to episodes of illness and they consequently give up. Primary health care in Zambia is not able to provide adequate care for disabled children, and their health needs are therefore assessed and managed within a family unit strongly influenced by poverty.

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2.3-025

Deworming benefits during the critical window of growth and development: reviewing the literature and identifying the research gaps for children 12–24 months of age

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INTRODUCTION As of 2002, WHO has recommended the inclusion of children 12–24 months of age in endemic areas where large-scale deworming activities are indicated. These children are in their most critical window of growth and development, at which time deworming interventions have important implications for short and long-term health benefits and nutrition and social outcomes. Therefore, the objective was to review the literature on deworming in early preschool-age children.

METHODS A search of relevant databases (e.g. Medline, Embase, Lilacs) was undertaken. Inclusion criteria of studies were: (i) randomized controlled trials (RCTs) with a placebo or usual care control group; (ii) growth and/or development outcomes; (iii) age <24 months at recruitment; (iv) use of WHO recommended drugs, albendazole or mebendazole; (v) healthy study populations.

RESULTS Five RCTs fit the inclusion criteria. Frequency of deworming ranged from once yearly to every 3 months. Two trials also included school-age children and two trials included children <12 months in their study populations. No trials focused on, or reported, age-disaggregated results for children <2 years of age. Anthropometric outcomes were reported as continuous and categorical measurements. Only one trial measured development, which was by parent's self-report. No adverse events were reported in any of the studies.

CONCLUSIONS A thorough understanding of deworming benefits in children 12–24 months of age has been limited by the: inclusion of children under 1 year of age, and a lack of age-disaggregated published data in studies including children >2 years of age; variable frequency of deworming administration; and heterogeneity in outcome measurement and reporting. An RCT on the benefits of deworming, including appropriate timing and frequency, on growth and development in children 12–24 months of age is currently being undertaken in Iquitos, Peru to help fill this important research gap.

2.3-026

A novel immunodiagnostic assay detecting serum antibody responses against selected fraction antigens of soluble egg antigen (sea) of *schistosoma japonicum*

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While there are multiple existing immunodiagnostic assays to detect the serological biomarkers in patients infected by *Schistosoma japonicum*, their use to follow the disappearance of parasite in patients receiving chemotherapy was not effective. We now demonstrate that soluble egg antigen (SEA) of *Schistosoma japonicum* can be separated into 23–28 fractions with different molecular weight which can be recognized by infected sera via Western blot analysis. Recognition to 107 and 121 kDa fractions disappeared in animal sera which were cured of infection. Using purified 107–121 kDa fractions as the coating antigens, a novel fraction antigen based ELISA (FA-ELISA) was developed which provided high sensitivity and specificity, with very low cross-reactivity in patients infected with other parasites such as *Clonorchis sinensis*, *Paragonimus westermani*, *Fasciolopsis buski*. This new assay can be an effective tool in following the efficacy of chemotherapy against *S. japonicum*.

2.3-027

Do prenatal and birth service characteristics predict exclusive breastfeeding initiation in the Dominican Republic?

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INTRODUCTION Some aspects of prenatal and birth services may increase the extent to which postpartum women initiate exclusive breast feeding, however, empirical findings are limited. The objective of this study was to determine whether prenatal and birth service characteristics predicted initiation of exclusive breastfeeding in the Dominican Republic, a country in which exclusive breastfeeding has been declining over time.

METHODS AND MATERIALS This study utilized data from the 2007 Demographic and Health Survey in the Dominican Republic to examine the relationship between prenatal and birth service variables and exclusive breastfeeding initiation (as measured by exclusive use of breast milk in the first 3 days of life) in a sample of women of reproductive age. Bivariate and multivariate analyses were used to examine these relationships.

RESULTS Of the 7512 women included in the analysis, only 44.8% initiated exclusive breastfeeding. Women who initiated exclusive breastfeeding had lower levels of education, lower income status, and were more likely to live in rural areas. Women who initiated exclusive breastfeeding were more likely to have had a vaginal delivery and received care in a public health center. OB/GYN involvement in prenatal care and during delivery was negatively associated with exclusive breastfeeding initiation. This negative relationship persisted after controlling for type of delivery, socioeconomic status and place of residence. Number of prenatal visits was a weak predictor of exclusive breastfeeding initiation; however, this relationship disappeared after controlling for type of delivery and socioeconomic status.

CONCLUSIONS Prevalence of exclusive breastfeeding initiation in the Dominican Republic is suboptimal. Findings that specialist health professional involvement in both prenatal care and delivery were associated with lower rates of exclusive breastfeeding initiation highlight the need for increased examination of breastfeeding promotion and support strategies within the organization of prenatal care and birth services in the Dominican Republic.

2.3-028

Impact of adherence to the PMTCT program at Saint Camille medical centre in Ouagadougou, Burkina Faso

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INTRODUCTION Despite the availability of interventions for the prevention of mother-to-child transmission of HIV (PMTCT), adherence to PMTCT program remains a challenge in resource-limited settings. Aim of our study is to evaluate the adherence to PMTCT program and its impact on the 12 months follow-up.

METHODS AND MATERIALS Data were retrospectively collected from clinical chards of HIV+ women and their children followed up to 12 months after delivery at the Saint Camille Medical Centre (SCMC) from 1 July 2006 to 15 January 2011. PMTCT protocol

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was based on WHO 2006 guidelines, with formula feeding or early weaning after 4 months, according to mothers' choice.

RESULTS The study concerned 229 mother-infant pairs, of whom 75/229 (32.8%) presented incomplete adherence to ARV regimen, timing of ARV initiation or right neonatal prophylaxis: 3/229 (1.3%) mothers with indications for HAART only underwent short-course prophylaxis, 1/229 (0.4%) didn't undergo PMTCT because of preterm delivery, 55/229 (24.0%) started ARV after 28th week and 19/229 (8.3%) newborns didn't receive rightly ARV prophylaxis. At the 12 months follow-up, living HIV-children were 138/154 (89.6%) in the complete-adherence group and 58/75 (77.3%) in the incomplete-adherence group ($P < 0.05$); dead children were 9/154 (5.8%) and 15/75 (20.0%) respectively ($P < 0.05$). Mother-infant pairs with complete adherence were 54/113 (47.8%) in the short-course prophylaxis group, 32/45 (71.1%) in the HAART started during pregnancy group, and 68/71 (95.8%) in the HAART before pregnancy group ($P < 0.01$). Other factors impacting the adherence to PMTCT were: mothers' age ($P < 0.01$), knowledge of HIV status before pregnancy ($P < 0.01$); gestational age at first antenatal visit ($P < 0.01$); number of antenatal visits ($P < 0.01$).

CONCLUSIONS The adherence to PMTCT impacts on the 12 months follow-up. Short-course prophylaxis regimen is complex and shows low compliance. Knowledge of HIV status before pregnancy is essential for the disease acceptance and management. Health system weaknesses constrain access to antenatal care, affecting adherence to PMTCT program and its effectiveness.

2.3-029

Beriberi, a persistent cause of infant mortality in 2010: community survey and case study in northern Laos

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INTRODUCTION Thiamine deficiency (Beriberi), a rare condition, was mostly described in vulnerable populations. Infant beriberi occurred mainly in breastfed infants of mothers with inadequate intake of thiamine. Young infants seen with cardiac failure from Lao hospitals suggest the persistence of beriberi as a possible cause of infant mortality. Traditional food taboos post-partum and nutritional habits may be the reason. WEe describe possible and probable cases of beriberi in northern Laos.

METHODOLOGY Three surveys were conducted in Luang NamTha Province from March to June 2010: a retrospective survey of all beriberi inpatients cases in the five hospitals of the province between 2007 and 2009; a cross sectional survey of all mothers with infants (1–6 months) in 22 villages where beriberi cases originated; and a verbal autopsy of all infants deaths, and an estimate of infantile mortality in these villages. Clinical possible and probable definition was used.

RESULTS From 2007 to 2009, 54 cases of infant beriberi were diagnosed with sudden severe cardiac failure, three died and 90% recovered after thiamine injection. Of 419 living births in 22 villages, 67 infants died during the first year of life (infant mortality 106 per thousand; 95% CI: 80–138) with a peak between 1 and 6 months. Of them, 20 had sudden death and suspected beriberi. During the cross sectional survey, of 167 mothers 20 (11.9%) had signs associated with probable beriberi and 20 (11.9%) probable beriberi. Of 167 infants, nine have symptoms compatible with latent BB.

CONCLUSION Beriberi remains a common cause of infant death in northern Laos. This survey, anecdotal reports from other regions, and research from Vientiane hospitals support the severity of this nutritional situation. This requires urgent investigation, prevention and response in Laos.

2.3-030

Iodine status of pregnant women in Lubumbashi, Dr Congo

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INTRODUCTION Surveys conducted among school-age children in DR Congo led to suspect an increased of adverse health problems induced by excessive iodine intake in the country. No data are currently available in Congolese pregnant women despite an increased need during pregnancy. Our study aim was to assess iodine status of pregnant women in Lubumbashi in Southwest of DR Congo.

METHODS A cross-sectional study was conducted in 2010 in maternities from urban, rural and semi-rural areas of Lubumbashi. We randomly selected 225 pregnant women attending prenatal consultation (75 at trimester T1, T2 and T3), 75 postpartum women, and 75 non-pregnant women, as controls. Urinary iodine concentration (UIC) was determined on random urine sample, using a modified Sandell–Kolthoff digestion method. Data are reported as median and Q1-Q3. According to WHO cut-offs on UIC (150, 250, 500 µg/l), women were classified as iodine deficient (ID), adequate, above, or excessive iodine intake.

RESULTS UIC significantly decreased during pregnancy, from 238 (115–387) µg/l at T1 to 129 (70–287) at T2 and 77 (27–222) at T3. This trend was similar across the three surveyed areas. In postpartum women, UIC was 144 (97–300) and it was 205 (95–331) in controls, similar to pregnant women at T1. Despite an excessive iodine intake in 7% (26/368) of women, 50% were ID, without significant differences across areas but with a significant increase with gestational age: 31%, 56%, and 68% at T1, T2, and T3, respectively.

CONCLUSION Despite excessive iodine intake reported in the general population, pregnant women are still iodine deficient in Lubumbashi. Efforts need to be made to achieve adequate iodine status in all segments of the population.

2.3-031

Detection of visual disorders during physical examination of Kenian school children

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OBJECTIVE Analyze the usefulness of childrenÂ′s examinations in schools in depressed areas of Africa . Screening for visual disorders in primary school children Chazon Children Center in Molo, Kenya.

DESIGN A health survey has been conducted at an elementary school during the months of November 2010 to January 2011. It presents descriptive study with health outcomes in a population of 215 children analyzed sex, weight, height, BMI, personal history and visual deficit.

METHODS Description of the sociodemographic characteristics of the 215 children in the school population. Absolute frequencies and proportions for qualitative variables; Measures of central tendency and dispersion for quantitative variables.

RESULTS The population is homogeneous in terms of sex (54.5% of girls and boys 45.6); the average age is 10.30 years (CI 9.93–10.67) and a standard deviation 2.79. Regarding family status 83.7% orphans, 16.3% of orphans. In relation to the vision though 74% have no visual defect, does note that a 1.4% have a

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severe and irreversible visual impairment and 23.7% moderate deficits correctable with lenses.

CONCLUSIONS It is important to carry out school health surveys in a systematic way especially in impoverished areas as visual defects can be detected. In some cases they are irreversible, and possibly could have been avoided if health surveys would have been carried out in the past.

Track 3: Chronic Diseases and Environmental Health

3.1 Chronic Diseases

3.1-001

Serologic evaluation of hepatitis B and D in patients with cirrhosis

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OBJECTIVE Fifteen million people in the world have chronic hepatitis D. This research is implemented on a number of cirrhotic patients with the aim to assess the prevalence of hepatitis D and B.

METHODOLOGY This study included 60 cirrhotic patients. The level of Anti HDV, AntiHBc (IgM) and HBsAg were measured by method of ELISA.

RESULTS In this study 60 cirrhotic patients were evaluated. Sixteen patients were HBsAg positive. In total of 16 HBs Ag positive patients, 13 patients were Anti HBc positive (IgM) and in total 13 HBc Ab positive patients, eight patients were HDV Ab positive.

CONCLUSION In present study 50% of cirrhotic patients with HBsAg positive, were Anti HDV Ab positive indicating concurrent infection with HBV and HDV in these cirrhotic patients. By timely screening of cirrhotic patients for HDV, patients could undergo additional management of treatments.

3.1-002

Genetic polymorphisms of toll-like receptor 9 in Brazilian patients with systemic lupus erythematosus

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INTRODUCTION Systemic Lupus Erythematosus (SLE) is a multi-systemic autoimmune disease associated with massive production of auto-antibodies. Toll-like receptor 9 (TLR9) recognizes unmethylated CpG-DNA and has been implicated in the development of anti-DNA auto-antibodies in murine model. We investigated the prevalence of TLR9 single nucleotide polymorphisms (SNPs) (-1237 C>T, +1174 A>G, +2848 G>A) in SLE patients and their association with clinical features.

MATERIAL AND METHODS The study enrolled 158 SLE patients attending the Rheumatology Service, Santa Izabel Hospital. All SNPs were identified by sequencing the TLR9 gene.

RESULTS Genotype frequencies in the studied population were 56%, 37%, and 6% for TT, CT and CC respectively, at position -1237, 39%, 41% and 20% for GG, GA and AA, respectively, at position +1174, and 23%, 46% and 31% for AA, AG and GG, respectively, at position +2848. The allele frequencies at position -1237 were 75% and 24% for T and C, respectively, at position +1174 they were 59% and 41% for G and A, respectively, at position +2848 they were 46% and 54% for A and G, respectively, and genotype distributions in all SNPs met Hardy–Weinberg's expectation. There was an association between homozygosis of allele C at position -1237 and central nervous system (CNS) involvement ($P < 0.05$). Psychosis was identified as a reason for this association ($P < 0.01$) rather than convulsion ($P = 0.83$). Likewise, homozygosis of allele G at position +2848 was associated with discoid rash ($P < 0.05$).

CONCLUSION These data show that TLR9 SNPs are associated with development of specific clinical symptoms in patients with SLE.

3.1-003

Prevalence of psychological distress and chronic health problems in urban hospital outpatients in South Africa

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OBJECTIVE The aim of this study was to assess the prevalence of psychological distress and associated factors among outpatients in an urban hospital in South Africa.

METHOD A sample of 1532 (56.4% men and women 43.6%) consecutively selected patients from different hospital outpatient departments were interviewed with a structured questionnaire.

RESULTS Based on the assessment of the Kessler 10 17.1% of the patients (15.5% among men and 19.4% among women) had severe psychological distress. Logistic multiple regression identified for men no income, poor health status, migraine headache and tuberculosis and for women lower education, no income, having been diagnosed with a sexually transmitted disease, stomach ulcer and migraine headache were significantly associated with severe psychological distress.

CONCLUSION The study found high prevalence of psychological distress among hospital out-patients in South Africa. Brief psychological therapies of adult patients with anxiety, depression or mixed common mental health problems treated in hospital out-patient departments are indicated. Accurate diagnosis of comorbid depressive and anxiety disorders in patients with chronic medical illness is essential in understanding the cause and in optimizing the management of somatic symptom burden.

3.1-004

Low HbA1c level as a predictor of increased mortality in type 2 diabetic patients with hip fractures

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BACKGROUND Tight glycaemic control, as evidenced by a target HbA1c < 7%, has been shown to reduce the incidence of diabetes complications. However, some controversy exists as recent evidence appears to show that very low HBA1C values appear to be associated with increased mortality. The present study evaluates the association between HBA1C level and mortality in elderly patients with type 2 diabetes mellitus (T2DM) admitted with a hip fracture.

METHODS This was a prospective cohort study of all T2DM patients ($N = 581$) on treatment admitted with a diagnosis of hip fracture from years 2005–2010 to Changi General Hospital. Primary end point was all-cause mortality. HBA1C levels was taken at admission. Patients were divided into two groups, HBA1C < 7% and? 7%. Mortality risk was compared using Kaplan–Meier survival curves and univariate Cox survival analysis.

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RESULTS The cohort (aged 76.5 ± 8.6 years, 73.3% females) had a median HBA1C level of 6.8% (IQR 5.2–8.4%). During a median follow-up of 20.8 months (range 0–75.9 months), 132 patients died (22.7%). Risk of all cause mortality was higher for patients with a HBA1C < 7%, when compared to patients with a HBA1C 7%, Hazards ratio 1.44 (95% CI: 1.01–2.07, P < 0.05), after adjusting for age, gender and ethnicity.

CONCLUSION A lower HBA1C level of <7% is associated with subsequent increased mortality risk, in elderly diabetic patients admitted with a hip fracture. Physicians need to exercise greater caution when treating elderly patients with DM.

3.1-006

Building the northern Australian workforce health promotion capacity to address chronic diseases

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INTRODUCTION The climate of reform and change is evident in the current Australian health care system where significant challenges are faced due to a growing burden of chronic disease, an aging population, workforce issues, and unacceptable inequities in access to services and health outcomes. Improved management of chronic conditions and a focus on health promotion and prevention are key priority action areas. It is vital that the health workforce has the appropriate knowledge and skills to work in a holistic approach that allows them to contribute to the downstream, midstream, upstream actions that will be required to address the future challenges. This presentation describes workforce health promotion capacity building initiatives developed in Northern Australia.

METHODS AND MATERIALS A range of courses have been developed to build workforce capacity including a 5-day Core Health Promotion Short Course and tertiary level courses including a postgraduate certificate, postgraduate diploma and Master of Public Health (Health Promotion).

RESULTS Between 2007 and 2011, fourteen 5 day short courses in health promotion were conducted for 254 participants. Follow up impact evaluation shows that the courses succeed in providing knowledge, skills, confidence and enthusiasm to undertake health promotion work but that a lack of understanding of health promotion from co-workers and managers, lack of organisational support and commitment, lack of resources, competing clinical priorities, and lack of time were barriers for undertaking health postgraduate courses commenced in 2010.

CONCLUSIONS There is strong support for workforce development in health promotion in north Queensland. Short courses and tertiary level training are one way to achieve this. However shifting health service delivery to a more upstream approach to address chronic disease requires broader capacity building within health services and systems including leadership, partnerships, resource allocation and organisational development.

3.1-007

Transient hepatitis C viremia without seroconversion among healthcare workers after occupational blood exposure in Cairo, Egypt

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INTRODUCTION With 14.7% of the general population (15–59 years) having anti-HCV antibodies (Demographic and Health survey 2008), Egypt is the country with the highest HCV reservoir. Healthcare workers are therefore at high-risk of HCV infection. Our aims were to study HCV transmission among healthcare workers exposed to HCV via occupational blood exposure (OBE) in Cairo.

METHODS The study was based on a surveillance system of OBE at Ain Shams University Hospital, Cairo. From August 2008 to September 2010, healthcare workers (HCW) were administered an in-person questionnaire and enrolled in a prospective cohort if they were anti-HCV negative at screening and had a positive anti-HCV index patient. During follow-up visits at weeks 2, 4, 8, 12 and 24 post-exposure, anti-HCV, qualitative HCV-RNA and alanine aminotransferase (ALT) were tested in Cairo. Transient viremia was defined as any detection of HCV-RNA in the serum during the follow-up followed by non-detection of HCV-RNA in serum for the remainder of the study. Cases of HCV transmission were confirmed via genomic sequencing of HCWs and corresponding index patients HCV isolates.

RESULTS During the study period, 596 HCWs (mean age (range) = 27 (16–59) years, male/female sex-ratio = 1.01), of whom almost half were physicians in training, reported an OBE to the clinics. Of the 70 exposed to HCV-RNA positive index patients, almost all (91%) reported injury with a hollow-bore needle. Nine (12.9%; 95% CI, 6.1–23.0%) presented transient viremia, the majority of which occurred within the first 2 weeks after exposure. No seroconversion occurred. Genomic sequencing is underway.

3.1-008

Therapeutic recourse for sickle cell disease in Abomey (Benin)

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In Benin, the family of Sickle Cell Disease patients resort to traditional medicine and/or modern medicine. There are several underlying reasons for this. Sickle cell disease lasts a lifetime and there is to treatment to cure it, hence the complexity of its management. This communication aims to present some therapeutic strategies and therapeutic pathways of Sickle Cell Disease patients in Abomey (Benin), mostly towards traditional medicine. This communication is based on a social anthropological approach to health comprising the analysis of observations and semi-structured interviews on life history from therapeutic use of Sickle Cell Disease patients and their family in Abomey. It is grounded theoretically on an analytical framework pertaining to symbolic interactionism (Blumer Herbert, 1969) and holism (MA Tremblay, 1982). Our results show that the disease is perceived as inherent in humans and requires a search for meaning and healing. There is no cure for sickle cell disease, but modern medicine enables people living with the disease to live long lives. Conversely, in traditional

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medicine, healers are unanimous that sickle cell disease can be cured. This explains the complete confidence that parents put in traditional medicine and especially the syncretism in the management of this disease.

KEYWORDS sickle cell disease, therapeutic recourse, modern medicine, traditional medicine

3.1-009

Health seeking behaviour of self-reported diabetics: an exploratory study of public parks in Dhaka

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By 2025, Bangladesh will be among the top ten countries in terms of the number of people living with diabetes (6.1%). In Bangladesh, most people seek care out of formal health system or rely on self-care. A better understanding of health seeking behavior and health care networks of diabetics is a new area of interest and needs exploration. A cross sectional survey was conducted to explore the health seeking behavior of self reported diabetics who use public parks in Dhaka. Two public parks representing two different socioeconomic areas (high and lower) were selected. Mixed method approach was employed. Participants were all male/female of 40 years and above and health providers at study parks were also included during 10–30 October, 2008. Majority of diabetics using the public parks were males of 50–60 years. Median duration as known diabetic was 8.7 years. Majority joggers were from high socioeconomic class. For majority, first and second choice for diabetic care was BIRDEM because of its popularity, easy access and free or subsidized treatment. None mentioned the public health sector at any point in care-seeking pathway. Participants mentioned changing diet, regular exercise, taking medicines and seeking spiritual support as lifestyle changes adapted after advice. Half reported spending more than 1000 taka per month for care. More than 60% reported seeking care from park providers. Reorientation of primary health care should be considered. Community-based care might be an effective strategy to tackle this issue by incorporating the informal health care providers into formal health system as institutional care is not enough. However, sufficient paramedical training for informal HC providers who are already providing services at parks and monitoring is important. Exclusion of females from these public spaces signifies the gender inequity and this should be taken into consideration in every program planning.

3.1-010

Factors associated with hypertension and compliance to treatment among the urban adult population in hospitals in Dhaka, Bangladesh

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INTRODUCTION Hypertension is a serious public health problem affecting 7.6 million premature deaths and approximately 6.0% of the global disease burden per year. Major contributor to these deaths and disease burden are Asians including India, Bangladesh and Pakistan. Present study aims to investigate the factors associated with Hypertension and compliance of patients to anti-hypertensive drugs.

METHODS AND MATERIALS It was a cross-sectional quantitative study conducted at an outpatient department (OPD) of a tertiary hospital in Dhaka. All subjects of age 20 years or more were considered eligible for this study. Study period was from November 15 2010 to January 15 2011. A total of 140 respondents (70 hypertensive and 70 non-hypertensive) were selected using systematic random sampling. Respondents were interviewed with a structured questionnaire. Medication Adherence Rating Scale (MARS) was used to know the rate of compliance of patients to anti-hypertensive drugs. Initially a univariate analysis followed by a bi-variate and finally a multivariate logistic regression were performed.

RESULTS Result from the logistic regression showed that family history (OR: 5.5, 95% C.I: 2.0–14.7, P = 0.00), smoking (OR: 3.8, 95% C.I: 1.1–12.6, P = 0.02) and alcohol consumption (OR: 8.8, 95% C.I: 2.0–38.5, P = 0.00) were significantly associated with development of hypertension. Compliance to treatment was 50% among the patients. It is noticeable that these three variables (smoking, alcohol and family history) were individually associated with the outcome in bi-variate analysis also. Though physical activity was not significant predictor in bi-variate analysis, this factor tended to be associated (OR: 2.5, 95% C.I: 0.96–7.1, P = 0.06) with marginal significance in logistic regression.

CONCLUSION The findings suggest that hypertension has several risk factors and compliance to treatment is low. These results could form the basis for designing effective interventions and consequently improve the compliance of patients to anti-hypertensive treatment.

3.1-011

Distribution of HCV genotypes in Palermo, Sicily

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Hepatitis C Virus (HCV) is present worldwide and shows an important eterogeneity among isolates. The distribution of HCV strains is characterized by a relative prevalence of one genotype. In order to assess the distribution of HCV genotypes in Palermo (southern Italy), an out Patients clinic-based cohort, collected prospectively, of 506 subjects with chronic HCV infection was surveyed, from 2004 to 2010. In the study cohort, 415 Patients were males and 91 females. Mean age was 33 years (range 18–61 years). Eighty-nine percent of the Patients were intravenous drug users, in the 5% the infection was related to sexual transmission, in the 6% the cause of the infection was not known. Fifty-eight patients were co-infected: 17 by HBV, 41 by HIV. Four hundred and seventy patients were sicilians, 29 patients were foreign people; the others seven were from southern Italy. The Results show that genotype 1b is predominant (30%), followed by genotypes 3a (27.6%) and 1a (26.4%). No evidence was found in support of a major increase in the prevalence of other genotypes, such as genotype 4, in relation to migration patterns.

3.1-012

Impact of ischemic cerebrovascular events on quality of life and functional performance in patients with chagas disease

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INTRODUCTION Besides the classic cardiac involvement, Chagas disease can result in neurological impairments and stroke is the most frequently reported. It is quite common that many stroke survivors remain with neurological sequelae ranging from mild to moderate. The association between these chronic and disabling diseases can lead to the emergence of depressive symptoms, contributing to poor functional performance and quality of life. Therefore, the aim of this study was to investigate the correlation between severity of stroke sequelae and depressive symptoms with functional performance and quality of life in chagasic stroke survivors.

MATERIALS AND METHODS Twenty patients were selected from a parasitary and infeccious diseases outpatient clinic. They were

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evaluated by an experienced examiner who classified the severity of stroke sequelae using the Modified Rankin Stroke Scale (MRSS). The examiner also assessed the level of depressive symptoms, functional performance and quality of life by Beck Depression Inventory (BDI), Barthel Index (BI) and WHOQOL-bref respectively. SPSS 19.0 for Windows was used for statistical analyses. A normal distribution of variables was tested and Pearson or Spearman correlation analysis was performed to evaluate the relationship among the scores on MRSS, BDI, BI and WHOQOL subscales. The level of $P < 0.05$ was accepted as statistically significant.

RESULTS MRSS shwoed correlation with BI (r = -0.663, P = 0.003), but it was not correlated with any WHOQOL subscale. Significant correlation was found between BDI score and all WHOQOL subscales (Physical: r = -0.733, P = 0.001; Psychological: r = -0.581, P = 0.012; Social: r = -0.713, P = 0.001; Environmental: r = -0.659, P = 0.003), however it was not associated with BI (r = 0.279, P = 0.262).

CONCLUSIONS The quality of life of such patients appears to be influenced by depressive symptoms than by stroke consequences. Nevertheless, the sequels seem to impair their functionality, while depressive symptoms did not.

3.2 Tobacco

3.2-001

Determinants of smokeless tobacco use among youth in Morogoro municipality, Tanzania

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Smokeless tobacco (SLT), like other tobacco products, causes illness and subsquent death to millions of people worldwide. In Tanzania, limited studies have been done on SLT compared to smoke tobacco, which have shown that SLT enclosed in sachets commonly known as 'Kuber Tobacco' is increasingly consumed by youth without knowing its health effects. This study aims to improve young people's health through establishing what social and economic factors influence them to use SLT. In addition, it assesses attitudes towards SLT and awareness of its hazards. A cross sectional design was employed using stratified and simple random sampling in selecting survey areas and systematic random technique and random numbers in selection of 300 respondents aged between 15 and 24 years. Questionnaire schedule and Focus Group Discussions were employed in data collection. Analysis employed summation scales, *F*-test, Chi-square and binary logistic regression using SPSS 11.5 software. Results showed a significant relationship ($P < 0.05$) between SLT use and the sex, age, peer influence, adult influence, religiosity, knowledge on SLT and its hazards. No relationship was observed between the number of years spent by youth in school and attitude towards SLT use. However, through logistic regression analysis, sex and peer influence were identified to have a stronger influence than other variables. It is, therefore, recommended that gender focused advocacy, and education programs through peers, religion and schools be established.

3.3 Cancer

3.3-001

Breast and cervical cancer screening: knowledge, attitudes and practice of health personnel in the region of Monastir (Tunisia)

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BACKGROUND Breast and cervical cancer are major contributors to morbidity and mortality among Tunisian women, who have low participation rates in cancer screening activities.

OBJECTIVE To describe the knowledge, beliefs, and practices of cervical and breast cancer screening among health personnel in the region of Monastir.

METHODS A cross sectional study exploring knowledge attitudes and practices of health personnel practicing in the primary health care centers of Monastir on breast and cervical cancer screening. Data were collected via a structured questionnaire containing 10 items on knowledge and attitudes towards the relevance and effectiveness of breast and cervical cancer screening. Data about the adherence of health personnel to screening activities were also collected.

RESULTS One hundred health personnel were included in our study. Midwives were the most represented (36%). Lack of education and staff training toward Clinical Breast Examination and Pap Smear Test were reported in 72.1% and 67.4% respectively. One out of two health personnel has positive cancer screening attitudes (52.4%). Health provider practiced Clinical Beast examination for 30.8% of patients and Pap smear test for 34.9% of them. In multivariate analysis, lack of staff training and the large number of consultations were the main factors negatively associated with systematic practice of breast and cervical cancer screening.

CONCLUSION More educational support and resources should be given to health care providers who must be aware of their own contribution in the promotion of breast and cervical screening activities.

3.3-002

The proportion of inflammatory breast cancer among breast cancer in the university hospital of Annaba (Algeria)

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OBJECTIVE Inflammatory Breast Cancer (IBC) is a lethal form of breast cancer, but its clinical diagnosis has not been standardized and its etiology is poorly understood

The objective of the study is to calculate the proportion of IBC among all breast cancers diagnosed and treated in gynecology ward of the university hospital of Annaba in Algeria.

METHODS The study enrolled cases of breast cancer with pathology diagnosis, admitted between January 2004 and December 2006 in gynecology ward of the university hospital of Annaba. Three hundred and fifty-two medical records of patients were reviewed. In addition to epidemiologic variables, clinical symptoms related to IBC diagnosis (warmth, erythema, peau d'orange, edema) were studied.

RESULTS Among 352 patients, 33 had an IBC (according to T4d stage) which corresponds to 9.4%. The mean of age was 55 ± 16 years old in patients with IBC vs. 48 ± 11 years old

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(P = 0.002). The proportion of patients with IBC and with an age at first birth <20 years was 14% vs. 6% in patients with non IBC (P = 0.06). The mean of the period of breastfeeding was 50 ± 56 months in patients with IBC vs. 34 ± 38 months (P = 0.09).

CONCLUSION 9.4% of IBC among all breast cancer cases is higher than founded in USA, but not more different from proportions seen in North African countries cases series as Tunisia and Egypt. More investigations were needed to explain these differences.

3.3-003

Artenimol-R in advanced cervical cancer

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Cervical cancer is a primary cause of cancer-related female mortality in developing countries. Human papillomavirus (HPV) infection is an essential factor in the development of cervical cancer. The artemisinin-derivates dihydroartemisinin (DHA) and Artenimol-R were shown to strongly inhibit HPV-induced tumor formation *in vivo*.

Given the safety of Artenimol-R in human, a first study is conducted to investigate the clinical benefit and the tumor biology of Artenimol-R on advanced cervix carcinoma. In a pilot study, ten women with advanced disease were treated with 100 mg daily during a week followed by 200 mg daily for 3 weeks. The clinical symptoms vaginal discharge and pain and adverse events were continuously followed up. Biopsy samples were taken at baseline, at day 14, and day 28 of treatment and were analyzed by immunohistochemistry. Patients were followed up and the time to the relapse of symptoms was determined. The Artenimol-R treatment induced clinical remission in all patients with median time for disappearance of clinical symptoms was 7 days (min 3 days, max 21 days). No adverse events with grade 3 or 4 occurred during the treatment period. At day 28 of treatment, the expression of tumour suppressor p53, epidermal growth factor receptor (EGFR), and proliferation marker Ki-67 as well as the number of blood vessels stained by CD31 antibody decreased, whereas the expression of TfR (CD71) increased. Following treatment period, the average time for clinical relapse was 6 months (min 4 months, max 8 months). An improvement of clinical condition and good tolerability supports the initiation of a second study that is aiming to determine the effect on tumor size and survival of a prolonged treatment period.

3.3-005

Dentistry and quality of life in patients with head and neck cancer since the diagnosis, pre, during and after cancer therapies

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Patients undergoing cancer chemotherapy or radiation therapy for head and neck cancer present significant oral complications that interfere with the patient's daily routine. As the oral complications induced by cancer therapy must be prevented and detected early, patients are referred for dental treatment at the Dental School of the UFMS before the start of chemotherapy or radiotherapy. Complications such as mucositis are perhaps responsible for the largest decline in quality of life, as it can lead to severe oral discomfort and associated factors of fear, anxiety and depression. Possible measures for its prevention is to adopt cytoprotective solutions. Prevention of deleterious effects and residual oncoterapia in the the oral cavity also minimises the risk of infection. At present besides medical treatment for treating disease, the patient should receive support from all areas involved. The interdisciplinary approach to cancer patients directly reflects the quality of patient survival.

3.3-006

Cancer, a neglected disease in Burundi, a low income country

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Cancers have, till recently, mainly associated with high income countries and to a lesser extend with middle income countries. Cancer in Burundi is not rare, but not well documented. We retrospectively observed 4161 cancer cases documented between 1974 and 2008. The source of data was the laboratory of anatomopathology of Kamenge university teaching hospital, the only of its kind in Burundi. 70% of the exams were performed before 1994. Malign tumors represented 10.6% of all anatomopathological analyses realized. Diagnosis was communicated 25.2 days after biopsy. about 51.6% of the cancer cases were male. Women were diagnosed mainly between 25 and 34 years and man between 45 and 54 years ($P < 0.05$). In man, stomach cancers (17.0%), soft tissue cancers and Kaposi sarcome (13.7%), metastases (12.7%), skin cancers (9.8%) and lymphoma (7.26%) were observed. In women, cervix cancer (15.4%), breast cancer (12.8%), stomach cancer (12.6%), les metastases (9.7%), skin cancers (7.6%) and lymphomas (7.2%) were observed. Detailed analysis showed that cancers had different epidemiological characteristics. This study shows that anatomopathological exams were less performed the last decade in Burundi, biopsies are made late but anatomopathological Results were delivered timely. Furthermore, despite high under five and maternal mortality, cancer in Burundi is prevalent and probably increasing. Moreover, some of the diagnosed cancers may be prevented by i.e. behavioral changes and vaccination. Health systems should be strengthened and resources should be put into place to diagnose and control these, mostly deadly, diseases.

3.4 Environmental Health and Climate Change

3.4-001

Contamination of hospital wards with pathogenic free living amoebae in Tehran, Iran

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INTRODUCTION Among the many genera of FLA, *Acanthamoeba* have been responsible for an increase in number of fatal diseases such as amoebic encephalitis. This is mainly due to an increasing number of susceptible hosts, including immune-suppressed patients being treated for HIV infection, leukemia, organ transplant, or excessive use of steroids. The aim of the present study was to screen for the presence of FLA in dust and biofilms from immunodeficiency wards in hospitals located in Tehran, using culturing and molecular approaches.

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MATERIAL AND METHODS A total of 70 dust and biofilm samples from wards of university hospitals were collected and examined for the presence of FLA using culturing and molecular approaches. Samples were filtered and cultured on non-nutrient agar covered with heat killed *Escherichia coli*. Based on the morphology of the amoebae in plate cultures, primer sets were applied for molecular identification. PCR was performed and sequences were analyzed against all eukaryotic nucleotide sequences archived in the GenBank database.

RESULTS Of 70 dust and biofilm samples from wards serving transplant, pediatric (malignancies), HIV, leukemia and oncology patients of five university hospitals, 37 (52.9%) were positive for FLA. *Acanthamoeba* belonged to the T4 and T5 genotypes, *Hartmannella vermiformis*, *Vahlkampfia avara* and *Vannella* spp were present. Presence of the T4 genotype on medical instruments, including an oxygen mask in an isolation room of an immunodeficiency pediatric ward, should be of concern for health authorities.

CONCLUSION These results highlight a clear need for greater attention to improved disinfection, especially where susceptible patients, such as those who are immune-suppressed, are served. To our knowledge this is the first report of these FLA in immunodeficiency wards in Iran, and also the first to identify *Acanthamoeba* T5, *Hartmannella*, *Vahlkampfia* and *Vannella* in moist habitats, such as biofilms, in this country.

3.4-002
Isolation of free-living amoebae from Sarein hot springs in Ardebil province, Iran

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BRIEF INTRODUCTION Free-living amoebae (FLA) are a group of ubiquitous *protozoan* which are distributed in the natural and artificial environment sources. The main aim of this study was to identify the presence of FLA in the recreational hot springs of Sarein in Ardebil Province of Iran.

METHODS AND MATERIALS Seven recreational hot springs were selected in Sarein city and 28 water samples (four from each hot spring) were collected using 500 ml sterile plastic bottles during 3 months. Filtration of water samples was performed, and culture was done in non-nutrient agar medium enriched with *Escherichia coli*. Identification of the FLA was based on morphological criteria of cysts and trophozoites. Genotype identification of *Acanthamoeba* positive samples were also performed using sequencing based method.

RESULTS AND CONCLUSIONS Overall, 12 out of 28 (42.9%) samples were positive for FLA which *Acanthamoeba* and *Vahlkampfiid amoebae* were found in 1 (3.6%) and 11 (39.3%) samples, respectively. Sequence analysis of the single isolate of *Acanthamoeba* revealed potentially pathogenic T4 genotype corresponding to *Acanthamoeba castellanii*. Contamination of hot springs to FLA, such as *Acanthamoeba* T4 genotype (*A. castellanii*) and *Vahlkampfiid amoebae*, could present a sanitary risk for high-risk people, and health authorities must be aware of FLA presence.

3.4-003
Relation between ambient temperature and mortality: a time series analysis approach in Tunis

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INTRODUCTION Climate change represents one of the major challenges of the 21st century. To date, the majority of research on weather and human health outcomes has been conducted in developed countries. No published analysis of the relationship between weather and health appear to have been carried out in Tunisia or more generally in North Africa. This study aims to describe the relationship between temperature and total mortality in the city of Tunis over a period of 3 years, from 2005 to 2007 using time series analysis.

MATERIALS AND METHODS The form of the relation (crude and adjusted) between mortality and temperatures was investigated using Poisson generalized additive models (GAM). Confounders included in the models were pollutant, trend, calendar month, day of the week, the period of Ramadan, and holidays.

RESULTS The whole study period suggested that the best fitting temperature variable, according the Akaike's Information Criterion (AIC), was for each day i the average of the mean temperature at day i, i-1, and i-2. The adjusted relationship between mortality and mean temperature with lag 0–2 was 'V' shaped with a steeper slope for low temperatures than for high temperatures. The shape of the exposure-response curve suggested a graphic breakpoint estimation (threshold) ranging between 25 and 27°C.

CONCLUSION This work provides a primary assessment of the relationship between climate and health in Tunisia, and should be further developed to refine our findings and to contribute to future prevention guidelines.

3.4-004
The impact of high temperature and air pollution on daily mortality in Tunis: 2005–2007

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INTRODUCTION With continued global warming, heat and heat waves are projected to increase in frequency and severity. An increase in daily mortality has been observed in association with heat thermal stress and air pollution across many regions in the world, mainly in the northern hemisphere. The objective of the present study was to estimate the impact of heat on daily mortality in Tunis, the capital of Tunisia, over a three-hot period (2005–2007) using a time-series analysis while controlling for the effects of air pollution. We also explored the confounding and synergistic effect of temperature and air pollution on mortality.

MATERIALS AND METHODS The temperature breakpoint above which mortality increases were estimated using a segmented linear regression. A Poisson Generalized Estimating Equations (GEE) model was then used to estimate the impact of heat on daily mortality. Potential confounders were pollutant, trend, calendar month, day of the week, the Ramadan period, and holidays.

RESULTS Over the three hot seasons studied, the temperature breakpoint was estimated at 27.4¢XC ('b 4.6). After adjustment

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for potential confounders, the daily mortality increased significantly by 4.0% [95% confidence interval: 0.8–7.3] for a 1¢XC increase in daily average temperature above the threshold. An increase of 10 mg/m^3 in nitrogen dioxide (NO_2) was associated with a significant increase in daily mortality [0.5% (0.1–0.9)]. No significant interaction was found between different temperature variable and the pollution indicator considered.

CONCLUSION This study highlights that heat can be a significant public health issue in countries with warm climates. Because North Africa is particularly vulnerable to heat waves, additional studies across this region are necessary to produce new insights to address this emerging threat under a changing climate.

3.4-005
Edible insects and insect consumption of Laos, a national survey in 2010

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INTRODUCTION Insects are regarded as a common food item and can contribute to food security and daily diet quality since they can provide 20–60% of their weight as protein values, and other nutrients of interest. This survey assessed the use and practices related to edible insects in the Lao population living outside of Vientiane.

METHODS We conducted a multistage sampling national survey between March and April 2010 among adults, children and vendors/collectors in all Lao provinces. We used three pre-tested questionnaires and a set of insect's picture.

RESULTS We enrolled 1629 adults, 316 children and 256 vendors/collectors in the survey in 96 randomised villages of 16 provinces and districts of Laos. Prevalence of insect consumption was above 95% among adults and children, in all provinces but two. Eggs of Weaver ant, Short-tailed Cricket, Cricket, Grasshopper, Cicada were the top five popular insects. Availability of insect was very different from North to South. Of 1059 people, <44% ever bought insects. Regular buyers were few (4.5%) with a mean expense on last time of 1.2 US dollars. Generally people will feed their children insect at a mean age of 34 months, and 85 very few ones (7%) will give to their infant at a mean age of 7 months. The majority (54%) will not advice insect feeding to infant.

CONCLUSION Insects contribute to the Lao diet and insect consumption is well accepted by adults and children. Developing insect farming may contribute to generation of income and decrease the impact on environment of extensive harvesting. More research is needed to assess the nutritional inputs of insects in the Lao diet and to evaluate how far this contribution can be extended.

3.4-006
Impact of climate change on the prevalence of gastroenteritis

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Global climate change has different effects on nature, natural resources and our life style. Some of these effects may also jeopardize our health as waterborne disease such as gastroenteritis. Warm weather or extreme climate seasoning allows the development of bacteria leading to infections related with contaminated food or water. Most of the times water is not correctly purified or stored and it may contain organic matter, including pathogen bacteria. The aim of this work is to find out if temperature change during the past 10 years in Mexico due to climatic change had influenced the prevalence of gastroenteritis in different regions of the country. Retrospective epidemiological data of gastroenteritis from the past 10 years and the temperature variation during those years on the different regions were analyzed. Total generation time change of enteric bacteria at different temperatures with and without organic matter was experimentally analyzed. Extrapolation into different regions of Mexico indicates that temperature is a determining factor on the prevalence of gastroenteritis in some of these regions that had reported warm temperatures being an average difference of 1.8°C just in 10 years (24.3–26.1°C). Environmental temperature is expected to increase almost 4°C in some regions of Mexico in the next 80 years. This kind of temperature change could be reflected on water temperature beside general outcomes. Incidence of gastroenteritis would affect gravely northern regions and the districts of the Gulf of Mexico. Our experiments with bacterial growth showed a generation time change of 164 min at 15°C compared to 72 min at 29°C in water with organic matter. The temperature and organic matter enhance the number of cells in contaminated water leading to reduce the amount of water to reach the minimal doses necessary to develop an infection and gastroenteritis.

3.4-007
Food security in Cuba is facing challenges of the climate change

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INTRODUCTION In Cuba, the protection of the environment has a constitutional status. Food security, which is essential to ensure the quality of life of the population, is significantly threatened by the climate change.

OBJECTIVES To describe the pattern of food and nutrition and health of the Cuban population and the possible negative impacts of the climate change.

MATERIALS AND METHODS A descriptive study of food and nutrition situation to identify the main problems and their possible association with the climate change.

RESULTS Changes in morbidity profiles are in correspondence with the trends and the climate changes. The frequency of droughts has increased significantly, despite its cyclical nature; the timing and extent have increased, especially towards the Eastern Provinces. The effects of the climate variability affect the productivity of crops, soil fertility and the change in agricultural areas. Are evident impacts such as reducing potential yields of crops potato, soy, beans, rice, cassava, maize and sugar cane and benefits for the growth of harmful species found influence on cattle grazing in areas predominantly with grass. Another of the great challenges that confront and increase the vulnerability is the high cost of food.

CONCLUSIONS The main challenge facing climate change has been accorded high priority to food security, an aspect that is manifested in the formulation of food and nutrition policy. These actions are based on the Cuban government's political will, intersectional work ensuring priority to the most vulnerable groups. Recognizing that climate change is becoming the defining issue of our era, the Cuban health system focuses its efforts on finding solutions to support lifestyles and economies with low

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carbon emissions, improving energy efficiency, alternative energy sources, forest conservation and sustainable consumption.

3.4-008

Study of weather related mortality in the Nouna HDSS area, Burkina Faso

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BACKGROUND Growing evidence points to emissions of greenhouse gases related to human activities as a key factor of climate change, which in turn affects human health and wellbeing. We studied the association between weather patterns and mortality in the Nouna Health and Demographic Surveillance System (HDSS) area from 2004 to 2008.

METHODS Meteorological data were obtained from 10 automatic weather stations located within HDSS area and linked to mortality data. Time series analysis methods were applied in R statistical software package to assess significant trends and associations between rainfall/temperature and mortality.

RESULTS Our results show a significant relationship between temperature and mortality, as well as a seasonal pattern in the mortality. The smooth function of precipitation effect on mortality describes a threshold with monthly rainfall below 100 mm leading to no apparent changes in mortality, while rainfall above 100 mm may lead to linear increase with up to seven times higher mortality that normal in the extreme case.

Overall, rainfall is the dominant variable explaining mortality patterns, while temperature has a minor effect in comparison with linear mortality increases during the colder months.

CONCLUSION Our study showed that mortality patterns in the Nouna HDSS tend to be closely related with climate/weather conditions, in particular extreme rainfall, and call for further investigation on cause-specific mortality patterns to better understand the particular effects of potential climate changes on population health.

KEYWORDS climate change, weather, rainfall, temperature, mortality, Nouna, Burkina Faso

3.4-009

Evaluation of the sensitivity of poultry workers to *Aspergillus fumigatus* and *Cladosporium carionii* antigens

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BACKGROUND One of the common causes of allergy in poultry workers comprises of fungal elements and most common fungi are: *Aspergillus*, *Fumigatus* and *Cladophialophora. carrionii.*

MATERIALS AND METHODS In the present study, both fungal extracts were prepared and after standardization, a crude antigens of *A. fumigatus* and *C. carrionii* Skin Prick Test (SPT) was conducted.

RESULT AND CONCLUSION SPT results showed: 55.2% of workers for *A. fumigatus*, and 39% for *C. carrionii* had positive ($P < 0.01$) The sensitivity and specificity for *A.fumigatus* allergens skin test was 95.9% and 80.4% respectively and for *C. carrionii* allergens was 94.9% and 93.9% as well. The skin test results strictly, correlated with the presence or absence of allergen specific IgE in serum. Serologic investigation with ELISA was done. The specific IgG, IgE and total IgE levels in the sera of workers were measured by ELISA technique for *A. fumigatus* and *C. carrionii antigens*. IgE levels raised in a greater number of workers (105 subjects) in compare to the control group (76 cases). Specific IgE in sera to *A. fumigatus* antigens Correlation Coefficients were determined with Pearson's Linear Regression analysis. A significant correlation between of the specific IgE in serum for both allergens was observed ($P < 0.001$). The same correlation was also seen for specific IgG. In addition, there was a significant correlation between specific and total IgE levels in serum for both allergens ($P < 0.001$).

KEYWORDS *A. fumigates*, *C. carrionii*, total IgE, specific IgE and IgG, ELISA test, skin prick test

3.4-010

Enumeration and identification of dust fungal elements produced due to inversion phenomenon in Isfahan city in December 2010

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BACKGROUND Inversion phenomenon occurs frequently in big cities. Many allergic diseases are caused by aerosols, especially fungal spore inhalation. As these spores are a resistant form of organism that can freely germinate in suitable conditions, evaluating the type and population of them will help the management of hygienic and control fungal disease.

MATERIAL AND METHODS One hundred and three dust samples were provided randomly in Isfahan in December 2010. All samples were mixed and homogenized, then 1 g of dust was diluted in distilled water containing antibacterial agents. Suspensions were cultured in standard media. Test was repeated for three times in order to obtain correct results. Isolated fungal colonies were sub-cultured on selective media and identified by their standard morphologic and physiologic criteria.

RESULTS The mean of total culture-able fungi in 1 g of dust sedimentation were about 52,500 colonies of hyaline, pheohyphomycete molds and also yeasts. *Aspergillus*, *Penicillium*, *Cladosporium* and *Rhizopus* were the most common fungi isolated from dust samples respectively. *Aspergillus* species with 41.1% and *Cladosporium Spp*. 27.8% were dominant fungi grown on Sc culture medium.

CONCLUSION This study shows the total culture-able fungi in dust. As there are many other fungal elements or others that were not able to grow under our study condition, further studies are necessary to identify all species of fungi elements in dust.

KEYWORDS enumeration, identification, inversion, weather, Isfahan

3.4-011

Epidemiology of animal bites and other potential rabies exposures and anti-rabies vaccine utilization in a rural area in southern Ethiopia

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The animal attacks on people are still a huge medico-social problem all over the world.

OBJECTIVES To determine the epidemiology of potential rabies exposures in a rural area in Ethiopia and to examine the utilization of anti-rabies vaccines.

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METHODS This is a retrospective and registry-based descriptive study including animal- related in a rural hospital in southeast of Ethiopia from 11 of September of 2006 to 10 of March of 2010.

RESULTS A total of 683 persons were included, 51.1% were female and 48.9% male; 73% were children. The median age of the study population was 9.0 years (range: 2 month–75 years). Seventy-three percent were children (38.2% <8 years old, and 35.2% between 8 and 15). The most common site of exposure was the leg (66.8%), followed by the arm (14.3%) and chest (11.8%). In children under 8 year the face was the most common site of exposition than other individuals (9.5% vs. 4.8%; P = 0.03). The main type of exposure was bites with bleeding (66.3%) followed by contamination of mucus membrane with saliva (19.7%). The primary source were dog bites (93.4%) while cat bites (2.6%) took the second place. Children (<15-year-old) were more common to be exposed to dogs (94.9%) than adults (88.7%) (P = 0.01). Fifty-seven percent of animal bites were pet owners. The most common contact to animals was 'walking by' (83.9%). The children got contact with the animals 'playing with' (10.7%) more than adults (1.1%) (P < 0.001). Unprovoked attack were more common in adults than in children (6.1% vs. 1.1%; P = 0.004). All of patients received an anti rabies nervous-tissue vaccine. Ninety-nine percent complete the treatment.

CONCLUSIONS Animal bites continue to be a problem in many parts of Ethiopia, and it is important to improve education about rabies prevention.

3.4-012
Dermatophytosis in the south of Tehran, Iran

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BACKGROUND Dermatophytosis is still considered as one of the major public health problems in many parts of the world. To identify the etiological agents of dermatophytoses in Tehran, a study was made from 2009 to 2010.

MATERIALS AND METHODS Specimens from skin, hair and nails were prepared using 20% KOH (potassium hydroxide) and were examined microscopically. In total, 315 positive samples were diagnosed. Samples were cultured on *Mycobiotic agar* and incubated at 30°; for 3–4 weeks. Colonies were examined macroscopically and microscopically to identify the species and in some cases physiological test were performed.

RESULTS *Tinea corporis* (45.7%) was the most common infection, followed by *Tinea cruris* (32.4%), *Tinea pedis* (7.6%), *Tinea unguium* (7%), *Tinea manuum* (3.8%), *Tinea capitis* (1.9%) and *Tinea barbe* (1.9%). The prominent isolated species was *Trichophyton verrucosum* with a rate of (29.5%), followed by *Epidermophyton floccosum* (27.6%), *T. mentagrophytes* (14.3%), *T. rubrum* (7.6%), *T. tonsurans* (5.7%), *Microsporum canis* (5.7%), *T. violaceum* (0.9%). 8.6% of cultures were negative.

CONCLUSION *Tinea corporis* was the most frequently observed clinical form and *Trichophyton verrucosum* was determined as the most frequently isolated dermatophyte in Tehran, Iran. These data can be used to ascertain the past and present trends in incidence, predict the adequacy of our current pharmacologic repertoire and provide insight into future developments.

KEYWORDS dermatophytosis, Tehran, Iran

3.4-013
Identification of the ethiological agents of onychomycosis in 800 patients in Tehran 2009–2010

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BACKGROUND Onychomycosis is a common nail disorder due to invasion of dermatophytes, yeasts and saprophytic moulds to the nail plates. Nearly 50% of all nail disorders are caused by fungi. In addition to the physical effects of onychomycosis, psycho-social consequences may interfere with individual's personal and professional life. The purpose of this study was to determine the prevalence of causative agents of onychomycosis in Tehran.

MATERIALS AND METHODS Eight hundred patients with prediagnosis of onychomycosis were examined during 12 months, both by direct microscopical observation of fungal elements in KOH preparations and culture to identify the causative agents. Specimens were cultured on SC and SCC media. The fungal species were identified by routine laboratory methods and the physiological tests were performed for differential identification.

RESULTS Out of 800 examined cases (320 fingernails, 420 toenails, 60 both), 386 (48.25%) were mycologically proven cases of onychomycosis. Among these positive cases, dermatophytes were diagnosed in 89 cases (23.05%), yeasts in 248 cases (64.24%) and non-dermatophytic moulds in 49 cases (12.69%). Among dermatophytes, Trichophyton mentagrophytes was found to predominant ethiological agent (68.87%). Among the non-dermatophytic moulds, *Aspergillus flavus* was the most prevalent species (40.55%). *Candida albicans* was the most prevalent yeast species (48.90%).

CONCLUSION The yeasts of the *genus Candida* were dominant cause of onychomycosis in compare to dermatophytes and non-dermatophytic moulds.

KEYWORDS ethiological agents, onychomycosis, Tehran

3.4-014
Popular restaurants, a potential source of transmission of food borne and infectious diseases in Bujumbura town, Burundi

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The second global water forum in The Hague stated that access to water, sanitation and hygiene is a human right. As small restaurants are highly frequented in Bujumbura, they represent a huge potential risk for transmission of food borne and other transmissible diseases. In Bujumbura town, we assessed the hygienic situation and sanitation of popular restaurants in Gihosha, a periurban popular quarter, and Rohera, a residential quarter. Between April and June 2010, we conducted a cross sectional survey and assessed randomly, from a pre-established list, 60 restaurants in each quarter. In Gishosha, 31.7% collected water from a collective water point, all others had an individual water tap. We observed that 41.7% of the restaurants had no garbage can and their waste was thrown in the environment (89.9%) or brought to a dumping ground (10.1%). 49.2% had a toilet of whom 82.7% were traditional. If present, 71.2% of the toilets were not maintained. A hygienic checklist revealed that 82.5% of the staff worked in (very) unhygienic conditions. Furthermore, in 96.7% of the restaurants, the utensils were not cleaned properly. In 81.7% of the restaurants, food and water were not covered. No restaurants participated in the annual medical control visit. The hygienic inspection services visited 26.1/year in the restaurants Gishosa and 29.5/year in Rohero. 76.7% of the managers found the inspections useful. Restaurant managers were in favor of more

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rigid inspections (30.8%), health promotion (50.8%) and improve water access. We conclude that restaurants may be an important source of transmissible and food borne diseases due to bad hygienic conditions. This situation is observed despite numerous hygienic inspections and an existing legal framework. However, managers were themselves not opposed to proactive measures.

3.4-015

Characterization of *Toxoplasma gondii* isolates from animals: multigenic analysis

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Toxoplasma gondii infection is widely prevalent in humans and animals worldwide. *Toxoplasma* is capable of infecting all species of warm-blooded vertebrates, and is an important opportunistic pathogen that causes severe disease in humans immunocompromised and congenital cases. *T. gondii* has an unusual clonal population structure consisting of three clonal lineages (types I, II and III), the current studies are aimed to determine its relationship with the epidemiology of the disease, therefore, given the necessary knowledge of the distribution of different *T. gondii* genotypes present in our environment this study aims to determine the genotypes of strains from animals. To establish the genotypes of strains associated with toxoplasmosis, and taking into account the difficulty of carrying out the isolation of the strain, was applied a genotyping method based on the analysis of multiple markers directly from clinical samples. Reference strains, RH (type I), MC-49 (type II) and C56 (type III), and 30 isolates from sheeps, pigs, goats, wild boars and foxes were analyzed. We proceeded to amplification of SAG1, SAG3, GRA6 and BTUB genes by nested-multiplex-PCR and the 3′ and 5′ ends of SAG2 gene by nested PCR. Subsequently, the amplification products were purified and analyzed by RFLP, digested with their corresponding restriction enzymes (Sau96I, HaeII, CfoI, Sau3AI, NciI, MseI, BsiEI and TaqáI), and definition restriction patterns for electrophoresis. The method, whose amplification and restriction patterns have been defined, allowed us the characterization of *T. gondii* directly from clinical samples without prior isolation in mice or cell culture, and even allowed the identification of the three major genotypes and atypical or recombinant strains.

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3.4-016

Mainstreaming climate change issues in Bangladesh

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The vulnerability to climate change (CC) in Bangladesh has been documented in IPCC reports. The scientific community and media have highlighted the impact of CC in Bangladesh importantly in different ways.

OBJECTIVE To assess how the issue of CC has been prioritised by Government, UN Organizations, donors, development partners and NGOs of Bangladesh in 2008–2009.

METHOD Published documents, reports, and events on the effects of CC in Bangladesh by Government, UN Organizations, donors, development partners and NGOs were analyzed for the period of 2008–2009.

RESULTS The Bangladesh Government has conducted a study on CC in 2009 and attended all conferences on CC management. A parliamentary committee for CC was formed. In 2008, UNFPA Bangladesh organized a policy dialogue on CC and released the State of World Population 2009 with a theme Population, Gender, Climate. UNDP published HDI Report 2007–2008, Fighting climate change: Human solidarity in a divided world- Risks, Vulnerability and Adaptation in Bangladesh. FAO, DFID, UNDP, EC, and the Government published its report in 2008 on Community Based Adaptation in Action: A case study from Bangladesh. UNHCR deals with climate refugees in Bangladesh. USAID's webpage title was Bangladesh – the Most Vulnerable Country to Global Climate Change. Dhaka office of EC has set a video clip in its webpage with a title: Climate Change- a call to action. Under the new long-term strategic framework, 'Strategy 2020', ADB focuses on responding to CC as part of the broader agenda of environmentally sustainable growth in Asia and the Pacific. ActionAid Bangladesh and the Embassy of Denmark implemented a project called Assistance to Local Communities on Climate Change Adaptation and Disaster Risk Reduction in Bangladesh and organized a conference in November 2009. One of its prime objectives was to generate, document and disseminate critical knowledge on community based CC adaptation for COP15. Bangladesh Center for Advanced Studies (BCAC) conducted its own research and has organized two conferences on Community Based Adaption. Bangladesh University of Engineering and Technology (BUET) organized an International Conference on Climate Change Impact and Adaptation Strategies for Bangladesh in February 2009. University of Dhaka also organized an international conference on Climate Change in 2009. Within the year 2008–2009 programs, projects, activities, reports, strategies of the Government of Bangladesh, different development partners, International/NGOs have replaced with the issue of climate change impact on Bangladesh very quickly.

3.4-017

Detection of seasonal changes and bacterial diversity of urinary tract infections in Sanandaj two major hospitals, Iran

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OBJECTIVE Urinary tract infections are among the most common infectious diseases and *Escherichia coli* is the most predominant causative agent. We aimed to investigate the seasonal variation of the commonly isolated bacterial pathogen of urinary tract infections.

MATERIAL AND METHODS This study was conducted at Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj, Iran. From 22 December 2006 to 22 March 2009, patients with urinary tract infection were referred to microbiology laboratories in Toohid and Beesat Hospitals and considered for analysis. Data were entered into a database using Microsoft Excel for pivot table analysis.

RESULTS From 10,000 clinical samples among referral patients, 498 bacterial isolates were identified, including: *E. coli* (266), gram-positive *cocci* (76), *klebsiella spp* (43), *Entrobacter spp.* (35), *Pseudomonas aeruginosa* (21), and *Pseudomonas spp.* (24). The highest rate of infection was in autumn (43.60%).

CONCLUSION This study indicates *E. coli* is a prevalent isolate and that diversity of bacteria is based on seasonal changes. It is therefore important to consider seasonal variation when empiri-

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cally treating diseases in our region and appropriate measures should be taken to determine factors associated with prevalence of such cases in order to design appropriate infectious control program.

3.4-018

Climate change and asthma: strengthening self-management and building resilience within individuals and healthcare systems

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Asthma is the most common chronic respiratory disease present in the population. In the UK, 5.4 million people are currently receiving treatment for Asthma of which 1.1 million are children. One in every five households has a person suffering from Asthma. Asthma is a multi factorial disease and most studies to date have looked at linking climate change with infectious diseases for which the etiology is known whereas the etiology for Asthma is complex and not well understood. Applying a socio-ecological model of health (Dahlgren and Whitehead, 1991), the research will examine individual and health system scale understandings of, and responses to, extreme weather events. The research will identify factors that make individuals and systems resilient (understood as adaptable, with good outcomes, in adverse circumstances), and set out ways which health professionals and healthcare organisations can boost resilience and self-management among its Asthma patient population and build preparedness within its systems. This presentation will outline results from the first phase of the research- literature review of 97 papers selected from databases (Scopus, Pub Med, Web of knowledge) linking environmental and social determinants to Asthma highlighting diverse perspectives (vulnerable populations, psychosocial stressors, environmental triggers) that influence the self-management and resilience of Asthma patients within their surroundings when faced with one or more extreme weather events as a result of evolving climate change impacts.

3.5 Cardiovascular diseases, diabetes and chronic diseases

3.5-001

Characteristics of type 2 diabetes mellitus in a tertiary health facility in Kumasi, Ghana

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Type 2 diabetes mellitus (T2D) is becoming common in sub-Saharan Africa. However, the potentially specific characteristics of T2D in African populations are inadequately defined. The aim of this hospital-based study in Kumasi, Ghana, was to characterize clinical, anthropometric, socio-economic, nutritional and behavioural parameters of T2D patients and to identify associated factors. In a case–control study conducted in 2007–2008, 1466 individuals were recruited from diabetes and hypertension clinics, outpatients, community, and hospital staff. T2D was defined by increased fasting plasma glucose (FPG). Serum lipids and urinary albumin were measured. Physical examination, anthropometry, and interviews on medical history, socio-economic status (SES), physical activity and nutritional behaviour were performed. Of the study participants, 675 (46%) had T2D (mean FPG, 8.31 mM). The majority of T2D patients was female (75%) and aged 40–60 years (mean, 55 years). Ninety-seven percent of T2D patients were aware of their condition and almost all were on medication. Nevertheless, many had hypertension (63%) and microalbuminuria (43%); diabetic complications occurred in 20%. Overweight, increased body fat, and central adiposity were present in 53%, 56%, and 75%, respectively. Triglycerides were increased in 31% and cholesterol in 65%. Illiteracy was high (46%) and SES indicators overall low. In multivariate analysis, T2D was independently associated with a family history of diabetes (aOR, 3.8; 95% CI, 2.6–5.5), abdominal adiposity (aOR, 2.6; 95% CI, 1.8–3.9), hyperlipidaemia (aOR, 1.8; 95% CI, 1.1–3.0), and with several indicators of low SES. In this hospital-based study from urban Ghana, DM2 affects predominantly female obese patients of rather low socio-economic status; hypertension and hyperlipidaemia are frequent. Prevention and management need to account for a specific risk profile in this population, and health policy and sponsoring bodies need to address the enormous challenges involved.

3.5-002

Association of socio-economic status with severity of diabetes mellitus among children attending a tertiary hospital in Dhaka city

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BACKGROUND Diabetes mellitus (DM) is the second most common chronic disease of childhood. In Bangladesh, diabetes has become a disease burden in children and adolescents.

OBJECTIVES To explore the association of SES with severity of diabetes mellitus among children attending in pediatric outpatient department of a specialized tertiary hospital in Dhaka city.

METHODOLOGY It was a cross-sectional study took place on pediatric outpatient department of BIRDEM hospital over 2 week's period. Total 144 diabetic children were selected by systematic random sampling. Structured modified questionnaire was used for collecting socio-demographic information. Child's height, weight and B.M.I for age were measured to determining the nutritional status. The wealth index was constructed by household asset information.

RESULTS/FINDINGS Adolescents (83.3%) had severe or uncontrolled diabetes and the level of HbA1c increases significantly with each year increase in age of child. There was female preponderance (53.5%) over male (46.5%) but diabetic severity was not associated with child's genders ($P = 0.525$). Mean HbA1c levels did not vary between SES, but varies with child's age. Diabetic severity in children was not significantly associated with SES ($P = 0.167$). SES varies with father's education but not with child's age, sex, and height or HbA1c level. Stunted children were found more severely or uncontrolled diabetic ($P < 0.050$). The majority of the children from rural area were from low SES. Among parents of diabetic children, majority of them had no formal schooling and were agricultural worker or farmer. Parents who had education less than primary level their children had significantly poor control of diabetes ($P = 0.05$) and with each level of increase in education significantly reduces the mean HbA1c level ($P < 0.05$).

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Conclusion Malnourished adolescents from less educated parents had more severe or uncontrolled diabetes which need an updated quantification of the growing public health burden of diabetes across the whole country.

3.5-003

Overweight and obesity among secondary high school students in Ho Chi Minh city, Vietnam

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Introduction Two previous surveys conducted in HCMC revealed an increasing prevalence of overweight and obesity among adolescents, from 5.9% and 0.7% in 2002 to 11.7% and 2.1% in 2004, respectively. This emerging health problem of obesity in youths from urban areas of HCMC led the city government to implement intervention programs aiming at reducing obesity among children. Our study was conducted in 2010 in order to assess the change in overweight and obesity prevalence among secondary high school students from HCMC.

Methods For a multistage cluster sampling method with random selection of schools, random selection of classes within schools, and all students in classes, the required sample size was 2056 students, assuming a 5% precision on expected prevalence. For all students, we collected age, weight, height, food frequency, and physical activities through interviews. Age- and sex-adjusted overweight and obesity were defined using International Obesity Taskforce cut-offs.

Results Of 2050 students, 17.5% were overweight, 3.27% were obese ($P < 0.001$), and 4.4% were underweight. These three prevalences were significantly higher in boys (6.5%, 22.5%, 5.4%) than in girls (2.9%, 12.9%, 1.32%, $P < 0.001$). Overweight and obesity were higher in students from wealthy districts (20.6% and 3.8%, $n = 1309$), than less wealthy districts (12.2%, $P < 0.001$ and 2.3%, $n = 751$). Underweight prevalence was significantly higher in less wealthy districts than in wealthy districts ($P = 0.012$).

Conclusion Overweight and obesity prevalence continues to increase among secondary-school students while underweight prevalence still remains. Therefore, more aggressive intervention programs are required in HCMC.

3.5-004

Assessment of the management capacity of NCDs in Zambia

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Background Non-Communicable Diseases (NCDs) are responsible for a lot of morbidity and mortality in developing countries. Most of these NCDs are preventable and in many cases can be treated with simple inexpensive interventions at the primary care level.

Methods A cross sectional survey was conducted using a structured investigator administered questionnaire in six out of nine provinces of Zambia from November 2008 to February 2009. Six general hospitals, four district hospitals, 10 urban health centres and 24 rural health centres were surveyed.

Results Of 44 facilities visited, 93.2% had sphygmomanometers, but only 52.3% used them routinely on their patients. Only 15.9% of the facilities had a management protocol for hypertension. Challenges in managing hypertension were cited as inadequate drug supplies 32.1%, (CI 24.2–40.8, $n = 131$), followed by inadequate staffing and diagnostic equipment respectively (22.1% and 22.9%, CI 15.4–30.2 and 16.0–31.1). For asthma, only 8 (18.2%) of the facilities agreed that they had and used protocols for the management of asthma. Challenges faced in asthma management were lack of drugs (38.3% CI 29.1–48.2% $n = 107$), staffing problems 14.0% (CI 8.1–22.1%), inadequate diagnostic equipment (10.3% CI 5.2–17.7%), poor drug compliance (9.3% CI 4.6–16.5%). Although the facilities had good supply of oral drugs for asthma, only 9.1% of them had inhaled corticosteroids. For diabetes, Only 17 facilities (38.6%) had a glucometre for measuring blood sugar, and these were found mostly in general and district hospitals. As in many developing countries, there are significant challenges in the management of NCDs at all levels of the health care system in Zambia. There is need for a new drive towards prioritizing NCDs through evidence based policy formulation and health system strengthening.

3.5-005

Patient involvement in care strategies for chronic diseases: the cases of diabetes and HIV/AIDS care in Mali. What lessons can be learned?

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Introduction The burden of chronic diseases dramatically increasing in developing countries, patient empowerment and patient-centred care are once again being promoted as key strategies for health system strengthening. In Sub-Saharan Africa, some progress has already been made in the field of HIV/AIDS, where patients are often involved in care management, but very few programmes have been developed for non-communicable chronic diseases so far.

Methods We focused on diabetes and HIV/AIDS in Mali. A literature review and interviews with national stakeholders and international donors were conducted to analyse national strategies for diabetes and HIV/AIDS, and to put into perspective the involvement of patients in the policy making process and care services.

Results The case of Mali is very emblematic as the first mobilisation of diabetes patients dates back to 1991. But their empowerment has since been limited by the lack of national political support, of financial and material resources, and by the little international concern. For HIV/AIDS, the first mobilisation of patients at the beginning of the 1990s emerged in a context of 'emergency' and relief care. Since HIV/AIDS has received close political attention, with the introduction of free antiretroviral drugs at the beginning of the 2000s, and donor funding has also increased. To date, the number of peer support programmes has multiplied, as has the number of patients' associations and local NGOs.

Conclusion Analysing the evolution of diabetes and HIV/AIDS care raises the question of how does the chronicity of a disease influence patients' empowerment. Also, the case of Mali clearly shows that patient mobilisation needs political leadership and international support to really succeed in influencing health policies and actively participating in care management. Lessons can be drawn on how to better involve patients in care and policymaking and improve care for non-communicable chronic diseases.

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3.5-006

Impact of diabetes on the socio-economic aspects and health seeking behaviour of patients attending coast general hospital Mombasa, Kenya

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BACKGROUND Diabetes mellitus is a chronic disease whose current global spread has the characteristics of a pandemic, type 2 is more common than type 1 in developing countries. The increase in incidence follows the trend of urbanization and lifestyle changes, perhaps most importantly a 'Western-style' diet. As a result people put on weight, making them more susceptible to chronic illness including, heart disease and cancer, adding to the strain on already overstretched health care systems.

OBJECTIVE To determine impact of diabetes on the socio-economic aspects and health seeking behaviour of patients attending coast general hospital Mombasa, Kenya

METHODOLOGY A descriptive cross sectional study which involved 246 respondents, who were purposively selected, was conducted. Quantitative and qualitative methods were utilized. Questionnaires and KII were used for data collection. Data from questionnaires were entered into SPSS and was analysed using Epi info version 3.3.2 while NVIVO version 7 was used for coding and thematic analysis of data from KII was described.

RESULTS Majority (70%) of diabetics were aged 50 years and above. Females were the majority (78.7%). Those with other chronic conditions alongside diabetes were (40.8%). Level of education was significantly associated with the type of hospital attended ($P < 0.001$). Patient knowledge on their status was significantly associated with the type of health facility attended ($P < 0.001$). Up to 97% of the patients informed us that they had diabetes medical services from the the public health facility, compared to only 57.5% of those who had not been informed that they had diabetes. Income levels and the age were not significantly associated with the type of hospital visited respectively.

CONCLUSION Investment in health education using various methods of information dissemination to reach out to the public is required. This would improve the public knowledge regarding diabetes prevention, management and care.

3.5-007

Estimation of the mortality caused by diabetes mellitus in Panama and its relationship to gender and cardiovascular risk factors

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Although diabetes mellitus is among the top five leading causes of death in the Republic of Panama, little information is available to its occurrence according to gender compared with other countries of the region. We compiled data from the National Survey of Quality of Life, Health and Living Standards Survey obtained in 25,000 individuals, data from the national population census of 2000 and 2010 and from the national registries of mortality from all causes spanning the years of 2001–2010. From these data sets we generated estimates of crude, adjusted and specific rates of mortality and compared these estimates with the presence of obesity, hypertension and hypercholesterolemia by gender in different regions of the country. After compiling and analyzing these data for mortality from diabetes mellitus for the years 2001–2009 and risk factors for we found that women in Panama have a higher mortality rate from diabetes and also higher prevalence of obesity and hypertension. We conclude that in the decade 2001–2009, women in Panama had a higher mortality from diabetes than men. Higher prevalence of certain risk factors explains in part these findings.

3.5-008

Knowledge of myocardial infarction in sample populations: a comparison of a developed and a developing nation

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OBJECTIVE To quantify and compare the knowledge of risk factors, symptoms and empirical treatment of Myocardial Infarction (MI) in sample populations presenting to hospitals in a developed and a developing country.

METHODS This hospital-based, cross sectional analysis of a convenience sample of population was conducted at waiting areas of Emergency Rooms of two comparable tertiary care academic hospitals A- 1395 bedded in the US and B- 1766 bedded in India. Participants aged 18 years and above of either gender were provided with standardized questionnaires comprising of demographic data and 13 items to assess their knowledge of risk factors, symptoms and empirical treatment of MI. Descriptive statistical analysis was performed using SPSS version 16.

RESULTS Of 434 participants (217 at each site) 63.5% were males with mean age 38.4 years (range 18–92 years). Symptoms such as chest pain, heaviness (83%), radiation of pain to arm (74.5%), risk factors- smoking (74.8%), obesity (75.7%) and hypertension (68%) were commonly identified by respondents. However, diaphoresis (59.5%), dyspnea (58.3%), diabetes (43%); empirical treatment – aspirin (46.5%), accessing nearest hospital (54.5%) and treatment with IV medications (32.8%) were poorly recognized. Diaphoresis and hypertension were more correctly answered at developing country site B ($P = 0.002$). Overall mean knowledge score at site A (8.64, max. 13) was significantly more than that at site B (7.16) $P < 0.001$.

CONCLUSIONS Overall inadequate knowledge about Myocardial Infarction was elucidated at the two sites. Sample population in a developed country is more likely to have a better knowledge base than a comparable sample population in a developing country. Awareness about empiric treatment, atypical symptoms, and diabetes mellitus as a risk factor should be effectively focused in educational interventions especially during waiting areas of physician offices/Emergency Departments to raise the awareness among the populations as primary and secondary prevention strategies to reduce the burden of MI globally.

3.5-009

Are the risk factors of type 2 diabetes and hypertension related to affluence and/or urbanisation. A study done in Blantyre and Mulanje, Malawi, June 2010

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Diseases of affluence are those thought to result from increasing wealth and ease of life. Hypertension and type II diabetes are regarded as such. However with the rise in the prevalence of hypertension and type II diabetes in developing countries, the 'diseases of affluence' paradigm was questioned. This only pointed out that economic-epidemiologic patterns are more complex than the 'diseases of affluence' paradigm would suggest. A cross-sectional study was conducted to assess the risk factors for hypertension and type II diabetes and to find out if they are associated with urbanisation and affluence in three hospitals in Blantyre and Mulanje with a total sample size of 153. This

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included enquiry into lifestyle risk factors and conducting measurements of blood pressure, total blood cholesterol, random blood glucose and height and weight (for Body Mass Index). The Results showed that most of the risk factors that were assessed were associated with neither urbanisation, increase in income nor affluence. Increased BMI and low levels of physical activity were the only risk factors associated with urbanisation and increasing socio-economic status. Therefore it was concluded that it is simply not affluence that affects the distribution of hypertension and diabetes and their risk factors and that these disease should therefore not be considered as merely 'diseases of affluence'.

3.5-010

Subclinical peripheral arterial disease in diabetic patients: do microalbuminuria and estimate glomerular filtration rate predict pathological Ankle-Bachial index?

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INTRODUCTION A 20–30% prevalence of peripheral arterial disease (PAD) has been reported in diabetic patients but little information is available on PAD risk factors in them. Chronic kidney disease (CKD) has been associated with an elevate prevalence of PAD.

OBJECTIVE To analyze the relationship between ankle-brachial index (ABI) and CKD, defined by either microalbuminuria or a reduced estimate glomerular filtration rate (GFR), in diabetic patients with no known atherosclerotic cardiovascular disease (ACD).

MATERIAL AND METHODS A cross-sectional observational study realized in a health care center including diabetic patients without ACD. The variables collected were age, gender, cigarette smoking and history of obesity, hypertension, dyslipidemia and diabetes, with blood pressure control and retina study. The analysis practiced included glycated hemoglobin (HbA1c), lipids, serum creatinine, GFR using Modification of diet in renal Disease (MDRD) equation and microalbuminuria in an overnight sample. ABI was calculated in all patients according to the AHA definition, after Doppler ultrasound probe, and defined pathological using 0.9 as a cut off point.

RESULTS We studied 115 diabetic patients, mean age 68.5 years, 60% males, 16.5% active smokers, 69.6% hypertension and 65.2% hypercholesterolemia, with 24.3% retinopathy and 38.4% HbA1c $\geq$ 7.5. Renal function study detected 1.7% creatinine $\geq$1.5 mg/dl, 16.5% GFR < 60 ml/min/1.73 m^2 and 24% microalbuminuria $\geq$20 mg/g. ABI < 0.9 was noted in 20% of patients. Statistical study showed concordance between ABI and microalbuminuria associated to retinopathy (κ = 0.38) more than microalbuminuria alone (κ = 0.22) and more than HbA1c (κ = 0.17), but not with GFR or creatinine. Correlation was also observed between ABI and microalbuminuria (P = 0.003).

CONCLUSIONS Relationship between CKD and ABI in diabetic patients is confirmed. Microalbuminuria is the renal function determination correlated to subclinical PAD in these patients. Microalbuminuria should be considered a PAD risk factor in diabetes and screening ABI should be performed to early detection of PAD and treatment.

3.5-011

Use of secondary preventive drugs in patients with acute coronary syndromes in Tunisia

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OBJECTIVES The survival benefits achieved by prescription of antiplatelet agents, B-adrenoreceptor antagonists (beta-blockers), angiotensin II receptor blockers (ARB), and lipid lowering agents in patients surviving the myocardial infarction (MI) have been well documented in large clinical trial. The main objective of this study was to evaluate the progress of cardiovascular secondary prevention practices in Tunisia.

METHODS In Tunisia, the Prevention of Recurrence of Myocardial Infarction and Stroke (PREMISE) study was conducted in two phases (2002 and 2009) during a period of 6 months in each year. Five hundred patients were recruited (250 in 2002 and 250 in 2009). The recruitment criteria were: previous MI, stable angina, unstable angina, percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft (CABG), and if their first cardiovascular event had occurred more than 1 month but not later than 3 years ago. We compared the total of both patient cohorts, using the prevalence of cardio-vascular risk factors (CVRF), MI characteristics, and the treatment prescribed at hospital discharge.

RESULTS A significant increase in the average age (61.5 ± 10.2 vs. 63.7 ± 10.36 years) and in thepercentage of women (27.2% vs. 46.4%) were found between 2002 and 2009. A significant increase was observed for statins (38.9% vs. 70.3%) and ACE inhibitors (49.3% vs. 69.9%), while non pharmacological prescriptions as healthy diet or tobacco cessation had opposite trends. Nevertheless, still in 2009, about 30.1% and 36.5% of the patients didn't receive respectively ACE inhibitors and statins, after (CHD) occurs. Despite this increase of prescriptions, adherence to treatment did not change substantially.

CONCLUSION Although the use of cardioprotective drugs had increased in CHD patients, there are still gaps in secondary prevention in Tunisia. The recommended strategies of secondary prevention need to be applied more intensively in clinical practice.

3.5-012

Cardiovascular risk factors control levels in peripheral arterial disease patients: a mediterranean primary health center study

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INTRODUCTION Patients with peripheral arterial disease (PAD) are at high risk of cardiovascular events and death. Secondary prevention in primary care can reduce cardiovascular event rates but risk factors control levels in PAD have not been reported enough.

OBJECTIVE To analyze the control level of traditional cardiovascular risk factors in patients with PAD attended in a primary health center placed in a Mediterranean area.

MATERIALS AND METHODS A cross-sectional descriptive study including patients diagnosed of PAD, with or without coronary and cerebrovascular disease, whose risk factors were controlled in primary attention despite vascular surgery surveillance. The variables collected were age, sex, cigarette smoking and history of coronary and cerebrovascular disease, arterial hypertension, diabetes mellitus and dyslipidemia. Blood pressure control and

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analysis in the last year, including glycate hemoglobin (HbA1c) and lipid serum levels, were reported. Risk factors control levels were evaluated according to Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II).

RESULTS We studied 174 PAD patients, mean age 71.47 years, 62.64% males, 21.83% associated to coronary disease, 10.91% to cerebrovascular disease and 2.8% to both vascular disease. Risk factors prevalence observed was 74.13% hypertension, 57.47% current smoking, 45.97% dyslipidemia and 41.95% diabetes. Risk factors control levels were 47.7% blood pressure ¡Ü130/80, 37.93% ex-smokers vs. 19.54% active-smokers, 60.34% total cholesterol <200 mg/dl and 54.02% low-density lipoprotein cholesterol <100 mg/dl. Diabetic PAD patients had 53.42% HbA1c < 7%.

CONCLUSIONS Risk factors prevalence in our PAD patients is similar to that reported in previous studies except in dyslipidemia, third in order but only referred in half of them. Risk factors control levels evaluated are worse than it was expected, especially lipid serum levels. A more intensive pharmacological treatment is needed. We encourage general practitioners to self-audit risk factors control levels in their PAD patients and improve secondary prevention.

3.5-013

An analysis of diabetes care in first line health facilities in Kinshasa, DR Congo

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INTRODUCTION Diabetes prevalence in Kinshasa is estimated at 5%, resulting in a caseload of about 200,000 adult diabetics. The study context is a 'network' of 60 first line health care facilities and four referral centres in Kinshasa offering a standardised package of diabetes care to approximately 7000 patients. Our research questions were: what is the present health status of diabetics? What is the current situation of the professional care provided to them and what is the extent of self-management by diabetics themselves?

METHODS AND MATERIALS Based upon predefined criteria, we selected three health centres. We conducted an observational study, in the form of a cross-sectional survey that included all diabetics routinely followed up. Data were retrieved from the central network register and individual patient records. Analysis was done at aggregate level and for each centre separately.

RESULTS AND CONCLUSIONS Three hundred and fifty-one Patients are being regularly followed up in the three facilities. Their median BMI is 25.6. Forty-one percent of all patients had normal glucose levels during their last visit. 1.6% was treated for a diabetic foot in the last year. Sixty-four percent also receive anti-hypertensive medication. Fourteen percent of the patients are on a dietary regimen only; 40% of all patients follows a regimen for glucose-control that includes insulin. People on insulin had less well controlled glucose levels. Only 50% of the patients managed to come for the last 3-monthly appointment, but 70% managed to undergo annual screening of risk factors. Few studies have been published about the practice and outcomes of diabetes care in sub-Saharan Africa. This study attempts to addresses this gap. Our results illustrate, in line with studies in Cameroon and Ethiopia, the possibilities and challenges. Further analysis is needed to see how diabetes care can be improved within the limits of the current means available.

3.5-014

Thymocyte nuclear protein 1: a novel autoantigen of heart valve lesion of rheumatic heart disease

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Autoantigens play a key role in mechanism of rheumatic heart disease (RHD), in order to investigating autoantigens of RHD patients which could be developed into potential molecular biomarkers of heart valve lesion, a RHD expression library with fibrosis heart valves was constructed and immunoscreened. In this research, a novel autoantigen relative to heart valves of RHD was primarily reported. First the total RNA of fibrosis heart valves tissues of RHD patients was extracted and mRNA was isolated, purified and reverse-transcripted to long cDNA. Phage was used to recombining cDNA to be expression library. The library was immunoscreened by serum of active rheumatic fever patients. An autoantigen positive clone was immunoscreened. The autoantigen gene was analysed by PCR, sequencing and bioinformatics. It also was subcloned and expressed in vitro. Western blotting was used to identify the expression protein. In this research, an expression library with heart valve tissues of RHD patients was successfully established. The titer of the primary library was 8.3¡Á106 pfu/ml, recombinant rate of it was 99% and 81% inserted segments were larger than 1 kb. Positive antoantigen genes were screened and one of it was homologous to thymocyte nuclear protein 1 (THYN1) which is firstly reported as an autoantigen from valve tissue of RHD patients. An unreported mutation C344T was found which induced Ser instead of Pro. The recombined vector could expression objective protein *in vitro* which could be reacted with sera of active rheumatic fever patients and rheumatic heart disease patients, but could not be detected by sera of health persons. This research implied that THYN1 was a candidate molecular biomarker of valve lesion of RHD. The function of THYN1 is rarely known and it's highly conserved among vertebrates and plant species and may be involved in the induction of apoptosis.

Track 4: Health Systems And Resources

4.1 Health Policy and Financing

4.1-001

Delphi expert survey on promoting research and development into drugs for neglected diseases

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INTRODUCTION Tropical infectious diseases are called neglected, because they are characterized by an R&D deficit. A similar deficit exists for rare (orphan) diseases that do not promise a sufficient return on R&D investment. For rare diseases, public health policy responded with financial and non-financial incentives for the pharmaceutical industry, so-called orphan drug regulations. Comparable instruments for neglected diseases (NDs) do not exist, which prompts the question whether a regulatory approach might benefit R&D into neglected diseases.

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METHODS AND MATERIALS In an international online Delphi survey conducted in two rounds, 117 (first round) and 56 (second round) experts of various professional backgrounds and affiliations were asked to rank the main causes for the R&D deficit and to assess the desirability and feasibility of a regulatory instrument to promote R&D into neglected tropical diseases. Orphan drug regulations and the draft Medical Research and Development Treaty were chosen as a frame of reference for measures to be included in such instrument.

RESULTS Poverty and a lack of (sustainable) funding were judged crucial for the R&D deficit into NDs in both rounds of survey. The majority of the respondents (88.4% first round, 86.8% second round) considered it desirable to have a regulatory instrument to promote R&D for neglected diseases; 77.9% (first round) and 79.3% (second round) also considered it feasible. Of the key provisions of orphan drug regulations, tax credits, fee waivers and protocol assistance were judged favorably also for R&D into NDs, while market exclusivity was viewed critically. A majority (87.1% first round, 77.2% second round) supported the concept of national funding obligations for neglected diseases, proposed by the medical R&D Treaty.

CONCLUSIONS The Delphi Survey confirmed a strong interest in a regulatory instrument to meet the challenge of promoting R&D into neglected diseases.

4.1-002

Targeted subsidies to improve enrolment to community-based health insurance among poorest households: why do we need to consider community perceptions and criteria of poverty during the identification process, Nouna Burkina Faso

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INTRODUCTION Most of Poverty analyses in Developing country are based on monetary poverty concept targeting incomes and consumption of households. Thus a household is regarded as poor if its income or its consumption level is low compared to a poverty line predefined. This conception ignore the own perceptions and aspirations of people; hence the development of community methods and criteria to define poverty in a given area.

OBJECTIVE To identify Community perceptions and criteria of poverty (CPCP)

METHODS The study was conducted in Nouna Health District in Burkina. A sample of heads of households was selected in each village to participate to the FGDs. Community wealth ranking was used as key method. FGDs around the concepts and perceptions of poverty were organized in 41 villages and seven sectors of Nouna town. Discussions were recorded, transcribed and analyzed using Nvivo8.

RESULTS We will retain that poverty is mainly deprivation of capacities, basis needs shortage, indecent conditions of life. Another important aspect is an absence of social capital ie a social relations network on which to refer to face the problems.

CONCLUSION Poverty is better felt by the one who is concerned or lives in the context than anybody. Therefore the community perceptions and criteria of poverty reflect certain realism in the way to appreciate the topic. CPCP are therefore determining if we want to undertake community development actions.

KEYWORDS self appraisal, poverty, community, rural, Burkina Faso

4.1-003

A progress report from score, the schistosomiasis consortium for operational research and evaluation

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SCORE, the Schistosomiasis Consortium for Operational Research and Evaluation, is moving forward on multiple different fronts to find research-based answers to questions that confront schistosomiasis control managers. This presentation will feature the several the findings of four very different portions of SCORE that have come to culmination over the last year. One is the ongoing randomized trials of drug delivery in Niger, Cote d'Ivoire, Kenya, Tanzania and Mozambique in areas with moderate to high prevalence of *Schistosoma haematobium* or *S. mansoni*. The second is the SCORE Data System, a newly developed cell phone-based data capture and management system. The third topic will be data comparing the use of a point-of-contact urine assay for *Schistosoma mansoni* infection in five different countries. Finally, the SCORE Rapid Answers Project, which uses evidence-based decision analysis in aid of policy development, will be discussed. For policymaking, the full benefits of anti-schisto MDA need to be well-studied, well-defined, and much more widely disseminated and promoted to achieve the worldwide goal of Schistosoma-related disease elimination.

4.1-004

Implementation of national and international health policies in two health districts in Benin: actors and practices

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Benin and its most important donors have signed the Paris Declaration on aid effectiveness. Benin has recently issued its national health policy (NHP) (2008–2018) as well as a 3-year plan for health sector development (2010–2012), which constitute the single policy framework for health development, supported by the International Health Partnership (IHP+) country Compact signed in 2010. The World Bank, Belgian Technical Cooperation and UNICEF have all signed the IHP+/Compact but their approaches to supporting the NHP do not fully converge. This communication aims to respond to the following question: Who are the actors and their practices in the implementation of national and international health policies in Benin? Using data collected at grassroots actor level in two health districts (Come and Bohicon) in South Benin, the communication presents the involved actors and analyses their practices, focusing on whether or not they are congruent with national and international health policies. The theoretical framework of the study is socio-anthropology of development interventions applied to public health. We found that the strategic actors who influence local health practices vary from one area to another, and that donor approaches differ, contrary to the principles of the Paris Declaration. Benin has issued national health policy documents but almost all agents intervening at operational level are unaware of their contents. This situation implies huge gap between the theoretical policies (be they national or international) and their implementation in the field.

KEYWORDS development aid, health sector policy, policy-implementation gap, Benin

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4.1-005

The evaluation of the Paris declaration on aid effectiveness: what does it tell us? What results for the health sector?

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Five years after the signing of the Paris Declaration on aid effectiveness by donors and developing countries, there is a climate of high expectations about the results achieved and the impact at sector level. Several evaluations and monitoring surveys have been conducted by the OECD to measure progress made so far in implementing the principles of ownership, alignment, harmonisation, results and mutual accountability. For the health sector, results have also been documented in the context of the International Health Partnership (IHP+). We critically reviewed all the aid effectiveness surveys and evaluations published since 2005 by the OECD and IHP+ so as to analyse what results have been achieved so far as well as the relevance of the frameworks used. To date, results of the implementation of the Paris Declaration are mixed. In the health sector, good progress has been achieved in terms of country ownership and coordination, while alignment and use of country systems, managing for results and mutual accountability lag behind. However, the results recorded often reflect different interpretation of the indicators depending on the respondent, the data available or the time of the survey. Making generalisations is therefore difficult and comparisons between country surveys and over time may not be appropriate. Linking progress in aid effectiveness with improvements in health outcomes is also controversial. Results from the evaluations of the Paris Declaration should be used with caution in the current debate about aid effectiveness. What do the indicators used actually tell us? How realistically can aggregated scores reflect complex issues such as aid effectiveness in different countries, by different donors and over time? Improved evaluation is clearly needed. Data collection should be more rigorous and at sector level contextual factors and behaviour change should be better assessed, over a longer term and through more qualitative comprehensive methods.

4.1-007

Performance of health insurance program in Nigeria: providers vs. insurers perspectives

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INTRODUCTION Health insurance scheme is a complex entity with different stakeholders. Performance assessment derived after 10 years of inception and 5 years of active implementation of the Nigerian Health Insurance Scheme serves as an impetus to redefining of policy and operation plans to meet stakeholders' needs and expectations. Both providers (HCPs) and insurers (HMOs) play major roles during implementation. However, there is the lack of studies that focus on their standpoint. We examined their perceived-performance of the scheme's reimbursement, referral matters and quality assurance arrangements. Factors that influenced their appraisal were identified.

METHODS AND MATERIALS Eight Focus group discussions (FGDs) with HCPs and five in-depth interviews with HMOs regional managers were conducted in Kaduna-Nigeria. Data was complemented with four in-depth interviews with HCPs management staff. In total 68 HCPs and 5 HMOs regional managers participated in the study.

RESULTS Providers and insurers rated well the overall performance of the scheme, but with complaints. Capitation payment was appreciated due to receipt of reimbursement while fee-for-service payment was criticized due to delay in authorization code to offer services to clients. Providers reported delays in approval to refer patients across levels of care. Insurers reported inconsistent quality assurance monitoring due to lack of transportation funds and shortage manpower. Factors that influenced their appraisal were the size of registered enrollees, frequency of referrals and providers/insurers funds availability. Facility type and previous encounter with HMOs influenced providers' appraisal, while for the HMOs were time of accreditation and human resources shortage.

CONCLUSIONS There is the need to improve on fee-for-service payment method and ensure unified time of capitation payment to providers. Patients' referral across levels of care and quality assurance arrangement could be improved by ensuring better communication, use of uniform quality assurance checks and strengthened monitoring.

4.2 Innovation and Technologies for Global Health

4.2-001

Networked data: a reality for researching, managing and benchmarking in healthcare

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OBJECTIVES The adoption of new technologies in healthcare processes generates large amounts of computerized data. Due to technological and budget limitations, data is only available to a reduce number of professionals within organizations and force most of the interchange of information to be based on calculated values, aggregating the original data. Our objective is to determine the impact in the decision-making processes of having access not only to the aggregated value, but also to the underlying data used and the knowledge of the exact calculation process.

METHODS To facilitate the access to data and analysis Bevalley network was developed and tested in a living lab. Bevalley is a worldwide network where users share, comment and tag data and analysis in real time. It is accessed easily through any computer with Internet connection. The usage of Bevalley is free of charge. Every professional or healthcare entity can upload easily any kind of tabulated data to Bevalley having the absolute privacy of the uploaded data guaranteed. Data can be stored within the entity's IT infrastructure. In Bevalley data can be analyzed using several tools. Both analysis and data can be shared.

RESULTS More than 20 healthcare entities and 500 professionals are using Bevalley network, from over 40 different countries. Research and management programs were built using the network where every participant had access to raw data used to calculate results and indicators and also created their own analysis that shared with other participants. Behavioral changes happened at several levels, some participants even developed spontaneous benchmarking systems to compare themselves with similar professionals/entities in the network.

CONCLUSIONS The usage of a shared data and analysis network not only provides a better way of making informed decisions in healthcare, but has also opened a window on new approaches on collaborative work and reputation measurement.

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4.2-002

Substandard medicines: a neglected plague?

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The global pharmaceutical market is characterized by multiple qualitative standards: patients in wealthy, strictly-regulated countries can generally be confident of the quality of medicines available to them, whereas a large majority of the population in resource-limited countries is at risk of receiving poor quality medicines. The reasons for this gap are multiple and include the lack of resources of regulatory authorities, the increasing complexity of the global pharmaceutical market, the ever-increasing pressure on prices, the lack of awareness of key-actors, the lack of transparent public information on the quality of medicines and the lack of political will at national and international level to address this structural problem. While a growing number of initiatives focus on counterfeit medicines, the problem of substandard medicines, which mainly affects-resource-poor countries and which has at least an equally deleterious impact the health of individuals and populations, remains insufficiently addressed. In addition, most current initiatives aiming at strengthening quality assessment for medicines focus on the fields of HIV-AIDS, malaria and tuberculosis, while there is little or no public guidance on the quality of medicines for other infective and chronic diseases. To address this technical and ethical problem, QUAMED (*) brings together Northern and Southern partners with the ultimate objective of fostering universal access to quality medicines. This will be achieved by performing technical assessments of products, manufacturers and distributors, and by analysing these findings as a basis for improving procurement strategies as well as developing evidence-based policies to improve quality of medicines in public health programs worldwide. We will present the preliminary findings from the first year of activities of Quamed, and we will discuss their possible short and long term implications, with special focus on the impact of quality of medicines on the performance of health systems.

(*) http://www.itg.be/itg/GeneralSite/Default.aspx?W-PID=705&MIID=528&L=E

4.2-003

Identification of electrophoretic proteins pattern of *Fuzarium verticillioides* isolates from maize by SDS-PAGE

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INTRODUCTION The *Fusarium* species is one of the most important toxigenic and allergenic fungi species. Some of them possess high allergenic components and some produce the various toxins such as fumonisins and T2-toxins. *Fusarium solani* is so important among allergenic *Fusarium* species and *Fusarium verticillioides* (*Gibberella moniliformis*) is intensely toxigen. This fungus produces B1, B2, B3 fumonisins on the crops such as maize, rice, cane, etc. The purpose of this study is identification of electrophoretic cytoplasmic proteins pattern of Iranian *Fusarium verticillioides* isolates from maize by SDS-PAGE.

METHODS AND MATERIALS In this study 20 isolates of this species were analyzed. The isolates previously were identified and confirmed in South Africa mycology center. Using Bradford method was measured protein range of each isolate and obtained its' molecular weight by SDS-PAGE.

RESULTS AND CONCLUSION The results indicated total 50 protein bands with molecular weight from 7 to 157 KD. Maximum protein bands were related to F4 and F10-c isolates with moderate toxigenicity and minimum protein bands to M2-a, K6 and A7-b isolates with low, moderate and high toxigenicities. The comparison of electrophoretic cytoplasmic proteins pattern of isolates with grouping based on toxigenicity did not show any correlation between their protein pattern and range of toxigenicity. It means that with this pattern we cannot classified these fungi from the point of view of toxigenicity.

4.2-004

Evaluation of IgG avidity ELISA test for detection of acute toxoplasmosis

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INTRODUCTION *Toxoplasma gondii* is one of the most prevalent parasites of human and warm- blooded animals. It is important to diagnose of the stages of toxoplasmosis in pregnant women and immunocompromised individuals. This study was performed to determine the acute toxoplasmosis by IgG avidity ELISA test in human.

METHODS AND MATERIALS One hundred serum samples were collected from different laboratories in Tehran and tested for detection of anti-Toxoplasma IgG and IgM antibodies with indirect immunoflurescent antibody test (IFAT) and ELISA test. The IgG avidity ELISA test was performed in duplicate rows of 96 well micro-titer plates, one row washed with 6 M urea and the other with phosphate buffer saline (PBS, pH 7.2), then the avidity index (AI) was calculated.

RESULTS AND CONCLUSION Sixteen out of 18 (88.9%) sera with acute toxoplasmosis showed low avidity levels (AI¡Ü50) and 76 out of 82 (92.7%) sera in chronic phase of infection showed high avidity index (AI > 60). Six sera had borderline ranges of AI. According to this study, the IgG avidity ELISA test could distinguish the acute and chronic stages of toxoplasmosis.

KEYWORDS acute toxoplasmosis, IgG avidity, ELISA

4.2-005

Extracellular production of silver nanoparticles by using three common species of dermatophytes: *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis*

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BACKGROUND The unique size-dependent characteristics of nanoparticles make them indispensable in many areas of human life ranging from industries to natural science. To develop a new green approach for biosynthesis of silver nanoparticles, myconanotechnology have represented a novel field of study in nanotechnology and has currently attracts a great deal of attention. In the present investigation, we have reported the extracellular synthesis, of highly stable SNPs using three more common species of dermatophytes including *T. rubrum*, *T. mentagrophytes* and *M. canis*.

METHODS To achieve the aim, clinical strains of three species of dermatophytes were grown in a liquid medium containing mineral salt. The cell-free filtrate of the each culture was then obtained and subjected to synthesize SNPs while expose with 1 mM of $AgNO_3$. Further characterization of synthesized SNPs was performed afterward. The reduction of Ag+ ions to metal nanoparticles was investigated virtually by tracing the color of the solutions that switched into reddish-light brown after 72 h. The UV-vis spectra

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demonstrated a strong quite narrow peak located between 422 and 425 nm was obtained for *T. mentagrophytes*. For *M. canis*, a fairly wide peak centering at 441 nm was observed and at last, such a weak spectrum to decipher was obtained for *T. rubrum*.

In case of *T. mentagrophytes*, the results of TEM demonstrated that the particles were formed fairly uniform, spherical, and small in size with almost <50 nm. For the other two species, TEM images showed the existence of small spherical nanosilvers.

RESULT AND CONCLUSION In this investigation, it was observed that species of the same genus of the fungi have the ability to synthesize SNPs extracellulary with different efficiency. So that *T. mentagrophytes* has done its best and produced fairly monodisperse SNPs in the range of 20–50 nm. Furthermore, the extracellular synthesis may make the process simpler and easier for following processes.

4.2-006
Invisible jungle

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Invisible Jungle is a radio program that promotes STEM education by providing interesting and relevant information to the public about the 'invisible' world of microbes. An interdisciplinary group of honors students manages all facets of research and broadcast production of the show, which started in the Fall 2009. To date, *Invisible Jungle* has produced and broadcast over sixty-five 2 min-long shows with a weekly listenership of 20,000 listeners across east central Texas. It has established itself as a regular presence within local radio and promotes access to materials via various e-media channels. We describe the genesis and development of the show, breaks with traditional STEM education banks, and our plans for expanding *Invisible Jungle* Radio and e-media into becoming a premier resource for educating the public about the microbial sciences.

4.2-007
Screening for hemoglobinopathies: benefits of a high-pressure liquid chromatography (HPLC) system

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INTRODUCTION Structural haemoglobinopathies and thalassaemias (thal) are the most prevalent monogenic disorders. They are associated with a protective effect against malaria. Migrational social changes in our country are resulting in a remarkable increase in these abnormalities. Within the province of Almeria (southern Spain), immigrants account for approximately 21.7% of the population (151,159 out of 695,560), most of whom come from the Maghreb and sub-Saharan Africa; a notably high figure compared to the national average estimated at 12.2% (5,708,940 out of 46,951,532).

OBJECTIVES To describe the haemoglobinopathies detected in immigrant patients seen in our Tropical Medicine Unit (TMU) from 2008 to 2011 after screening structural haemoglobin variants using a HPLC (High Pressure Liquid Chromatography) system Hi-AUTO A1c 8160 (Menarini®). In cases of microcytosis/hypochromia not associated with iron deficiency, Haemoglobin (Hb) A2 and Hb Fetal were dosified for beta-thal diagnosis, using the same HPLC system. If they were normal, genetic analysis for alpha-thal was carried out when suitable.

RESULTS A total of 1054 immigrant patients coming from sub-Saharan Africa seen in the TMU were evaluated; average age 29.3 years (range 7–71); 967 male (91.7%). A total of 213 (20.2%) hemoglobinopathies were detected – 110 (51.6%) Hb AS (sickle cell trait); 35 (16.4%) HbAC; 13 (6.1%) heterozygous alpha-thal; 12 (5.6%) homozygous alpha-thal; 10 (4.7%) beta-thal; less frequent, HbAS+non-tipified microcytosis (7), non-tipified microcytosis (5), HbAS+homozygous alpha-thal (3), HbAS+heterozygous alpha-thal (3), Hereditary Persistence of Fetal Haemoglobin (3), HbHope (3), HbCC (3), HbSS (1), HbSC (1), HbO Arab (1), HbH (1), deltabeta-thal (1) and triplication alpha-thal (1).

DISCUSSION The gradual increase in the proportion of immigrant patients in our area makes the implementation of screening programs for hemoglobinopathies necessary. The HPLC system offers a fast, simple, affordable and reliable method, highly effective in the detection of haemoglobinopathies.

4.2-008
Data management in tropical medicine: a call for uniformity

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INTRODUCTION Booming applications of informatics and telecommunication had a major impact on society over the last decades. This Information Age is creating a new frame in which all sectors, including Tropical Medicine, can collect data at high speed and in large volumes. Research projects become more ambitious but must achieve appropriate standards on data quality, leaving a number of challenges for Data Management (DM).

METHODS In December 2010, a network of clinical data managers and database developers from Belgium, Burkina Faso, Cambodia, the Democratic Republic of Congo, Kenya, Nepal, The Netherlands, UK and Zambia met at the Institute of Tropical Medicine in Antwerp, with the aim of setting up a platform for knowledge sharing and support on DM activities

RESULTS Problems commonly met by clinical data managers include underestimation of the workload by study coordinators and donors; short timelines, which have an impact on the quality of deliverables; late involvement in projects, making streamlining of DM with project purposes difficult; low position in hierarchy of research groups, causing communication problems during projects. In addition, in the absence of colleagues and broader networks, a data manager is often left alone to choose appropriate technical solutions (e.g. software or validation methods adapted to the research constraints), without consulting fellow colleagues.

CONCLUSIONS The above shortcomings illustrate the growing pains of DM, which is not always recognized within the research team. We recommend that clinical data managers working in tropical medicine gather in formal networks, where they can introduce common tools and working methods and create uniform templates and SOPs. This will facilitate communication, knowledge sharing and collaboration both in and among institutions in similar resource settings. In the long term each network member will upgrade his/her level significantly, with a better involvement in research projects and a positive influence on quality of research.

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4.2-009

The monitoring and evaluation system of the fight against aids in Benin: a critical reading

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Before the advent of the Paris Declaration on aid effectiveness in 2005, Benin had already started to implement an approach for rationalizing the fight against HIV/AIDS, which called for, inter alia, the establishment of a single monitoring and evaluation (M&E) framework. Despite efforts to this end, the field of HIV/AIDS remains highly fragmented and so characterized by poor governance. This situation inspires the question, what are the constraints related to the practice of M&E that limit the use of retro-information for improving the design of policies to fight against HIV/AIDS. The constraint analyses are based on the review of M&E policy documents in the sector. That descriptive and historicist approach has been complemented by empirical data collected among 74 stakeholders involved in community medical follow-up evaluation through interviewing techniques and their analysis in an interactional perspective. This study showed that the M&E system of interventions against HIV/AIDS, which is supposed to bring more coherence in the sector, is actually not consistent on its own. Under the influence of international policies and their massive funding flows, M&E mechanisms have become procedural and led by a routine and quantitative logic, which has favored its diversion towards other purposes by almost all stakeholders. The case studies showed that a systemic, constructivist and empathetic intervention approach produces more social performance than the project/program approach with an M&E device. We conclude that international and national policies should reorient their intervention approaches to achieve better governance and efficiency.

KEYWORDS monitoring and evaluation, HIV/AIDS, governance, Benin

4.2-010

Cutaneous Leishmaniasis international observatory: an initiative for evidence-based global health

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BACKGROUND Cutaneous Leishmaniasis (CL) is a neglected mosquito-borne protozoan disfiguring and stigmatizing infection that causes a great suffer of the patients and their families. It is a global increasing public health problem but still does not receive the necessary attention. It is a disease that is present in 82 countries and its incidence is estimated at 10 million of affected people. An international team has developed with the support of the WHO, three Cochrane systematic reviews focused on the role of control strategies for CL.

OBJECTIVE To develop a Cutaneous Leishmaniasis International Observatory (CLIO), as a non-for-profit model of knowledge management, based on the scientific evidence and for improving the lives of people with CL.

METHODS Our main need is to seek contacts, ideas and interest of institutions, organizations, companies and individuals to lead and develop this action and to help complete the project in the best conditions. Of particular interest is to periodically maintain and actively disseminate a comprehensive and rigorous analysis and synthesis of the information derived from updated Cochrane reviews.

RESULTS We have collected the opinion and support of different people and organizations. We have developed a strategic plan for the commitment of CLIO to reach real and significant impact in guiding and promoting practice, policy decisions and in helping to identify research priorities around the world. We want that healthcare decisions in CL were informed by reliable, up-to-date, scientifically defensible and relevant evidence.

CONCLUSIONS The major interests of CLIO are the update of the information and to develop networking activities and communication actions for actively disseminate what is best known about the effects of control interventions in CL.

4.2-011

Targeted intervention to reduce communicable diseases and increase access to health care among long-distance truck drivers and truck stop sex workers in Sub-Saharan Africa

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Long-distance truck drivers, sex workers and transport corridor communities are more likely to contract and transmit STIs, HIV and other communicable diseases like tuberculosis (TB) and malaria. These populations have limited access to healthcare services and targeted interventions are lacking. North Star Alliance (North Star), a public-private partnership formed by TNT and the World Food Programme in 2006, and joined by UNAIDS, the International Transport Workers' Federation and ORTEC, provides access to basic healthcare as well as HIV, TB and malaria preventive and treatment services. North Star's network of 22 Roadside Wellness Centres (RWCs) operates out of custom-fitted shipping containers, staffed by a clinician and health educator, which are located at border posts, truck stops and ports. RWCs offer primary healthcare, STI treatment, HIV counseling and testing, and a robust referral mechanism. HIV treatment and TB diagnosis and treatment are gradually being introduced. This corridor-based, decentralized approach is strengthened by a standardized consultation system supported by an IT 'Corridor Medical Transfer System' (COMETS) that tracks key performance indicators for monitoring and evaluation. Patients are able to access their confidential health information anywhere in the network. As of April 2011, RWCs have provided general health services to 124,000 men and 72,000 women, distributed over 750,000 male condoms and 13,000 female condoms, and treated over 20,500 STIs. Each RWC provides clinical services to 25 people a day plus dozens who request information and counseling. The expanding network and high utilization show the success of this innovative partnership. North Star responds to the health needs of key populations and provides a platform for operational research to measure effectiveness. COMETS can analyse disease patterns on both a corridor and regional basis and the RWC model invites innovative technologies like GeneXpert to diagnose TB and Health solutions to improve HIV and TB treatment adherence.

4.2-012

Coartem: a 10 years experience of patient-centric approach to fighting malaria

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INTRODUCTION Over 400 million treatments of Coartem (Artemether–Lumefantrine), the gold standard artemisinin-based combination therapy (ACT) for malaria have been deployed to endemic countries for the last 10 years.

METHODS Our focus shifted from providing a quality medicine in public/private partnership with WHO to a holistic, 'patient-centric' approach, focusing on educating caregivers and patients to

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ensure, timely treatment and adherence to full course of medication, involving multiple partnerships.

RESULTS A dispersible formulation was developed jointly with Medicines for Malaria Venture (MMV) to meet the specific needs of children. Coartem Dispersible tablets can be given dispersed in a small amount of liquid and are sweetened to mask the bitter taste which is typical of most antimalarials. Currently, we are evaluating novel approaches that may be of use in malaria elimination strategies. A study assessing the utility of Coartem in mass screening and targeted treatment for malaria in entire village populations, including carriers of the malaria parasite that are asymptomatic has been undertaken in an effort to reduce parasite transmission. New strategies to expand access to ACTs have also been implemented: the Affordable Medicines Facility – malaria (AMFm) initiative, where funds from donors will be used as subsidies to lower the price of ACTs at retail outlets, and the SMS for Life initiative, part of the Roll Back Malaria (RBM) program, a tool for supply chain management based on electronic mapping technology and short text messages sent via mobile phones.

CONCLUSION These initiatives go beyond a mere deployment of drugs, while maintaining and further evolving a patient-centric approach, and are essential for achieving a sustained health benefit in developing countries. Sharing these learnings with relevant stakeholders may allow developing strategies that achieve similar results also for other diseases, worldwide.

CONCLUSIONS Community participation in planning, implementation and evaluation in the Cuban Health services increased in the study areas, indicating an empowerment potential of the CPPE methodology in the Cuban context.

4.2-013

'Consult online': the Dutch telemedicine system for tropical doctors

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INTRODUCTION In April 2008 the Association of Dutch Tropical Doctors in Training set up a Telemedicine System in the Netherlands. Their aim was to provide expert advice to Tropical Doctors working abroad. By sending cases by email to 'Consult Online' their questions are forwarded to a number of consultants of a requested specialty, who also have experience working in developing countries. Advice is sent back and the Tropical Doctor decides whether and how to use this.

METHODS AND MATERIALS All consultations since 2008 have been stored in the email inbox of 'Consult Online'. These data have been put into a database, from which the following data were obtained: country of origin, number of cases (per specialty), number of specialists replying, number of reactions per case, reaction time.

RESULTS Fifty-five specialists from 16 different specialties are participating in 'Consult Online'. From April 2008 to April 2011, 85 consultations took place, originating from 11 countries and three continents. Dermatology was the most popular specialty with 31 consultations, followed by Surgery (17) and Gynaecology (14). On average, there were three reactions per case (maximum 10). Over 50% of the emails were answered within a day, over 75% within 2 days. The result of the advice could not be evaluated, since the Tropical Doctors using 'Consult Online' most often did not communicate the outcomes of their cases.

CONCLUSION 'Consult Online' provides Tropical Doctors with expert advice and feedback. On average, they receive three reactions per case and over 75% of the cases are provided with advice within 2 days. Whether and how this advice should be used is up to the Tropical Doctor and his or her patient.

4.2-014

The center of infectiology charles mérieux in Madagascar (CICM): a tool to fight against infectious diseases

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INTRODUCTION In Madagascar, fight against infectious diseases is a challenge for Public Health and country development.

OBJECTIVES Establisha reference pole in infectiology in Madagascar and be able to bring a help to the diagnosis and to the treatment in this field by setting up a laboratory of molecular biology offering modern diagnostic capacities; Participate in the training of the local students; Become a meeting place in the field of the research in infectiology.

MATERIALS AND METHODS Partnership between the Fondation Mérieux, the Ministry of Public Health and the University since 2007. 2009–2010: construction of the center of infectiology including a laboratory of molecular biology answering the international standards; Development of research projects in the same frame with those designed by the Ministry of Health.

RESULTS Inauguration in April 2011 in the presence of Ministers for Public Health, of Higher Education and Research and of M Alain Mérieux First project initiated in 2010 on the Etiology of non malaria fever in children under 5 years in collaboration with Ministry of Public Health Participation to the GABRIELnetwork (Global Approach for a Biological Research on Infectious Epidemics in Low income countries). This network includes laboratories from the developed, emerging and the developing countries. Current project started in 2010: multicentric study on the surveillance of pneumonia in children under 5 years of age. Current training: within the framework of an international project, the CICM participated to different training programs at the RodolpheMérieux Laboratory (Molecular diagnostic and assurance quality courses).

CONCLUSIONS AND PERSPECTIVES The CICM by developing its local and international partnerships will contribute to fight against emergent and re-emergent infectious diseases as well as neglected diseases in Madagascar. The Centre will also contribute to train local scientists to reinforce their biological potential.

4.2-015

Www.saludentreculturas.es: a new tool for promoting migrants' health

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INTRODUCTION There are many barriers for migrant population to access preventative programmes (mobility; administrative, linguistic and cultural barriers). Our aim was to create a multilingual/multicultural interactive website especially aimed at professionals from health and social fields (general practitioners, NGOs professionals…) and their users.

MATERIAL AND METHODS The unequal distribution of migrant population in Spain, their cultural-linguistic diversity as well as their social and working conditions were taken into account. Professionals from different fields (health sciences, computing, anthropology, psychology, intercultural mediation and translation) collaborated in its design from January 2010.

RESULTS The website combines four areas of information: Health: information (in many cases supported by audiovisual tools) about

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pathologies with special relevance to individual and public health such as HIV, tuberculosis, Chagas disease, vaccine-preventable diseases and travel-related diseases, and other issues such as mother and child health and health-related resources in Spain.

MEDIATION Information and papers about mediation, migration, culture, health-related resources and translation/interpreting resources. Spoken health messages: brief messages about HIV, tuberculosis and malaria. Each kind of message is available in the language of the target population (e.g., malaria messages available in two African languages). These messages enable migrants to get health advices no matter what their educational level or their level of Spanish, English or French is. Schedule of activities: updated dates about conferences, talks, events and courses on health-related topics, culture, mediation and migration, mainly performed in Spain.

The website is available in three languages: Spanish, English and French. Spoken health messages are available in Wolof, Bambara, Arabic, Romanian, Bulgarian and Chinese.

CONCLUSION Through this interactive website, health and social professionals and their users from other cultures will be able to access reliable and relevant information about health and intercultural mediation, as well as health-related resources in Spain. Thus, it could help migrants to improve their health-related practices, overcoming linguistic barriers as well as time/space availability.

4.2-016

Can the measurement of carbon monoxide be a simple, cost effective method to measure air pollution exposure?

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Recent studies have found that air pollution is an important risk factor for cardiovascular disease (CVD), a growing problem in developed and developing countries. Respirable particulate matter (PM10) is often measured as a proxy for overall air pollutant concentrations but its measurement is often time consuming and expensive, as extensive monitoring and laboratory facilities are usually required. Passive color dosimeter carbon monoxide (CO) tubes may be a more economical and simpler proxy measure of air pollutant concentrations. This study was conducted in the semi-urban area of Nouna, Burkina Faso. Area concentrations of PM10 and CO from the kitchens and sleeping rooms of case and control households were measured over 24-h. Passive, real-time samplers were used to measure area PM10. Area and personal CO concentrations were passively measured with color dosimeter tubes. Interviews and on-site inspections were also conducted to assess the fuel and stove types used. These assessments were part of a larger case-control study examining the relationship between biomass smoke and malaria risk. In comparisons of overall PM10 and CO area concentrations within households, the two measures were found to be significantly correlated ($r = 0.79$, $P < 0.0001$, $n = 181$). Overall PM10 area and CO personal concentrations were also significantly correlated ($r = 0.27$, $P < 0.0001$, $n = 224$), although not as strongly. A significant but weaker correlation was also present between overall area CO and personal CO concentrations ($r = 0.21$, $P = 0.0047$, $n = 183$). Area CO could be a simple proxy measure of area PM10 concentrations; however, this relationship may not be consistent across all stove and fuel types. Conversely, personal CO concentrations may not be a reliable measure of area PM10 concentrations, since personal exposures are not likely to occur continuously but instead experienced intermittently. Carbon monoxide tools may used as a general but cost effective alternative to PM10 area monitoring, although further testing under different settings is needed.

4.2-017

Comparison of two molecular techniques, PCR and real time PCR, for detection of giardia duodenalis in faecal samples from companion animals

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Giardia duodenalis is an intestinal parasite that affects humans and a broad spectrum of mammals. Giardia presents seven genotypes or assemblages (A-G), two of which (A and B) are found in humans. It would be interesting to study the presence of Giardia in companion animals because they can be a reservoir and source of infection to man. Detect infection in these animals is important for diagnosis, epidemiology and control of giardiosis. Microscopy is the primary method for diagnosing giardiosis, but it depends on the excretion of Giardia cysts, experience and ability of practitioner. In the last decades, molecular methods have made the diagnostic applications easier and faster. The aim of the present study was to evaluate molecular methods to improve the diagnosis. Moreover, three handling samples methods (sucrose-gradient separation, ether and no concentration method) previous to DNA extraction were also evaluated. DNA extraction was performed with the Qiagen stool kit. Faecal samples from dogs ($n = 96$) and cats ($n = 4$), that had been previously positive to Giardia by microscopy, were analyzed to compare two different conventional PCRs (one with the gdh gene as target and the other with the ß-giardin gene) and real time PCR (SSUrRNA gene). Of the total of samples, 90 (90%) were positive by real time PCR, 61 (61%) by seminested-PCR (gdh) and 41 (41%) by nested-PCR (ß-giardin). We observed that real time PCR showed better Results and greater sensitivity than conventional PCRs for the diagnosis of giardiosis. However, the conventional PCRs followed by RFLP analysis allow further characterization of the genotype or assemblage of Giardia involved. Therefore; these techniques (PCR-RFLP) are interesting for the parasite characterization studies, although so far, its diagnostic sensitivity is low.

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4.2-018

Mortality in Nouna health and demographic surveillance system (NHDSS): assessment of the validity of verbal autopsy results

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INTRODUCTION Three of the millennium development goals require specific mortality measures, highlighting the need for valid data on mortality. Verbal autopsy (VA), which collects information on signs, symptoms and treatment pathway, experienced before death addresses these goals. The objectives of this study are twofold: Firstly to compare causes of deaths patterns using VA and health facility-based assigned causes in Nouna Health District (NHD) and secondly match with socio economic profile of deceases.

MATERIALS AND METHODS Two years, 2008–2009 VA data and NHD facility based data was analyzed and compared. Economic status of decease persons was measured by an 'asset index', i.e. a composite indicator constructed by aggregating data on asset ownership and housing characteristics. Analysis was done using Stata 10 and Excel 2007.

RESULTS In total 1022 deaths collected in the HDSS were diagnosed by physicians using VA during the 2 years period and malaria is the leading cause with 37.87%. Similar results are found by the

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health facilities data and malaria is the first cause of death with 40.70% out of 489 deaths registered. In the age group of below 1-year, the causes of death are identical in the two data sources. Malaria, pneumonia and meningitis are leading causes. In adult group (older than 15 years) with the VA data and HIV represents 3.15% of causes against 8.01% in the health facility data. The standard of living analysis on VA data shows that malaria is common in poorest group (43.08%) than the others. Meningitis is about 5.13% in poor group, 3.65% in middle income and 1.94% in rich category. For HIV, we have 0.97% in rich group, 3.65% in middle and 2.05% in poor one.

CONCLUSION In this study <50% of death was registered in the health facilities reinforcing the importance of HDSS in death information collection in countries where most of the deaths occurred out of health facilities. Comparison of causes of death patterns using VA and facility-based data doesn't show any statistical significance difference supporting the relevance of VA for ascertaining cause of death in poor setting. The under-estimation of HIV by VA is understood as opportunist infections are often identified as the cause of death and not HIV. The analysis of standard of living shows that meningitis and malaria are high in poorest group than rich one. The results of this study confirm the relevance of VA but also indicate also that improvement are needed to better approach the real causes of death for better health policies.

4.3 Health Systems, Human Resources and Access to Care

4.3-001

A blueprint of Brazilian competencies status to improve the care in the neglected tropical disease

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INTRODUCTION The so-called neglected diseases, threaten more than 1 billion people worldwide and about 230 million in Latin America. In Brazil it is estimated between 10% and 20% of the population with the incidence of most of the 17 neglected tropical diseases (NTD) are listed by WHON. The structuring of knowledge management in the twentieth century and the development of industrial policies, Brazil has advanced in technology and science, yet the gap in risk management to innovate in order to identify the scientific and business competencies is still a challenge because it has permeated many factors, including the cooperative management and that adds value to technological development, especially in the public health.

METHODS Consultation and analysis of results in the database of the Brazilian Innovation Portal and the National Council for Scientific and Technological Development (CNPq) and bibliography indexed in the Virtual Health Library, Scielo and Lilacs.

RESULTS Through the Brazilian Innovation Portal has been possible to identify 40,770 competencies for neglected diseases in the national territory, yet by productivity index were 2633 scientists from CNPq. Although there is a 'communication' of these bases, extracting the data still needs greater effectiveness in knowledge management.

CONCLUSIONS Cannot identify, in a practical and fast, all Brazilian scientists who can contribute to the development of treatments for NTD and effectively in the industries. Over the past 30 years, the DTN as Chagas, dengue, leprosy, leishmaniasis, malaria and tuberculosis are included in discussions of the government agenda. Suggest the more effective use of 6.5% of the scientists with maximum level of the CNPq and selected and organized use of competencies identified as a source of consultation for the public and private sector.

4.3-002

Implementing a laboratory in a tropical remote area or what can be done with little funds and no electricity access

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Medical biology is often ignored or misconducted in developing countries especially in rural remote areas. However, basic clinical laboratories may be implemented with little means and without regular electricity access. This presentation will show many useful laboratory tools that do not require electricity to be performed and share algorithms that have been developed in Madagascar to valorize simple biological data. It will also highlight the fundamental importance of having a strong and reliable management and accountability. The solutions proposed are: (i) having reliable and educated human resources with a reliable laboratory management; (ii) implementing useful tools that can be performed without electricity: gram and MGG staining and all what can be concluded from their examination: algorithms have been built to help physicians Interpreting Laboratory Test. Results and orientating their antibiotic choice according to gram staining and available bioresistance data; MGG staining is usefull for the diagnosis of leukemia, anemia but also leishmaniosis, filariosis, donovan bodies… – direct examination will help for the diagnosis of Tina capitis, Tinea corporis, vaginosis, vaginitis, fecal parasites, ectoparasitosis – rapid test is a revolution for developing countries: they are stable, easy to practice, cheap and may be used for screening: (i) biochemical disorders : urine test, HCG, cardiac markers; (ii) infectious diseases: HIV, B hepatitis serologies, malaria, cholera, *Helicobacter pylori*... – RPR test is still a first step in the diagnosis of Syphilis though Vidal test should not be performed anymore for the diagnosis of Typhoid Fever; (iii) having autonomous and proper accounting practices.

4.3-003

Monitoring drug effectiveness in Kala-azar in Bihar, India: cost and feasibility of periodic random surveys vs. a health service – based reporting system

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INTRODUCTION Visceral leishmaniasis (VL) is a major public health problem in the Bihar state, India. A regional VL Elimination Initiative was launched in 2005 based on the use of oral drug miltefosine. However, concerns were raised about development of drug resistance. Drug effectiveness cannot be assessed accurately by the current recording and reporting system of health facilities (PHCs). In 2009 a random survey was conducted in Muzaffarpur district to document the clinical outcomes of VL patients treated by the PHCs in 2008. We analyze the operational feasibility and cost of such periodic random survey as compared to health facility based routine monitoring.

METHODS A random sample of 150 patients was drawn from registers kept at PHCs. Patient records were examined and the patients were located at their residence. Both patients and physicians were interviewed with the help of two specifically designed questionnaires by a team of physician and social scientist.

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Costs incurred during this survey were properly documented and vehicle log books were maintained for present analysis.

RESULTS Only 115 (76.7%) of the patients could be located in the first effort and finally 11 patients were not traceable on account of erroneous recording of patients' characteristics and addresses at the PHCs. Per patient follow-up cost was US$ 15.51 and on average 2.27 patients could be visited per team-day. Human resource involvement constituted 75% of the total cost whereas involvement of physician costs 51% of the total cost.

CONCLUSION A random survey to document clinical outcomes is costly and labor intensive, but gives probably the most accurate information on drug effectiveness. A health service based retrospective cohort reporting system modeled on the monitoring system developed by tuberculosis programs could be a better alternative.

Involvement of community health workers in such monitoring would offer the additional advantage of treatment supervision and support.

4.3-004

Are integrated services networks of privately delivered health care a good option for lmic? The prosalud experience in Bolivia

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INTRODUCTION PROSALUD is the largest Bolivian NGO in the health sector, created by USAID to serve poor and middle income populations. Vowed to be self-financed through a fee-for-service and out-of-pocket payment basis, it aimed at becoming independent from financial support from USAID. Most international organizations viewed PROSALUD a successful, replicable experiment in private delivery of Primary Health Care. This article evaluates PROSALUD e.g. comparing its costs, accessibility and quality of care to Bolivian public services.

METHODS We compared a sample of four PROSALUD/MoH-paired health centres (HC) during the period 2005–2007. Their operational costs were established on HC accounts. We reviewed the literature, observed health activities and interviewed HC staff. A sensitivity analysis permitted to assess the stability of the conclusions reached.

RESULTS PROSALUD better integrated health care but its quest for self-financing harmed care quality and affordability. Its HC produced consistently fewer preventive care and more curative care than MoH HC. Despite being an NGO, the managerial rationale was to a large degree a profit-making one. PROSALUD HC unit costs were consistently higher than those of MoH. Prices at PROSALUD were not affordable for at least two thirds of the Bolivian population.

CONCLUSIONS The sub-standard quality of curative care in MoH HC results from long term, steady deterioration of the public sector, by a long-term policy paradigm preventing its HC to deliver versatile curative care and to compete with the private sector. PROSALUD services are accessible mainly to wealthier urban Bolivians. The PROSALUD model is probably not replicable in settings where poor populations predominate owing to costs and prices charged. In wealthy neighbourhoods, it could harm national solidarity. Its health care and organization model, however, could usefully inspire the design of public HC.

4.3-005

Health service delivery, access to care, costs of health care and coping mechanisms: a snapshot from three central Lao provinces

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OBJECTIVE to assess uptake of selected maternal and child health services, breastfeeding practices, ability to access curative care, associated out-of-pocket expenditure and coping mechanisms amongst the socioeconomically worst-off and best-off in three central provinces.

METHODS Cross-sectional survey in nine districts using a pre-coded structured questionnaire amongst 1010 women who delivered within 18 months before interview. Data were stratified according to socio-economic quintile defined by principal component analysis.

RESULTS The 40% worst-off of the study population were statistically significant disadvantaged for uptake of nearly all types of preventive health services as well as quality of these services. Antenatal care attendance and its quality were positively correlated with institutional delivery. Sick adults of the poorest 40% were less likely to seek care and to consult a hospital than best-off adults. However, costs associated with care seeking were similar for both groups (US$ 12.7 for children; US$30.7 for adults) with prices at the public sector exceeding those charged in the private sector. Treatment failure rates were considerable. A fifth of the interviewees were indebted as a result of care seeking for previous illness episodes with an average debt of US$126 per household. Of all interviewees reporting a care seeking episode, 30% had insufficient cash on hand whereby 82% of them (25% of total) resorted to borrowing for which 81% had to pay interest.

CONCLUSION To cover the equity gap and to enable access to health services, a comprehensive, targeted approach is required

4.3-006

Help seeking for children with severe pneumonia and diarrhoea in rural Guatemala

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BRIEF INTRODUCTION Globally, about 10 million children under five die every year, almost all of them in low income countries. Pneumonia is the leading single cause of mortality (19% of all deaths) in children under five, followed by diarrhoea (17%). In Guatemala, in 2000, under-5 mortality was 55.7 per 1000 child years, but in rural areas it is estimated to be at least 80 per 1000 per year. The potential impact of improved access to health care on under-5 mortality in Guatemala could be considerable. In particular, prompt seeking of care from a formal qualified health provider is seen as an imperative.

METHODS AND MATERIALS In 2008/2009 a survey of a representative sample of 1605 households with children under 5 years was conducted in the rural Guatemalan municipalities of Comitancillo and San Lorenzo. The survey interview referred to the recent health of youngest child. Demographic and socio-economic data were also collected. Over 95% of the same households were surveyed again in late 2009. As part of the structured interview schedule, a 14-day calendar was used to record all recent help seeking and treatment.

RESULTS Period prevalence rates for severe pneumonia and severe diarrhoea during the survey were 11.6% and 15.3% respectively. Multilevel logistic regression identified mother's perception of severity and recognition of key warning signs as independent

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predictors of seeking formal care for severe pneumonia. Formal help seeking for severe diarrhoea was significantly associated with perceived severity, knowledge of the government-sponsored Emergency Plan and proximity to main health facilities.

CONCLUSIONS Regardless of socio-economic and cultural context, higher rates of formal help seeking for severe childhood illness may be achieved via educational interventions. Such interventions should include increasing awareness of early warning signs for severe childhood illness.

4.3-007

Lessons for building and retention of a stronger health workforce: job engagement among thriving nurses in Uganda

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BACKGROUND In response to the global shortage of health workers, which is a severe problem in sub-Saharan Africa (SSA) due in large part to talent drain to other continents, there is an urgent need to highlight factors that promote job engagement in SSA nurses, who are the backbone of SSA health care. Recent nursing research in Norway illuminated job engagement as a process driven by the meaning in life that nursing may impart. Job engagement can help nurses cope with the rigours of nursing and stay on the job in their own countries.

AIM This study examined the job engagement experiences of Ugandan nurses known to thrive on the job.

METHODS A qualitative, in-depth interview study was conducted in two Ugandan districts between March and May 2010, producing data from 15 nurses with reputations for thriving despite having difficult working conditions. The nurses were selected from private and public health units.

RESULTS Job engagement in nursing is a process driven by a search for, the experience and maintenance of meaning and meaningfulness. Calling to the nursing profession triggers a process that is mediated by reflection and introspection about their work life enabling thriving nurses to adjust and cope better on the job. Deep attention to values, ethical issues, and meaning attached to oneâ€™s work are key factors in promoting nursing job engagement.

CONCLUSION During their training and also on the job, nurses should learn the skills for, and practice habitual introspection and reflection about the satisfactions they derive from their work, to help them retain a high level of job engagement despite the adversities of the nursing profession. To strengthen the human resources for health workforce in SSA, training institutions and health care managers ought to empower nurses with vital skills to ensure retention and satisfaction on the job.

4.3-008

Use of mobile phone: communication barriers in maternal and neonatal emergencies in rural Bangladesh

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BACKGROUND A toll free mobile telephone intervention is being tested in one sub district of Bangladesh to assess the utility of mobile phone in increasing communication for maternal and neonatal complications. Prior to initiating this new model a formative research was conducted among the Community Skilled Birth Attendants (CSBAs), mothers and their husbands to explore perceived advantages and disadvantages of mobile phone communication for first stage rapid management of maternal and neonatal complication.

METHODS AND MATERIALS Semi structured in-depth interviews were conducted among twelve CSBAs and fourteen mothers along with their husbands. The mothers were selected from different socio-economic status – low (monthly income <100 dollars), medium (in between 100 and 200 dollar) and high (equal or more than 200 dollars) purposively. Thematic analysis and triangulation of responses from different groups of respondents were conducted.

RESULTS All husbands expressed supportive attitude in their wives accessibility to their mobile phone, but wives' interviews revealed disparity in free access to husbands' mobile phone. The advantages identified to mobile phone communications were reduced cost of consultation and transportation to appointments with skilled providers, time saving during emergencies and controlling barriers to pregnant women's mobility. Other direct benefit is consultation between CSBAs and health professionals to increase the confidence of the CSBAs in handling maternal emergencies. Respondents sensed that determining the management without examining the patient might affect the appropriateness in providing treatment when the communication mode is only mobile phone. The mothers and their husbands who communicated through mobile phone with providers for their health issue had noted some other barriers: irritability from provider's side and sometimes the phone was found switched off.

CONCLUSION Willingness and active participation of the service providers along with the acceptability and accessibility by mothers and their family are necessary to make this mobile phone communication initiative successful.

4.3-009

Perception and expectation of poor urban communities of Kinshasa/DRC on health care services

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It is important to improve health care availability to poor populations to prevent manageable health problems as vulnerable populations increase in number. It is not the lack of effective existing interventions but their low use which constitutes the main hindrance to achieve the Millennium Development Goals. However, well documented strategies involving the population have successfully improve the use of health care in rural communities. Our goal is to investigate the perceptions and expectations of the urban poor communities in Kinshasa in terms of accessibility to health care to establish specific and effective health care strategies, in those communities. It is a cross sectional study conducted by focus group interviews of the communities in eight districts of four poor urban areas in the city of Kinshasa. Concerning access to health care, the perception of the communities is that the number of health centers and their distance is not the problems. However, the cost of services does not motivate people to attend health centers. The structures providing the best care are out of reach of the population and the ones they can afford provide poor quality services. The expectation of the communities is that the Ministry of Health should provide public access to primary health care and drugs for free or low cost. Communities also suggest their involvement during health campaigns organized by the Ministry of Health (ITNs distribution, poliomyelitis immunization, Vitamin A supplementation…) for a better cover and acceptance of those interventions. These results show that health care strategies involving the urban poor communities in Kinshasa would be

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greeted by the population and would improve poor urban community's access to health care.

4.3-010

'What counts': health workers' preferences for public health facilities or church-run health facilities in Tanzania

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INTRODUCTION Shortage of qualified health workers and geographical imbalances require that the national health sector management address issues related to the distribution of health workers across various types of health facilities. This article addresses health workers' perceptions and experiences of differences between working conditions in the public health sector and in church-run health facilities in a rural district in Tanzania.

METHODS The study has a qualitative study design to elicit in-depth information. The data comprise ten focus group discussions (FGDs) and 30 in-depth interviews (IDIs) with nursing staff and clinicians in the public health sector, and in a large church-run hospital in a rural district in Tanzania.

RESULTS The financial aspects of the working conditions were pointed out as very important for decisions on where to work. In particular differences in the pension paid upon retirement were consistently argued to be of major importance, and the governmental scheme was consistently experienced to the best among informants from both sectors. Other identified factors were workload, type of disciplinary actions, access to training, allowances and housing. Health facility infrastructure and availability of medical equipment and drugs were also pointed out as aspects of high relevance when considering where to work. Also family concerns and age were determining factors.

CONCLUSIONS The study identified a complex set of factors that impact on health workers' decisions regarding where to work. The pension scheme in the government sector led to a clear preference for employment in the government sector over church-run health facilities. The findings of this study are important in a context where church-run health facilities provide a vital part of the health services in Tanzania.

4.3-011

Catastrophic out-of-pocket health costs due to inefficient management of the health-care system: an evidence-based case study of tuberculosis control program in rural Burkina Faso

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BACKGROUND In low-income countries, illness-related costs incurred by patients constitute a severe economic burden for households. The weak uptake and poor international authorities.

METHODS AND MATERIALS In 2008–2009 we conducted 242 in-depth interviews among smear-positive pulmonary tuberculosis cases enrolled in the national tuberculosis program in rural Burkina Faso. We assessed cost burden by collecting data on household income and direct and indirect costs related to tuberculosis.

RESULTS Median direct and indirect costs were €1135 and corresponded to more than 6 months of household income (IQR: 2.9; 10.8). Through a better rationalisation of the demand and supply of healthcare, half of the households could save up to 75% or more of their direct expenses related to TB and could reduce significantly their opportunity cost. Catastrophic health costs that patients have to cope with are largely unjustified and thus potentially avoidable. The major relative positive margin could be saved during both intensive and continuation phases of the DOTS-treatment by rationalising the utilisation of x-ray, sputum test and other medical examination, treatment-expenses.

CONCLUSION The cost burden related to tuberculosis in rural Burkina Faso is due mainly to bottlenecks in the health system (i.e.: multiplied encounters to several health-care professionals, etc). To reduce undue tuberculosis-related costs, health-system strengthening mechanisms must focus on improving health services. This is a priority because too many people are still facing catastrophic health expenses.

KEYWORDS cost-of-illness, out-of-pocket expenses, tuberculosis, households, Burkina Faso

4.3-012

Travel health information system at the Valencian community

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INTRODUCTION Vaccination is a main topic in the Public Health services at the Valencian Community, therefore it has been developed the Vaccine Information System (SIV), computerized health system information, which provides nominal information of the status of vaccination. Vaccination for travellers is also part of the vaccination programs, and it belongs to the pre-travel consultation. This activity is done at the International

Vaccination Centre (ICV), where the physician provides to the traveller appropriate advices to help reduce the risk of illness during the travel. One of the pillars of SIV is maximum accessibility and integration with other Health System Information like medical records of the primary and specialized care, pharmacology-vigilance, and epidemiological surveillance.

METHODS AND MATERIALS SIV is composed by five applications: Nominal vaccine registration, Vaccine Storage Registration, Backoffice, Indicators and Consulting traveller (CONVI), the latter has been implemented since May 2011. CONVI allows recording the trip itinerary; regions to be visited, dates and length of travel, purpose of travel, types of accommodations; traveller medical records, vaccination records, adverse events, medications, allergies, pregnancy and breastfeeding. Selection, administration, and documentation of vaccinations required and recommended vaccination and prescription of preventive medications (malaria chemoprophylaxis).

RESULTS Currently, seven IVC are running at the Valencian Community, and 31 health care professionals are involved. The health care workers of IVC have access to SIV, and the information registered is available among the users.

CONCLUSIONS SIV is a Health System Information that allows health care workers to access a unique database from different IVC. CONVI will improve the quality of health assistance, as well as advice for travellers.

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4.3-013

Community empowerment in the planning, implementation and evaluation of health interventions in four Cuban health areas

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INTRODUCTION We understand community empowerment as people working together to increase their power and control on factors that affect their lives and their individual and collective health. We present the achievements in terms of empowerment of an action-research that used a Comprehensive Participatory Planning and Evaluation (CPPE) approach – adapted to the Cuban context – for planning, implementation and evaluation of local health interventions.

MATERIALS AND METHODS For 3 successive years (2008–2010) participatory planning workshops were progressively introduced in three municipalities. The interventions designed in the workshops were implemented the following year and then collectively evaluated. Workshop participants were a mix of formal leaders from the health and other sectors and informal community leaders. Quantitative and qualitative output and process indicators of empowerment were systematically documented in standardized case study reports for each of the planning exercises and interventions implemented. The reports were elaborated on the basis of workshop reports, evaluation forms, project documents, interviews and focus group discussions with the different actors involved. Data analysis was supported by the NVivo8 software.

RESULTS Twelve local health interventions were planned and implemented in various communities. These achieved different levels of empowerment through (i) addressing health determinants according to felt needs; (ii) involving their leaders in the decision-making process; (iii) increasing their capacities in terms of planning and implementing; (iv) increasing their feeling of ownership towards the interventions. Contextual factors that play an important role as enablers or barriers in the process and for results attained were identified. Finally, high levels of satisfaction with the experiences and a willingness of actors to sustain the process over time was documented.

CONCLUSIONS Community participation in planning, implementation and evaluation in the Cuban Health services increased in the study areas, indicating an empowerment potential of the CPPE methodology in the Cuban context.

4.3-014

Health related quality of life in nine municipalities of Cuba 2010

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Health related quality of life (HRQL) is the value assigned at different stages, the duration of life changed by the deficiency, functional status, perception of health and social opportunity due to illness, accident, treatment or specific policy directly or indirectly related to health. This value is individual and depends on the subjective perception of the individual and their political, social and economic development. There are several different questionnaires to measure; in this case the EuroQol was used in order to determine the HRQL in nine municipalities of Cuba. Descriptive and transversal study with a stratified cluster sampling was carried out. The universe was the population 15 years and more of the nine municipalities. We used the demographic variables age, sex and marital status, and EuroQol variables: mobility, personal care, daily activities, pain/discomfort and anxiety/depression. Most people had their mobility, personal care and daily activities preserved. Anxiety-depression was the dimension of smaller proportions of people without problems in the municipalities. Percentage of persons without affectations at the five dimensions was decreasing for older persons in all municipalities. Males showed higher percentages in the five dimensions, the same as single and married. Camagüey municipality was the best percentage by age group, sex and marital status. We conclude that young men, single or married had a higher level of HRQL and mobility, personal care and daily activities were less affected dimensions.

4.3-015

Financial incentives for health worker motivation: the case of health services in Tanzania

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INTRODUCTION Health systems worldwide face the challenge of making the best use of the health workforce, often in contexts of limited resources. Health worker motivation as a determinant of the quality of health services has received increasing attention in recent years. One approach to increase motivation is to offer financial incentives for good performance. In Tanzania a results-based bonus system is planned implemented to reduce the high levels of maternal and neonatal deaths. The aim of the present study was to explore health workers' perceptions of potential benefits of the planned results-based bonus system.

METHODS Qualitative in-depth interviews (IDIs) and focus group discussions (FGDs) were employed in the data collection. The collected material consists of 22 IDIs and 8 FGDs carried out at public health facilities in a rural district in Tanzania. Relevant public documents were also systematically reviewed to obtain information on the modalities of the planned results-based bonus system.

RESULTS The health workers addressed a wide range of issues of importance for motivation. The expected bonuses payable under the planned results-based bonus system were pointed out as a motivating factor. The study however found a range of other factors important for motivation. The lack of resources at the health facilities was repeatedly referred to as cause low motivation. Moreover, recognition of performance was pointed out as crucial for health worker motivation. The planned financial incentives thus emerged as only one part in a complex scenario of factors influencing motivation.

CONCLUSIONS The important role placed on other types of motivational instruments than financial incentives raises questions about whether results-based bonuses alone will yield the desired improvement in health worker motivation is a resource poor setting. The paper demonstrates that good working conditions with adequate resources and recognition of good performance are perceived as central for work related motivation.

4.3-016

Human resources for health for the laboratory

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INTRODUCTION Health care delivery in resource poor setting is hampered by the insufficient number of skilled health workers.

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Strengthening laboratory services, including the laboratory workforce (LW), in low and middle income countries is recently stressed in declarations as those of Maputo, Lyon and Yaoundé. This study aims to assess existence and implementation of policies and strategies to develop and manage the LW.

METHODOLOGY We performed a desk review of National Health Policies and Strategies of 18 Sub-Saharan Countries. The analysis addresses – among others – the availability and type of Human Resource Development and Management strategies for the LW, such as pre-service training, incentive packages, continuous professional development, and issues related to the high proportion of low educated laboratory workers.

FINDINGS Fifteen out of 18 countries address the laboratory services in general in at least one national health document. Four out of 18 countries have strategies related to the LW eight out of 18 countries have a national HRH plan. Seven out of eight national HRH plans address the LW. Four out of seven national HRH plans that address LW have strategies to increase/retain the LW. One out of the seven national HRH plans with attention to LW have a costed LW strategy. Four out of 18 countries have a national laboratory services strategy (NLSS). All NLSS address the LW by strategies to increase/retain LW. None of the NLSS have costed strategies. All countries have a HIV strategy of which 12 address the laboratories in general and 10 address the LW. Only two plans formulated strategies to strengthen LW in areas broader than HIV. None of the strategies are costed.

CONCLUSION Our results highlight important gaps regarding human resources for laboratory in current national health documents in Sub-Saharan Africa.

4.3-017

Acces to health care of immigrant population in public policies: comparative analysis

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BACKGROUND High proportion of immigrants in Spain poses new challenges for the healthcare system, which must provide care to a diverse population. International studies show an inadequate access to healthcare for migrant populations. Despite the development of specific immigration health policies, comparative analysis of these policies is scarce and not focuses on access to adequate healthcare. The objective is to analyse the elements that influence the immigrants' access to an adequate healthcare from the viewpoint of the health policies.

METHODS A descriptive comparative study of the immigration health policies at national and regional (Andalusia and Catalonia) level was conducted through content analysis. The dimensions of the analysis were: definitions of immigrant, access and quality of care, policy principles and objectives, strategies and results' evaluation.

RESULTS Few policies include the definition of immigrant and none of them of access and quality. At national level, the aim is to guarantee the right to health protection and include guaranteeing access to care. At regional level, the objectives follows the national guidelines but more specifically. Strategies are addressed to access' determinants related to immigrants – provision of information, immigrants' recruitment, and language training (only in Catalonia); to the entry into the healthcare system such as improving service supply and, only in Andalusia, at geographical access; and to access of quality care –communication improvement, services' organization model, professional training, knowledge of disparities of health and access. Related to the system it focuses on the bureaucracy to obtain PHC. Differences in the type of strategies were found between the communities.

CONCLUSION Health policies address access barriers to healthcare for immigrant population identified by the literature. However, persistent problems in accessing healthcare together with the lack of definitions, absence of budget for policies and the poor definition of the strategies could require a careful implementation. Care processes that observed in other health areas. The ignorance of the language is presented as the main barrier to proper care, which fits with the available evidence. There would be no significant ethnographic difference on the demand expressed in comparison with the native population, except in those aspects related to the population pyramid. There is a lack of associations o immigrants, despite the professional development in a cultural context, only a small percentage of community pharmacists perform specific training in this field. Probably this is due to have no real difficulty for effective care.

4.3-018

Community pharmacy and immigrants: facts and trends in pharmaceutical care in non-native population in Barcelona (Spain)

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INTRODUCTION Catalonia has the highest migration rate in Spain. Currently there are 180 different nationalities and more than 20 languages are spoken. It is a high magnitude, multicultural and heterogeneous phenomenon. This growth has occurred in a short period of time, <5 years. Several authors have found differences related to health care for immigrants due to cultural barriers, language, beliefs concerning health or lack of standards for using the health system in the host society. The community pharmacy is often the first entrance point to the healthcare system in the host societies because of its accessibility, regional balance, social proximity and easy recognition.

OBJECTIVE Knowing the extent of pharmaceutical care of immigrants and identifying the main barriers to care as well as the profile of demand for care in this context.

MATERIAL AND METHODS Validated survey administered by telematic support to a representative sample of pharmacies in areas with the highest percentage of non-native population.

RESULTS One hundred and twenty-one valid records were obtained in the province of Barcelona in a period of 3 months. 111 (91.7%) were pharmacies in urban areas, including parts of the city of Barcelona, and 10 (8.3%) were rural. 50.5% of them attended a percentage of migrants from 30% to 60% of its users. American, North African and Asian communities are most prevalent, accounting for 96.7% of clients served. The lack of co-official languages is perceived as a barrier to proper care for 77.7% of the participants. Cultural differences to 64.4% and lack of loyalty it was for 51.2%. On the demand side, 82.6% were requests for drugs not on the market and 66.9% of 'home-made' remedies. 97.5% received drug requests to send to relatives in the country of origin. In 86.8% of cases were required to care for urgent health problems. The explanation of prescribed treatments (56.2%), on administrative procedures (52.1%) and diagnostic tests (17.4%) were aspects of healthcare information required. 90% of pharmacists were unaware whether there were associations of immigrants in your area. Only 16.5% had undertaken refresher training on issues related to cultural or health care internationally

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in the last 3 years. No significant differences were found on the main demands of pharmaceutical care in comparison with the native population except for mental health, gerontology, preventive immunizations, nutrition and natural therapies that were greater for the natives ($P < 0.005$) while family planning, it was for the allochthonous ($P < 0.005$). No differences were found for any of the variables related to the type of pharmacy and years of pharmacist practice.

CONCLUSIONS Although the rate of immigrants treated in some community pharmacies has a high and heterogeneous distribution, this factor has subjectively less impact on care processes that observed in other health areas. Ignorance of the language is the main barrier to proper care, which fits with the available evidence. There would be no significant ethnographic difference on the demand expressed in comparison with the native population, except in those aspects related to the population pyramid. There is a lack of associations of immigrants, despite the professional development in a cultural context, only a small percentage of community pharmacists perform specific training in this field. Probably this is due to have no real difficulty for effective care.

4.3-019

Palliative care in Sub-Saharan Africa: a systematic review of the qualitative literature

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BACKGROUND In the context of HIV/AIDS in sub-Saharan Africa, the provision of palliative care is an important public health issue, but it is still lacking a sound evidence-base. This review aims to appraise the qualitative research literature on palliative care in sub-Saharan Africa in order to inform policy, practice and further research.

METHODS Relevant literature was identified through eight electronic databases AMED, British Nursing Index & Archive, CINAHL, EMBASE, IBSS, MEDLINE, PsycINFO, the Social Sciences Citation Index and hand searches. Inclusion criteria were: qualitative or mixed-method studies in sub-Saharan Africa, about palliative care. The methodological quality of studies was assessed with a standard grading scale.

RESULTS The review identified 39 relevant articles, 17 came from South-Africa. The majority (28) of the articles focused on HIV/AIDS, seven were on multiple or unspecified conditions and four on cancer. Participants across studies were: informal carers (19) health professionals (16), patients (11), Community members (3) and bereaved relatives (1). Informal carers were typically women, the elderly and children, providing total care at home, and lacking support from professionals or the extended family. The studies informed about how home-based care programmes function in practice and what is needed to make these effective. The data showed that patients and carers preferred institutional care but this needs to be understood in context. Studies focusing on culture informed about good and bad death, culture-specific approaches to symptoms and illness and the bereavement process.

CONCLUSION The review questioned some of the assumptions held about palliative care in Africa that the extended family takes on the care for the sick, and people prefer to be cared for at home. This review mapped the areas covered by qualitative research, highlighted novel findings, and identified areas relevant for a research agenda on socio-cultural issues at the end of life.

4.3-020

Effect of user-fee removal on utilization of caesarean section services in Boeny region, Madagascar

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BACKGROUND Maternal mortality ratio in Madagascar is estimated as high as 510 per 100,000 live births in 2005. Malagasy government has created a new initiative of removal of user-fee for caesarean section and medical treatment for babies up to 6 months of age. We have described the changes in access to emergency obstetric services after the introduction of the initiative in Boeny region.

METHODS We have employed an Unmet Obstetric Need indicator, which has been proposed by De Brouwere, in order to assess the access. The indicator focuses caesarean section performed only for selected life-threatening events (absolute maternal indications, AMIs), such as uterine rupture, placenta praevia, abruption placentae, and a group of complications (foeto-pelvic disproportion, transverse/facial/front presentations) that ultimately cause uterine rupture. Optimum caesarean section rate for AMIs is estimated between 1.1% and 1.3%. Data on caesarean sections in the region was collected and compared before (May 2007 until April 2008) and after (May 2008 until April 2009) the initiative.

RESULTS The numbers of confirmed caesarean sections for AMIs among the residents in the region were 100 and 133 before and after the initiative, respectively. The estimated needs of caesarean sections for AMIs ranged 296–350 and 305–360; therefore the deficits were 196–250 and 172–227 in the two periods. There was certain decrease of the deficits after the initiative; 23–24 in the region. However the decrease has been observed only in an urban city. A little decrease or even increase was confirmed in other rural districts.

CONCLUSION Beneficiaries of the initiative were limited only to the people in urban area. It was partially because that the initiative did not support transportation fee and there is no effective means of ambulance system. Therefore, there would be economical and geographical barriers, even certain portion of direct and indirect costs were exempted.

4.3-021

Evaluation of a peer-education program for diabetes and hypertension in rural Cambodia

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The prevalence of diabetes in Cambodia is high, and the current healthcare system is unable to provide adequate care for the people affected. Where care is available it is often unaffordable and many patients are pushed into poverty because of high out-of-pocket expenditures. MoPoTsyo is a Cambodian NGO providing care for poor people with diabetes and hypertension by engaging a peer educator in their own community. The focus of the education is on self-measurement of glucose levels and adaptation of life style, including nutrition and daily exercise. In addition, the NGO provides financial assistance to the poorest. Our research was performed in Takeo province, one of the poorest provinces in Cambodia, where the NGO has been working since 2007. We used a mixed-method approach: (i) a retrospective cohort study assessing perceived improvement in health, ability to control the disease and adherence after joining the program by using structured questionnaires. A random sample of 150 patients was

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taken from the 226 patients that were in the program for at least 2 years. Basic biomedical data were collected; and (ii) in depth-interviews with 14 patients and two peer-educators were carried out to get a greater understanding of the challenges patients faced before and after joining the program. A total of 134 patients completed the questionnaires. There was a significant drop in Fasting Blood Glucose (mean drop 44.44 mg/dl; $P < 0.001$) and BP (mean drop 10/8 mmHg; $P < 0.001$) compared to baseline, but not for BMI. Overall, most patients reported improved outcomes on the questionnaires, but the in-depth interviews showed that many still faced substantial financial difficulties. Peer-support interventions have the potential to greatly improve care for chronic diseases without putting additional strain on the health workforce. Poverty remains one of the major problems faced by patients with chronic conditions.

4.3-022

Assessing the implementation of social health insurance in Nigeria: where are the missing links?

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INTRODUCTION Achieving universal coverage of health insurance has been the goal of the long term health development plan in Nigeria. National Health Insurance Scheme (NHIS) was established in 1999 as part of health sector reform. The scheme aims to provide health insurance which entitles enrollees and their dependants to good quality and cost-effective health care services, but active implementation began in 2005. We assessed the experiences encountered and factors that have helped or hindered the expansion of coverage. This study tends to provide information on experiences within the Nigerian context.

METHODS AND MATERIALS Stakeholders analysis which encompass SWOT and PEST analyses were employed. It was complemented with reviews of NHIS data reports from 2001 to 2010. Synthesis of government official documents which dealt with the implementation of NHIS in Nigeria was used.

RESULTS The major turning point of the health sector in Nigeria began in 2004 alongside with other governmental and economic sectors within the context of the overall Government macro-economic framework referred to as the 'National Economic Empowerment and Development Strategy (NEEDS)'. Only 5.3 million Nigerians are enrolled. The beneficiaries are civil servants in Federal employment and two States including the 600,000 pregnant women and children under the MCH Project. Benefit package is comprehensive to ensure certain equity. Interest of participation from the private sector involvement made success of implementation. Legal frame and political willingness at local levels presents the greatest challenge towards universal coverage expansion. States, local governments, and the informal sector which constitute the highest proportion are yet to be protected by the scheme. Skepticism and public apathy still remains a big challenge.

CONCLUSION Legal frame which established the scheme could be amendment to facilitate expansion of coverage. Active political support and commitment at the local levels is highly required through mobilization and sensitization.

4.3-023

Determinants of health service utilization in Centro Habana, Cuba

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INTRODUCTION The Cuban health system assures universal coverage and is widely accessible. However, in itself, this does not guarantee uniform use of services. To possibly improve utilization, we studied health seeking behavior, identifying the importance of predisposing and needs factors based on the framework of Andersen and Newman.

METHODS AND MATERIALS Between April and June 2010 we interviewed the head of a random sample of 408 households (1244 individuals) in the 'Marcio Manduley' health area of Centro Habana. We collected data on general family characteristics and, for each household member, on sickness episodes in the preceding month, health service utilization and individual level predisposing factors. Enabling health service factors, being equal at the level of the studied health area, were not included in the questionnaire. We analyzed the data in contingency tables and with logistic regression. The dependent variable was 'use of any formal health service in the preceding month'.

RESULTS Three hundred and twelve of 1244 individuals included had experienced a health problem and of these 69% had consulted a health service. In univariate analysis we found a significant ($P < 0.05$) association of service utilization with the predisposing factors sex, age, civil status, schooling and occupation and with the need variable 'number of illness episodes'. In the logistic regression model only female sex [OR 1.9 (95% CI 1.2; 3.1)] and need [OR 8.8 (95% CI 6.9; 11.3)] remained independently associated with services use.

CONCLUSIONS Health need is by far the most important determinant of health services utilization. Of the examined predisposing factors, only gender exerts a moderate effect. The latter finding merits further study.

4.3-024

Population health and its determinants in Cuba: disparities study 1989–2010

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INTRODUCTION Since 1990, Cuba has suffered an important economic crisis, linked to the disappearance of the Soviet Union. Globally, the country's holistic and integrated health approach has limited negative health consequences. Nevertheless, geographical health differentials within Cuba and their determinants need further study.

METHOD We looked into differentials between the 14 Cuban provinces for the period 1989–2010. We studied health services determinants (19 variables, such as health expenditure, doctors/population, immunization coverage), socio-economic determinants (15 variables, such as population density, educational level, race), and health outcomes (19 variables, such as life expectancy, mortality rates, quality of life index, infant mortality rate, maternal mortality).

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RESULTS Structural health services variables showed only small differences between provinces, in line with the Cuban health system characteristics of universal coverage and accessibility. With regard to social determinants, we observed quite important differentials for 'population density' and 'economic production', that followed a well-defined geographical pattern: high, average and low population density, urbanization, and industrialization had a strong spatial correlation. For the health outcome indicators, only 'maternal mortality' showed important differences between provinces, but they were independent of socio-economic factors. However, economic productivity and living conditions were inversely related to minor differentials in other health outcome indicators, which could be explained by age structure of the population, sanitary problems and more stressful living conditions in urban areas.

CONCLUSIONS Geographical differences in maternal mortality need further analysis, possibly at the level of smaller geographical units.

4.3-025

Equity of access to health care in the health systems of Colombia and Brazil

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INTRODUCTION Both Brazilian and Colombian 90's market based health system reforms were led 'to improve efficiency and equity of health services provision' but were structurally different. In Brazil, the tax based national health system was decentralized to state, federal and municipal levels. In Colombia, the General Social Security System in Health comprised a contributory regime for the working/able to pay population and a subsidized regime for the poor. Brazil contracted private networks while Colombia established a competition among insurers and health providers. No comparative analysis on their results has been conducted so far. The objective is to analyse in a comparative way factors that determine access to the continuum of care in Brazil and Colombia and the level of equity of access in both countries.

METHODS A cross-sectional study was conducted, by means of a population survey based on face-to-face interviews to individuals who had had at least a health problem in the previous 3 months and resided in the areas of study. Data were collected from February 2011 to June 2011 applying a structured questionnaire based on a review of the literature, existing questionnaires and meetings with experts. The area of study was made up of two municipalities in each country, selected applying the following criteria: predominantly urban character and encompassing different socioeconomic levels. A systematic random sample of 4500 individuals was selected.

RESULTS Preliminary results reveal several obstacles along the continuum of care in both systems, specific to each healthcare model. In Brazil, barriers are rather related to services availability (e.g. waiting lists and shortage of professionals). In Colombia they are related to insurance's authorizations and copayments. Horizontal equity doesn't appear to exist in any of the two countries although the Brazil should more than Colombia since coverage is theoretically universal while in Colombia it varies with the type of insurance.

4.3-026

Access to quality healthcare in Spain: immigrants' voices

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BRIEF INTRODUCTION The increase of immigrants in Spain poses new challenges for the delivery of healthcare. International studies show an inadequate access healthcare for migrant populations. In Spain, access is mostly analyzed as service utilization from the providers' perspective, and few studies focus on the immigrants' perspective. The objective is to analyze the elements that influence access to quality healthcare from the viewpoint of immigrants.

METHODS AND MATERIALS A descriptive and qualitative study with a phenomenological approach was carried out by means of semi-structured individual interviews to users and potential users from Morocco, Rumania and Bolivia (96). Areas of study had a high concentration of immigrants and different foreign nationalities in Andalusia and Catalonia. A narrative content analysis was conducted, segmented by themes.

RESULTS Informants agreed that they resort to the services when they perceived their health problem to be serious. Free healthcare at the point of delivery, local language knowledge and social support were elements that facilitated access. According to some informants lack of social support linked to family commitments hamper services utilization. Other hindering factors that emerged were: working conditions (fear of asking permission), problems with registering in the city council, long waiting time in primary and emergency care, work overload of professionals and use of technical language. Some informants feel that they are treated differently because of being immigrants. Fear of being deported or stigmatized emerged as a potential factor that could prevent undocumented immigrants from seeking care for some diseases, such as TB.

CONCLUSIONS Despite the Spanish NHS offers universal coverage to residents in the country, interviewees identified different barriers to obtain care. While the informants coincided in some barriers, they also showed some differences. Some of the barriers are faced once they have entered the system and point out the insufficient adaptation of the services and professionals.

4.3-027

Investing in human resources quality for better physical accessibility: a case study from East Nusatenggara, Indonesia

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BRIEF INTRODUCTION The Australia Indonesia Partnership for Maternal and Neonatal Health (AIPMNH) Program in East Nusa Tenggara province has a strong focus to build the capacity of human resources for health to improve competency and improve health service provision. This approach is in line with local policy in NTT of 'MCH Revolution' which aims to shift the skilled birth attendance to facility birth, local government are keen to increase the quality of maternity service by improving the availability of clinical staff at first and second line service. We support the policy with a program that ensure the clinical competency of key health staff from in service training through regular supervision to monitoring the implementation of newly trained skills on the workplace.

METHODS AND MATERIALS This is an evaluation of a project that was started in 2008 and conducted in 14 selected districts from the

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total of 21 districts in East Nusatenggara which see one outcome of health system performance on accessibility. Mixed methods were used to collect data including document review, observations and review of routine health information.

RESULTS The complimentary approach which focus the HR development on quantity and quality has shown a positive result with 33% of first line facilities have an appropriate staff according to local standard. After a series of training and regular evaluation, a post training evaluation report shown an average percentage of staff who is competence on basic maternity skills is 43–67%. Preference of mother in using a midwife for basic maternity service has increase to 72.5% (from 36.5% in 2007). Province wide facility birth in 2010 is 24.2%, while facility birth on the supported area has reach 87%.

CONCLUSIONS Place for maternal and newborn death are still dominated by non facility based. Ensuring the availability of key health human resources at the first line level, could increase the confident and acceptability by community. But with the high demand of midwives from East Timor, there is a need for proper HR planning to ensure a longer retention.

4.3-028

Lessons learned from an organisation-wide policy change within an international non-governmental organisation: process and issues linked to the removal of user fees within msf supported health services

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INTRODUCTION With its main focus on crisis, Médecins Sans Frontières (MSF) intervenes also in situations of post-conflict or extreme health gaps, with 60% in Sub Saharan Africa. In 2003 MSF adopted a policy change, committing to provide care without requiring patient payments. The experience of how to conduct a fundamental and organisation-wide policy change on user fees can be of use to other international organisations.

METHODS AND MATERIALS A description of the process of policy change is made, with identification of key elements and moments, organisational changes required, operational consequences and impact on interventions and health status. During the eighties and nineties, co-payment was accepted in most public health services supported by MSF, if certain pre-conditions and implementation modalities to assure equitable access were present. Following increasing problem reporting in terms of accessibility, affordability and perverse effects on quality of care, a policy change was formalised in 2003. This policy paper stated the abolition of direct patient payments in all MSF supported health care.

RESULTS The following tools proved useful in the process: (i) Systematic review of accessibility situation in MSF supported health services to obtain an objective measure of the degree of problems; (ii) Practical support during implementation in terms of organisation and planning of additional resources; also monitoring tools were provided; (iii) Intensive briefings and discussions to obtain organisational buy in at all levels; (iv) At project and country level argumentation briefs and scientific literature were provided for use with health authorities and other organisations. Lessons learned from the process: (i) Population based measurement of access was key to obtaining a realistic perspective of access, as only way to measure non-use of available health services. The usual classification based on crisis situation showed its limitations. Post-conflict contexts showed prolonged high mortality and financial access problems, but so called 'stable' areas, without any history of conflict – showed equally bad under five mortality indicators; (ii) Many assessments focus on affordability of care in terms of willingness to pay or avoiding catastrophic health expenses. MSF's experience showed the importance of exclusion/deterrence from utilisation and the important financial obstacles linked to relatively small fees for primary health care; (iii) Increased utilisation rates allowed better assessment of the real disease burden in the community; (iv) Several population assessments post-abolition of user fees, show a significant reduction in general and child mortality.

CONCLUSIONS For international organisations that support existing public health services, assurance of financial access is crucial, as it is key to reach those people most in need of care. Without this, additional resources mobilised by or through the organisation, are trapped in inaccessible health facilities, channelled to the better off. For reasons of medical quality, effectiveness at population level and accountability, abolition of user fees is an important policy decision for international health organisations.

4.3-029

Reorganization of provincial level of health system in North Kivu and oriental Kasaï, democratic Republic of Congo

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INTRODUCTION In the framework of implementation of health system straightening in DR Congo, and in a context of decentralization dictated by the National Constitution, reflexion work has been initiated based on essentials functions of health system's provincial level in two different provinces in the country.

METHODS Two years multidisciplinary action research led in four steps: (i) socio-anthropologic investigation on plans put in place in health provincial division (HPD); (ii) reflexion and analysis workshop around specific plans; (iii) joint elaboration (experts and HPD) of a new organization chart based on four specific professions; and (iv) definition of this four professions (contains and organization).

RESULTS This action research gave rise to function plans put in place by HPD in order to develop a more participative management and to compensate for the weakness of structural organization. Experts backed HPD for the implementation of a new structure likely to institutionalize this new participative management. The latter is based on four professions: (i) health district support; (ii) control and inspection; (iii) information, communication and research and (iv) management. HPD and experts drew up profession definition, competences description. Results were presented at national level and have been integrated in the new health development plan.

CONCLUSIONS Apart concrete result obtained, additionally, adopted approach – focused on apprenticeship and organizational development – has contributed to dynamize provincial level essentials functions. Two major challenges have to be taken up: (i) support HPD transformation from actual situation to the new model and (ii) spread this new model to the others provinces, according to the same participative approach, successful condition to adjust the organization chart at the context.

4.3-030

Health systems obstacles in the delivery of quality care to people with chronic diseases (PWCD): a case study from urban India

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INTRODUCTION India is undergoing a rapid epidemiologic transition with chronic diseases now constituting a leading cause of death and suffering. Delivering an appropriate response to this rising

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burden implies health systems strengthening. We analysed the structure and functioning of the Local Health System (LHS) in the poor urban neighbourhood of KG Halli (Bangalore) in order to identify its principal weaknesses in providing quality care to PWCD.

METHODS We applied the health-system analysis framework developed by Van Olmen et al. to the situation of KG Halli using data generated over a period of 2 years through (i) a household census (n = 9299, response rate = 98.5%) using a questionnaire on socio-demographic characteristics, illness profile, health seeking behaviour, and healthcare expenditure; (ii) mapping and interviewing healthcare providers (n = 24); and (iii) observational field-notes. Document analysis and STATA were used to analyse data.

RESULTS The KG Halli LHS faces poor operational and administrative integration across public and private healthcare providers. Most resources including trained personnel, laboratory and pharmacy support lie in a poorly regulated private sector that cares-on a fee-for-service basis – for more than 80% of PWCD. Lack of gate-keeping at the primary care level leads to hospitals managing almost 60% of PWCD. These inefficiencies contribute to have one out of four families experiencing catastrophic expenditure (>10% of household income) in ambulatory care alone. All the preceding factors, plus poor referral and information systems, negatively affect continuity and effectiveness of care, with a high hospitalisation rate of 191.4/1000 per year in PWCD, pointing to delays in receiving adequate care at the primary care level. The geographical delimitation of the population of responsibility of the public facilities in the KG Halli area is inappropriate; among the private providers, the notion of a 'population of responsibilityâ' is simply not taken into consideration. Contextual factors like rapid urbanisation and internal migration pose additional challenges.

CONCLUSION The KG Halli LHS not only responds poorly to needs of PWCD but also causes them considerable impoverishment. Rising chronic disease burden provides an opportunity to re-examine organisation and performance of health systems. A structured analysis allows identification of the main obstacles to overcome.

4.3-031

Chinese immigrants in a southern area of Madrid: hospitalization pattern and comparison with other immigrant groups

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BACKGROUND Chinese immigration to large cities in Spain has increased in the last decades. It is believed that they use national health care facilities less frequently and differently to other immigrants. The aim of this study was to describe the pattern of hospitalization among Chinese as compared to other immigrant groups in a general hospital attending a large proportion of foreign population.

METHODS Review of hospitalization of Chinese immigrants at the University Hospital Fuenlabrada, Madrid, period January 2006–June 2009. Description of patients' characteristics and hospitalization pattern using variables included in the Minimum Basic Data Set and the Diagnosis Related Groups (DRG) classification. Comparison with Latin-American (LAm), African (A), and European (E) immigrants.

RESULTS Chinese (159), LAm (1705), A (1931) and E (1022) accounted for 4817 (8.7%) of all admissions. Average age: Chinese 30 years (LAm 33; A 34.1; E 35 all P < 0.01), 88% women (LAm 75%; A 78%; E 69% all P < 0.05). Departments: 79% obstetrics (LAm 48%; A 52.6%; E 45.3% all P < 0.01), 4.4% general surgery (LAm 10.9% P = 0.09; A 5.3% P = 0.6; E 7.4% P = 0.16) 4.4% internal medicine (LAm 14.54%; A 17.1%; E 18.8% all P < 0.01) 3.1% gastroenterology (LAm 2.8%; A 2.6%; E 4.1% all P > 0.5). Main DRG's: Vaginal delivery (VD) without complications (C) (40.9%), (VD) with (C) (27%), cesarean section without (C) (6.1%), (VD) with sterilization (1.9%), disorders of pancreas except malignancy (1.9%), gastrointestinal hemorrhage (1.3%), kidney/urinary tract infection(1.3%). Mean length of stay 3.17 days (LAm 4.2; A 5.1; E 4.85 all P < 0.01)

CONCLUSIONS The hospitalization pattern of Chinese patients in our setting differs from that of other major immigrant groups. They are young women admitted to obstetric wards during labour, resulting in vaginal deliveries and short lengths of stay. Admission to internal medicine and general surgery is significantly less frequent as compared to all groups and to LAm, respectively.

4.3-032

Sustaining community midwives for quality maternal services: an experience from a project in a rural area of Bangladesh

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INTRODUCTION Bangladesh has been a serious shortage of qualified health workers at all levels. The shortage of nurses and midwifery staff is particularly acute. In view of this shortage and huge demand for services, informal providers emerged as the largest group. In 1994 ICDDR, B initiated a community based primary health care project in Chakaria, a remote rural area of Bangladesh in an attempt to ensure quality services to the villagers.

MATERIAL AND METHODS The project involved in training of locally recruited 13 females as community health workers for disseminate health messages which they did till 1998. In 1998 to 2001 they were trained as community midwives in five batches through a residential course of 3 months. On the job training on midwifery also continued by the project physician twice a month. The community midwives provided services from seven village health post established by the villagers 6 days a week, performed ANC, home delivery and PNC, refer complicated cases to the physician attending once a week at village health post and other public/private providers. In January 2006 the community midwives were released from ICDDR, B paid service with an arrangement that ICDDR, B will only reimburse for safe delivery services they provide to the women from lowest two asset quintiles. From 2009 ICDDR, B has discontinued reimbursement for the service provided by the community midwives. Data from the Chakaria Health and Demographic Surveillance System of 2005 and 2010 were used for comparing the performance of midwives.

RESULTS As of now, all midwives could sustain at their profession without further support from the project. HDSS data shows the performance of midwives remains same in 2010 compared to 2005.

CONCLUSION The most important factors that contributed in sustaining midwives in their profession included community involvement in the process, quality of training they had received and services they provide, raising their profile and providing professional and financial support by ICDDR, B, for an initial period of time, continuous linkage with ICDDR, B physician for consultation mostly through mobile phone, and clear vision of making them sustainable from the beginning. In settings with acute shortage of health manpower locally recruited females should be trained to fill up the shortage of maternal service providers.

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4.3-033

Adding a reference wing to an unstructured public urban hospital: impact on the organization of health district in Kintambo, DR Congo

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INTRODUCTION Most public reference hospitals in health districts in Kinshasa, DR Congo, are characterized by a lack of management and an absence of healthcare rationalization. As a result staff are demotivated, financing is lacking, and the target population is dispersed to unregulated private health centers which offer the same services. The consequence is healthcare inaccessibility. Kintambo health district presents all this difficulties: the objective of this experiment was to propose a quality reference system which would be able to improve healthcare within the unstructured environment.

METHODS In December 2009, a reference wing was established in the reference hospital in Kintambo. Building rehabilitation, essential medicine and allowances were available. Resources rationalization and competence strengthening in the field of management were put in place. Evaluation was done after 1 year.

RESULTS In 2010, within Kintambo hospital which had 200 beds and 800 staff, the reference wing has proposed 18 beds of internal medicine, pediatrics and surgery. These beds were under medical surveillance round the clock. Three medical doctors, seven nurses and a pharmacist assistant make the medical team. Mortality rate was 4%, while it could reach 25% in the others departments. The reference wing was opened to the referred outpatient within the health district: that situation has strengthened links between healthcare levels of sanitary pyramid.

CONCLUSION In a context of hospital disorganization, staff excess and lack of financing, it was possible to put in place an adapted and relevant reference wing.

4.3-034

The influence of social networks on the health of older people: review of literature and research opportunities in Africa

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INTRODUCTION Aging is a universal and total social fact. It is also a public health problem in the sense that it is the source of disability. This requires a range of services through formal and/or informal care and support for elder people. We performed a critical literature review to clarify the prospects for Research on this theme in Africa.

METHODS This literature review used 'pubmed' and targeted two types of articles published over the last 15 years in Europe, America, Asia or Africa. The first type of articles targeted the relationship between the elements or characteristics of social network and health of the elderly. The second and those proposing theories or models on this theme.

RESULTS The first type of studies, searching for social causation have focused on the causal links between a predictor and a dependent variable indicating the health of the elderly. They are developed in a deterministic logic and do not take into account the complex interactions between social network and health of the elderly. The Results varied from one study to another, and sometimes in the same study. The second type of studies focus on explanatory theories developed in European, North American or North America context. Their validity and anchoring in an African context has rarely been studied.

CONCLUSION The literature review highlights the need to develop theories grounded in African reality to better understand the complex interactions between the social network and health of the elderly.

4.3-035

Functional disability among elderly people in Bobo-Dioulasso (Burkina Faso)

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INTRODUCTION A significant increase in functional disability in elderly is expected in the world including Africa, where elderly healthcare are already inadequate. This will increase pressure on health and social care systems if the good management and prevention strategies for of elderly disability are not identified, adopted and evaluated now. This study was conducted to describe the multidimensional functional disability in elderly people in Bobo-Dioulasso (Burkina Faso).

METHODS This is a descriptive transversal study, which took place in Bobo-Dioulasso. We used a systematic random sample of elders. Each participant was interviewed using the questionnaire 'PRISMA7' which identified elderly with functional disability. To describe disabilities, elders who obtained 4 or more than 4 score were interviewed with the questionnaire 'SMAF'. Data analysis was performed using Stata. The study protocol has obtained the approval of the Burkina Faso National Ethics Committee for Health Research.

RESULTS A sample of 362 individuals was interviewed using the PRISMA7. Forty-two per cent of them were identified as being with functional disability and 25% expressed the need for supervision or assistance. This need of assistance varies according to the five areas of SMAF and in between items of each areas. The means SMAF score is 20 out of 87. The rate of functional impairment is zero because each elderly people identify a resource to assist them in case of disability The family is the primary resource that manages permanently elders' disabilities.

CONCLUSION In Bobo-Dioulasso, a quarter of elders needs help that are covered by social support network However, social and Health policy must take into account that prevision consider that elders needs will increase and resources will decrease from now to 2050.

4.3-036

Social capital for strengthening community-based malaria control in Palawan, the Philippines

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Palawan is the remaining highly malarious Province' in the Philippines. The social mobilization thrust of the province malaria control program-Kilusan Ligtas Malaria (KLM) meaning 'movement against malaria in Tagalog', is envisioned to sustain the local implementation of malaria control. In keeping to this goal, the malaria control strategies are community-based. However, sustainability of malaria control remains in question. 'Can social mobilization lead to its ultimate goal of people and community empowerment and thus ensure sustainability of malaria control?' It is in this light that we examined social relationships and networks, collective norms, individual and ecological trust, and

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access to resources, the so-called Social Capital (SC) that will support the social mobilization efforts of KLM. A qualitative research was conducted through a series of focus group discussions among health care providers and health service beneficiaries in selected communities of the province. The study describes the SC existing in these communities in terms of the nature and extent of the villagers of personal social network and the relationships of formal and informal organized groups in the community. The Results of the study showed that group activities and individual participation were influenced not only by available public resources but also by the level of development and maturity of the community and its members. The level of community participation was still limited, especially among the indigenous peoples, partly owing to marginalization secondary to socio-cultural and economic differences. The study identified the benefit of involving existing groups in the community. Social cohesion, collective action and cooperation in implementation of malaria control activities were particularly noted. The concept of SC is relatively new to the field of health. Considering that SC is still an emerging interest in malaria control, we will further utilize the Results of this qualitative research in strengthening community-based malaria control.

4.3-037

Unmotivated health workers in pakistan: the tip of an Iceberg

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AIM Developing countries suffer the greatest burden of disease managed with a relatively smaller health workforce. This is compounded by low health provider motivation, which leads to emigration and compromised health service delivery and quality. Few studies have examined this issue in developing countries, especially among physicians. This part of the study aimed to explore physicians' motivation, preferences and plans regarding their jobs.

METHODS A stratified random sample of 360 physicians, with equal male and female participation, was selected from public (primary, secondary and tertiary) and private tertiary health facilities in the Lahore district, Pakistan. Pretested, semi-structured, self-administered questionnaires were used. Physicians ranked their current job motivation level on a five-point Likert scale and reported their preferences regarding work in Pakistan.

RESULTS Overall, 58% physicians were fairly/highly motivated, 27% were neither motivated nor demotivated and 15% were less motivated/demotivated (higher in primary and secondary setups). Most (71%) preferred to stay and work in Pakistan and 19% wished to work abroad. More male physicians (29%) than females (13%) preferred to work abroad. Over half (54%) of the physicians planned to continue and 42% to leave their current jobs (higher in private and public primary setups).

4.3-038

Health worker motivation in tertiary hospitals in Pakistan

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AIM Considering the human resource crisis in developing countries, low health worker motivation is a neglected yet important problem in health service delivery and quality. In this study, we aimed to identify factors affecting physicians' motivation in public and private tertiary hospitals in Pakistan.

METHODS A stratified random sample of 300 physicians working in the largest public and private tertiary hospitals in the Lahore district, Pakistan was selected. Pretested, semi-structured, self-administered questionnaires were used. For this part of the study, physicians reported their five most important work motivators and demotivators in their current jobs. Open-ended responses were coded according to emergent theme, classified as intrinsic, organizational and socio-cultural and frequencies calculated.

RESULTS Public tertiary physicians reported more intrinsic and socio-cultural motivators. Serving people, respect and opportunities for higher qualifications were important in both setups. In private setups, more organizational factors were reported like financial incentives other than pay and good working conditions. Demotivators across setups were mostly organizational. Less pay was reported most frequently. Less personal safety and poor working conditions were important in the public sector, particularly among female physicians.

CONCLUSIONS In developing countries like Pakistan, addressing physicians' motivation is important to minimize brain drain and health worker shortages. The important motivators in this study were mostly intrinsic and socio-cultural, which are difficult to affect. However, demotivators were largely organizational, which can be addressed even at local levels (e.g. less personal safety and poor working conditions), especially in public setups. Small scale changes may substantially improve physicians' motivation and the quality of care.

4.3-039

Are doctors safe at work in Pakistan?

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AIM Few studies have considered the importance of a safe environment for health providers, particularly in developing countries. In resource-limited settings, safety maybe compromised exposing workers to greater disease risks like tuberculosis, hepatitis and AIDS. Lack of physical safety can also be a strong demotivator, especially in conflict areas. We explored satisfaction about safety and occupational health from doctors' perspectives.

METHODS A stratified sample of 360 doctors with equal male and female participation was randomly selected from public (primary, secondary and tertiary) and private tertiary health facilities in the Lahore district, Pakistan. The study used pretested, semi-structured, self-administered questionnaires. In this part of the study questions were asked about doctors' satisfaction about the level of physical and health-related safety as well as general occupational health in their current jobs.

RESULTS Regarding physical safety during work, 58% of doctors were less satisfied/dissatisfied with more females reporting dissatisfaction (82%). Fewer doctors in private setups (11%) compared public setups (74%) were dissatisfied. Regarding health safety measures against diseases, 79% of doctors were less satisfied/dissatisfied. Concerning general occupational health satisfaction, 70% of doctors were less satisfied/dissatisfied.

CONCLUSIONS Less personal safety was very important for public sector doctors, especially females. Recent security issues in the region may be compounding the issue. More importantly, prevention and compensation for both the physical and health-related safety of doctors, particularly for those working in low income countries and regions of conflict, should be considered to reduce illnesses, burnout or the emigration of doctors, particularly women, from an already burdened health system.

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4.3-040

Exploring rehabilitation systems for children and youths with disabilities: integrating specialised health services and community based rehabilitation in Tanzania and Sudan

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INTRODUCTION Poverty, malnutrition and infectious diseases repeatedly result in congenital and acquired disabilities among children (WHO, 2011). Rehabilitation services in most low-income and post-war situations are largely insufficient, while the impact and costs affect whole families. Adequate rehab services often imply both specialised health services and Community Based Rehabilitation. There is a need for research relevant to rehabilitation policy, systems and services' development in contexts where poverty and conflict are affecting children even more. Potential 'best practices' and challenges need to be studied locally.

METHODS AND MATERIAL A qualitative design including multi-sited anthropological fieldwork was applied, building on previous research and collaboration with rehabilitation institutions in Tanzania and Sudan. The NGOs have long-standing experience with specialised and Community Based Rehabilitation (CBR). Literature and documents on rehab policies, rehab institutions' and CBR programmes' strategic plans, registers etc. were analysed, and interviews and focus group discussions with key-informants performed, whereof rehab managers and professionals in each country.

RESULTS Satisfactory rehabilitation implied in both countries early intervention, and continuity between hospital-based and CBR services. Everywhere, national and international donors played a considerable role in financing services. Awareness, rural/urban location, referral and transport were significant in facilitating access. Quality of services depended on availability of specialised and motivated personnel, sometimes working voluntarily. Priorities in rehabilitation also depended on the type of impairment and on donors and consumer-organisations involved. Professional task-delegation and CBR volunteers required follow-up.

CONCLUSIONS Adequate rehabilitation of children with disabilities depends on the chain of rehabilitation services available from community level to specialised health centres. Private models of service-provision and financing dominate; yet, increased involvement of the public sector in integrated services and in promoting the education of specialised rehab professionals and training of volunteers is needed. The role of international and national donors and consumer organisations remains vital in comprehensive CBR.

4.3-041

Three clinical research platforms in Africa and Latin America to build sustainable research capacities

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Drugs for Neglected Diseases initiative (DNDi) integrates capacity strengthening in all of its projects to support registration, uptake, and sustainable access of new treatments for neglected diseases. Supported by DNDi, three regional disease-specific platforms in Africa and Latin America collaborate in R&D programmes: (i) HAT Platform (Human African Trypanosomiasis, or sleeping sickness) (ii) LEAP (Leishmaniasis East Africa Platform, for visceral leishmaniasis) (iii) Chagas Clinical Research Platform (Chagas disease). The platforms bring together regional and international actors: ministries of health, national control programmes, regulatory agencies, academia, clinicians, civil society groups, and pharmaceutical companies with a common vision of addressing patient needs in the local and national contexts where the diseases are endemic. They utilize, capitalize upon, and reinforce clinical capacities in endemic regions, and address infrastructural requirements where necessary. They provide on-site training in clinical research in sometimes very remote settings, which are the most challenging research environments. The HAT Platform has trained site investigators and clinical monitors, implemented two clinical trials, and facilitated the implementation of the first new, improved treatment for stage 2, Nifurtimox-Eflornithine Combination therapy (NECT). LEAP coordinates clinical trials and trains staff, and has contributed to the rehabilitation of several treatment centres (Sudan, Ethiopia, Kenya, Uganda). In 2010, DNDi and LEAP delivered SSG&PM, a new shorter-course combination therapy for visceral leishmaniasis, recommended by the WHO Expert Committee on the Control of Leishmaniases as first-line treatment for VL in East Africa. The Chagas Clinical Research Platform, which held its first meeting in 2009, will strengthen infrastructure and training, establish and foster joint research projects, expand community participation, and improve evaluation and delivery of new treatments. These three platforms are part of an innovative model for sustainable research capacity in neglected disease endemic countries, providing a constructive and effective environment for regional collaboration on developing treatments for neglected patients.

4.3-042

Fear of deportation may limit legal immigrants' access to HIV/AIDS-related care – a survey of Swedish language school students in Northern Sweden

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BACKGROUND The increasing rates of HIV infection and AIDS cases that are currently being reported in high-income countries can be partly explained by migration from high prevalence countries. Yet, early diagnosis of HIV/AIDS in immigrants remains a challenge. This study investigated factors that might be limiting immigrants' access to HIV/AIDS-related care.

METHODS AND MATERIALS Data were collected over 1-year period through a survey questionnaire completed by 268 legal immigrant students of two Swedish language schools in Northern Sweden. Participants were aged between 16 and 63 years and originated from 133 countries. Descriptive and logistic regression analyses were performed.

RESULTS Thirty-seven percent reported reluctance to seek medical attention if they had HIV/AIDS. At the bivariate level, reluctance to seek care was positively associated with youngest (16–24 years) age (OR = 4.53, CI: 1.23–16.69, $P < 0.05$), being from the Middle East (OR = 16.8, CI: 2.14–132.24, $P < 0.01$), having 6 years of education (OR = 4.2, CI: 2.07–8.52, $P < 0.001$, having low/poor knowledge of HIV/AIDS (OR = 4.7, CI: 2.50–8.77, $P < 0.001$), having stigmatizing attitudes towards those affected (OR = 8.0, CI: 3.66–17.58, $P < 0.001$), being afraid of disclosure (OR = 14.5, CI: 4.39–47.86, $P < 0.001$) and deportation (OR = 8.2, CI: 4.57–14.59, $P < 0.001$). Fear of deportation (Adjusted OR = 6.6, CI: 3.08–14.23, $P < 0.001$) emerged as the most important determinant of reluctance to seek care among this sample of 'settled' immigrants after adjusting for socio-demographic factors, knowledge level, stigmatizing attitudes and fear of disclosure. Other predictors included fear of disclosure (Adjusted OR = 5.5, CI: 1.26–23.60, $P < 0.05$) and lowest level (0–6 years) of education (Adjusted OR = 3.3, CI: 1.19–9.06, $P < 0.05$).

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CONCLUSIONS Targeted interventions should consider the heterogeneity of migrant communities and the complex interplay of various factors which may impede access to HIV-related services. The myth about deportation because of HIV/AIDS should be countered.

4.3-043

The international master in medical and veterinary entomology

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INTRODUCTION The International Master in medical and veterinary Entomology (IME) is a teaching programme for 2nd year Master students on vector systematics, biology and ecology, population genetics, genomics and control. The IME's objective is to prepare future researchers in areas of public health, veterinary, teaching and research.

MATERIALS AND METHODS Candidates must have a Bachelor of Science (BSc) or equivalent. CVs and applications are evaluated by a scientific committee. The IME consists of three parts: (i) theoretical courses delivered by top-notch experts from each field in Ouidah, Benin; (ii) laboratory and field-work practices in endemic regions of West Africa; (iii) a 5-month research training at IME-collaborating laboratories mainly in Africa and Europe. A written report on the research training is presented at the end of the academic year.

RESULTS Since its creation in 2007, the IME has welcomed 60 students from 21 nationalities and three continents (Africa, Europe and South America). Fifty-nine students have successfully earned their dual Master degree issued by the UAC-Benin and UM2, France. Only one student did not attain the expected level of quality. Accomplished students have pursued different research and public health interests: Up to 29 of them have enrolled in research-oriented PhD studies and the remaining work on national control programs or research institutions in vector control, predominantly in Africa and the Indian Ocean.

CONCLUSIONS Thanks to an international effort, the IME provides highly specialized training to an average of 16 students per year since 2007. Education is the key to progress. We provide an equal opportunity education on medical and veterinary research areas affecting all of us. Our gratitude to the IRD, French Cooperation, WHO, Institute Pasteur, CIRAD, Centre for scientific research and surveillance on emerging diseases in the Indian Ocean (CRVOI), USAID, MRTC, Vector Control Industry, ANR, TWAS, FP7-European Commission and many more.

KEYWORDS Entomology, master, training, vector control, capacity building

4.3-044

Structure and performance of the integrated disease surveillance system in the 33 districts of Maharashtra in India

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INTRODUCTION After successful implementation since 2004, the Integrated Disease Surveillance (IDS) system is now in its early stages of independent operation in the state of Maharashtra, India. It is now necessary to document the baseline performance of the system to identify areas for improvement and to allow future monitoring. This paper presents the findings of a baseline assessment of the structure and performance of the IDS system in all the 33 districts of Maharashtra from January 2010 to January 2011.

METHODS AND MATERIALS The CDC updated guidelines for the evaluation of surveillance systems and the WHO Protocol for assessing surveillance and response systems were modified and used to design the overall evaluation. The system was assessed along four key areas – structure, core functions, support functions and surveillance quality. Data was collected using questionnaires, record review and group interviews. We present findings at the state level in this paper.

SELECT RESULTS Standardized case definitions and trigger levels were well defined for all the 21 priority diseases. Annual trend analysis was available for all diseases for all 33 districts for all the 52 weeks. Of the 138 positions sanctioned in the state, 44 (31.4%) were vacant; none at the SSU. The frequency of communication between districts, state and national level was inconsistent. Over 29,138 (93%) technical and non-technical staff was trained in epidemiology as of May 2011 including all staff at the SSU. The system reported 263 outbreaks [majority of Acute Diarroheal disease (48%)] in the year of which 71% were reported late. The consistency of reporting ranged from 19% to 85% for 'P forms'; 0–97% for 'S forms' and 25–85% for 'L forms'. Majority of the districts (40–50%) preferred to send the forms by Email as against the data portal. The system did not meet the meager threshold of at least one private reporting unit per region (Taluka).

CONCLUSIONS Although the IDS system in Maharashtra is functioning satisfactory, the following areas require strengthening. The discrepancies within the districts for timeliness and completeness are large. There is a lack of independent data analysis and monitoring of indicators at district level. The data portal needs to be re-vitalized. Trained contractual staff should be retained and offered permanent positions for sustainability. Mandatory feedback sessions at the state and district. Appropriate incentives to private reporting units to ensure their active participation in the system.

4.3-045

Access barriers to effective hiv treatment linked to integrated service delivery through inequitable health services: the case of rationing arv initiation through patient payments for health care in Kinshasa, DRC

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INTRODUCTION Repeatedly it has been shown that patient payments for HIV treatment lead to worse outcomes in terms of initiation, adherence, survival. Patients suffering chronic diseases are particularly prone to catastrophic health expenditures. Subsidies targeted at assuring health care free of charge have shown better access and better coverage potential for illnesses with a public health impact. Nevertheless patient fees are still required in several countries; mostly ARVs are (at least in theory) free of charge, but the burden of other costs linked to treatment often fall on patients. This analysis shows how patient payment for these linked costs excludes a large proportion of patients from accessing free ARV.

METHODS AND MATERIALS Systematic review of patient payment required in public, faith based and NGO supported health structures that offer HIV treatment and receive international

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funding. This information was collected by interviews of key informants and of in situ checks of applied tariffs. Patients already on treatment transferred from NGO facilities applying free care to public structures applying patient fees were traced to verify loss to follow up. Funding availability and pre-conditions concerning patient payment were checked with all major international funding agencies. Rationing mechanisms as consequence of the funding shortfall were explored through interviews with key informants among health workers and patients.

RESULTS Although in theory ARV are provided free of charge in public health structures and most non-governmental or faith based health structures, costs linked to other elements of care can be real barriers to initiation and continuation of treatment. Patients that start late treatment and/or face specific complications or co-infections face proportionally higher financial obstacles. Specific financial obstacles were identified at steps in the treatment process linked to laboratory examinations, such as CD4-count, Viral load and other biochemic tests needed to assure adequacy of the regimen used. Cost per CD4 test would be as high as 15–20 usd and viral load testing would could around 60 usd. This contrasts strongly with the average revenue of households in Kinshasa; with the majority of the people gaining <1 usd per day, such costs are deterring health care seeking and exclude all but the few better off. Functioning costs of public and faith based health structures are mainly financed by patient fees; NGOs with insufficient international funding for recurrent costs also rely on user fees. HIV patients referred to public health structures for initiation or continuation of ARV treatment, often abandon treatment because of financial reasons Donor funding mostly covered the purchase of ARV, but not of OI drugs, nor consultation costs. Although laboratory reagents are internationally funded, personnel costs are not included and the shortfall of funding opens up the possibility to charge patients for these tests.

CONCLUSIONS Although certain donors require the implementation of free access to treatment for patients in structures they support, in reality – because of insufficient financial means – most patients face deterred or delayed treatment, leading to low coverage and unsatisfactory results. Non-calibrated application of the national health systems policy of organizational and financial integration leads to financial exclusion, limited coverage and unsatisfactory patient and programme outcomes. There is an urgent need for international subsidies targeted at improved financial access of all key elements of HIV treatment.

4.3-046

The question of adequate utilisation rates for outpatient and inpatient activities in DRC for planning and resource allocation: a lack of standards?

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INTRODUCTION Recent provision of basic health care free of charge has resulted in several African countries in a significant increase in utilisation rates of primary and secondary health services. The reported increases often meet reactions indicating surprise or interpretations of 'exaggerated' or irrational use of services.

METHODOLOGY We reviewed literature and in particular WHO documentation on specific indicators for acceptable utilisation rates both for OPD and IPD. We inquired which reference values were most frequently used for planning and evaluation in country.

We also compiled reports of utilisation rates in various health structures in DRC with some indications on financial and geographic access. Where available, health structure based utilisation rates were compared to population based morbidity reports.

Additionally we analysed utilisation and hospitalisation rates from MSF supported health services in other countries in Sub Saharan Africa.

RESULTS The WHO standards that are referred to most often provide for OPD utilisation 0.6 or 1 new contacts per inhabitant per year respectively in rural and urban areas. However these values refer to recorded utilisation rates in real life, i.e. under cost recovery schemes. This probably led to serious access barriers and therefor underestimation of adequate utilisation rates. The 0.6 contacts per inhabitant per year is therefor of limited use as standard. For hospitalisation rates there were extremely few reference figures. The ones available describe existing reality rather than adequate use.

In DRC planning and resource mobilisation is based on the general averages proposed by WHO for the OPD utilisation rates; this leads to probably false perceptions of over-use or irrational use by the population. There is little population based information to check reported health service utilisation under care free of charge; the available ones indicate an important gap between morbidity, health seeking behaviour and actual service use. The gap of reported morbidity based on health service reports in areas with and without patient fees was analysed for specific health problems such as Sexually Transmitted Diseases in OPD and caesarean sections in IPD.

A preliminary analysis of figures on hospitalisation rates and OPD utilisation rates in MSF supported structures in SSA countries is also provided.

CONCLUSIONS There is an urgent need for updated information on OPD and IPD utilisation under service provision free of charge to patients. The currently used norms are not adapted to new strategies such as provision of care free of charge; as a consequence they set lower targets than needed on the base op epidemiological and morbidity information. This significantly limits the planning basis of adequate resource allocation and mobilisation. The coverage by curative OPD services has also some implications for preventive care offered through existing health structures.

4.3-047

'The doctor just writes what i tell him'. dilemmas experienced by tanzanian health care staff in the implementation of imci in the consultation room

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Evaluations of Integrated Management of Child Illness (IMCI) have shown that when the intervention is fully implemented and receives adequate health systems support and supervision, it is cost effective and have the potential to significantly reduce child mortality and morbidity. This presentation reports from an anthropological study of facility-based implementation of IMCI in Tanzania and present a perspective on IMCI from the consultation room. The study concludes that IMCI trained health care staff only rarely examine children physically during consultations. Instead the consultations consist of a brief oral exchange between the health care staff and the child's caretaker which leads to a prescription. As one mother expressed it: 'The doctor just writes what I tell him'. The presentation explores the social organisation of the clinical encounter which assigns meaning to the conspicuous absence of the diseased body. Furthermore, the presentation explores the dilemmas health care staff experience when expected to enact policies and guidelines in practice.

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4.3-048

How much and where we do pay for coping with our health problems

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BACKGROUND Bangladesh is a low-middle-income country with limited resources and setting but with huge threat of many upcoming challenges including the effect of climate change on settlement, agricultural production, economic development, human health and so on.

OBJECTIVES The study has conducted to know the coping strategies for the health problems induced by climate change. How much people spent for it and where they spent money for treatment.

METHODS The study was a cross-sectional survey of household heads from two villages one from northern part and another from southern part of Bangladesh. A total of 450 households (53% males and 47% females) selected through a multistage sampling techniques, completed a semi-structure questionnaire.

RESULTS On an average TK. 10,000.00 per year (US$1 = TK.72) was spent by every household. Eighty-three per cent household took treatment from village doctors, pharmacists as their first priority. One hundred per cent of them do not have any formal degree from medical science. One hundred per cent money was out of pocket payment (OOPP).

DISCUSSION Rural people are spending a lot for their health care but not getting quality care as 83% going to village doctors for health care. There is no fund pulling (FP), community funding (CF) or health insurance (HI) program at rural areas to support the burden of health cost of the poor people. Immediate and effective policy actions are needed either to improve the quality of the rural providers to ensure quality health services. Need initiatives from government NGOs and development partners to introduce FP, CF, HI at rural areas to reduce OOPP, improve quality health services and to achieve universal health coverage in this climate vulnerable areas.

4.3-049

Health research policy in Southern Sudan

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The overall responsibility for health research in South Sudan falls under Division of Research, Monitoring and Evaluation and has been the remit of the Directorate of Planning and Coordination in the Ministry of Health Government of Southern Sudan. Scientifically unsound research involving human subjects is by definition unethical as it may expose participants to risk or inconvenience for no benefit. Even if there is no risk of injury, expenditure of subjects' and researchers' time in unproductive activities represents a waste of valuable resources. Mechanisms, therefore, exist to ensure the scientific validity of health research. This is usually the responsibility of research ethics committees. An ethics committee can verify that a competent body has judged the research to be scientifically sound. The existing structure of the research department includes research data hub, ethical committee and the research secretariats. The National ethical committee composes of seven (7) people and from various different fields and not necessarily all from ministry staff. National ethics committee (NEC) will undertake the independent, competent and timely ethical review of research proposals. There are two standard application forms for health care research conducted in Southern Sudan; one for Social and Behavioral Research and another for Medical and Clinical Research. Researchers (principal investigators) also have to submit a covering letter from their academic institution, evidence of REC approval from their own institution, a detailed research protocol and the data collection tool. Seven hard copies of the application are required. Southern Sudan is emerging from a decade's long civil war, which has degraded the country's social and physical infrastructure. The Health research policy have presented the Government of South Sudan with the opportunity to start building a nation and to regulate and monitor research in the country.

4.3-051

Hernia surgery in Subsaharan Africa

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INTRODUCTION This is a review of hernia surgery in sub-Saharan Africa.

METHODS The database of the author was screened for hernia operations done in the South Kivu province of the DRCongo (Kamituga) during the period Nov '08–May'09. Likewise the database was checked for hernia operations done Feb–May 2011 in north-eastern Nigeria (Jimeta-Yola).

RESULTS The data from the Congo showed 70 hernia operations out of a total operation frequency of 286 (24%). There were 38 inguinal hernia repairs; 25 adults (two females, median age 56 years) had a Bassini repair. There were three bilateral repairs and five revision operations. Thirteen children (all boys, median 1.25 years) underwent herniotomy. Epigastric hernias were seen in 16 patients (13 women, median age 45).Nine incisional hernias (eight females, median 31 years) were found mostly post caesarean section; and there were six umbilical hernias and one femoral hernia. Within the Nigerian setting there were 62/236 operations (26%) attributed to hernia repair. Inguinal hernias contributed 51 cases: there were 35 adults (three females, median 50 years) and 16 children (all boys, median 1.5 years). Acute obstruction was present in seven patients. Eight had umbilical hernias, of which three presented as an acute abdomen. Finally, there were two incisional hernias and one epigastric hernia.

CONCLUSIONS Hernia surgery contributed in both settings approximately a quarter of the overall workload for the general surgeon.

Surprisingly, acute presentation of hernias were not seen in Kamituga(Congo), but in 10/62 (16%) of the case load in Jimeta-Yola(Nigeria).

More than one-third were epigastric (16/70) and incisional hernias (9/70)in Kamituga (Congo),both were rarely seen in Jimeta-Yola (Nigeria).

Suturing the various hernia types includingtheBassini technique for inguinal hernias in adults is the preferred treatment option.

4.3-052

The attencion to internacional travelers in a unit of primary care

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INTRODUCTION The increasing number of travellers to the tropical and subtropical region has raised the risk of suffering from cosmopolitan and tropical illnesses . In that sense, it is essential a specialized medical counselling which may decrease the consequences of that kind of trips.

AIM To describe the 5-year experience in a Unit of Primary Care attention to the international traveller.

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MATERIALS AND METHODS The team was formed by five doctors and four nurses, coming and shared by Primary Care Units and especially trained in Tropical and placed in two different outpatient offices. All travellers are visited by one doctor and one nurse simultaneously. The Unit offers careful counselling before travelling depending on the country, means of transport and accommodation. Vaccines required are prescribed and administered, as well as malaria chemoprophylaxis if needed and sanitary counselling about water, food and dressing. Moreover, electronic accessibility during the travel is always feasible, some visits for light and mild pathology has been performed, at the same time that support is given to general practitioners in terms of International Health.

RESULTS Ten thousand six hundred forty three visits have been carried out, 19 839 vaccines have been administered and 3510 malaria chemoprophylaxis have been prescribed. Twelve questions have been answered through electronic mail.

CONCLUSIONS As a whole, specialized visits for the international traveller are feasible from Primary Care Units whenever physicians and nurses are correctly trained. Besides, these units play an important role when counselling immigrants at their time of coming back to their origin countries for personal reasons. At the end, specialized units may also stand as a strong relief for hospital units, nearing Primary Care to the people when International Health Counselling needed.

4.3-053

The need for palliative care in sub-Saharan Africa

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BACKGROUND In many Western countries the need for the improvement of palliative care has gradually been recognized.

METHODS A scoping exercise of the literature to assess the need for palliative care in sub-Saharan Africa. Relevant literature was identified through eight electronic databases: AMED, British Nursing Index & Archive, CINAHL, EMBASE, IBSS, MEDLINE, PsycINFO, and the Social Sciences Citation Index; and hand searches were conducted in three palliative care journals.

RESULTS In sub-Saharan Africa 22 million people were infected with HIV in 2007, 67% of the global total, and the region remains the most heavily infected worldwide. The burden of HIV is exacerbated by cancer and other non-communicable diseases: in 2008 there were 667 000 cases of cancer and 518 000 cancer deaths. With an aging population, the incidence of cancer has been predicted to grow substantially. In this context, the provision of sustainable palliative care is important. However, there are PC services in only 26 out of 47 countries, and only in Uganda, South Africa, Kenya and Zimbabwe are these to some degree integrated into the existing health system. Moreover, due to lack of resources, PC is generally limited to urban centres and their immediate surroundings. Only a third of the population of sub-Saharan Africa has access to essential drugs.

CONCLUSION There is the knowledge and experience to control pain and diminish suffering significantly at the end of life but for most of the population in Africa palliative care is not available. The greatest need is in countries where healthcare resources are the scarcest. The WHO addressed these needs by pioneering a public health approach for palliative care. For interventions to be effective, the strategy must be owned by the community and finding ways to achieve this is a priority.

4.3-054

Improving health workers capabilities; an impact evaluation of a postgraduated diploma in tropical paediatrics

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INTRODUCTION The dire shortage of health workers in many countries is among the most significant constraints to achieve the Millennium Development Goals . Many postgraduated studies try to give the right training to the right people to create an effective workforce for the delivery of health care. Evaluation of these courses is essential to know their real impact. In 2003 the Diploma in Tropical Paediatrics was created by the Spanish National Centre of Tropical Medicine- ISCIII to strength the health workers capacities. The aim of this study is to evaluate this Diploma and its impact in the last 4 years.

METHODS AND MATERIALS Based on the Four Level Model the evaluation analysed: Reaction: participants opinion of the learning process. Learning: knowledge and skills acquired. Performances: participants' ability to transfer knowledge and skills acquired and changes in their professional career.

RESULTS The extent to which the results of the three levels accomplish with the course's objectives. The first two levels were analysed using data collected by the Escuela Nacional de Salud referring to participant results and feedbacks sheets. Percentages and means were calculated. A survey was sent to the students to evaluate the third level.

PRELIMINARY RESULTS Since 2007, 79 professionals have been trained: 57% were from LAC, 39% from Spain and 6% from Africa. The three study units better evaluated by the students were HIV Infection (9.1); Community Paediatrics Health (8.8) and Nutrition (8.5). The highest grades obtained by the students were in Tropical Clinical Paediatrics (8.7); Nutrition (8.6) and Paediatrics Parasitology (8.4) Preliminary.

CONCLUSIONS The Diploma is highly valued by students, none of the seven study units have ever been evaluated under eight. There is not direct relation between students' grades and their study units preferences showing that the subjects of these units are really relevant to the practitioners trained.

4.3-055

Integrating home-based environmental interventions (IHIP): a community-randomised trial to improve indoor air pollution, drinking water quality and child nutrition in rural Peru

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INTRODUCTION Today, 8.8 million children die annually, 18% attributed to pneumonia and 15% to diarrhoea. The combination of simple, effective interventions is cost-effective and synergistic effects on health outcomes might reduce disease burden. We developed and evaluated the impact of delivering an Integrated Home-based Intervention Package (IHIP) that would reduce acute lower respiratory infections (ALRI), diarrhoeal diseases and improve nutrition.

METHODS We conducted a cluster-randomized control trial in rural Cajamarca, Peru. 534 households with children aged 6–35 months from 51 community-clusters were randomized to receive a home-hygiene intervention package including OPTIMA-improved stove, kitchen sink, hand-washing, and SODIS-household water treat-

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ment, or a psychomotor-stimulation package as control intervention. During 12 months surveillance, we collect diarrhoeal illness, ALRI and child growth outcomes. In a sub-sample we determined the level of faecal contamination of drinking water, hands and kitchen utensils and the prevalence of diarrhoegenic *E. coli* in stool specimen.

RESULTS The study arms were similar at enrolment. The intervention arm we observed 287 diarrhoeal episodes or a mean 1.7 episodes per child per year at risk, while in the control group 365 diarrhoea episodes or a mean 2.3 episodes per child per year at risk were seen. The prevalence of cough was 7.3% in both study arms; of cough and fever it was 2.4% in control and 2.2% in the intervention groups. The durations of days-spent-ill were with 20 *vs.* 15 in the control and intervention groups. Average number of days for health care seeking was 3.3 *vs.* 2.9 days in control and intervention group.

CONCLUSION The integrated home-environmental intervention package (IHIP) was successfully accepted and rolled-out in 51 rural communities of a large community-randomised trial in Peru. Comparable at baseline, preliminary analysis showed unexpected modest health impact of the IHIP comparing study arms, but analysis of pneumonia as key impact indicator is pending.

4.3-056

Human resource investment for schistosomiasis control in a low-transmission area: the example of Wuhan, P.R.China

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Schistosomiasis is a neglected parasitic disease common in subtropical and tropical areas . Control has been successful in a number of endemic countries, including the People's Republic of China (PRC), where control has always been high on the public health agenda. Since the 1950s the number of cases has been reduced by over 90%. Today, China is a low-transmission area with low endemicity and low infection intensity. The question arises what is the cost, in terms of continued human resource investment, to keep incidence at the current low level and whether or not this investment is justified. Data was obtained from the Center for Disease Control Wuhan (population 8.35 million). This included information about schistosomiasis prevention and control measures, annual population screening rates, average infection rates and the number of personnel employed in schistosomiasis prevention and control. Schistosomiasis infections are largely based on serological screenings and selective stool examinations for seropositive individuals. In 2008, 1216 patients in Wuhan were diagnosed with schistosomiasis (average infection rate 0.85%). For that same year, 516 personnel were available and employed part or full time in schistosomiasis control on the city, district and town levels. This resulted in a very high health care worker-patient ratio of 1:2.4. The majority (78%) of these were professional health workers involved in diagnosis or treatment. Few were dedicated to other control measures, such as health education. A considerable human resource investment in schistosomiasis control is being made in Wuhan on all administrative levels. However, without constant population screening, proactive case detection and the early treatment of cases, disease transmission might increase and even greater resource investments in terms of control and treatment would be required in the future.

4.3-057

Health system strengthening and disease specific programs in Democratic Republic of Congo

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INTRODUCTION During the last decade , the overall health sector in Democratic Republic of Congo (DRC) was mainly oriented towards specialized programs. The multiplicity and the importance of such programs have contributed to fragment the health system with incoherent and unstructured functioning at all levels. In reaction, the ministry of health has reoriented its efforts and interaction between interventions focusing on disease control and Health System Strengthening (HSS) is now at stake. We intend to explore the possible scenarios to achieve an overall HSS through disease specific programs.

METHODOLOGY The assessment at national level was conducted by interviews with key informant persons and by document review on health system strengthening, financing and health programs. The information obtained from a complete situation analysis at national level was analyzed to identify how specific programs could achieve an overall health system strengthening.

RESULTS The different scenarios proposed focused on interactions between the support to three disease specific programs (HIV, Immunization, and diabetes), the MOH direction of disease control, the MOH financial management unit, the provincial health department and the health development agencies. This should help in reflecting about the expected characteristics of these interactions.

4.3-058

Perception of a new occupational health service by the health care workers of the provincial hospital of Tete, Mozambique

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INTRODUCTION An occupational health (OH) program designed as a convenient , confidential and comprehensive screening and care service for hospital care workers (HCW) was launched in April 2008 in the Provincial Hospital of Tete. After 2 years of activity, about 50% of all invited 423 HCW had attended the service. We aimed at investigating the users' satisfaction as well as the reasons for non-adherence to the program.

METHODS In November/December 2010, we conducted a qualitative survey using semi-structured individual interviews with three categories of randomly selected participants: (i) HCW who had attended the OH service, (ii) HCW who had never consulted, and (iii) key informants of the provincial hospital and health authorities. Interviews were recorded with full respect of privacy and transcribed for analysis with the Atlas.ti software.

RESULTS For the first (n = 20) and the third (n = 14) groups, the OH consultation was perceived as a very useful service with clear and well-defined objectives of morbidity screening and care in a risky professional environment. HIV-infected HCWs greatly appreciated this dedicated service (compared to the overcrowded and less confidential general HIV consultations) and some reported that it had increased their follow-up adherence. Among the second group (n = 18), uncertainty about the 'comprehensive care' design of the program and the perception that it focused merely on HIV

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screening and care were prevailing. Other frequently reported barriers for adherence were: (i) fear of confidentiality breaches between peers, (ii) lack of clear information, and (iii) high service provider rotation and unexpected delays for some consultations.

CONCLUSIONS The new OH program was favorably perceived by hospital workers, and particularly by HIV-positive HCWs. Confusion existed however among hospital staff about the comprehensiveness of provided care. Communication on the program objectives and confidentiality issues could still be improved. Stability of the service provider team and attendance schedule should be reinforced.

4.3-059

Pre and post intervention patient tracking survey at fevers unit of Korle-Bu teaching hospital

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INTRODUCTION Korle-Bu Teaching Hospital Fever's Unit sees over 16 000 HIV clients. With the increasing number of clients at the unit with fixed infrastructure and dwindling human resource, the quality of service being delivered is being compromised. Clients were dissatisfied with the long waiting hours. We carried out pre and post intervention patient tracking survey to determine the mean waiting time and the effect of the interventions

METHODS AND MATERIALS We randomly selected 100 patients who visited the clinic and tracked the patient flow to ascertain the average time spent at each station using unique identification numbers and synchronized watches to record the time an activity was carried out. Certain bottle necks were identified and appropriate interventions put in place. The survey was repeated post-intervention to find out whether the average waiting time that has changed. At the end of each survey, the recordings were collated. These were entered into an excel spread sheet and analyzed in SPSS version 16 software comparing pre and post intervention waiting time at the clinic.

RESULTS A total of 200 clients were tracked, 100 in pre-intervention and 100 post intervention tracking survey. Prior to the intervention, the average waiting time from the time the patient picks the folder till leaving the hospital was 4 h 50 min and post-intervention was 3 h 35 min. The average waiting time to check weight has reduced from 1:45 to 0:16 min, and waiting to see the doctor has reduced from 1:20 to 0:54 min pre and post intervention respectively.

CONCLUSION We have reduced patient waiting time by 1 h and 15 min, and improved service and patient contact time. Doctor patient contact time has increased from 0:08 to 0:19 min so has pharmacist patient contact time has increased from 0:05 to 0:11 min.

4.3-060

Maternity care for (im)migrant women in Germany, Canada and the UK: the German perspective

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BACKGROUND Efforts to identify ways in which maternity care access and experiences can be improved for migrant/minority women in Western countries mostly lack international comparison. A study group from Germany, the UK and Canada compared the healthcare setting for maternity care offered to migrant women in the three countries. We present the results from a German perspective.

MATERIAL AND METHODS A desk-based analysis of documentary sources (policy, professional bodies, and experts) was undertaken, in which implied assumptions or underlying rationals in the wider political context and in the healthcare field were identified. Also, workshops were facilitated in August 2008 with immigrant women, immigrant support agencies and maternity service providers to establish the key areas of concern.

RESULTS Germany's policy orientation was until recently characterized as strictly anti-immigration and contemporary health policy and practice is characterized by significant tensions and ambiguities. The website of the German College of Gynaecologists and Obstetricians only mention migrant women related to HIV/AIDS, genital mutilation, breast cancer and gestational diabetes. The German College of Midwives has recently been renamed the 'Association of Midwives in Germany', acknowledging those midwives practicing in Germany who have other nationalities. Research focuses on Turkish women instead of the entire range of migrant women in Germany. Some concerns were shared with the other two countries such as ineffective cross-cultural communication; lack of supportive services to enable particularly new migrant women to effectively navigate the health system and exercise choice; failure to recognize and respond acceptably totrauma, isolation, poverty and an insecure residency status.

CONCLUSIONS Germany has less service offers for pregnant migrant women than Canada and the UK. Measures that the other two countries provide such as audits, particular education of healthcare providers and participation of immigrant consumer groups in recommendations and guidelines may improve maternity services for migrant women in Germany.

4.3-061

Health professionals migrating to the US and Canada

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INTRODUCTION The US and Canada are experiencing an increased demand for licensed health professionals currently both have shortages and maldistribution of these professionals. This is the result of several factors; the greatest being increased demand for services due to an aging population, access to and demand for technological advancements, and too few licensed health professionals being educated. Both the US and Canada will continue to face shortages of licensed health professionals for the next 15 years, at minimum. Neither country's educational pipeline projects enough new graduates to meet the demand. Therefore, health professionals from other countries will continue to be a part of meeting the demand.

METHODS As part of a larger project (Mobility of Health Professionals), an online survey collected information from over 500 licensed health professionals who have migrated to either the US or Canada. Links to the survey were placed on web sites of organizations that these individuals work with.

RESULTS The Results of the survey showed that the reason health professionals come to the US and Canada are: better working conditions (US 88%, CA 78%), higher earnings (35%, 42%),better employment benefits (70%,30%), job growth opportunities (60%, 51%), training and educational advancement (74%,60%), resources and advanced technologies available to complete job tasks (46%, 34%), improved lifestyle conditions (86%, 100%), political stability (46%, 52%), friends or family

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live there (66%,38%). Data was also collected on reasons for leaving home country.

CONCLUSIONS Issues that must be addressed are difficulties immigrant health professionals face in getting licenses approved, partnering with foreign universities to train health professionals, and providing educational grants for immigrants at universities (in US or Canada). Additional strategies that must be employed are increasing the number of health professionals trained, effectively using advanced technologies such as telemedicine, and task shifting among health professionals. While doing so, both countries must make every effort to adhere to the World Health Organization's Code of Practice on the International Recruitment of Health Personnel.

4.3-062

Technical assistance for health system strengthening in fragile state: learning from experiences through systemic approach

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INTRODUCTION The Democratic Republic of Congo (DRC) is in the process of implementing an ambitious health sector reform with the aim of strengthening their health system. In the same time, political reform is also taking place. Decentralization at provincial level is one of the pillars of this process that should play a central role in the strengthening of local health system. Specifically, organizing technical assistance or 'coaching' of health districts by province is of particular importance. The proposed study will explore how to support and facilitate an efficient technical support coming from various external actors.

METHODS We developed a case-study in the future province of Kwilu, Bandundu, DRC, where the health reform implementation is beneficiating of the Belgian Cooperation support. We viewed technical assistance in health systems as complex interventions in a complex adaptive system. Situation's exploration can then use system thinking developments and tools to approach the complexity. This presentation reports the development of a systemic approach derived from Checkland's soft systems methodology.

RESULTS The expected result of the approach is to provide an in-depth understanding of the contributions and meanings of technical assistance at provincial level. It should contribute to a more effective design of technical assistance to strengthening the health districts in DRC. This work aims also to feed the aid effectiveness debate and to give insights to Belgian cooperation and specifically provide insight on the role of international technical assistance.

Track 5: Global Migration, Conflicts and Population Health

5.1 Global Population Movements: Visiting Friends and Relatives (VFR), migratory flows and pilgrimages

5.1-001

Hepatitis a in immigrant population and their children

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The prevalence of hepatitis A in low income countries is very high. Immigrants and their children returning home to visit friends and relatives (VFRs) are at high risk of developing the disease.

MATERIAL AND METHODS We included in this study the cases of hepatitis A in immigrant population observed from January 2006 to December 2010 in Barcelona South Metropolitan area. The population of this area is about 700,000 inhabitants with 12% of immigrants. The serological diagnosis was made in the Laboratori Clínic L'Hospitalet of the Catalan Institute of Health by detection of IgM antibody to Hepatitis A virus using the chemiluminescent immunoassay (Vitros® Johnson & Johnson). Risk factors were analyzed according to the information collected by the Epidemiological Surveillance Unit. Outbreak is defined as the presence of two or more cases epidemiologically related in time or space.

RESULTS There were 23 cases of hepatitis A diagnosed among immigrants and their children. Age of patients ranged from 2 to 38 years, with 16 children and seven adults. In reference to risk factors, 69.6% (16) patients were VFRs, 13% (3) were men who have sex with men (MSM) and in 17.4% (4) it was not possible to identify risk factors. Among the VFRs, 13 were children and the regions visited were: South America (8), Morocco (7) and India (1). There were 30.4% (7) of the cases associated with epidemic outbreaks, four of family household and three in individuals with sexual behaviour with increased risk (MSM related to outbreaks in diverse European countries in 2009).

CONCLUSIONS In immigrant population the main group at risk for developing hepatitis A, are children who travel to visit friends and relatives, probably because the lack of protective antibodies. It should be considered the reliability of vaccinating them before the established schedule vaccination age.

5.1-002

VFRS characteristics treated at the tropical medicine and international health drassanes unit in 2010

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INTRODUCTION The UMTSID (Tropical Medicine and International Health Drassanes Unit) had 15,002 pre-trip consultations in 2010. The study focuses on VFRs (travelers visiting relatives/friends). The objectives of the study were: (i) To compare the percentage of VFRs addressed in 2010, according to yearly quarters. (ii) To compare gender, age, travel duration and anticipated days before pre-trip consultation, of the VFRs by geographical area of destination.

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Methods Descriptive study: The pre-travel consultation was organized into two agendas. The inclusion of passengers in either of the agendas was random. The agenda of the study included 6460 travelers (43% of all consultations). Variables: type of trip (VFR), age, sex, duration, time and destination (Africa, Asia, America). Source of information: clinical history. For all comparisons a Chi-squared test was performed with a significance level of 0.05.

Results (i) Of all travelers 15.4% (995) were VFRs, of total VFRs 60.4% were Africans; (ii) Significant differences between the percentages of VFRs traveling in each quarter of the year, 44.6% occurring during the third quarter; (iii) Significant differences between the three geographical areas visited in terms of sex (Africans and Asians with high male predominance) and how many days prior to the trip the consultation was made: 34.6% of Africans come with <7 days before trip, 20.8% of Asians and 11.9% of Americans; (iv) The percentage of children under 18 years is significantly higher among Americans (52.5%) and Asians (45.4%) than in Africans (21.6%); and (v) The percentage of travels lasting more than 60 days is significantly higher in Africans (40.8%) and Asians (33.8%) than Americans (13.6%).

Conclusions The UMTSID should: (i) Create a prevention plan (with emphasis mainly on the 3rd quarter) to motivate African VFRs to schedule their pre-trip visits earlier; (ii) To further analyze VFR consultations of Asian and American adults.

5.1-003

Imported infectious diseases in immigrants who travel to visit friends and relatives (VFRS) attended at a tropical medicine unit in Spain

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Introduction In 2009 VFRs (immigrants from a developing country, who return to their region of origin to visit friends and relatives), comprised approximately 27% of worldwide international travellers. They have been identified as a special risk group for travel-related preventable diseases, especially infectious diseases, when compared with other types of travellers. Recently, a new definition of VFRs has been proposed: VFRs are those travelling to visit friends and relatives, regardless of immigrant status/ethnicity, and where an epidemiological risk gradient exists between host and destination country.

Methods Retrospective, descriptive study of demographic variables and infectious diseases diagnosed in immigrant-VFRs attended at the Tropical Medicine Unit, Ramón y Cajal Hospital (Madrid) (January1989–June2010). Demographic variables: age, gender, duration of travel, country/area of travel. Reason for referral classified according to 10 syndromes: febrile, hematologic-eosinophilia, acute diarrhea, chronic diarrhea, dermatologic, neurologic, musculoskeletal, respiratory, genitourinary and cardiovascular. Standard diagnostic techniques were used. Frequencies of syndromes and diagnoses were calculated for the total population and according to areas of origin and were compared between most frequent origins (sub-Saharan Africa-SSA and Latin America-LA).

Results Three hundred and thirty-two immigrants-VFR, 209 (57.1%) from SSA, 142 (38.7%) LA, 12 (3.3%) Asia, 3 (0.8%) North Africa Most frequent countries of travel: Equatorial Guinea, Ecuador, Bolivia, Nigeria 54.8% females, median age 36 years (Interquartile-range IQR: 28–45), median length of travel 1 month (IQR 0.6–2). Febrile and neurologic syndrome more frequent in SSA; Chronic diarrhea more frequent in LA ($P < 0.05$). Malaria, latent tuberculosis infection, filariasis and chronic viral hepatitis more frequent in SSA, dengue, intestinal parasites and Chagas disease more frequent in LA ($P < 0.05$).

Conclusions Immigrant-VFRs may have infections acquired during VFR-travel and others acquired before migration. Differences in diagnoses have been found according to areas of origin. Immigrant-VFRs who attend a medical consultation post-travel may also be screened for other chronic and asymptomatic infectious diseases based on area of origin.

5.1-004

Profile of migrants attending the rapid HIV test service at primary care centres in Madrid

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Introduction Forty-eight per cent of new HIV diagnostics in the Comunidad de Madrid were born abroad. In 2010, a culturally and linguistically adapted HIV Prevention and Early Diagnosis Service (EDS) were established in four primary care centres. Five mediators do pre and post-test counselling, refer positive outcomes for confirmation and provide information about this anonymous and confidential service in migrant meeting places.

Aim To describe HIV-related vulnerability factors and prevalence of HIV in the migrant population going to the EDS.

Methods Sociodemographic data, sexual history, source of knowledge about the EDS and rapid test result. Descriptive statistical analysis from 29 April 2010 to 31 March 2011.

Results Of 723 patients, 255 were migrants (34%) from Latin America (51%), Western Europe (19%), Greater Maghreb (9%), Eastern Europe (6%), Indian Subcontinent (6%), Subsaharan Africa (5%), Middle East (1%), North America (1%) and Central Asia (1%) with the following characteristics: 66% men; age: 31 (26–39); time in Spain: 5 years (2–9); level of education: (primary: 11%, secondary: 44%, higher: 45%); never did an HIV test: 46%; no health insurance card: 14%; history of STIs: 20%; practice prostitution: 5%; see prostitutes: 15%; consume intravenous drugs: 1%; have sex after consuming alcohol: 19%; 167 men: men who have sex with men: 32%. Customary condom use: 69%; unprotected anal or vaginal penetration in the past year: 75%; people in window period: 51%; number of sexual partners in the past year: 2 (1–4); stable partner: 64%. Tests done: 248; only counselling: 7; reactive results: 10 (4%). Confirmation: positives: 7 (five Latin Americans); negatives: 1; unconfirmed: 2. Most frequent reasons for using the service: speed (51%), anonymity (12%). Heard about the service: mediator (26%), internet (22%) and word of mouth (19%).

Conclusions A culturally adapted EDS helps to improve migrants access to early HIV diagnosis, reducing the vulnerability of the target population facing the infection.

5.1-005

Emergency admissions of illegal immigrants on arrival: a 3 year experience

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Arrivals of illegal migrants increased sharply at Greece's land border with Turkey. This probably reflects the fact that the Greek–

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Turkey border had become for many a safer and cheaper route to the EU rather than crossing the Mediterranean. Based on the cases admitted to the two local hospitals near the Evros River – one academic and one state hospital – this paper explores, for the last 3 years, the disease pattern of illegal immigrants on arrival. After an initial health and psychological care at the detention centers, 283 patients were referred for hospitalization: 106 (37.5%) in 2008, 70 (24.7%) in 2009 and 107 (37.8%) in 2010. Common medical conditions were: tuberculosis, acute gastroenteritis, dehydration, rhabdomyolysis and pneumonia. Common surgical diagnoses were: frostbites, bone fractures and signs of physical violence. Over the years, mean duration of hospital stay was significantly decreased from 11 days (2008) to 4.9 days (2010). Although the incidence for medical, surgical and psychiatric patients did not change over the years, the relative incidence of the pediatric patients significantly increased from 2.83% (2008) to 16.82% (2010). This study clearly documents that illness problems of illegal migrants at arrival are mainly linked to the travel itself.

5.1-007

Implementation of preventative actions for VFR migrants living in Madrid (Spain)

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INTRODUCTION A qualitative study about risk perception in VFR migrants carried out in Madrid in 2009 proved the importance of implementing prevention activities aimed at VFR living in Madrid and gave us the key points to reach migrant population.

METHODS 2009: Creation of multilingual and fully illustrated leaflets about: health risks when travelling to the Tropics, preventative measures (before, while and after travelling) and contact information for seeking medical advice and assistance. 2009–2011: Individual advice given by intercultural mediators in the waiting room of the Tropical Medicine consultation. Gathering of sociocultural data and risk perception before the counselling. 2010–2011: Two annual informative campaigns (one before Christmas and one before summer holidays) in places where migrants go before travelling.

RESULTS Sixty-four migrants (43 men, 21 women) from 16 tropical countries received individual health counselling for VFR. They came especially from Equatorial Guinea (16%), Guinea Conakry (14%) and Senegal (12%). Time in Spain: 36 months (median). Main vehicular languages: French (47%), Spanish (17%) and English (15%). Up to 17% didn't know where to go for vaccinations nor for seeking medical advice. Up to 37% had scheduled trips to their countries (12% coming from rural areas): 61% though there were no health risks. The most mentioned risks were related to malaria, water, indigestion, dengue and headache. One thousand two hundred and sixty leaflets and 60 posters (in English, French and Spanish) distributed in: healthcare centres, embassies and consulates of 21 countries located in Tropical Areas of Africa and Latin-America, hairdressers, restaurants, call centres, travel agencies and NGOs. Free online access to leaflets: www.saludentreculturas.es

CONCLUSIONS To understand the risk perception of VFR migrants and their social, linguistic and cultural characteristics is essential to establish the best timing, location and communication strategies for preventative actions. Qualitative research and intercultural mediators are key tools to develop effective prevention programmes.

5.1-008

Changing epidemiology of imported filariasis in France (39 cases)

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INTRODUCTION Few data concerning imported filariasis in France are available. We present the cases diagnosed at the parasitology unit of Tenon-Saint-Antoine teaching hospitals, Paris, over a 10-year period (2001–2010).

METHODS Microfilariaemia was detected with fresh peripherical blood sample, thick and thin peripheral blood smears and centrifugation of blood haemolysed with saponin. When onchocerciasis was suspected skin snip test was performed. Serological methods included immunofluorescence replaced by ELISA after 2008, and electroimmunodiffusion as confirmation method.

RESULTS During the study period, 39 filarial infections were diagnosed. All patients, 23 men and 16 women, were adults. The mean age was 39.2 years (range = 19–77 years). Thirty seven were immigrants or visitors native from endemic filarial regions. The majority (28/37) came from Central Africa, essentially Cameroon (22/28). Only two patients were French travellers. Loiasis was the most common diagnosis (27/39), associated with Mansonella perstans infection in two cases. Only 12 upon 27 presented with clinical symptoms due to Loa-loa infection: Calabar swelling (7/12), generalised pruritus (5/12) and/or eyeworm (3/12). One Calabar swelling was observed in a French traveller infected after a 7 days stay in rural Cameroon 9 months before. Loiasis was discovered because of a screening for eosinophilia in seven patients and fortuitously with the discovery of microfilariae in 8. Of note, one observation of Loa-loa microfilariae in the follicular fluid followed an oocyste retrieval for *in-vitro* fertilization in a 19-year old African women. Four patients had onchocerciasis. There were African natives (Senegal, Sierra-Leone and Cameroon) presenting with onchodermatitis. One was a French traveller with an onchocerciasis-associated limb swelling. Eight months previously he had spent 6 weeks in Central Africa. Lymphatic filariasis was diagnosed in three immigrants, two from Comoro islands and one from Haïti. Two were asymptomatics and one had a tropical pulmonary eosinophilia. Isolated asymptomatic Mansonella perstans infection was found in four patients and unspecified filariasis in one.

CONCLUSIONS The epidemiology of imported filariasis has changed in France for the last decades. Thirty years ago onchocerciasis was the most frequent imported filariasis, mainly observed in immigrants from Mali. The disappearance of this infection in new immigrants from this country indirectly supports the success of the Onchocerciasis Control Program (OCP) in West Africa. Loaiasis has become the most common imported filariasis, symptomatic in less than half of infected patients. Imported filariasis, even rare in travellers, can be acquired during short stays and clinicians should be aware of this diagnosis in short term travelers with eosinophilia and/or characteristic presentation.

5.1-009

Trends and features of imported dengue infections in Spain

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INTRODUCTION Dengue viruses (DENV) are the most widely distributed arboviruses in the world . DENV circulate in either

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human or non-human primates in two different cycles (urban and sylvatic) and it is transmitted by *Aedes* mosquitoes .The four different serotypes of DENV infect humans, causing frequent epidemics and severe disease. The disease is endemic in the Americas, Africa, Asia and Oceania, although in the summer of 2010 was also described autochthonous circulation in South Europe (France and Croatia). Here we report the surveillance of imported dengue infection in Spain from 2008 to 2010.

MATERIAL AND METHODS Samples from suspected cases of dengue infection, sera and/or whole blood were processed by molecular (PCR), virological (viral isolation) or for serological methods (IgM,IgG and antigen detection),according to the onset of fever. Epidemiological analysis was conducted at the Spanish National Centre for Epidemiology.

RESULTS From 2008 to 2010 196 acute dengue infections were detected. Less than 30% of these infections were viremic; and 10% of them resided in areas infested with *Ae. albopictus*. Dengue with alarm symptoms was present in < 2% of the total acute infection. The dengue serotype most frequently found was DENV 1. Two relevant events were detected in Africa, the first description of DHF caused by a sylvatic strain and the introduction of DENV 3 in Burkina Faso.

DISCUSSION Mosquito surveillance in the Mediterranean basin, including Spain, demonstrates the presence of *Ae. Albopictus* and highlights the risk of initiation of local transmission cycles for both Dengue and Chikungunya viruses. According to our results, Spain, like South France and Croatia is a potential scenario for dengue re-emergence. Consequently, it is highly necessary to reinforce surveillance in travelers and to prepare primary health care settings for detecting autochthonous cases in areas infested with the vector.

5.2 Health Care Policies and Social Determinants of Health in Migrants

5.2-001

Social determinants of health: a new challenging attitude in parasitic infections control in developing countries

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SDH is a broad concept, influencing the entire aspects of human life on the earth. A large number of debilitating illnesses have been disappeared along with the gradual improving of social and economical standards. Despite the general consensus on the subject, providing a quantitative evaluation of SDH effects on specific health item is extremely intricate. From the perspective of epidemiology, elimination and/or transmission interruption of the most parasitic infections in developed and developing countries are evidently indebted to the growing level of social determinants of health. Nowadays, a great number of world populations are suffering from chronic consequences of different parasitic infections. Malaria, Schistosomiasis, Elephanthiasis and Soil Transmitted Helminthiasis are still of major concerns facing to health programmers mainly for those who are planning in poor countries. At this time a considerable contrast exists between the present global assets and the basic requests of the poor nations. Accordingly an appropriate concept adjustment is highly needed. Access to the diseases elimination should be managed along the promotion of life. Successful records of some programs in infection control are an extremely reliable motivation to socialize our professional attitude. Nearly elimination of Urinary schistosomiasis using improved agricultural irrigation system and the significant decline of soil transmitted helminthiasis following utilization of chemical fertilizers during the past three decades in Iran, are exemplary in this regard. Assessment of successes in national infection control programs which are attributed to social determinants of health can be practically recommended to those nations confronting similar public health problems.

5.2-002

Do health services adequately cater for the needs of migrants? epidemiological case study from Germany

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BACKGROUND Access to, utilization of, and outcome of health care should be equal for migrants and the majority population. In Germany, the proportion of migrants receiving an invalidity pension is higher than that of Germans. Lower utilization and effectiveness of medical rehabilitation are presumed to be the main causes. We examined whether differences in utilization and effectiveness of medical rehabilitation between Germans and migrants are attributable to differences in socio-demography, socio-economic background and health status.

METHODS Utilization of rehabilitation was analyzed for household members aged 18 years or above enrolled in the German Socio-Economic Panel in 2002–2004 (n = 19,521). Effectiveness of rehabilitation was defined by the occupational performance at the end of rehabilitation. It was examined by using an 80% random sample of all completed medical rehabilitations in the year 2006 funded by the German Statutory Pension Insurance Scheme (n = 634,529).

RESULTS Foreign nationals utilize medical rehabilitation less often than Germans (OR=0.68; 95%CI=0.50; 0.91). For those who do, medical rehabilitation is less effective (OR for low occupational performance = 1.50; 95%CI = 1.46; 1.55). Both findings are only partially attributable to socio-demographic, socio-economic and health characteristics: After adjusting for these factors, ORs for utilization and low occupational performance were 0.66 (95%CI = 0.49; 0.90) and 1.20 (95%CI = 1.16; 1.24), respectively.

CONCLUSION Differences in the utilization and effectiveness of medical rehabilitation between Germans and mirgants cannot be explained only by socio-economic differences or poorer health before rehabilitation. In addition, factors such as the ability of the rehabilitative care system to accommodate clients with differing expectations, and migrant-specific characteristics such as cultural differences, play a role.

5.2-003

Inequalities in the access to social and medical care for migrants in three European countries

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Despite the efforts of the European law to harmonise healthcare access policies throughout the member States, differences could still exist for both legal and undocumented migrants. The aim of this work is to compare the legislative framework on social and medical care granted to migrants among three countries in the Schengen area. A literature review was conducted in April 2011 using European, national and regional information sources from official or associative contents. The European law considers health and social coverage as a universal right and promotes full accessibility to healthcare to everyone (especially to marginalized

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populations). France, Italy and Spain provide their citizens with universal health coverage, through mostly centralized (France) or decentralized (Italy and Spain) health systems. Legal migrants are granted the same healthcare as country citizens. A negative discrimination is present based on the legality of the stay but undocumented migrants are at least granted urgent care in public hospitals, coverage for maternity and children. In France and Italy, they are also granted care for some infectious diseases and preventive measures. In France, the social coverage for undocumented migrants (less complete) depends on the duration of the stay. In Spain, without economic resources, they benefit from the same health services than Spanish citizen with the necessity to justify from an address. The European law, so far, has not managed to reduce the inequalities and to introduce equity among migrants, and among these countries. Undocumented migrants have different conditions of access to healthcare compared to European citizens. This study should be extended to other European countries to underline other disparities and then to perfect the established coverage system. A reflection on migrant care in term of global welfare could benefit both migrants and European citizens. Also, the European Union should take charge of the management and welfare of migrants.

5.2-004

Migrant communicable disease screening programmes: an analysis of the translation of policy into practice

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INTRODUCTION Numerous countries employ migrant communicable disease screening programmes, with differing emphases on public health protection and the health of the individual. Criticisms have been levelled at screening policies internationally, however, little is known about the translation of policies into practice. In Ireland a voluntary screening programme is in place and national screening guidelines were produced in 2000, 2001 and 2004. This study reports and analyses factors influencing the implementation of the guidelines.

METHODS AND MATERIALS In-depth interviews were conducted with 18 healthcare workers who have been involved in screening across Ireland. Screening services were present in 14 of the 32 local health office areas with several services covering multiple areas. A framework analysis was conducted.

RESULTS Various forms of local guidance, adapted from the national guidelines, were followed. Factors influencing the implementation of the guidelines were identified under four categories: (i) Individual level factors specific to the complex needs of the population group, for example, offering HIV/AIDS tests to traumatized individuals; (ii) Broader health system factors, such as a lack of Mantoux testing and polio screening due to resource limitations; (iii) Intersectoral policies, primarily the dispersal of asylum seekers around Ireland; (iv) Contextual factors, for example, the A (H1N1) pandemic leading to the reallocation of staff. The absence of a national coordinator and management was particularly noted. Influences such as limited resources resulted in reduced service coverage nationally, while intersectoral policies resulted in interrupted continuity of care.

CONCLUSIONS The national guidelines are broadly implemented in practice, where screening services remain. However, factors outside the narrow remit of the guidelines greatly influenced their implementation and therefore service provision. The resulting service fragmentation, heterogeneity and sub-optimal continuity of care impact upon migrant screening and could have consequences not only for public health but critically for meeting the health needs of vulnerable migrants.

5.2-005

Health of humanitarian entrants in Queensland, Australia

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INTRODUCTION Australia welcomes about 13,000 people on humanitarian visas – refugees, women at risk, etc. each year. In 2010–2011 about 1600 of these were settled around Queensland. In 2008 the Queensland government funded a new statewide health service to support access to health care for these entrants. This service consisted in a 'hub' (service manager and medical officer) and six 'spokes', around the state. The spokes function in a variety of organisations with very different models of care.

METHODS AND MATERIALS This presentation will give an overview of the statewide service and the different models of care used. Each provides a nursing assessment of the new arrival; some spokes also provide a medical assessment, immunisations, pathology testing and referrals to other services. One regional service provides extended support to the entrants and health care providers within the community, sometimes for more than 1 year.

RESULTS Humanitarian entrants to Queensland receive varied support for their health care on arrival depending on where they settle. There has not been any evaluation of the different models of care provided for the more than 3000 entrants to Queensland since the start of the statewide service.

CONCLUSIONS The refugee health service in Queensland is a good start to support the health care of new arrivals. However without the extra support that is provided in Townsville most of the entrants would be unable to negotiate the Queensland health system and would therefore not have their health issues dealt with appropriately.

5.2-006

Ecological factors associated with reproductive health behaviors among unmarried female migrants in China

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BRIEF INTRODUCTION The current family planning policy in China mainly targets the married couple instead of unmarried population. Along with the social economic development and the treads of globalization and urbanization, the reproductive health (RH) issues among unmarried migrants are getting on the research agenda in China. The aim of this study is to explore the ecological factors associated with RH behaviors among unmarried female migrants from rural to urban cities.

METHODS AND MATERIALS Cross sectional anonymous self filing survey method were used in the study. One thousand two hundred and ninety-three unmarried female migrants from manufacturing, restaurants and commercial/service enterprises were surveyed. Six hundred and one of them were from Shanghai and 692 of them are from Guangdong. Logistic regression was used to analyze ecological factors associated with premarital sexual and contraceptive behaviors.

RESULTS For premarital sexual behavior, the predisposing factors included sexual and RH knowledge, the attitudes towards premarital sex and pregnancy and contraception during sex; the enabling factors included living condition, commercial/service enterprise environment, the availability of contraception, different original place they came from and the place they live now; the reinforcing factors included fixed boyfriend, extent of commu-

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nication with parents, and educators from the workplace. For contraceptive behavior, the predisposing factors included knowledge of condom to protect from STDs, self efficacy to negotiate with boyfriend on condom use and the experience of using contraceptives at first sex; the enabling factors included the availability of contraception; peer influence may be a reinforcing factor.

CONCLUSIONS Higher educational level, good family education, higher RH knowledge level and good communication with parents may protect the female from premarital sex. The knowledge of condom to protect from STDs, the self efficacy to negotiate with boyfriend on condom use and the experience of using contraceptives at first sex and the availability of contraception may help to keep good contraceptive behaviors.

5.2-007

Adaptation and implementation of a framework for the analysis of health care quality of the immigrant population in Spain

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BACKGROUND The high proportion of immigrants in Spain has created new challenges for the health system that must provide care to a heterogeneous population. International studies reflect barriers in access to quality healthcare to this population. The objective is to analyze the access to quality healthcare of immigrants by developing a conceptual framework and application to the existing literature in Spain on access to quality care.

METHODS First stage was conducted a review of conceptual frameworks on access and quality of health services and developed a framework adapted to the immigrant population. Second stage, was developed a systematic review of articles that looked at access to quality healthcare for immigrants from databases in health and social sciences and was applied the framework developed in the first phase.

RESULTS The conceptual framework for analyzing the quality of care proposes two dimensions: accessibility and effectiveness evaluated in three categories: structure, process and outcome. Studies examining healthcare quality in the immigrant population are scarce. Identified as determinants of accessibility, factors related to immigrants: predisposing-knowledge of the system, job insecurity, education, culture, religion, and language – enabling to use the system and needs attention. Factors relating to suppliers: predisposing-administrative rules, attitudes and beliefs of staff, work load-enabling-office hours, time for consultation and-professional needs – continuity of care loss, limited knowledge in tropical medicine, limitations on intercultural approach. Among those on the effectiveness are the effectiveness of clinical care, proper care and treatment-the effectiveness of interpersonal care – language used/interpreted, acceptance of personaland status health: outcome, cure or not.

CONCLUSIONS The application of the conceptual framework to analyze the access to quality care in Spain reflects that the evidence on access to health services in Spain is scarce. Likewise, studies that take into account variables related to quality are almost nonexistent.

5.2-008

Feasibility and acceptability of a community based migrants' health cohort in Catalonia

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INTRODUCTION Cohort studies can provide comprehensive information about migrants for understanding patterns and factors underlying their health. This study examined the feasibility and acceptability of establishing a community based cohort for longitudinal surveillance of changes in migrants' health over time.

METHODS Between September 2010 and May 2011, 18 organizations working with migrants in Barcelona were identified, 15 selected based on their representativeness and accessibility, and 10 declined participation. We performed four focus group and interviewed 265 migrants at least 18 years old, using a structured questionnaire.

RESULTS The interview response rate was 38.0%. Half (50.2%) were men and the mean was age 35.8 years. Most (62.3%) were Latin Americans and 25.7% from Maghreb/Sub-Saharan Africa. The majority (81.5%) were willing to participate in a longitudinal study but showed mistrust of the use of the information provided. Incentives would not be accepted by 56.0% since they were perceived as a commercial commitment, whereas 19.4% would accept money. They acknowledge that sensitization prior to the study would help in recruiting participants. The primary health centre was identified as the preferred place to be interviewed and tested (45.0%), given its appropriate sanitary conditions. The greatest perceived benefit of participating was to know their health status (52.9%). As contact tracing information, 72.5% reported that they would give their mobile phone number and 24.9% an identification number.

CONCLUSIONS Although migrants showed positive attitudes toward participation in a community based cohort study, the current economic restrictions that community organizations are facing and the reluctance of migrants to be identified exacerbates the usual operational difficulties that cohort studies pose. Offering activities with a perceived benefit for both migrants and their organizations as part of the study, such as health education, might facilitate their participation. Nevertheless, the use of incentives deserves caution since the perception of the establishment of a commercial relationship might negatively affect participation.

5.2-009

The power of epidemiological evidence in advocacy strategies (in the framework of democratic states) used by MSF to impact health outcomes of mobile populations through policy change

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The oral/paper aims to highlight the significance of epidemiological advocacy used by MSF to impact health outcomes of mobile populations through policy change in democratic states. A case study of MSF's operations with mobile populations in South Africa will be utilized to highlight: how epidemiologic evidence has been used to recognise vulnerability and expose discriminatory policies that have a negative impact on health outcomes of mobile populations; how epidemiological evidence has been used to advocate for the change in policy in South Africa, and how

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epidemiological advocacy has effectively had an impact on the health outcomes mobile populations. The oral/paper will draw on case examples in MSF operations since 2007 with migrants in South Africa, including: deportations; xenophobic violence; lack of status; stigma against migrants; cholera outbreak on the border of South Africa and Zimbabwe; border crossing; and hidden migrant populations in Johannesburg. Concluding that there must be recognition that there are national and international policies needed to promote positive health outcomes for mobile populations and that the creation of these policies can be influenced through the contribution of targeted epidemiological evidence using advocacy strategies.

5.2-010

Mortality of pandemic influenza A (H1N1) in Markazi Province of Iran

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INTRODUCTION Influenza is an acute respiratory disease caused by influenza virus infection. Influenza epidemics are reported every year and worldwide pandemics occur with varying frequency. Most mortalities are due to underlying diseases and its side-effects. In this study, we evaluated 10 fatal cases caused by established type A influenza (H1N1) infection in Markazy Province of Iran in the pandemia of 2009–2010.

MATERIAL AND METHODS This Mortality survey was compiled by a review of deceased patients' files. We assessed different variable such as: demographic data, underlying diseases, secondary infections, delayed commencement of therapy, and non-medication. Collected data were analyzed by measures of central tendency and dispersion using Excel software.

RESULTS Among the 10 deaths that were due to established H1N1 virus in the Markazy Province the median age was 30 years and 90% had underlying diseases. Ninety percent of deaths occurred during October and November and the rest took place in December and February.

CONCLUSION Most patients were adults and the main reason for death was delayed commencement of antiviral treatment. This emphasizes the importance of timely treatment in high risk patients. In flu pandemics, physicians should swiftly start specific therapy in at-risk groups to reduce mortality rates.

KEYWORDS influenza A virus H1N1 subtype, mortality, pandemy

5.2-011

Parent's healthcare utilization patterns for children's severe symptoms in a rural hospital in Ghana: is there a disadvantage for girls?

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INTRODUCTION Previous studies suggested that parents in rural areas in Ghana may be less willing to pay for hospital care for daughters compared to sons. We examined the association between the child's gender, health-related factors and parental socio-demographic characteristics in children presented in hospital for the symptoms fever (malaria suspect or sepsis suspect), productive cough or diarrhoea.

MATERIAL AND METHODS Data were collected (2009–2010) in a rural hospital in the Ashanti region, Ghana. In total, 2211 parents presenting their child to the hospital reported that it had one of the relevant symptoms. Chi-squared tests and multivariate regression analyses were conducted.

RESULTS Girls were slightly overrepresented (51.6% vs. 48.4%, $P < 0.01$). In bivariate analyses, the mean distance to the hospital was higher for parents of boys than for parents of girls ($P < 0.05$) but there was no difference regarding the height of fever and the duration of diarrhoea episodes. Girls more often had health insurance, were of Christian religion and their fathers more often had a medium income (30–100 cedis), (post-) secondary education, and were literate. Boys more often were Moslems or of other religions and their fathers more often had a non-formal or primary education, a higher income (>100 cedis) and their mother more often was literate. In multivariate logistic regression analyses with all socio-economic and health-related characteristics, only Christian religion (OR = 2.00; 95% CL = 1.03, 3.89) and other religions (OR = 3.28;95% CL = 1.05, 10.25) were related to presenting a girl to hospital compared to presenting a boy. The child's health and insurance status and parental age, education, income and hospital distance were not related to the child's gender.

CONCLUSIONS Cultural aspects may contribute to the parentsâ€™ decision to present a boy or girl in hospital. Future studies should highlight the underlying factors that may contribute to the association between healthcare decisions and social context in a culturally sensitive way.

5.2-012

Qualitative research about eating habits in Latin-American migrants living in Madrid, Spain

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INTRODUCTION Spain has the largest number of migrants from Latin America after the US, and a high index of childhood obesity. Studies have showed that childhood obesity is more common in migrant children. Latin-American migrants request information about nutrition and eating disorders in NGOs. Our research aims to describe the mechanisms influencing the diet of Madrid-based Latin-American migrants and to create an informative leaflet using the Results obtained.

METHODS Qualitative research with phenomenological approach (Husserl) consisting of 16 semistructured interviews in Madrid NGOs. Participation criteria: to come from Ecuador, Colombia, Bolivia or Peru (4/5 of the main Latin-American nationalities in Spain); to have lived in Spain for <6 years.

RESULTS Interviewees: nine women and seven men. Countries of origin: Bolivia (six), Peru (five), Ecuador (four), Colombia (one). Educational level: secondary (11) and primary (five). Participants came mainly from urban areas (11). Median age: 37.6 (range 31–45). Seven unemployed (of which, five were men). Main Results: (i) Economical factor was the most important for choosing food. (ii) The available cooking-time is short and they tend to choose fast-food (ready or frozen meals). (iii) They consume high-calorie food not just because of hunger but also due to stress and sadness. (iv) The importance of a well-balanced diet and of child nutrition is evident. (v) Childhood obesity is linked to the time children spend alone (due to working conditions of their parents), scarce physical exercise and high-calorie food. (vi) Women see their body image more negatively than men, causing either increased ingestion (obesity) or decreased ingestion (anorexia).

CONCLUSION Despite the majority of Latin-Americans being knowledgeable about food and the requirements of a balanced diet, several people showed needs for further information about the topic. Hence the importance of providing culturally adapted information based on qualitative researches with the target population.

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5.3 Infectious Diseases (HIV, TB, Chagas Disease) and Mental Health

5.3-001

Factors affecting access to treatment for PLHA: a review of the role of stigma and factors affecting PLHA accessing treatment in Juba, South Sudan

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Background South Sudan (SS) is emerging from 22 years of war and conflict. HIV prevalence is estimated to be higher than in the North (3.1%, 2.3% respectively), yet the HIV response is limited and knowledge of HIV extremely low: Only 9.8% of respondents in a SHH Survey1, were able to identify two modes of HIV prevention. ARVs were introduced in 2006. Regardless of a free drug supply by WHO, there is poor access across SS. UNAIDS estimate that only 1% of total eligible PLHA are receiving ARVs in Sudan. The main venue that supplies ARVs in Juba is Juba Teaching Hospital. Much of SS has no access to ARVs.

Aim of the Study: What needs to be done; an overview of causes of stigma and factors contributing to poor access to treatment for PLHA in Juba/SS.

Methods Qualitative and quantitative methodologies were used with more emphasis on qualitative. The quantitative information captured the socio-demographic characteristics of participants to help identify the most affected group and design solutions and recommendations accordingly. Questionnaires were completed with 46 PLHA and nine health workers, and follow-up semi-structured interviews were conducted with 21 PLHA and three health workers.

Results All PLHA have experienced stigma in various forms, but women face higher levels of stigma than men. Stigmatizing attitudes from health workers, families and the community were identified as the major barrier preventing people from choosing to know their status and, if positive, from accessing treatment in Juba. Other factors affecting access included lower socio-economic status and distance to health facilities. Good quality voluntary counseling and testing and health care provision increases understanding and successfully encourages PLHA to seek and adhere to treatment.

5.3-002

Inflammation and *T. cruzi* virulence: effects of treatment with etanrecept and steroids on acute Chagas disease

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Chagas disease is caused by the intracellular protozoa *Trypanosoma cruzi*, which causes myocarditis and organomegaly. The pathophysiological mechanisms associated to virulence are still unclear, concretely the role of inflammation related to parasite replication. The aim of this work is to determine the effect of the TNF blocker, etanrecept (Enbrel®) and, the corticosteroid dexamethasone (Decobel®) on parameters of the acute phase such as survival, sickness behavior and allodynia. NMRI mice (30 g) were infected intraperitoneally with 1000 tripomastigotes per gram of a virulent Venezuelan isolate of *T. cruzi*. Mice were as follow: (i) infected untreated group (n = 21), (ii) infected treated once with 0.8 mg/kg etanrecept at 7 days post-infection (n = 9), (iii) infected treated daily from 7 day post-infection with 3 mg/kg of dexamethasone for 5 days (n = 10), (iv) healthy treated with etanrecept and dexamethasone (n = 10 and 8 respectively). The horizontal and vertical motility was determined during 3 min in an activity cage and, it was measured daily during the second week post-infection. The mechanical allodynia was recorded in a plantar aethesiometer during the first and second weeks post-infection. The results show that etanrecept increase the survival time whereas dexamethasone causes a decrease. In animals treated with both drugs were observed a reversion of the motility decrease during the second week vs. to untreated group. Mechanical allodynia was increased during the first week post-infection but not during the second week in untreated infected mice and was reverted by dexamethasone and etanrecept treatment. In conclusion, sickness behavior and allodynia are associated to acute Chagas disease and, can be improved both dexamethasone and etanrecept treatment, but only etanrecept is able to increase the survival time. The inflammation associated to TNF could be responsible of high virulence in Venezuelan *T. cruzi* strains.

5.3-003

Screening of Chagas disease by non-conventional serological tests using excreted-secreted superoxide dismutase antigens in blood donors in the east of Andalusia (Spain)

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Trypanosoma cruzi is the causative agent of Chagas disease which is recognized as a global public health issue in non-endemic countries (outside Latin America) by congenital transmission, blood transfusion, organ/tissue transplantation. In this scenario, many research efforts are being leaded to standardize and establish a biological diagnosis for implementing strict guidelines on control measures for blood banks and organ transplant systems to eliminate the risks of Chagas disease transmission. In the present study, we evaluate the performance of the use of serological tests based on a protein excreted by the parasite (SODe-CRU) and estimate the prevalence of *T. cruzi* in blood donors in south-eastern Spain. A total of 550 donor sera samples were essayed by a commercial test (CHAGAS ELISA IgG + IgM, Vircell), by an in-house ELISA and Western-blot against the antigenic SODe-CRU. Overall seroprevalence resulting by the commercial test was 1.27%, but blood donors confirmed positive by the two in-house serological tests were 3.45% from the total sample. Moreover, the sera positives by the commercial test were also positives by the in-house serological tests performed. Further studies are necessaries to obtain reliable epidemiological data because it may be that commercial test underestimate the *T. cruzi* seroprevalence. The utility of SODe antigen has been demonstrated by its high immunogenicity and sensibility/specificity, making it a useful molecular marker for the detection of antibodies to *T. cruzi* and useful for the screening blood banks donors.

5.3-004

Imported infectious diseases in immigrants by region of origin: results from the Spanish network on imported infectious diseases by travellers and immigrants (+Redivi)

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Introduction Immigration has ¡ increased in Spain, currently representing 12.2% of Spanish population. Local series about imported infections, often disclosed a partial view while country-wide networks provide a more representative picture. We describe

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imported infections by immigrants by region of origin based on ad-hoc national network.

METHODS AND SUBJECTS +Redivi is composed of 11 medical centres, which attend travellers and immigrants throughout different regions in Spain. We report on data collected January 2009–March 2011. There was identified three main regions of origin: Sub-Saharan Africa (SSA), Latin America and Caribbean (LAC) and Meridian Asia (MA).

RESULTS Data were available from 1943 immigrants. Demographics: SSA: Median age 29 years, 32.8 female, from Equatorial Guinea (29.2%) and Senegal (15.2%); LAC: median age 33.3 years, 61.8% females, from Bolivia (69.8%) and Equator (9.6); MA median age 23.5 years, 25.5% females, from Pakistan (62.3) and India (21.7). Immunosupression was mostly due to HIV infection (95.5%). Most Subsaharians and Latin-Americans were initially asymptomatic, whereas Meridian Asiatics had mainly Eosinophilia. Most frequent final diagnosis were Eosinophilia (15.6% in SSA, 14.6% in LAC, 52.4% in MA), mostly Strongyloidiasis, Hookworms or Schistosomiasis. Latent tuberculous infection was diagnosed in 12.8% of SSA, 7.2 of LAC, 10.4% of MA) and amoebiasis in 3.7% of SSA, 1.9% of LAC and 7.6% of MA. 30% of LAC were diagnosed of Chagas disease. HIV infection of new onset was diagnosed in 4.2% of SSA, 0.6% LAC and 0.3% MA. There were cosidered healthy 15.2% of SSA, 15.9 of LAC and 5.7% of MA.

CONCLUSION The analsis of immigrants according to their area of origin shows differential characteristics in terms of demographics, most frequent health related problems and final diagnosis. These differences should be considered in the clinical management in order to provide an individualized.

5.3-005

Interest of TESA blot for the diagnosis of chronic/indeterminate Chagas disease in Europe

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INTRODUCTION Chronic/indeterminate Chagas disease is emerging outside of endemic areas. More than 4000 cases have been confirmed in Europe. According to the WHO 2002 recommendations the diagnosis is based on serology performed with two different reagents. We have evaluated the interest of TESAcruzi blot (TB) as confirmation test to resolve serologic discrepancies.

MATERIAL AND METHODS Three hundred and eighty-five samples were studied: in retrospective study 214 sera from the laboratory's bank tested with IIF (indirect immunofluorescence assay, biocientifica) and three different ELISA kits (BioMérieux, Biokit, Wiener Lab). In prospective study 171 sera sent to the laboratory for serological diagnosis of Chagas disease from 25 March to 28 June 2010 tested with IIF and ELISA Wiener lab. All these sera were tested with TB.

RESULTS Retrospective study: For 84 of the 85 patients considered as positive TB was positive as well. For one patient treated for acute Chagas infection in 2007 the TB was negative 3 years after. TB was negative for 129 non infected patients; 28 of these cases have one ELISA or IIF positive, and two are positive with both ELISA and IIF with low index reactivity. Prospective study: 10 patients had a positive serology (WHO criteria) only three with negative TB; these patients had: one acute malaria, one HIV primo infection and one had been treated for Chagas 4 years before. One hundred and thirty-seven patients were considered as negative, TB was negative except one case equivocal. Twenty-four patients considered as healthy have ELISA or IIF positive but TB negative.

CONCLUSION TESAcruzi may be a gold standard for the diagnosis of chronic/indeterminate Chagas disease. Specificity is 100% if equivocal cases are classified as negative. The WHO 2002 criteria may cause false positive classification of patients which are correctly classified by TES TESAcruzi. Further studies are necessary to evaluate the negativation of TESAcruzi after treatment as criterion of recovery.

5.3-006

Evaluation of recombinant P30 (SAG1) P22 (SAG2), and P43 (SAG3) antigens for the diagnosis of toxoplasmosis by ELISA

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Toxoplasma gondii is an important human pathogen with a worldwide distribution. Diagnosis of infection human toxoplasmosis is very important, especially in pregnant women and immune suppressed individuals. The use of recombinant antigens obtained by molecular biology techniques is an alternative way for the detection of serum antibodies. In this study we have evaluated and compared the diagnostic usefulness of the SAG1, SAG2 and SAG3 *T. gondii* recombinant antigens together and with traditional ELISA antigens. A 1158, 957 and 560 bp fragments of P43 gene, P30 and P22 gene, respectively were confirmed by PCR for recombinant plasmid, plasmid vector and genomic DNA of the parasite JM109 strain of *E. coli* was used as host cell for protein expression. Recombinant proteins expressed and purified by affinity chromatography. One hundred and fifteen anti *T. gondii* IgG positive sera, 30 negative sera, 30 sera from patients with other parasitic and viral infections and 30 anti *T. gondii* IgM positive were collected. Sera were tested using a commercial IgG and IgM ELISA kit (Euroimmun, Germany) and with recombinant SAG1, SAG2 and SAG3 proteins. Sensitivity and specificity of the recombinant SAG1, SAG2 and SAG3 in comparison with commercial ELISA assay were 93.9% vs. 93.3%, 100% vs. 88.3%, and 98.2% vs. 93.3% respectively. In conclusion recombinant SAG1, SAG2 and SAG3 produced in *E. coli* can be used for detection of IgG specific antibodies against *T. gondii* while SAG2 (P22) with sensitivity and specificity of 64.2% and 83.3% is more suitable for detection of IgM specific antibodies against *T. gondii*.

5.3-007

Risk factors of Toxoplasma infection in Chaharmahal va Bakhtiyari Province, southwest of Iran

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INTRODUCTION AND AIM Toxoplasma infection is one of the most prevalent parasitic infection all over the world, but prevalence rates of infection vary from place to place and even in different regions of a country due to reasons that remain largely obscure. The present study was carried out to investigate factors affecting Toxoplasma infection in Chaharmahal va Bakhtiyari Province of Iran.

METHODS Nine hundreds and ninety serum samples collected from the individuals referred to clinical laboratories of the province were examined for anti-Toxoplasma IgG antibodies using the enzyme-linked immunosorbent assay method. Demographic information was also collected through a questionnaire and data

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were processed and analyzed using the software SPSS ver.12 and Chi-square test, *t*-student test and regression logistic model, respectively.

FINDINGS Age range of the individuals was between 1 and 85 years (mean 27.89 ± 18.23 years). Out of 990 serum samples examined, anti-Toxoplasma IgG antibodies were found in 339 samples (34.2%). Although there was a significant correlation between the factors, age, residence, job, education level and the seroprevalence of infection, but no such relationship was observed between the variables, sex, cat keeping, consumption of vegetables and Toxoplasm seropositivity.

CONCLUSION The study indicated that the vast majority of the population are susceptible to Toxoplasma (66%) infection and they could be infected during their future life. Also, according to the findings of this study, factors such as age, location of residence, job and educational level could be considered as the risk factors of Toxoplasma infection in this region of the country. Therefore, health policies should be directed toward the promotion of population knowledge about Toxoplasma infection, the parasite's transmission routes and the risk factors affecting the infection.

5.3-008

Hookworm infection among immigrant population living in Barcelona

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Hookworm is a soil-transmitted helminth worldwide spread. Due to the last migratory flows in our country, this infection has appeared in our environment.

METHODS We studied retrospectively the intestinal parasites cases observed in a Primary Healthcare Laboratory in Barcelona from 2000 to December 2010. The diagnosis of hookworm infection was made by microscopic examination of concentrated faeces after centrifugation. Anaemia was defined as values of Hb <13 g/dl in men and <12 g/dl in women and children. Eosinophilia levels were classified as: mild ($0.5–1 \times 10^9$ eos/ml) moderate ($1–3 \times 10^9$ eos/ml) and severe ($>3 \times 10^9$ eos/ml).

RESULTS A total of 72 patients were shedding eggs of Ancylostoma/Necator. The first case was diagnosed in Mars 2007. There were 15 cases in 2007, 11 in 2008, 29 in 2009 and 17 in 2010. Only 8 (11%) patients presented anaemia, that was mild, but a 8 years old patient had a moderate anaemia (Hb 9.2 g/dl). Among the 72 patients, 62 (86%) presented different levels of eosinophilia: 26 patients (36%) had mild eosinophilia, 33 (46%) moderate, and three (4%) severe. Concerning the origin of patients infected with hookworm 57 (77%), came from Latin America; 10 (14%), were from Western Africa; four (5%) from Southern Asia and finally one (1%) came from Caribbean region. Age of patients was from 8 to 70 years, 67 were adults and five children. Parasitic co-infection was present in 25 (34.7%) patients. The parasites co-infecting were: *Trichuris trichiura* (nine), Strongyloides (seven), Entamoeba histolytica/dispar (four), Hymenolepis nana and Giardia lambia (three patients each).

CONCLUSIONS Among the immigrant population in Barcelona, there was hookworm infection with a high percentage of eosinophilia but not anaemia. Most of those patients were young adults from Latin America, the largest immigrant group in our environment.

5.3-009

Implication of immigration in tuberculosis (TB) in a metropolitan area of Spain

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INTRODUCTION Endemic TB in Catalonia (Spain) is higher (19.9/105 inhabitants/year) than in other parts of Europe. In addition, the recent demographic changes have slowed the decline in TB rates. We assess the importance of immigration in presentation, resistances and epidemic control of TB in a metropolitan area.

METHODS We reviewed cases of TB detected (2000–2010) in our district hospital, that assists about 105 people of Badalona city (14% not Spanish-born). We compared immigrants with native population and we analyzed the contact trace follow-up in both groups. PPD + (5 mm–natives;15 mm–incomers) and chemoprophylaxis according consensus recommendations.

RESULTS TB was diagnosed in 146p of which 37 (25.3%) immigrants (Sub-Saharan 3; Morocco 9; S. America 13; SEAsia 9; East Europe 3) The estimated incidence 13.3/105/year. In native population, men (72.9%) were more affected than women, while in immigrants gender was balanced (45.5%); $P < 0.05$. Mean age was lower in foreigners (32.8 10.3) than in Spanish-born people (49.7 21.4); $P < 0.05$. Comparison of extra-pulmonary/pulmonary TB didn't reached differences between groups, although the former was more frequent in immigrants16/20 (44.4%) than in autochthons 30/73 (29.1%); $P = 0.09$. We reported one XDR-TB in a Russian man, one Moroccan woman resistant to pyracinamide while one Spanish patient resistant to hydrazide. Although 6p in every group required DOT, the percentage was significantly different; $P < 0.05$. We followed 347 contacts: the median number 4 (IQR 3–6) in Spanish-born and 2 (IQR 0–5) in foreigners; $P < 0.05$. Mean age of contacts 37.115.9 and 24.8 9.8, respectively; $P < 0.05$. PPD (+) in 43.7% of Spanish-born contacts (nine conversions) and 54.1% in foreigners; $P = 0.07$. New cases of TB were detected in one immigrant but in six natives; $P = 0.08$. There were not significant differences in chemoprophylaxis comparing medians in natives vs. incomers: primary 0 (IQR0–2) vs. 0 (IQR0–1); $P = 0.1$; secondary 1 (IQR0–3) vs. 0 (IQR0–2); $P = 0.1$.

CONCLUSIONS Immigrants of both genders with TB were younger than Spanish people, who were mainly men. We observed a trend of more extra-pulmonary cases in incomers. Even though infrequent, resistances were a worrisome issue, particularly XDR-TB. We detected more clusters in native contacts.

5.3-010

Communicable disease screening services for migrants in Ireland: a mapping study

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INTRODUCTION Effective communicable disease prevention and control strategies for migrants are essential, both for public health and the health of the individual; one such strategy is migrant screening. In Ireland, although the overall tuberculosis (TB) crude notification rate remains relatively stable, the percentage of TB cases in foreign-born persons increased from 8.3% in 1998 to 43.6% in 2008. This study aimed to map the current structures and processes of the voluntary communicable disease screening service for migrants in Ireland.

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METHODS AND MATERIALS Telephone interviews, with a sample of individuals working in the screening service, were conducted between May and September 2010. Purposive and snowball sampling techniques were used and enquiries made in each of the 32 Local Health Office (LHO) areas.

RESULTS The screening service coverage was fragmented and heterogeneous; a screening service was not in place for migrants with irregular status, migrant workers or students. For programme refugees the International Organisation for Migration provide pre-departure 'Fitness to Travel Assessments'; after arrival, the Office of the Minister for Integration report screening is organised, however, a designated healthcare worker responsible for the screening was not identified. A dedicated screening service for asylum seekers was identified in under half (43.8%) of the LHO areas; all offered screening for TB, hepatitis B and HIV/AIDS. Services were absent in two counties where high numbers of asylum seekers reside (excluding the reception centre). Mantoux tests could be offered by 78.6% of the services (not necessarily routinely) covering only 34.4% of the LHO areas. Polio screening was offered by 7.1% of the services, covering only 3.1% of the LHO areas.

CONCLUSIONS Screening service coverage was incomplete and heterogeneous. This could have a major impact on communicable disease control efforts in Ireland and importantly on the health services meeting the needs of the individual migrant.

5.3-011

International migrations and HIV care in the rural district of Nanoro, Burkina Faso

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INTRODUCTION West Africa Countries account for almost the half of the estimated 20,000,000 international migrants in the African Continent. In the frame of the scaling up of HAART, our study aims to identify specificities of HIV care for migrants patients in Burkina Faso.

METHODS AND MATERIALS The Health District of Nanoro (140,000 inhabitants) covers a rural area in the North-West of Burkina Faso where about 30% of adult males migrates to the cocoa plantations in Ivory Coast. HIV prevalence is estimated around 1%. Data were collected from the medical files at the Nanoro District Hospital.

RESULTS From 1st January 2007 to 31st May 2011, the District Hospital of Nanoro followed 422 HIV/AIDS adult patients, 175/422 (41.5%) males and 247/422 (58.5%) females. Migrants were 135/422 (32.0%), 82/175 (46.9%) among men and 53/247 (21.5%) among women (P < 0.01). Mean age was 38 Â ± 10 years among migrants and 39 Â ± 10 years among resident (P = ns). At baseline, 109/135 (80.7%) migrants and 173/287 (60.3%) residents were in WHO clinical stages III or IV (P < 0.01). The average CD4/microl at baseline among migrants and residents were respectively 285 Â ± 255 and 332 Â ± 250 (P = 0.05). During the first semester of HAART, the mortality rate was 14/104 (13.5%) among migrants and 15/183 (8.2%) among residents (P = ns). As many as 14/104 (13.5%) migrants and 7/183 (3.8%) residents were transferred to other centers (P < 0.01) and 5/104 (4.8%) migrants and 5/183 (2.7%) residents were lost to follow up (P = ns). The average CD4 count increased from M0 to M6 ranged from 199/mm^3 Â ± 188 to 403/mm^3 Â ± 304 among migrants and from 213/mm^3 Â ± 122 to 399/mm^3 Â ± 197 among residents (P = ns).

CONCLUSIONS For migrant patients, access to care seems to be delayed. The high frequency of migrants under HAART transferred for follow up to the destination country requires an improved cooperation among the health systems of the African Countries.

5.3-012

Usefulness of a western blot method for serodiagnosis of Chagas' disease in cases of inconclusive serological results

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BACKGROUND A single dose of 500 mg mebendazole is one of the recommended treatments for soil-transmitted helminth (STH) infections by the WHO. Based on randomized, placebo-controlled trials, mebendazole shows high cure rates for *A. lumbricoides*, but not for *T. trichiura* and hookworm. We evaluated the impact of mebendazole treatment on STH infections in Cuban schoolchildren under field conditions.

METHODS During a 3 year period, we followed up 268 STH-positive Cuban schoolchildren (5–16 years) at 6 months intervals, and assessed the effect of selective periodic treatment with a single dose of 500 mg mebendazole on STH infections. Infections with *A. lumbricoides*, *T. trichiura*, and hookworm were diagnosed by stool examination (two 25 mg Kato-Katz examinations). Common risk factors related to STH were assessed by parental questionnaire.

RESULTS A significant reduction in the number of STH infections between 58.7% and 93.9% was obtained after 3 years with the highest reduction for *T. trichiura* and the lowest for hookworm. Between two consecutive follow up periods we found cure rates between 56.3–82.6%, 52.4–76.9%, and 44.4–76.7% for *A. lumbricoides*, *T. trichiura*, and hookworm, respectively. After two treatment rounds, approximately 75% of the children infected were cured, with important differences between helminth species (95.2% for *A. lumbricoides*, 80.5% for *T. trichiura*, and 76.5% for hookworm). At the end of the study, cumulative cure rates were almost 100% for all three STHs. No differences in common risk factors for helminth infections were observed between children who were cured after one treatment and those who were still helminth positive after at least four treatments.

CONCLUSION Our results indicate that periodic selective treatment with a single dose of 500 mg mebendazole is effective in reducing the number of STH infections in Cuban schoolchildren, although not equally for all helminth species, and only after at least two rounds of selective treatment.

5.3-013

Analysis of probable correlation between *Toxoplasma gondii* and schizophrenia: a seroepidemiological longitudinal investigation from 2002 to 2007 in Suzhou and Wuxi regions, Jiangsu, China

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Toxoplasma gondii is a species of parasitic protozoa that has been associated with various psychiatric conditions. In recent years, increasing evidence supports the hypothesis that *T. gondii* infection may be an etiological factor for the development of psychosis in some patients. There is no longitudinal study in literature that investigated the association between *T. gondii* infection and schizophrenia. Therefore, this study aimed at longitudial method to investigate if *T. gondii* infection is a possible cause of schizophrenia in patients from 2002 to 2007, and the

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analysis of investigation indicated the probable correlation between *T. gondii* and schizophrenia. The prevalence of anti-*T. gondii* IgG and IgM antibodies were detected by enzyme-linked immunosorbent assay on 1720 patients with schizophrenia and 1552 controls during 2002–2007 in Suzhou and Wuxi regions, Jiangsu China. The overall anti-*T. gondii* IgG prevalence in the study population with schizophrenia was 17.97% and was significantly higher than that in controls 7.35% (χ^2 = 81.74, OR = 2.76, P £ 0.001). Seropositivity rates for anti-Toxoplasma IgM antibodies in schizophrenia 6.22% was insignificantly higher that in controls 5.28% (χ^2 = 1.29, OR = 1.19, P £ 0.05). In addition, the ratio of seropositivity to *T. gondii* antibodies in population with schizophrenia and controls was remain stable in during 2002–2007. In conclusion, our data suggest that schizophrenia associates with *T. gondii*. The data of positive *T. gondii* antibodies in individuals of schizophrenia vs. a control group demonstrated the infectious ratio has relatively stabilized on yearly basis from 2002 to 2007.

5.3-014

Surveillance of Chagas disease in pregnant women in Madrid (Spain), 2008–2010

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INTRODUCTION One of the most important modes of transmission of *Trypanosoma cruzi* infection in areas where it is not endemic is vertical transmission: from mother to child. Currently, the only way to control this is determining the presence of *T. cruzi* antibodies in pregnant women from endemic areas. The aim of this report is therefore to assess the efficacy of different programmes of serological screening to monitor infection by *T. cruzi*/Chagas disease in pregnant Latin American women living in Madrid (Spain).

METHODS A retrospective study was undertaken in seven hospitals in the Autonomous Community of Madrid. Serological screening programs were classified in two main strategies, a selective one (pregnant women from Bolivia) and a universal one (pregnant women from Latin America). The data were collected via a form designed for the analysis of aggregate data.

RESULTS A total of 3839 pregnant women were tested, and the overall prevalence was 3.96%. The rate of congenital transmission was 2.6%. The 95.4% of seropositive mothers were from Bolivia and the overall prevalence in pregnant Bolivian women was 11.4%. The current monitoring programmes have variable coverage ranging between 26% (selective screening) and 100% (universal screening).

CONCLUSION Monitoring of pregnant women from Latin America only reaches full coverage if universal screening of pregnant women is carried out at any moment of pregnancy, including at delivery. A common of national regulation is necessary in order to ensure homogenous implementation of screening.

5.3-015

Compliance of health care workers with suggested pandemic influenza vaccine and vaccine side effects

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INTRODUCTION In our country influenza A H1N1 vaccination for health care workers started in November. In our study we investigated acceptibility of vaccination, side effects and their incidence among health care workers.

METHODS Records of the vaccinated health care workers between 20 November 2009 and 1 December 2009 were examined for acceptence and side effects prospectively.

RESULTS Six hundred and sixty-nine of total 1185 healthcare workers were vaccinated voluntarily with Pandemic influenza A H1N1 vaccine. Vaccination rates were 84.3% (291/345) for doctors, 52.9% (198/374) for nurses and 38.6% (180/466) for other personel. 261 (39%) healthcare workes reported side effects in 14 days. One hundred and ten patients reported only systemic side effects while 62 patients suffered from only local effects. Both systemic and local side effects were reported by 89 patients. Most common side effects were pain on injection site (17.3%), dizziness (7.6%) and head ache (6.7%).

CONCLUSION In situations concerning public health as Influenza H1N1 pandemia, proper education and informing of healthcare workers is important in order to prevent prejudice and to establish an effective vaccination campaign which will provide control of infection.

5.3-016

Imported infectious diseases in asymptomatic immigrants attending at a tropical medicine unit in Spain

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INTRODUCTION In 2010, there were 214 million international migrants worldwide. Migrants may be infected with diseases endemic in their country of origin but may be asymptomatic. Identifying and treating imported infectious diseases among asymptomatic cases may have an important impact both for the individual concerned and for public health.

METHODS Observational, descriptive, comparative and retrospective study of asymptomatic sub-Saharan (SSA) and Latin American (LA) immigrants (ASYM-IMM) attended at the Tropical Medicine Unit, Ramón y Cajal Hospital (Madrid, Spain) (January 2000–January 2011). Demographic variables: age, gender, country/area of origin and time from arrival in Spain to consultation (A–C). Routine screening included: HIV, HBV, HCV, rapid plasma reagin (RPR for syphilis) serology, Mantoux skin test (PPD), stool parasites, PCR for malaria, Chagas serology for LA and schistosomiasis serology if risk factors.

RESULTS Six hundred and thirty-three cases: LA 350, SSA 283. Main countries of origin: Bolivia (41.9%), Senegal (7.9%), Nigeria (6.5%), Ecuador (5.5%) and Cameroon (4.6%). Median age: 29 years (IQR: 23–36), 54% were males. HIV+: significantly more frequent (SigMO) in SSA (2.3%), median time A–C (MTA-C), 2 months (Interquartile range-IQR: 0.3–11). Chronic HBV infection: SigMO in SSA (13.6%), MTA-C, 7 months (IQR: 2.75–13.5). Chronic HCV infection: SigMO in SSA (1.1%) MTA-C, 2 months (IQR: 1–2). RPR: no differences between ASS (2%) and LA (1.6%), MTA-C, 8.5 months (IQR: 1.75–35.25). PPD: SigMO in SSA (72.1%), median time A–C 9 months (IQR: 2–35.5).

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Intestinal Parasites: no differences between SSA (2.5%)-LA (2%) MTA-C, 9.5 months (IQR: 3–28). Chagas disease: In LA (47.2%), MTA-C, 44 months (IQR: 27–62). Malaria: SigMO in SSA (5.8%), MTA-C, 5 months (RIQ: 2–12), 80% due to P. falciparum, one case diagnosed 28 months A-C. Schistosomiasis: In SSA (6.9%), MTA-C, 4 months (IQR: 2–15).

CONCLUSIONS A targeted screening program for infectious disease in ASYM-IMM may include: HIV, HBV, HCV, RPR, PPD, PCR for malaria, Schistosomiasis if risk factors in SSA and HIV, HBV, HCV if risks factors, RPR, PPD, Chagas disease in LA. The low prevalence and clinical significance of intestinal parasites questions the need for performing routine stool analysis

5.3-017

Access to and effective use of HIV and other health services for international migrants and ethnic minorities in Northern Thailand

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Thailand has about 3 million international migrants, mostly from Myanmar. Migrants' malaria and TB rates are reported to be 10 times higher, while HIV rates groups equal or exceed rates in the general Thai population. However, migrants make less use and less effective use of services than the general Thai population. Thai Government health services are free for all citizens; some Government services, including antenatal care are available for international migrants. Absence of Thai citizenship, health insurance, and Thai language ability, and knowledge of disease and health services constrain access to available services for international migrants and ethnic minorities. Many migrants in Northern Thailand are members of ethnic minority groups. They often live in the same communities as members of the same minority groups who are Thai citizens. By 3 June 2011 our native speakers of respondents' languages had censused five Lahu communities and in ine Chinese + Shan community. They had interviewed 364 randomly selected women who had delivered within 5 years. Interviews focus on antenatal care, the main entry point for HIV testing, and on constraints to care. Lahu communities include significantly higher proportions of Thai citizens (some of whom were migrants) than in the Chinese + Shan community; Lahu non-citizen migrants are significantly less likely than Lahu citizens to have health insurance, to be able to speak Thai, to have used antenatal care, or to have been counseled or tested for HIV. Migrant Lahu are significantly more likely than Chinese + Shan migrants to report 'lack transport', 'lack Thai language', 'don't know how to talk with doctor', 'ineligible for services', and 'fear harassment from government officials' as reasons for delay or non-use of services.

Significant heterogeneity of migrants implies that detailed knowledge is needed to plan and implement effective interventions to increase effective use of available services.

5.3-018

The burden of the TB patients especially for migrants during the courses of diagnosis and treatment in Shanghai

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BRIEF INTRODUCTION China is one of the 22 highest TB burden countries in the world. The information from China TB Information Management System has indicated that there is high proportion of migrant among notified TB cases in big cities ranged from 40% to 80%. In Shanghai, the number of migrant from other provinces was account for 26% of total population as estimated in population sampling survey in 2005. Migrants are generally vulnerable in living and working, suffering TB might lead to high burden. In 2007, Shanghai implemented the program of deduct and free anti-TB treatment for migrant cases same as local residents. This study is aimed to describe and analyze how the burden of the TB patients especially for migrants equity to get diagnosis and treatment in Shanghai.

METHODS AND MATERIALS Information on general TB management was collected from seven districts CDC. 714 TB patients completed treatment from seven districts in Shanghai were interviewed applying questionnaire in summer of 2010, among of them, 294 cases were migrants.

RESULTS The medium cost of confirmed diagnosis and completion of treatment was 5200 yuan for local residents and 3037 yuan for migrants; and 2150 yuan was reimbursed by both TB program and insurance for local residents, 1435 yuan were reimbursed by the TB program for migrants; while 2600 yuan was paid out of pocket and account for 43% for local residents, and 1841 yuan was paid out of pocket and account for 56% for migrants.

CONCLUSIONS The cost deduct and free program for TB cases could help to relieve some burden for patients, but the self-payment is still relatively high, especially for migrants who have no insurance. Further study on the rational benefit package for migrants to equity accessing services and cost control would be needed.

5.3-019

Chagas disease among the Latin-American population attending at a non-endemic area: our experience in a regional hospital in Catalonia

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BRIEF INTRODUCTION Estimated 12 million persons in Latin-America and thousands of immigrants living outside are infected with *T. cruzi*. Fundació Hospital-Asil Granollers is a district hospital with a catchment area of 300,000 inhabitants, 12% of whom are immigrants. People from Latin America represent the second largest group of immigrants after the North African ones. Chagas disease has become the most prevalent imported disease in our area.

METHODS AND MATERIALS A descriptive-study carried out among the Latin American population diagnosed of Chagas is presented. Patients were recruited between June 2007 and March 2011. We designed a coordinated pathway between primary care, internal medicine, gynecology and pediatric departments, to diagnose and follow-up all cases detected. The main outcome was characterization of clinical, epidemiological features, and treatment's side-effects. All patients had been screened for *T. cruzi* by two different serologic tests, plus PCR in pediatric patients. A blood examination, ECG and echocardiogram were made. Barium swallow and enema, Holter and cardiac MRI were requested at symptoms.

RESULTS $N = 92$ were positive for Chagas infection, 81% adults. Typical adult patient profile was 40 year old Bolivian female without evidence of visceral involvement arriving at Spain 3 years before. 52% had previously family Chagas history. Most of them had come to our service from primary care, other than obstetrics, cardiology and emergency department. Thirty-five percent were asymptomatic, 24% complained about symptoms, 25% had been previously diagnosed, 5% blood donors, and 9% pregnant. Eight patients had additional medical condition: two HIV, two cancers,

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three presented with other parasitological infections, one HbsAg positive. After physical examination, history taken and complementary tests, 67% were diagnosed as indeterminate chronic stage, 28% and 5% cardiac and digestive chronic involvement. Most patients with cardiac form presented mild ECG features without symptoms. Dilated myocardiopathy and complete branch block were the major features detected among symptomatic patients. In pediatric group, only three were confirmed for Chagas disease during follow-up (two congenital cases). Twenty-five patients had received treatment until now with benznidazol with poor tolerance. Major side-effects: skin hipersensibility, gastrointestinal upset, fever and arthritis. Three patients with cardiac involvement needed pacemaker device.

Conclusions Prevalence of Chagas disease in our area is significant. Its diagnosis, and follow-up require an interdisciplinary work between hospital services and Primary Care. We should emphasize the need for early diagnosis among patients with accurate epidemiological background who consult for cardiac-symptoms. Despite the incidence of treatment's side-effects, its efficacy reducing vertical transmission should let us to have an active case research.

5.3-020

Congenital Chagas disease: the importance of screening before the delivery. Our experience in a regional hospital in Catalonia

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Brief Introduction Prevention, control and treatment of Chagas disease in non-endemic areas has become a challenge in our Health Care System. Congenital transmission of *T. cruzi* in these areas is becoming an increasing problem.

Methods and Materials A descriptive study of congenital transmission of *T. cruzi*, carried out among Latin American pregnant women who came at a regional hospital for birth in 2010.

Results We attended 2021 pregnant women at birth during 2010 (Latin American ones were 9%). We diagnosed of Chagas disease seven Latin-American women during pregnancy, without previous screening for *T. cruzi* infection. We report two-cases of congenital transmission. Case 1: Newborn from 40 year old Bolivian woman, without symptoms for Chagas disease. Mother's diagnosis confirmed in first trimester. The newborn was delivered at 35 weeks of gestation by caesarean section because of abnormal brain Doppler, and breech presentation. Apgar score was 2/6/9. On physical examination hypotonia, petequies, systolic murmur and hepatosplenomegaly were found. The child was referred to a terciary level because of maintained severe hypoglycaemic and coagulation disturbances. Chagas disease was confirmed later by PCR and microhematocrit. Treatment with Benznidazol was started. Although PCR became negative at 18 days old, child showed no response to any medical effort to correct hypoglycaemia and colestatic pattern. Due to these reasons, treatment was weaned off at 30 days old. Patient was discharged at 38 days with good clinical course. During follow-up, PCR, microhematocrit and IgG serology was negative at 7 months old. Case 2: Newborn from 16 years old Bolivian woman, diagnosed from Chagas disease during delivery. The newborn was delivered at 37 weeks of gestation by eutocic vaginal birth. Apgar score was 9/10. Asymptomatic with normal birth weight. Chagas disease was confirmed by PCR amb microhematocrit. Benznidazol was started during 60 days without side effects.

Conclusions Congenital Chagas disease in non-endemical areas is probably underestimated. Although most children infected vertically will follow an asymptomatic clinical course, diagnosis must be suspected in any ill infants from a Latin American endemic mother. It is important its early detection and treatment in order to avoid complications. A short treatment (30 days) maybe is enough for cure infection.

5.3-021

Chagas disease in a tropical medicine unit in Spain

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Background Chagas disease is endemic throughout Central and South America and its distribution is rapidly changing as million of person at risk have moved to developed countries. Diagnosis and outcomes of treatment are based on serologic tests; a negative serology post-treatment corresponds to cure, but this situation usually occurs after several years.

Method Descriptive study conducted in a specialized unit of a tertiary hospital in Valencia (Spain). All cases of Ech attended between November 2003 and May 2011 were included. Demographic characteristics, stage of disease, laboratory findings and serological response in treated patients were recorded. Statistics: percentages, mean, standard deviation, median and percentiles 25 and 75.

Results Two hundred and eighty-two patients were included, 203 of them in active monitoring. Mean age 38.4 ± 9.86 years, 196 (69.5%) females and 95% were from Bolivia. Median stay in Spain until the diagnosis was 4 (3–6) years. One hundred and ninety-four patients were referred from the Blood Transfusion Centre, 58 from Primary Care, 17 from Specialized Care and 13 after screening program during pregnancy. Epidemiological factor related to the transmission mechanism was the recognition of the vector in 90.8% of cases. 63.1% of patients were asymptomatic and six patients were immunocompromised, two of them were HIV-infected. One hundred and forty-seven patients (51.8%) were classified as indeterminate form of chronic phase. Among determinate form: 81 patients (72.3%) had cardiomyopathy, 22 patients (19.6%) had digestive involvement and nine patients (8%) had a mixed form. Benznidazol treatment was given to 156 patients (55.3%), 60 (41.4%) of them showed toxicity to this drug; skin disorder was the most common. After follow-up, six patients reached a negative serologic test for *T. cruzi*.

Conclusions In health care units dedicated to the study of imported diseases, Ech is one of the most prevalent. It is important to evaluate the response to treatment in non-endemic areas.

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5.3-022

Sensitivity and specificity of the simple/stick chagas (Operon S.A.) for the serological screening of Chagas disease using different samples (serum, peripheral blood and capillary blood)

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INTRODUCTION *Trypanosoma cruzi* infection is an imported parasitic disease currently on the increase in Spain due to immigration from Latin America. As most of infected people are in the chronic phase of the disease, the diagnosis is mainly carried out by serologic tests. Simple/Stick Chagas (Operon S.A.) is a rapid immunochromatographic test used to detect anti-*T. cruzi* antibodies. The aim of this study was to compare the sensitivity and specificity of this test on different biological samples: serum, peripheral blood and capillary blood.

MATERIAL AND METHODS Well characterized serum samples and non-characterized specimens were included in the study. Well characterized serum samples were from chagasic patients (63), non-chagasic individuals (95), visceral leishmaniasis patients (38) and malaria patients (55). Non-characterized specimens were from Latin American immigrants and individuals at-risk with clinical and/or epidemiological background: serum samples (450), peripheral blood (94) and capillary blood (251). The gold standard was considered to be the concordance of the Results of both ELISA and IFAT, in house conventional tests.

RESULTS The sensitivity and specificity of the Simple/Stick Chagas test in well characterized samples were 100% and 97.9%, respectively. Cross-reactivity with samples from visceral leishmaniasis patients was not found. In contrast, 27.3% of false-positive Results were observed with samples from malaria patients. The sensitivity of the rapid test in serum or plasma, peripheral blood and capillary blood samples was 100%, 92.1%, and 86.4%, respectively, and the specificity was 91.6%, 93.6% and 95%, respectively.

CONCLUSION The Simple/Stick Chagas test had a variable sensitivity depending on the kind of sample. It performs better when serum or plasma samples are used. Therefore, it could be used for serological screening combined with any other conventional test.

5.3-023

Parasite burden in blood donors infected with *Trypanosoma cruzi* living in Madrid

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INTRODUCTION Chagas disease is an imported parasitic infection in Spain which must necessarily be ruled-out in blood donors (Royal Decree 1088/2005). To date, at least six cases of transfusional Chagas disease have been reported. The aim of this study was to determine the parasite burden in blood donors infected with *Trypanosoma cruzi* and its relationship with the antibody levels.

METHODS Forty-nine blood donors from endemic areas residing in Madrid were included in the study. All of them had anti-*T. cruzi* antibodies detected by four serological tests: CerTest, Biokit, CNM-ELISA and CNM-IFAT. Parasitemia detection was carried out by conventional PCR and blood culture. Real-time PCR was used to quantify the parasite burden. Blood samples were taken at two or three different time points, according to the layout of infected individuals. Thirty milliliters and 3–6 ml of blood were used for culture and PCR, respectively. Hemoculture was done only in the last blood collection.

RESULTS Thirty-two (65.3%) out of the 49 donors showed positive DNA amplification at least in one of the blood samples taken. Fifteen donors were positive by culture, parasites in culture were observed by microscopy (7) or detected by PCR (8). The parasite levels ranged between 1 and 10 parasites/ml. Taking into account the parasitemia detected by PCR, three categories of seropositive individuals were recognized, individuals with: (i) non-detectable parasitemia, (ii) intermittent parasitemia, and (iii) persistent parasitemia. The antibody levels in individuals with detectable parasitemia were higher than in individuals with non-detectable parasitemia.

CONCLUSION The parasite burden in individuals with *T. cruzi* infection residing in Madrid was similar to those reported in individuals living in endemic areas. It is necessary to sustain the serological screening of blood donors at-risk as a control measure for preventing *T. cruzi* transmission in non-endemic areas.

5.3-024

Chagas disease

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INTRODUCTION Chagas disease is caused the protozoan infection *Trypanosoma cruzi*, endemic in Central and South America. The increasing Latin American immigration to Spain made Chagas an emerging disease in our environment, creating a need for study and control.

OBJECTIVE to evaluate clinical features, epidemiology and evolution of patients with Chagas'disease treated at the Hospital Son Espases, Palma Mallorca (Spain).

MATERIAL AND METHODS A retrospective study of patients seen by Chagas disease in the years 2006–2011. Patients were identified in the Microbiology Department (*T. cruzi* serologic tests positive). Epidemiological, clinical, diagnostic end evolutive data were reviewed. Statistical description: SPSS18.0.

RESULTS In the 5 year period 43 patients were seen, 14 men and 29 women. Eighty-six percent came from Bolivia. The first serology was made, origin country: 17 patients, in Hospital: 17 patients. PCR performed in 25 patients (13 positive and 12 negative). Heart symptoms in 30.2% patients and gastrointestinal in 25.6%, the most common: chest pain (14 people), with constipation (8), with GERD (8).16.3% of patients with eosinophilia. Normal ECG in 31 patients, four with BRDHH and four BRDHHHAI. ECHOCARDIOGRAM 32 patients: 28 normal and four with dilated cardiomyopathy. Treatment: 19 patients were not treated. Twenty received benznidazole, only two had to change to nifurtimox. Other therapies: a pacemaker, an ICD and a pacemaker implant pending. Second PCR after treatment in seven cases, all negative. By gender, 60% asymptomatic men and 71.4% women affected. No significant differences. Sixty-six percent of patients younger than 45 years and older showed symptoms were symptomatic in 71.4%. There are no differences.

CONCLUSIONS Majority of the people of their country of origin is Bolivia. Are younger patients. Much have symptoms, usually mild and inconspicuous. The elderly often present with more severe disease. All patients with PCR positive treated had a PCR negative after completion. The treatment was well tolerated in most of the cases only two suspended.

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5.3-025

Chagas disease: congenital transmission and treatment success in a health department of Valencia (Spain)

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OBJECTIVE To describe congenital transmission and treatment success of Chagas disease in Latinamerican immigrants in our Health Department 07-La Fe, in Valencia (Spain).

METHODS We collected sera from Latinamerican women in reproductive age who attended our hospital from January 2008 to December 2010. Samples were tested for *T. cruzi* serology and PCR (infected patients). Sera and umbilical cord blood from newborns from infected mothers were tested for *T. cruzi* at 0-1-6-12 months old. Infected children (two positive PCR and/or positive parasitological examination) were treated with benznidazol (3.5–7 mg/kg/day–60 days). Infected women were also treated with benznidazol (5 mg/kg/day–60 days) and followed up at 3-6-12 months after treatment.

RESULTS Out of 1403 tested women from endemic countries, 137 were positive for *T. cruzi* antibodies (prevalence 9.7%). All infected women except one came from Bolivia, mostly from Santa Cruz (40.1%) and Cochabamba (28.1%). All were asymptomatic (chronic stage). Fifty (36.5%) were initially treated and 41 (82%) of them completed treatment, persisting *T. cruzi* IgG antibody detection (IFA values over 1/512) up to 12 months after treatment. Of all women who completed treatment, 14 had positive PCR prior to treatment which was then negative before 6 months of therapy. In 23 women (46%), benznidazol had some type of adverse effect and three of them (6%) had to interrupt treatment. Five newborns had congenital transmission and were treated. Only one of the babies had symptoms at birth (dilated cardiomyopathy and a neuroblastoma). Benznidazol treatment was well tolerated by all babies. All of them were seronegative with negative PCR before 16 months-old.

CONCLUSIONS (i) Prevalence of infection in Latinamerican women is similar to other reports. (ii) Infected women were mainly from Bolivia and asymptomatic. (iii) PCR is essential during acute phase diagnosis but new disease markers are needed in order to measure treatment efficacy in chronic phase. (iv) Treated women had not tolerated benznidazol as well as newborns.

5.3-026

Health and complexity. The migration of Chagas disease: an interdisciplinary approach

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Due to the growing migration flows from endemic countries, Chagas disease has become a health priority also in non-endemic areas. Italy is the second European country in terms of number of Latin American migrants; however, no specific health policy for Chagas disease diagnosis and treatment has yet been adopted. Since 2009, the Centre for International Health of the University of Bologna is carrying out an action-research project in the Emilia-Romagna region, where migrants proceeding from endemic areas reach 300,000. Adopting a multidisciplinary, multi-method and participative approach, framed in the theory of social epidemiology, the project aims at assessing Chagas disease prevalence and investigating the social, economic and cultural characteristics of Latin American migration in the territory. The first regional health service for Chagas disease diagnosis and treatment has been recently created and, to date, more than 60 people have been tested for *Trypanosoma cruzi*. Thanks to the information and training provided, health professionals are more aware of Chagas disease and of the broader determinants of health related to migration. The ethnographic research has shown that Latin American migration in the area can be divided into two broad groups. The larger one comprises mainly economic migrants coming from Peru and Ecuador, who experience difficulties in accessing health care services and have little or no previous knowledge about Chagas disease. The smaller one comprises people coming mainly for political reasons from Brazil and Argentina, who are aware of Chagas disease and tend to distance themselves from the associated stereotypes of poverty and marginalisation. The adopted strategy allows an ongoing exchange between quantitative and qualitative research Results and health intervention, as well as a shared evaluation by all involved stakeholders. The approach appears very promising in dealing with complex health issues in which biological, socio-economic, cultural and political determinants are tightly linked.

5.3-027

Global migration and tuberculosis trends in non-endemic country

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The World Health Organization considers tuberculosis (TB) to be a global, uncontrolled, fatal, contagious epidemic urgency since 1993. International migration and immigrant population growth observed over the last three centuries. Within one decade an increase had occurred by 10 million immigrants to industrialized countries such as UK, France, and USA. Imbalances between e-migration push factors and in-migration pull factors and exposure-re-activation related has changed TB epidemiology in non-endemic countries. Here we assessed suspect TB cases in a sample of 180 new immigrants to Kuwait using four-diagnostic tests and evaluated the trends of TB morbidity/mortality/fatality status by gender and nationality in Kuwait during 26-years-period (1984–2009) using governmental data records. TB Morbidity rates show a predominance of males and non-Kuwaitis' were higher than those of their counterparts and TB Mortality (cause-specific MR) and case fatality rates were three-times higher in Kuwaitis' and females in particular than non-Kuwaitis'. Worldwide inter-related risk factors impair 'migration-TB control' and result in negative public health complications. TB stigmatization ('fear of infection') and un-employment risks decrease health-seeking behaviour. Fatality rates rises in non-endemic populations can be related to MTB genotypic differences. Difficulties in migration control and weakness of health system measures to detect latent TB result in a continued risk of introduction of TB. A two-diagnostic test policy for all immigrants' to- and from TB endemic regions and a policy to reduce the stigma associated with TB are recommended.

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5.3-028

Drug resistance amoung tuberculosis patients in central province-Iran (2005–2010)

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OBJECTIVE Today, one of the basic problems of tuberculosis treatment is drug resistance issue. Study to determine the drug resistance of Mycobacterium strains isolated from patients with pulmonary tuberculosis to Antituberculosis drugs (isoniazid, rifampin, ethambutol, streptomycin, pyrazinamide) was done.

MATERIAL AND METHODS All patients with pulmonary tuberculosis resistant to treatment during 2005–2010 culture and antibiogram standard method (proportional) was completed.

RESULTS The rate of resistance were overall in patients with smear positive 7/3% and the rate of MDR-TB equivalent to 4/3% and 0/5% of smear positive patients were resistant to five drugs. Among the most resistant strains resistant to isoniazid (68/8%), respectively, and then rifampin (62/5%), Pyrazinamide (25%) ethambutol (21/9%) and streptomycin (21/9%), respectively. highest rate in the age group 15–45 years ($P < 0.001$) and incidence of resistance in men, were significantly more than women ($P = 0/008$) the amount of Resistance grade was significantly associated with smear positivity ($P = 0.015$) as well as resistance was significantly associated to the relapse of TB and HIV infection. and the death rate in patients with resistant TB were higher compared with other patients, but from the standpoint of nationality and residence were not significantly associated with resistance.

CONCLUSION The review of drug resistant *Mycobacterium* strains studied over 6 years show the growing process and therefore close attention to prevent the creation and dissemination of resistant strains is very essential and for this the strategy of 'DOTS II' is the best method.

KEYWORDS *Mycobacterium tuberculosis*, antituberculosis drugs, resistance, antibiogram

5.3-029

The migrant health guide – a tool to support health care professionals caring for migrants in the UK

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INTRODUCTION The last UK Census in 2001 showed that 8% of people living in the UK had been born abroad. Whilst the majority of these migrants are young, healthy adults, surveillance data shows that the greatest burden of infectious diseases (TB, HIV, malaria, enteric fevers) in the UK falls on those who were born abroad. In addition, primary care practitioners in the UK may encounter infections or other health concerns in migrant patients which they are not familiar with from their experiences with the general UK-born population.

METHODS The migrant health guide was developed as an online tool with the aim of bringing together available information (such as data on the prevalence of diseases and nutritional deficiencies in different countries), guidelines, and translated patient information in a format that is easy to use and can be quickly accessed during a standard consultation. It was launched by the UK Health Protection Agency in January 2011 at www.hpa.org.uk/migranthealthguide.

RESULTS The guide is divided into four main sections; General Information, Countries A-Z, Health Topics, and Assessing Migrant Patients. Countries A-Z is the largest section of the site and gives relevant information about children's health, infectious diseases, nutritional and metabolic concerns and women's health relating to over 130 different countries of origin. Global disease prevalence data has been used to indicate countries of origin for which testing or awareness of infections such as HIV, TB, hepatitis, malaria and Chagas disease is recommended.

CONCLUSIONS Feedback from users of the guide so far has been positive and the guide is continuing to evolve as new topics and resources are added. Awareness of the existence of this resource amongst health professionals needs to be further raised and its impact formally evaluated in primary care.

5.3-030

Imported strongyloidiasis diagnosed in an outpatient tropical medicine center

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INTRODUCTION Strongyloidiasis is a soil-transmitted helminthiasis which may persist in the host for indefinite periods. Most infected individuals are asymptomatic or may present unexplained eosinophilia. In case of immunosuppression, strongyloidiasis may become a disseminated, life-threatening disease. The aim of this study is to describe 108 cases of strongyloidiasis in our center.

MATERIALS AND METHODS A retrospective chart review of 108 cases of *Strongyloides stercoralis* identified through microbiology laboratory records from 2008 to 2010. Data were analyzed with SPSS 16.00.

RESULTS Men were 62/108 (57.4%). All them were immigrants, VFR (visiting friends and relatives) 30/108 (27.8%). Areas of origin: Latin America 64/108 (59.2%), Sub-Saharan Africa 38/108 (35.2%) and Asia 6/108 (5.6%). Main countries: Bolivia 46/108 (42.6%) and Equatorial Guinea 21/108 (19.4%). Mean of age 34.7 ± 12.2 years. Mean of length of stay in Spain 2.9 ± 2.8 years. Reason of first visit: screening 36/108 (33.3%), eosinophilia 41/108 (38.0%), screening for Chagas disease 19/108 (17.6%). Diagnose was made from the examination of a single stool sample in 36/106 (34.0%). Two or more samples were nedeed in 52/106 (49.1%). Cultures were positive having the previous stool exams negative in 18. Serology was made in 14/108 (13.0%) and it was positive in all cases. Absolute eosinophilia was present in 88/101 (87.1%) and hyper-IGE levels in 62/77 (80.5%). None of them was immunocompromised. Main other diagnoses: Chagas disease 21, trichuriasis 20, hookworm disease 20, latent tuberculosis infection 15, mansonelliasis 6. Treatment was ivermectine in 88/108 (81.5%), albendazol in 16/108 (14.8%) and some didn't come for treatment 4 (3.7%). Patients who came for the complete follow-up after treatment were 42/108 (38.9%).

CONCLUSION Screening for *S. stercoralis* should be done in all immigrants, especially if eosinophilia or hyper-IgE is present. *S. stercoralis* culture is highly recommended when examination of stool is negative. Serology is a reliable option for diagnosis.

5.3-031

Perceptions of TB among migrant population living in Madrid (Spain)

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INTRODUCTION In the Community of Madrid, being foreign-born represents a higher of risk of contracting TB than being HIV infected. The objective of this research is to investigate the perceptions of TB among migrant population in order to create an effective preventative program.

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Methods Qualitative study based on Ethnography research with method and investigator triangulation. Study period: September–November 2009. Tools: five Focus Groups and nine in-depth interviews with migrants from Sub-Saharan Africa (Nigeria, Ghana, Cameron, Senegal, Guinea Conakry, Kenya, Rumania; Morocco, Latin America (Colombia, Bolivia, Ecuador, Peru and China).

Results Total of participants: 47-knowledge: TB transmission and symptoms were known. However, there is no clear distinction between latent and active TB. Factors thought to cause TB were: smoking, alcohol, poverty, jobs as mining industry, overcrowding, coming from a developing country, having a bad cold virus. Some migrants though TB could be transmitted through saliva, physical contact or that it could be hereditary. Mantoux is sometimes thought to be a vaccine. Participants had heard about the existence of medical treatment, they also emphasized the importance of traditional medicine (Africans) and religious beliefs (Maghribian). TB imaginary: TB was considered an old disease related to poor countries but that still filled with fear because of social rejection. Relations with Healthcare service: Migrants mentioned communication problems in hospitals (especially Chinese people), lack of trust in medical staff and a feeling of perceived discrimination. Grandparents are seen as a reference for healthcare in Latin Americans and Sub-Saharan migrants.

Conclusions Results were very helpful to create preventative strategies and materials tailored to each migrant population. Multilingual and fully illustrated leaflets were created and distributed among migrant population living in Madrid. Individual and group prevention activities were organized with the collaboration of medical staff, intercultural mediators and NGOs.

5.3-032

Differences in knowledge and attitudes related to HIV among migrants from Sub-Saharan Africa and Latin America living in Spain

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Introduction This research aims to describe and compare the HIV-related knowledge and stigma in a cohort of migrants living in Spain, coming from Sub-Saharan Africa (SSAM) and Latin America (LAM).

Methods Eight hundred and sixty-four SSAM and LAM filled in a Knowledge, Attitudes and Practices (KAP) survey before attending an HIV prevention course. Study period: 2009–2010. Location: NGOs. Statistical analysis: univariate pattern, SPSS program. All shown Results had statistical significance ($P < 0.05$).

Results Surveyed participants: 282 LAM, 582 SSAM. Main African countries: Senegal (25%), Mali (17%), Cameroon (12%). Main Latin-American countries: Bolivia (40%), Ecuador (19%), Peru (11%). SSAM: 87% men; LAM: 57% women. Median age: 26 yaers (SSA), 32 years (LAM). Most SSAM were Muslims (61%) and most LAM, Christians (70%). SSAM: 39% have completed ≥secondary education vs. 85% LAM. Time in Spain: SSAM 12 vs. LAM 36 months. LAM knew better about sexual (LA 97%, SSA 82%) and blood transmission (LAM 94%, SSAM 89%). Misconceptions about transmission: kissing (LAM 24%, SSAM 17%); sweat (SSAM 16%, LAM 9%); mosquitoes: (SSAM 37%, LAM, 26%). More SSAM (12%) didn't want to know if they were HIV-positive compared to LAM (5%). However, facing a positive result, 85% of SSAM will talk to their sexual partner vs. LAM 79%; and 52% will talk with their boss vs. LAM: 21%. SSAM would accept more to share the house (77% vs. LAM 67%) or a glass (58% vs. LAM 31%) with a HIV-positive person.

Conclusion In our cohort, LAM showed a higher level of knowledge about HIV and SSAM fewer stigma, maybe due to their socio-demographic characteristics, such as the educational level. Thus, it is important to emphasize issues related to stigma in all HIV-prevention programmes.

5.3-033

Providing rapid HIV testing to migrants in non-clinical settings

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Introduction In Madrid, 47.4% of new HIV diagnosed patients are foreign-born. Testing and counselling should address linguistic and cultural needs of users.

Objective To describe the migrant population accessing rapid HIV testing in NGOs.

Methods After an HIV prevention course in NGOs, a multidisciplinary team offers an on-site rapid HIV testing, assuring anonymity and confidentiality. We observed the proportion of people deciding to stay for the rapid HIV test. Counselling is performed before and after test and a questionnaire is filled in with each person. A descriptive statistical analysis of the questionnaires filled in between 2 December 2009 and 25 April 2011 was performed. Results with $P > 0.05$ had no statistical significance (ns).

Results Rapid HIV testing performed in 81 people. Socio-demografic data: Latin America 48%, Sub-Saharan Africa 20%, Eastern Europe 28%, Maghreb 1%, Spain 3%. Men (56%); education level: medium-high (86%); without health card (10%). Sexual history: Heterosexuals (99%), stable partner (63%), never/sometimes using condoms (77%), suffered from previous sexually transmitted infections (4%), alcohol and drugs abuse during sexual relations (5%). HIV test: Never got tested before (68%); main reason to get tested: curiosity (47%), unprotected sex with occasional partners (27%), mistrust of one's stable partner (10%), previous surgery (3%). tests Results: 100% negative. Acceptance rate in NGOs: 44% (184 people in the course/81 getting tested). This percentage varies according to the population [Eastern Europe: 41%, Sub-Saharan Africa: 46%, Latin America: 50% (P = ns)]. Gender: 49% of women, 41% of men got tested (P = ns). Education level: medium-high: 50%, low: 26% ($P = 0.000$).

Conclusion Rapid HIV testing in NGOS is a welcomed initiative. However, the main users are people with high education level. Most of the users are heterosexuals and have no risk practices. Therefore, it is necessary to find more strategies for getting easier access to the test for migrants with more HIV-related vulnerability

5.3-034

Chronic chagas' heart disease in a non-endemic area

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Brief Introduction Twelve million persons in LatinAmerica and thousands of immigrants living outside are infected with *T. cruzi*. It's estimated that 20–30% of infected people will developed symptomatic heart disease at some point during their lives.

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Fundació Hospital-Asil Granollers is a district hospital with a catchment area of 300,000 inhabitants, 12% of whom, are immigrants. Chagas chronic disease has become the most prevalent imported disease in our area.

MATERIAL AND METHODS A descriptive, prospective-study carried out among the LatinAmerican population diagnosed of Chagas chronic cardiac disease is presented. Patients were recruited between 7 June and 11 March. The main outcome was characterization of signs and symptoms most frequently associated with these patients, thus electrocardiogram, chest X-ray and echocardiogram findings. All patients had been screened for *T. cruzi* by two different serologic tests. Holter and cardiac MRI were requested at symptoms.

RESULTS We've reported 92 cases of Chagas disease, 81% adults. Twenty-two adults were diagnosed of Chronic Cardiac disease due to clinical features and/or electrocardiographic and echocardiographic abnormalities. Typical adultpatient profile was 44 year old Bolivianfemale, major presented with mild ECG features without symptoms. Atypical precordial pain, homesickness, effort dyspnea and syncope were the symptoms most frequently associated with Heart disease. One and 10 patients had no abnormalities on electrocardiogram and echocardiogram. Most common electrocardiographic disorders were complete right branch block (N = 12), ventricular extrasystolia (N = 4), sinus node dysfunction (N = 4), atrioventricular block (N = 3), atrial fibrillation (N = 2). Major echocardiogram features detected were dilated cardiomyopathy (N = 6), dilation and disfunction of right ventricle (N = 3) and systolic disfunction (N = 3). Three patients with cardiac involvement needed pacemaker device. Isquemic heart disease in one patient and Cardiac mixoid liposarcoma in another were diagnosed as additional medical condition. No thromboembolic event found.

CONCLUSIONS Prevalence of Chronic Chagas Heart disease in our area is significant. We should emphasize the need for early diagnosis among patients with accurate epidemiological background who consult for cardiac-symptoms. We need to improve understanding of Chagas heart disease by physicians in order to detect, follow-up and treat the disease.

5.3-035

Epidemiology and clinical profile of imported Chagas diseases in Alicante (Spain)

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OBJECTIVE The aim of this investigation is to describe the clinical profile of a series of Chagas diseas (CD) attended in Alicante (on the Mediterranean Coast).

METHODS This study was performed in four general hospitals in the Alicante province between January 2002 and May 2011. Theses centres were not a referral institution for imported diseases, tropical medicine or International Health.

RESULTS A 128 patients with *T. cruzi* infection were studied: female (63.3%) and male (37.7%). The mean of age was 35.9 + 11.2 years. Three (2.3%) was neonatal cases, and 96.8% were adults. Distribution of infected people was: Bolivia 101 (78.9%); Paraguay, 11 (8.6%); and Argentina, 7 (5.5%). The main cause of doing the *T. cruzi* test was: asymptomatic screening (38.2%), and pregnant women screening (27.6%). One patient was infected with HIV (0.8%). PCR for *T. cruzi* were performed on 100 (78.1%), and was positive in 66 patients (66%). 53.2% of patients referred symptoms related with CD: palpitations (42.7%); chest pain (28.4%); constipation (20.4%); dyspnoea (16.3%); and syncope (10.7%). Chest X-ray was performed on 78 of, and showed cardiomegaly in 14.1%. Electrocardiogram was done in 98 patients; 20.4% showed disorders: Right bundle branch block (6.1%), supero-anterior hemiblock (5.1%), left bundle branch block (3.1%), ventricular extrasistolia (4.1%), and auricular block (2%). Echocardiography was performed on 63 patients (28.5% was abnormal. A barium X-ray was anormal in three of 25 cases (11.5%). 75 (58.6%) patients were treated with benznidazol, and 21 (28%) patients were stopped by a secondary side effects. 35 (46%) patients presented secondary effects: cutaneous rash (33.5%), gastrointestinal symptoms (5.3%), polyneuropathy (4%); and hepatitis 2 (2.7%). PCR against *T. cruzi* after treatment was negative in all of cases that were performed.

CONCLUSIONS It is important to improve clinical and epidemiological knowledge of CD in non-endemic countries and to developed appropriate clinical, diagnosis and treatment protocols in these setting.

5.3-036

Effect of artesunate-mefloquine fixed-dose combination in malaria transmission in Amazon basin communities

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BACKGROUND Studies in South East Asia have suggested that early diagnosis and treatment with artesunate (AS) and mefloquine (MQ) combination therapy may reduce the transmission of Plasmodium falciparum (Pf) malaria and the progression of MQ resistance. The effectiveness of a fixed-dose combination of AS and MQ (ASMQ) in reducing malaria transmission was tested in isolated communities in the Amazon region.

METHODS AND MATERIALS Priority municipalities were selected according to pre-specified criteria: monthly average of >20 cases of Pf malaria; proportion of imported cases<15%; cooperation of local health authorities. Routine national malaria control programmatic procedures were followed. Existing health structures were reinforced and health care workers were trained to perform early diagnosis and to treat all confirmed cases with ASMQ. A local pharmacovigilance structure was implemented. Effect estimates and 95%CI for the indicator variables of years (trend), months (seasonality), and intervention (ASMQ) were evaluated on three monthly outcomes from January 2004 to December 2008: Incidence Rates (Falciparum), Ratio Falciparum-Vivax, and Hospital Admission Rates (malaria).

RESULTS The total population who received ASMQ between June 2006 and December 2008 numbered 23,845. A significant effect of the ASMQ intervention was observed in all evaluated outcomes [Incidence Rate 0.34 (0.20–0.58); Ratio Falciparum/Vivax 0.67 (0.50–0.89); Admissions 0.53 (0.41–0.69)], with a decrease in the mean level of the time series, adjusted for the trend and seasonality. Interaction effects between months and intervention were also evaluated. An elimination of the end of the year seasonal malaria peak was seen post-intervention. No serious adverse events relating to the use of fixed-dose ASMQ were reported.

CONCLUSIONS In the remote region of the Juruá valley, the early detection of malaria by health care workers and treatment with

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fixed-dose ASMQ was feasible and efficacious, and significantly reduced the incidence and morbidity of multidrug-resistant Pf.

5.3-037
Clinical case of cerebral schistosomiasis

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BRIEF INTRODUCTION Schistosomiasis affects 207 million people after contact with contaminated water. Cerebral schistosomiasis is not a usual presentation with no specific signs or diagnostic imaging techniques, and a great variability on its presentation.

METHODS AND MATERIALS Malian, 31 year old men. In Spain since 2002 no previous illness. No recent trips. Head injury, on the left side with amnesia. Brain-TC: peripherical lesions with oedema. Contrast captation in front, left side lesion. Seven days later Brain-RMN: Bilateral hemorragical lesions in peripheria. Oedema without enhancement after contrast. It is not possible to rule out metastasis or parasitic lesions. *Ac. Schistosoma* positive Chronic VHB VEB, VHS past infection, rest of serologies negatives. Stool and urine studies negative. No urine schistosomas Inmunologic blood test (including tumoral indicators) normal Toracoabdominal TC: normal Mantoux 5 mm. Booster 15 mm Lumbar punction: normal Main differential diagnosis Cysticercosis: Infrequent in Islamic countries RMN: central and peripherical distribution. Praziquantel treatment. Tumoral Lesion: no coherence with this case and studies. No response to praziquantel. Toxoplasmosis: Negative serologies. Brain-RMN: similar pattern with captation of contrast. No response to praziquantel. Tuberculoma: Brain-RMN commonly one lesion, without oedema. Opistorchiasis: Asia. Contaminated fish Cerebral Schistosomiasis: Difficult diagnosis, assymptomatic and great clinical and image variability. Epilepsy is a frecuent after-effect. Epidemiologic history and recuperation after empiric treatment lead us to diagnosis. Histopatological examination of nerve tissue is the final diagnosis Due to no evidence for a final diagnosis, we oriented the case as convulsions most probably caused by cerebral schistosomiasis. We started treatment with praziquantel and corticoids, with favourable results.

RESULTS After 3 months: Brain-TC: normal. Brain-RM: important disminution of number and size of lesions.

CONCLUSIONS Cerebral schistosomiasis even if it is not a usual presentation must be included in the differential diagnosis for immigrant patients with cerebral lesions from an endemic country.

5.4 Health in conflicts and Refugee populations

5.4-001
Lampedusa, between Africa and Europe

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The recent socio-political events in the Maghreb have had and will continue to have an impact on migration in the Mediterranean; the rates of boat landings on our southern coasts have already risen abruptly. The thousands of migrants who arrived in recent weeks on Sicily's southern coasts are irrefutable evidence of the failed policies of outsourcing of border controls, which Italy has been using to stop migrants before they reach our shores, including those who meet the requirements to apply for political asylum in our country. The National Institute for Health Promotion of Migrant Populations and to Combat Diseases of Poverty (NIHMP) has undertaken activities in Sicily that aim to improve the accessibility of health facilities and the awareness of the right to health and the right to treatment through various projects. The recent events at Lampedusa have been crucial for the activation of NIHMP Sicily (with the ASP, Red Cross, UNHCR and MSF), in order to carefully organize a structured plan for health interventions and reception in emergency areas. The health of migrants is also of strategic importance in order to ensure the health of the entire community. The socio-medical model includes, upon arrival of the ships, a reception and triage at the dock of Lampedusa. Subsequent work is carried out at the NIHMP healthcare clinic, which is specially equipped and connected to regional health care facilities. The implementation of this effective clinical reception has been possible thanks to a team of specially selected doctors, nurses, cultural mediators, psychologists, and lawyers. Data from April 12 to May 30, 2011 in Lampedusa demonstrate the absence of disease in three-fourths of the observed population, not accounting for area of origin. Diseases linked to the 'trip' (including hypothermia, dehydration and malnutrition) were found in just under a quarter of the population.

5.4-002
Humanitarian aid and health care when development goes 'backward': MSF field-based perspectives from Ivory Coast and Liberia

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In recent years, protracted crises and fragile post-conflict settings have challenged the co-existence or even a linear continuum between relief and development. Forced migration tests humanitarian and development paradigms where sudden-onset emergencies, violence, and displacement arise alongside extensive development work – as recently in Ivory Coast. There, the presence of refugees and displaced in open settings-among host communities in Liberia and in villages or the bush in Ivory Coast-challenged emergency interventions centered on camps and towns even as populations became more vulnerable, facing continued violence and declining resources. Ongoing development and stabilization projects in both countries delayed aid responses to refugees and displaced people on both sides of the border-despite available resources and opportunities to assist in secure areas closer to the border and conflict flash points. As in Ivory Coast, post-conflict countries in development often risk 'backsliding' to conflict, and aid must adapt to address this recurring phenomenon. Many have now recognized that the debate around access to healthcare remains inseparable from humanitarian aid responses in countries straddling emergency and recovery. In contexts like Ivory Coast, healthcare is often inaccessible to the most vulnerable even in stable periods-with renewed crises exacerbating ongoing needs and consequently, lack of access to medical care. During the recent emergency, the Ivorian government decreed a temporary suspension of user fees for healthcare, renewing debate about access to healthcare and user fees, long a concern in this context. Drawing on MSF interventions in the region and examining aid and healthcare responses to displacement in Ivory Coast and Liberia, this paper points to the challenges for aid when facing 'backsliding' from development to emergency-and, in exactly such cases, the opportunity to improve access to medical care that is so much more urgently needed in times of crisis, including suspension of user fees.

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5.4-003

Policy brief: the health needs of refugees in Tunisia

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INTRODUCTION The recent conflict in Libya has resulted in an estimated 140,000 refugees crossing in to Egypt and Tunisia. This is around a 1000% increase from the estimated refugee population of August 2010. There are estimates that around 2500 people per day are still fleeing the conflict, furthering the refugee emergency situation. This is clearly a huge population with little access to health care, coupled with desperate need for assistance. This article aims to outline the health issues faced by these refugees, review current Tunisian health and refugee policy, and recommendations for action.

METHODS A literature search of published materials, grey literature and policy documents was conducted.

CONCLUSIONS The health system is not prepared for the massive influx of population; there is no legislation to protect the rights of non-citizens, no procedure for seeking asylum and women are directly discriminated against. Legislation needs to be developed to provide clear guidance for the treatment and management of refugees and the asylum seeking process and ensure a non-discriminatory approach. A National Refugee Commission needs to be established to govern refugee services, to ensure effective and accountable action, and act as an access port for donor agencies wishing to contribute towards the refugee relief effort. Refugee health programmes need to be integrated into national or regional health programmes to account for the likely protracted nature of refugee situations experienced in the age of globalisation. Finally, health infrastructure and human resources need to undergo reform to reduce bureaucracy and increase efficiency.

5.4-004

Who can afford health care? Evaluating the socioeconomic situation in a post-conflict area in DR Congo

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INTRODUCTION Since 2008, the north-eastern districts of the Democratic Republic of the Congo (DRC) are facing a guerrilla war of the Lord's Resistance Army (LRA) with insecurity and massive displacement. Since then, Malteser International (MI) is assisting with free health care for internally displaced persons as well as the general population in Aba and Faradje health zones (ECHO grant). Before the uprising the health system financing was entirely based on user fees. The aim of this study was to identify the socioeconomic status of the population and to assess their capacity to contribute to healthcare.

METHODS In 2/2011 MI carried out a three-stage, cross-sectional study interviewing heads of 552 randomly selected households in 23 clusters in Aba and Faradje health zones using a standardized questionnaire.

RESULTS The living conditions of the population in the two zones are homogenous (95.5% (527/552) live in a hut; 86% (476/552) use a pit latrine; 97% (537/552) cook with firewood). Major source of income is agriculture (57%, 457/779) and 46.7% of the households earn less than US$ 5.4/week. Major expenses are food (36%, 474/1308), healthcare (29%, 384/1308), and school fees (16%, 213/1308). Lacking sources of income is the main problem (39%, 369/944). Nevertheless, 38% (210/552) of the households estimate their current living situation as fair. Asked for the affordable maximum fees for healthcare, 58% (314/542) mentioned US$ 0.16 for consultation fees and 73% (398/541) US$ 0.34 for drugs. 6.1% (33/542) opted for free consultations and 19.3% (104/538) for free drugs.

CONCLUSION AND RECOMMENDATIONS Almost 80% of the population is living under the poverty level of US$ 1.25/day. Still they are able to contribute to healthcare. Since 4/2011 the population is paying a lump sum of US$ 0.55 for outpatient treatment. Thus transition to full contribution to health care is prepared once the emergency funding phase is over by 2012.

5.5 Travel Medicine

5.5-001

Skin disorders among travellers returning from tropical and non-tropical countries consulting a travel medicine clinic

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INTRODUCTION The present study evaluates the causes and risks for skin disorders among returned travellers.

METHODS AND MATERIALS Data of 34,162 travellers returning from tropical and non-tropical countries and presenting at the out-patient travel clinic of the University of Munich, Germany, between 1999 and 2009, were considered for this study. Among them, 4158 travellers (12.2%) were diagnosed with skin disorders. Their demographic, travel (destination, duration and type of travel), and clinical (causative agents resulting in skin disorders) data were analysed.

RESULTS The main destinations visited were Asia (40%), Africa (27%) and Latin America (21%). Tourism in the form of adventure travel/backpacking (47%) and package holiday (23%) were the most common purpose of travel. The leading causes that resulted in skin disorders were of arthropodal (23%), bacterial (22%), helminthic (11%), protozoan (6%), viral (6%), allergic (5%), and fungal (4%) nature. The 10 most frequently diagnosed specific skin diseases with significantly elevated proportion among travellers returning from a certain destination were insect bites (17%, Southern Europe), cutaneous larva migrans (8%, Asia and Latin America), cutaneous leishmaniasis (2.4%, Mediterranean Region/Middle East), dengue fever (1.5%, Asia), rickettsiosis (1.3%, Africa), myiasis (0.8%, Central America), filarioses (0.7%, Africa), tick bites (0.6%, Central/Eastern Europe), schistosomiasis (0.6%, Africa), and tungiasis (0.6%, Africa).

CONCLUSIONS As more than 20% of all skin disorders among returned travellers were caused by arthropods and about 50% by infectious pathogens, pre-travel consultations should consider specific prophylaxis concerning this matter and the travel destination which was assessed to be the most important risk factor for the majority of imported skin diseases.

5.5-002

Influenza in Southeast Asia-transmission, burden of disease, vaccination

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Travellers to tropic areas in Southeast Asia are exposed to seasonal and pandemic influenza. Several criteria needed to be established to evaluate the risk of transmission and the need of vaccination: During what seasons there is the highest risk of transmission? How high is the risk compared to areas in climatic zones with defined summer and winter periods? What is the best time to vaccinate and

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what vaccine should be used? Several countries in Southeast Asia were regularely visited between 2006 and 2011 and data collected regarding seasonal and pandemic influenza. National sources as well as international institutions (WHO, CDC, Institute Pasteur) and hospitals were involved in data collection. At the same time during the period between January 2009 and April 2010 own data in the clinic of the German embassy in Jakarta were collected to estimate burden of disease in expatriats and European tourists. Clear periods of high influenza transmisson in tropical countries in Southeast Asia were defined, less transmission rates in travellers and expatriats compared to their home countries were found and finally periods of vaccination in different areas are suggested.

5.5-004

Travel related shigellosis. An analysis of risk factors

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CONTEXT Travel-related shigellosis is not well documented in Canada although it is frequently acquired abroad and can cause severe disease.

OBJECTIVES To describe the epidemiology of shigellosis acquired during travel for Quebec (Canada), and to identify high-risk groups of travelers.

METHOD AND DATA SOURCES: We performed a random sampling of 335 shigella cases (from a total of 760 cases) notified in the provincial database of reportable diseases from January 1st 2004 to December 31st 2007. Each case was analyzed according to information available in the epidemiological questionnaire. Total number of trips by region from Statistics Canada was used as denominator.

RESULTS Annually, between 40% and 51% of cases were notified in travelers, 46% of whom were aged between 20 and 44 years. Children under 11 years accounted for 16% of cases, but represent only 4% of travelers. Most cases were serogroups *S. sonnei* (49%) or *S. flexneri* (45%). Almost 31% of cases were notified between January and March. The majority (64%) were acquired in Central America, Mexico or the Caribbean. However, Indian subcontinent, Africa and South America had the highest ratio of number of cases per number of trips. Tourists represented 76% of the cases; 62% of cases traveled for <2 weeks. At least 15% of cases in travelers were hospitalized.

CONCLUSIONS Shigellosis is frequent in Quebec travelers taking short trips to a 'sunshine destination'. Since no vaccine is available, counselling patients on food and water precautions, and to bring along a broad-spectrum antibiotic for self-treatment is important.

KEYWORDS travel, infectious diseases, shigella, Quebec

5.5-005

Prevalence of extended spectrum beta lactamase (ESBL)-producing *Escherichia coli* fecal carriers returning from tropical and subtropical countries

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INTRODUCTION ESBL-producing Enterobacteriaceae has become a worldwide endemic problem during the last decade. The most widespread enzymes among the ESBLs are the CTX-M family. The prevalence of ESBL faecal carriers observed in Spain in 2007 was 8.2%. The aim of this study was to investigate the prevalence of ESBL-producing *E. coli* in stool samples from travellers returning from tropical and subtropical countries.

METHODS Stool specimens from patients with travelers' diarrhea were screened for ESBL Enterobacteriaceae using agar plates with medium selective for cephalosporin resistance (ChromID ESBL, BioMerieux) from December 2009 to April 2011. MALDI TOF was used to identify *E. coli* strains. ESBL production was confirmed using double-disk synergy test. PCR and sequence analysis for genes encoding ESBL (blaCTX-M, blaTEM and blaSHV) was performed.

RESULTS A total of 444 patients with travellers' diarrhoea were screened; 66 were found to be ESBL-producing *E. coli* faecal carriers, representing a prevalence of 14.9%. CTX-M type was the most prevalent enzyme in 90.9% (65.1% CTX-M-15, 12.1% CTX-M 14, 6.1% CTX-M-27, 7.6% others); six patients carried a SHV type enzyme which represented 9.1% (4.5% SHV-5 and 4.5% SHV-12). Among faecal carriers who had travelled to India: 80.6% carried CTX-M-15, 9.7% CTX-M-14, 3.2% CTX-M-1, 3.2% SHV-5 and 3.2% SHV-12; Indonesia: 33.3% CTX-M-15, 33.3% CTX-M-3 and 33.3% CTX-M-27; Mozambique: 66.6% CTX-M-15 and 33.3% CTX-M-9 and Peru: 66.6% CTX-M-15 and 20% CTX-M-65.

CONCLUSION In this study on patients with travellers' diarrhoea the prevalence of ESBL-producing *E. coli* was almost 2-fold higher than that normally observed in Spain, being CTX-M-15 the most prevalent enzyme. These ESBL-producing strains may increase the burden of ESBL-producing *E. coli* in our geographical area.

5.5-006

Helicobacter pylory infection in immigrant population

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INTRODUCTION H pylori infection is the most common infectious disease in the world. Nearly 50% of the world's population is estimated to be infected. While the prevalence of the infection has dropped significantly in many parts of North America and Western Europe, no such decline has been noted in the developing world. The prevalence of Helicobacter pylori infection varies across different regions and racial/ethnic groups. We described the prevalence of *H. pilory* infection in a immigrant population atended in Tropical Medicine Unit of Hospital Universitario Central de Asturias between 2007 and 2009.

METHODS Al patients attended at the Tropical Medicine Unit diagnosed of *H. pilory* infection were retrospectively reviewed. Using epidemiological questionnaires, information was obtained on past and present medical history, smoking and drinking habits, and place of birth, as well as the consumption of fruits, vegetables and unsafety water. *H. pylori* infection was diagnosed using serum enzyme-linked immunosorbent assay. In all positive patients a commercial stool enzyme-linked immunosorbent assay was performed.

RESULTS We studied 693 patients between 2007 and 2010. Sixty-eight patients (10%) had positive Helicobacter pilory serology (62% males, mean age 37 years) and 40 had positive stool enzyme-linked immunosorbent assay. The mean time on Spain was 1122 days (limits 21–3657 days). The countries of origin were: Senegal (28%), Ecuador (12%), Equatorial Guinea (6%), Sahara Occidental (6%) and Bolivia (5%). All patients had taken unsafety

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water and vegetables. The most frequent symptom were epigastric pain. Twelve patients were asymptomatic. In twenty patients we performed a gastric endoscopy and in 15 patients a chronic gastritis were diagnosed.

CONCLUSIONS *H. pilory* infection is prevalent in immigrant population. The most frequent place of origin is Subsaharin Africa. It's a important cause of abdominal pain in this population.

5.5-007

Morbidity in front-line humanitarian aid workers

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INTRODUCTION Humanitarian workers frequently face health risks, psychological stress, and moral tensions created by a number of new circumstances, such as: exposure to environmental and health hazards, unfamiliar socio-cultural contexts, isolation from usual support networks, insecurity, suboptimal living and working conditions, uneasy group dynamics, and difficult professional ethical choices.

METHODS (i) Prospective compilation of staff medical cases managed in a humanitarian NGO, (ii) literature review.

RESULTS Based on a compilation of 113 cases seen over a 10 month period 2010–2011, health problems affected 14% of expatriate staff, occurring mainly during their missions. 62% were due to medical issues; 21% were due to occupational accidents or illnesses; and 17% called for psychological support. Malaria was the most common diagnosis accounting for 20% of medical issues. There were 22 medical transfers (evacuations or repatriations) to a higher level of care. Occupational accidents were primarily accidental exposure to blood or body fluids (AEB) in medical staff.

CONCLUSIONS Despite systematised individual preventive interventions before mission including vaccination, malaria prophylaxis and briefing on health risks, there is significant morbidity in humanitarian workers in the field. Whilst some morbidity is coincidental, malaria is the commonest cause of morbidity and is largely preventable. Occupational accidents (AEB) are frequent due to high patient loads, many interventions (intravenous access; surgery; obstetrics), suboptimal working conditions and fatigue. The risk of blood-borne pathogen infection may be higher as projects are often in countries with a relatively high prevalence of HIV and hepatitis B. Psychological morbidity is commonly the result of cumulative stress and less often due to acute traumatic stress following a critical incident. Reducing morbidity and occupational accidents in humanitarian workers requires more emphasis on pre-departure preparation and advice (including adherence to malaria prevention advice), psychological and ethical preparation and strong preventive and mitigation systems in the field.

5.5-008

A novel antigen could be a potential biomarker for serodiagnosis of *Angiostrongylus cantonensis*

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Angiostrongylus cantonensis, a diet source parasite, is the most common pathogen that causes eosinophilic meningitis. Outbreak of angiostrongyliasis cantonensis, as new emerging infectious disease, was reported in transpacific country, Northern America, Australia and Europe. Due to its great threat to human health, angiostrongyliasis cantonensis has been considered as one of the top 100 global threaten infectious diseases of 21 centuries. But at present, there were still no efficient and specific diagnostic methods available, and especially lack of specific antigen for serodiagnosis. In this research, the construction and screening of cDNA expression library was firstly applied to investigate *A. cantonensis* antigen. mRNA of adult A. cantonensis was purified and constructed into cDNA expression library. By screening with serum, a cDNA clone encoding a novel antigen with a predicted 47.6 kDa protein was isolated. It belongs to intermediate filament family proteins analyzed by bioinformatics, western blotting and mass spectrometry. The recombinant antigen was identified to be IgG4 specific to *A. cantonensis* by western blotting with serum samples respectively infected by other six kinds of parasites. Immunohistochemistry assay showed that the antigen locates in cytochylema of intestinal canal wall. In a conclusion, a gene encoding a specific antigen was obtained, and the antigen has specificity for serodiagnosis of angiostrongyliasis cantonensis.

5.5-009

Trends of imported infectious diseases seen in travelers returning from tropical and non-tropical countries 1999–2010

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BRIEF INTRODUCTION The number of international travels was increasing 50% from 1999 to 2010. In that time period, certain infectious diseases – such as dengue fever, chikungunya, and acute HIV-infections – were more frequently imported by travelers, especially from the tropics and subtropics. However, the number of other travel associated infectious diseases – such as malaria – was decreasing in the last years.

METHODS AND MATERIALS This study analyzed data of 24,363 patients consulting the outpatient travel medicine clinic of the University of Munich between 1999 and 2010 after returning from tropical and non-tropical countries. The aim of the study was to evaluate up and down trends of imported infectious diseases and to assess variables which have been correlated to these trends.

RESULTS The proportion of very young (0–19 years; 5.7%) and elderly (>64 years; 4.8%) travelers were significantly increasing over time period. The majority (86%) of travelers were born in Germany (German origin). The most travelers returned from Asia (42%), whereas that proportion was significantly increasing in the last 12 years. The following most frequent destinations were Africa (33%) and Latin America (18%), whereas its proportions were significantly decreasing. The proportions of cases with dengue fever (0.89%), salmonella enteritis (0.82%), acute HIV infections (0.15%), and chikungunya (0.06%) were significantly increasing over time period, whereas it was significantly decreasing for amebiasis (0.98%) and malaria (0.95%).

CONCLUSIONS The spectrum of infectious diseases imported by travelers returning from tropical and non-tropical diseases has changed in the last 12 years. As shown in many other studies, the number of imported cases of dengue fever, chikungunya, and acute HIV-infections increased and the number of imported cases of malaria decreased, but – surprisingly – some other trends on cases of salmonella enteritis, amebiasis, and mononucleosis were found in this study.

5.5-010

Profile of the international travellers attending the foreign health medical service in Barcelona (2010)

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INTRODUCTION The Foreign Health Department, belonging to the Spanish Ministry of Health, do different tasks and one of them is

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the vaccination and advice to the international traveller. Its office in Barcelona attended 5829 people in 2010. We want to describe the profile of these travellers and the characteristics of their travels.

MATERIAL AND METHOD We did a descriptive study. We got the information from our clinical files and analyzed 5724 travellers. We did not consider people when more than one variable were missed out. We built a data base with Excel 2003 and we carried out the analysis with the program Epi Info.

RESULTS Month of the visit: almost 40% in June, July and August. Place of origin: Spain 72.1%, Africa 20.9%, Europe 3.9%, America 1.4% and Asia 0.8%. Sex: 3177 males and 2547 females (ratio = 1.25). If we excluded African travellers (82.1% males) the ratio decreased (0.93). Age: 58.6% adult population (31–60). 23.5% between 21 and 30. 7.4% older than 60 and the rest younger than 20. Destination: Africa (49.9%) followed by Asia (31.6%) and America (17.2%). 1.2% more than one continent. The main touristic destination was Asia (43.9%) followed by Africa (35.9%) and America (19.4%). Purpose and duration of the travel: Tourism: in more than 80% of the travellers coming from Spain and Europe. 44.4% of the travels lasted from 16 to 30 days VFR: 97.9% of the Africans, 88.6% of the Asian and 81.3% of the American. 46.4% of the travels lasted from 1 to 3 months Work: 6.4%. 26% of the travels lasted <8 days Humanitarian Aid: 2.6%. 37.7% of the travels lasted more than 3 months.

CONCLUSION Almost 25% of our travellers are emigrants who require an special attention so it is possible that we should consider to carry out additional preventive messures for improving their sanitary education.

5.5-011

Comparison of travelers treated in the pre-travel consultation at the tropical medicine and International Health Drassanes Unit in July 2003, 2007 and 2010

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INTRODUCTION The UMTSID (Tropical Medicine and International Health Drassanes Unit) is amongst the leading European institutions in the provision of medical attention for travelers in terms of pre-travel consultation.

OBJECTIVES (i) Compare age, type of trip, duration and destination between July 2003, 2007 and 2010. (ii) Compare malaria chemoprophylaxis given in July 2003, 2007 and 2010, by geographical travel destination.

METHODS Descriptive study, carried out during July of 2003, 2007 and 2010. The study included a statistically representative sample of travelers during this month for each year; n = 560, 761 and 565 respectively. Variables: age, type of trip, duration, destination (Africa, Asia, America) and malaria chemoprophylaxis. Source of information: clinical history. For all comparisons a Chi-squared test was performed with a significance level of 0.05.

RESULTS Significant increase of VFRs: 3.6% (2003) and 10.2% (2010). Significant increase of travelers over the age of 60: 2.7% (2003) vs. 6.1% (2010); No differences in trip duration: median of 20 days. Significant change on destination with increased visits to Asia (22.6% (2003) to 41.9% (2010), decreased visits to America (34.6% to 19.4%) and visits remaining constant to Africa (41% and 38.4%) It is important to note the significant decrease in malaria chemoprophylaxis indication between 2007 (64.3%) and 2010 (45%), due to a lower percentage of infection in Asia (51.7% and 19.9%) and America (43.9% and 10.4%) respectively. No significant increase in emergency self-treatment 5.7% (2007) and 8.3% (2010).

CONCLUSIONS Study confirms what was observed throughout daily practice: significant increase in VFRs and over 60 years of age, which may increase the complexity of pre-travel consultation. Several institutions, mainly central European, have considered Asia and America to have a low risk of Malaria since 2008. The results from UMTSID study confirm this criteria is agreed upon.

5.5-012

Imported malaria in Catalan teaching hospital inpatients from 1990 to 2010

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INTRODUCTION Each year 125 million international travellers visit areas at risk of malaria transmission. In Spain malaria cases occur among mobile population as immigrants or travellers returning from endemic areas. The aim of this study was to describe the epidemiological characteristics of imported malaria in Catalan teaching hospital inpatients from 1990 to 2010.

MATERIAL AND METHODS A retrospective study was performed based on a case review of all patients diagnosed with malaria microbiologically confirmed from 1990 to 2010, and hospitalized at a Catalan referral teaching hospital. The data was obtained from clinical records, basically focused on demographic and epidemiological information related to the trip as well as clinical and microbiological data.

RESULTS Fifty-two patients suffering from malaria were studied. The majority were young (37.29 years, SD) men (71.2%) visiting friends and relatives (48.1%) without taking any antimalarial chemoprophylaxis (80.8%) to sub-Saharan countries (Ghana 15.4%, Equatorial Guinea 13.5%) and coming back to Spain in summer time. The most frequent symptoms were fever (100%), followed by headache (59.6%) and jaundice (28.8%), requiring few of them ICU admission (15.4%) and causing two fatalities (3.8%).

The diagnosis was confirmed by microscopic examination in 51 cases (98.1%) and antigen detection in 24 cases of 26 made (92.3%), being the Plasmodium falciparum the most frequent specie (71.2%) with low level of parasitemia.

CONCLUSIONS Malaria in our region is imported from endemic areas due to P. falciparum and more frequent in young male travellers visiting friends and relatives, without chemoprophylaxis and coming from African countries.

5.5-013

Descriptive analysis of yellow fever (YF) vaccination of travelers from greece visiting developing countries

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INTRODUCTION Our aim was to identify patterns of YF vaccination of travelers visiting YF endemic countries.

METHODS AND MATERIALS A prospective study was conducted from 1 Januar 2009 to 31 December 2010 in all (57) health departments. YF vaccine is only available at these departments which are the official travel medicine providers in Greece. Data were collected using a standardized individual form per traveler including demographic characteristics, travel variables and travel counseling information from travelers seeking pre-travel medical advice.

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RESULTS Two thousand one hundred and twenty-three travelers attended the health departments during the study period. YF vaccine was recommended to 67% (1424) of all travelers. Of those, 65.2% (914) were men. 44.8% (638), 23.2% (330), 4.7% (67), 1.6% (22), traveled to sub-Saharan Africa, Central and South America and Caribbean, South East and East Asia and Indian Subcontinent, and North and South African countries respectively. 11% (157) of them traveled worldwide. 43.2% (615) traveled for recreation, 44.2% (630) traveled for work, 58.1% (73) for visiting friends and relatives. According to duration of travel 58% (827) stayed <1 month, 9.8% (140) 1–3 months, 13.7% (195) 3–6 months, and 13.2% (188) >6 months. According to area of travel, 39.3% (559), stayed in urban areas, 32.4% (461) in urban and rural areas, and 4% (58) in rural areas. According to place of residence, 54.1% (770) stayed in hotels, 24.2% (344) on cruise ships, 13.9% (98) at local people's homes, and 3.1% (46) in camping.

CONCLUSIONS The main destination of a large number of travelers who received YF vaccine was sub-Saharan Africa, where the vaccine is required and/or recommended, and Central and South America where the vaccine is recommended for some areas. Our results show that there is a need for improvement regarding correct and selective recommendation of vaccination for travelers to YF endemic countries seeking pre-travel advice.

5.5-014

Travelers' vaccination – a study at sete rios international vaccination center in Lisbon, Portugal

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INTRODUCTION Vaccination offers the possibility of avoiding dangerous infections that may be encountered abroad. Vaccines for travelers include those that are used routinely, those that are mandatory, and those that may be advised before travel. This study aims to determine (1) the prevalence of prescription of tetanus/diphtheria vaccine, hepatitis A, typhoid fever, and yellow fever; (2) what myths and misunderstandings are held by international travelers about vaccines.

METHODS AND MATERIALS A cross-sectional survey was undertaken at Sete-Rios International Vaccination Center, Lisbon. Travelers were invited to participate in the study while they waited their turn. Only travelers aged at least 18 participated. The interviews were conducted during January–February 2009.

RESULTS A total of 347 travelers participated in the study. The average age was 39.74 years (SD = 11.68 years); 69.6% were male. From the total, 67.1% traveled to Angola, 31.5% to other tropical destinations, 68.5% had already been in tropical countries, 23.3% were to be vaccinated <1 week before departure and 29.8% between 1 and 2 weeks before departure, 47% were going to be in the tropics more than a month (an half of them more than 6 months). 6.4% of the travelers would not take tetanus/diphtheria vaccine, 57.7% hepatitis A, 43.9% typhoid fever. 4.3% of the respondents had medical prescription to take yellow fever vaccine but they were not going to an endemic area. For 14.4% of the study group non mandatory vaccines do not need to be taken (20.6% did not know the subject), 12.1% believed that the protective effect develop just following administration (28.6% did not know), 21.1% believed that vaccines fully protect (20.9% did not know).

CONCLUSIONS A large proportion of travelers do not take the appropriate vaccinations and many do it in a close time before departure. Both travelers' knowledge about vaccines and medical prescription must be improved.

5.5-015

Oral anticoagulation in expatriats and long term travel – algorithm and checklist in German embassies abroad

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INTRODUCTION Vitamin K antagonists, e.g. warfarin, are prescribed as a long term treatment for an increasing number of patients worldwide. There is a lack of evidence based guidelines for long term travellers or expatriats on oral anticoagulation. However, like other national or international institutions sending their employees abroad for months up to several years in areas with special climate and health related hazards, the German Foreign Office has to balance between potentially lethal complications, which are rather unlikely to be manageable in some resource limited countries, and each individual pursuit to proceed in their career.

METHODS We searched not only Pubmed for published data on risk factors on hemorrhagic complications, in particular those attributable with long term stays in countries with special climate conditions, but looked also on the assessment of specific regional issues e.g. access to safe blood products, reliable ambulance infrastructure, etc., provided by the network of regional medical doctors in Africa, Asia and the Americas affiliated to the German Foreign Office.

RESULTS Comprehensive studies on anticoagulation and long term stays abroad are scarce, guidelines virtually non-existing, despite a variety of known risk factors for hemorrhagic complications, e.g. nutritive-toxic or climate specific. Unlike other subgroups of expatriats, those working at embassies in capitals tend to have rather appropriate access to reliable emergency facilities. However, there is an intolerable risk remaining at some specific embassies and due to a severe underlying disease of the patient, respectively.

CONCLUSION We tried to implement an evidence-based algorithm and checklist for our employees on oral anticoagulation prior to their departure to an embassy abroad, e.g. reliable access to INR-measurements and no departure within the first 3 months of anticoagulation or to identified embassies in areas where hemorrhagic complications are more likely to be fatal.

5.5-016

Traveler profile of Japanese encephalitis vaccine during 2010

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INTRODUCTION Asia and the Pacific are the world's second tourist destination after Europe, with 204 million in 2010. Many travelers to Asian countries are backpackers who spend long periods in rural areas endemic for Japanese encephalitis. Although it's a rare disease among travelers (1/20,000–1/50,000 weeks stay), the risk may be increased depending on the area and exposure degree. Vaccination and barrier measures are the best way of prevention.

METHODS AND MATERIALS We conducted a retrospective study of 309 travelers vaccinated against Japanese encephalitis from January to December 2010. For vaccine indication we followed the UMTSID protocol for travelers, guided by the WHO. Data were obtained from medical records and analyzed by SPSS 18.

RESULTS Men were the majority with 51.5% of the total sample, 78.6% from Spain, 8.7% from other European countries. 92.9% were between 20 and 40 years old. The length of stay was over

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30 days in 75.1%. Regarding the geographical areas visited: 33% visited one area, 40.8% two or more areas, being the most visited Southeast Asia (89.9%). When they traveled to a single country, India was the most visited with 10.7%, Thailand 3.9% and Indonesia 3, 2%. Duration of the trip in days: 90 days over 55%, 30–90 days was 34.6%. The group between 25 and 35 years. was the one who made longer stays (64.7%). Purpose of travel was free tourism 81.6%. Vaccination schedule was completed in 74.1%.

CONCLUSION A large part of voyagers ask for pre-travel advice early enough to assess at least the risk and possibilities of vaccination. Compliance of an appropriate vaccination schedule was high. A significant number of vaccinated travelers make free and long-term trips at risk areas. The case reported in May 2011 in a tourist who visited Bali, suggests that the risk may be higher than expected and might consider a revision of the vaccination protocol.

5.5-017

Hepatitis B status in travelers seen in the Boston Area Travel Medicine Network (BATMN)

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BACKGROUND Hepatitis B virus (HBV) infection can cause severe morbidity and mortality in both acute and chronic stages. Individuals born or lived in countries with d2% HBsAg prevalence (HBV risk countries) may have unrecognized chronic HBV infection and/or travel-associated exposure. We reviewed records of individuals presenting to BATMN travel clinics from 12 June 2008 to 31 July 2010 to determine how their HBV status was assessed and managed in order to identify their need for immunization and chronic HBV infection, and describe characteristics of those tested.

METHODS Demographic data and trip information were collected and entered into a secure database (CS-Pro) for all travelers seen in BATMN clinics. Data was analyzed for HBV risk individuals, including HBV test Results if available. We defined four mutually exclusive categories: HBV-infected (HBsAg+), immune (anti-HBs+, HBsAg), susceptible (anti-HBs-, HBsAg-, anti-HBc-), possible HBV (lone anti-HBc+).

RESULTS Among 13,732 records in the database, 2134 (16%) were born in HBV risk countries. 532 (25%) of these travelers had previous HBB testing and 230 (11%) were tested during their pre-travel consultation through BATMN. Among 230 travelers born in HBV risk countries tested during the pre-travel visit, 7 (3.3%) were HBV-infected, 95 (43.6%) were immune, 116 (55.5%) were susceptible, and 10 (5.5%) had possible HBV exposure. Characteristics of these travelers were: 50.5% male, mean age 43.9 years, mean trip duration 44.2 days, 45.3% reported a non-English primary language. Subjects tested were most commonly Asian or black, traveling to visit friends and relatives, staying in home/local residence, and most commonly born in Asia and Africa.

CONCLUSIONS About half of foreign-born travelers tested during their travel encounter were not immune to HBV and may benefit from HBV vaccine. Pre-travel HBV test identified previously unrecognized chronic HBV infection. The travel clinic setting provides an opportunity to address an unmet health need (screening for hepatitis B and immunizing when appropriate) in immigrant travelers.

5.5-018

Traveler's vaccination in eight tertiary care hospitals in the valencian community, 2008–2009

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BACKGROUND/OBJECTIVE In recent years, the frequency of international travels has increased, and therefore the number of imported infectious diseases has increased as well, reaching 25% annually. The objective of this study is to analyze the profile of people who are vaccinated against the most common diseases in travellers of the Comunidad Valenciana (CV).

METHODS A descriptive study was performed. We Used the data from the, Vaccine Information System (SIV) of the CV, which has all the vaccines administrated in eight tertiary care hospitals from 2008 to 2009. The variables analyzed were sex, age, hospital, departmental affiliation and timing of vaccination.

RESULTS Vaccines were administered to 1210 travellers, of whom 89.8% were vaccinated against typhoid fever, 8.2% against the meningococcal A + C, 1.3% yellow fever, cholera 0.7%, and 0.1% against Japanese Encephalitis. In 2009, were administrated 35.3%, it is less vaccines administrated to travellers than the previous year. 53.5% were women; the age group with the highest number of vaccination was between 25 and 34 years for both, men and women. It Was observed statistical significance (P = 0.01) between sexes in the age group between 35 and 44 years, the percentage was higher among men (19.2% vs. 13.8%) from February to July was the period with the highest demand for international vaccination at these hospitals. In the province of Valencia were administered 71.8% of vaccines for travellers, while the province of Alicante was 14.4% and 13.8% Castellón.

CONCLUSIONS Most of the users of the international vaccination centres of the hospitals in CV were women aged between 25 and 34 years, and were born in Spain. The province of Valencia administrated most of the vaccines for travellers in the CV. The typhoid fever vaccine was the most often used to protect travellers.

5.5-019

Epidemiological survey of the prevalence of intestinal parasites among schoolchildren in Sari, Northern Iran

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Parasitic infection is highly prevalent throughout the developing countries of the world. Surveys on the prevalence of various intestinal parasitic infections in different geographic regions are a prerequisite for developing appropriate control strategies. A cross-sectional study was carried out to determine the prevalence of intestinal parasitic infections among schoolchildren of the public primary and secondary schools of the urban areas of Sari, Mazandaran province, northern Iran. The study was conducted from November 2005 to June 2006. A total of 1100 stool samples from 607 males and 493 females of 7014 years were examined by direct wet mounting, formaline-ether concentration, Ziehl-Nelsen and trichrome permanent staining methods. A parental questionnaire for a number of common risk factors was completed for all of participants. One or more parasite species were detected in 264 (24%) of the children (22.6% in males and 24.3% in females). Different species of intestinal parasites were detected: Blastocystis hominis seemed to be the most frequent parasite (10.6%), whereas *Giardia lamblia* had a prevalence of 6.4%, *Entamoeba coli* 3%, *Endolimax nana* 1.5%, *Enterobius vermicularis* 2.2%, *Trichostrongylus* sp. 1.7% and *Strogyloides stercoralis* 1.2%. The prevalence of intestinal parasite infections in females was slightly higher than male without statistically significant difference. No age

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association was detected, and a slight lower in positivity with increasing age was observed. A significant association was observed with contact with soil, washing hands before meals and washing raw vegetables before consumption. Although Paediatric pathogenic intestinal parasite infections are no more prevalent in this geographical area, an improvement in personal hygienic conditions and behavioral characteristics is important to completely control the parasitic infections in schoolchildren in northern Iran.

5.5-020

Lesson from workshop training method on the syndromic approach of imported diseases

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The so called COCOOPSI, a Spanish group consisted of family physicians concerned about international health, implemented an educational intervention to primary care centers of Catalonia in 2008 aimed to provide information about preventive activities in the traveler and the knowledge of common pathologies in this setting. The intervention, consisted of a 2-h workshop based on classic clinical case reports discussion, were carried out in 21 centers in Catalonia.

The objective of this study was to evaluate the satisfaction of participants after this educational intervention by means of a standardized survey

METHODS AND MATERIALS Transversal observational study. Population Study: health staff who completed the satisfaction survey after attending to workshop. Data collection: survey of satisfaction

RESULTS In total, 239 physicians (62.5%), 135 nurses (35.2%), two dentists (0.5%) and seven pharmaceutical (1.8%) participating in 21 training courses were included and responded the survey. All but three items were rated over 8 on a scale from 0 to 10 whereas the duration of the workshop (7.2), quality of the notes (7.8) and the extent to which expectations have been met on the workshop (7.9) were qualified below 8. The most repeated comment in the surveys was the inadequate time for the workshop.

CONCLUSIONS Workshop intervention is a very well accepted method to provide information, however the survey emphasizes the necessity of dedicate more time to discussion. The different teaching skills could have an outstanding influence on the accomplishment of the learning expectative of the participant.

5.5-021

Profile of people vaccinated against Japanese encephalitis in the Valencian community in 2010

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INTRODUCTION For some travellers who will be in a high-risk setting of Asia , Japanese encephalitis vaccine is recommended for reducing the risk of infection. The vaccination to travellers is recorded through the Vaccine Information System (SIV) in the Valencian Community. The vaccination is realized though two different centres; the Health Centres that belong to the Ministry of Health and Social Policy, and the International Vaccination Centres that belong to the Valencian Agency of Health.

METHODS A descriptive analysis was performed with the cases vaccinated against Japanese Encephalitis reported in the SIV in the Valencian Community in 2010. The statistical analysis was done by health centre, region (Valencia, Castellón and Alicante) and health professional.

RESULTS Five hundred sixty eight vaccines of Japanese encephalitis were reported, representing 2.4 per thousand of the total vaccines administrated. About 56.3% of the vaccines administrated were in men. About 46.3% of the vaccinated population was aged between 15 and 29 years. About 41.8% were aged between 30 and 44 years and 4% aged over 60 years. A statistical significance was found between male and female in the group aged between 15 and 29 years. 94% of the vaccines administrated were reported at the health centres that belong to the Ministry of Health and Social Policy in Valencia and Castellón, and 5.3% of the vaccine administrated to CVI dependent of the Valencian Agency of Health By region, 89.3% vaccines administrated were reported in Valencia and 7.2% in Castellon. Nursing reported 98.4% of the vaccine against Japanese encephalitis.

CONCLUSIONS The highest percentage of cases reported were women aged between 15 and 29 years. Most of the vaccine administrated was reported through nurses working in the health centres of Ministry of Health and Social Policy. It is an underreporting of the vaccines administrated in Alicante.

5.5-022

Pharmacokinetics, dosing and tolerability of the anti-malarial mefloquine in small children

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INTRODUCTION Most studies of imported malaria show that children of migrants who visit friends and relatives (VFR) in Africa are particularly at risk of malaria and most in need of an inexpensive, simple dosage, anti-malarial chemoprophylaxis. The use of anti-malarial drugs in children is hampered by a paucity of pharmacokinetic data and paediatric drug formulations.

METHODS We did an intensive study of the literature and reports (previously submitted to Health Authorities) on file at F. Hoffmann-La Roche to collate data on the use of mefloquine in young children weighing < 20 kg. We reviewed paediatric chemoprophylaxis and treatment data with a focus on pharmacokinetics, dosing and tolerability of mefloquine.

RESULTS Chemoprophylaxis data: A simulated mefloquine plasma profile in an older study of Fansimef (mefloquine-sulfadoxine-pyrimethamine MSP combination) prophylaxis showed that mefloquine doses to achieve protective chemoprophylaxis levels of the drug in children should be at least 5 mg/kg. A blood concentration of mefloquine of approximately 620 ng/ml is considered effective against *Plasmodium falciparum* malaria. For small children 62.5 mg weekly (5 mg/kg) mefloquine (equivalent to of a tablet) achieves protective levels of mefloquine. This simulated plasma profile in children corresponds to that seen for adult travellers using weekly prophylaxis doses of 250 mg. This reinforces current practice of using weight-based dosage for children. Treatment data: The findings of the treatment studies done in children indicate a predictable pharmacokinetic profile in children, similar to that observed in adults. The main age related difference in pharmacokinetics is that clearance per body weight is higher in older children, aged 5–12 years, compared to younger children aged 6–24 months. The stereoselectivity of mefloquine in children was similar to that observed in adults. Tolerability data: There are few data on the paediatric tolerability of mefloquine chemoprophylaxis except for the MSP studies which reported good tolerability. In mefloquine treatment studies, vomiting was a problem at high doses (25 mg/kg).

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INTERPRETATION Currently available data provide a scientific basis for the use of mefloquine in small children weighing > 5 kg in the chemoprophylaxis setting. Weight based dosage is appropriate.

5.5-023

Case-control study on infection-induced thrombocytopenia among returned travelers

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INTRODUCTION Thrombocytopenia is a frequent diagnostic finding among travelers returning from tropical and subtropical regions.

METHODS AND MATERIALS This case-control study analyzed data of 19 473 returned travelers presenting at the University of Munich between 1999 and 2009.

RESULTS Out of them, 732 (3.8%) travelers were diagnosed with thrombocytopenia (cases). Thrombocytopenia was significantly more frequent among patients with malaria (63%), acute HIV infection (48%), dengue fever (47%), mononucleosis (23%), paratyphoid/typhoid fever (14%), and rickettsiosis (12%). Malaria and dengue fever caused 25% of all cases and 75% of all cases of severe thrombocytopenia. The most frequent travel destinations were Asia (42%) and Africa (33%). Travelers to sub-Saharan Africa (high risk for malaria) and to South/South East Asia (high risk for dengue fever) had the highest relative risk for thrombocytopenia.

CONCLUSION Platelet count among returned travelers is an essential screening parameter, as thrombocytopenia is highly correlated with important infectious diseases, particularly with malaria and dengue fever.

5.5-024

The diagnostic value of leucocytosis in imported diseases among returned travellers

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BRIEF INTRODUCTION Leucocytosis is a common, but unspecific finding among travellers presenting at travel clinics.

METHODS AND MATERIALS At the Department of Infectious Diseases and Tropical Medicine at the University of Munich, data from 21 024 patients in the time period 1999–2010 with and without leucocytosis were analyzed. Moderate leucocytosis (ML) and pronounced leucocytosis (PL) were defined adapted to age. The aim of this study was to enhance the prediction of specific imported diseases in returning travellers with leucocytosis by considering additional demographic, travel, clinical and additional laboratory data.

RESULTS Overall, 812 (3.9%) patients were found with ML and 203 (1.0%) with PL. For ML patients, no specific diagnosis could be made in 31.3% (n = 254) respectively 22.7% (n = 46) for PL patients. The most frequent clinical syndromes at presentation were enteritis (n = 226, 27.8%) in ML patients and respiratory tract infections (n = 41, 20.2%) in PL patients. The prevalence of particular pathogens differed substantially between patients without leucocytosis, ML and PL patients. Diseases specific for tropical regions only formed a small fraction in both groups. Several diagnoses were made in 9.4% (n = 76) of ML patients in comparison to 10.8% (n = 22) of PL patients. For most clinical syndromes, few pathogens accounted for the major disease burden. The proportion of leucocytosis among travellers not only depended on certain pathogens but also on several other epidemiological variables.

CONCLUSIONS Although co-infections are common and a large number of pathogens are potentially causative for leucocytosis, WBC count is an elementary laboratory parameter for the diagnostic work up in returning travellers. This study shows that the prediction of certain imported diseases among returned travellers with leucocytosis can be considerably improved by involving demographic, travel, clinical and additional laboratory data in the analysis.

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