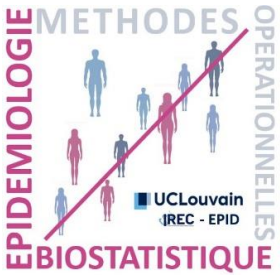
UCLouvain
Institut de recherche
expérimentale et clinique



Intestinal parasitic infections in adults living with HIV in the department of Cochabamba, Bolivia

Jorge L. Avilés Sarmiento

Promotors: Prof. Jean Cyr Yombi

Prof. Annie Robert

Thesis submitted in fulfilment of the requirements for the degree of Doctor in Public Health Sciences

Belgium, April 2023

Members of the jury

President	**Professor D'HOORE William** Institut de Recherche Santé et Société Faculté de Santé Publique Université catholique de Louvain, Bruxelles, Belgique
Promotor	**Prof. YOMBI Jean Cyr, Chef de clinique** Chef du service des maladies infectieuses Cliniques Universitaires Saint Luc Institut de Recherche Expérimentale et Clinique Université catholique de Louvain, Bruxelles, Belgique
Promotor	**Prof. ROBERT Annie** Chef du Pôle Epidémiologie et Biostatistique Institut de Recherche Expérimentale et Clinique Université catholique de Louvain, Bruxelles, Belgique
Supervisor In Bolivia	**Dr. EROSTEGUI Revilla Carlos** Instituto de Investigaciones Biomédicas IIBISMED, Universidad Mayor de San Simón (UMSS), Cochabamba, Bolivia
Secretary	**Prof. PEÑALOZA BAEZA Andrea, Chef de clinique** Chef du service des Urgences Cliniques Universitaires Saint Luc Pôle Epidémiologie et Biostatistique Institut de Recherche Expérimentale et Clinique Université catholique de Louvain, Bruxelles, Belgique
External members	**Prof. BONTEMPS, Patrick** Gastroentérologue, Directeur de service Hôpital universitaire de Bruxelles, Université Libre de Bruxelles, Bruxelles, Belgique

Prof. DRAMAIX WILMET Michèle
Département de Biostatistique
Centre de Recherche en Épidémiologie, Biostatistique et Recherche Clinique
Ecole de Santé Publique
Université Livre de Bruxelles, Bruxelles, Belgique

Prof. DARCIS, Gilles
Centre VIH, Centre hospitalier universitaire Sart Tilman,
Université de Liège, Belgique

Prof. GOFFARD, Jean-Christophe
Centre de VIH, Hôpital universitaire de Bruxelles
Université Libre de Bruxelles
Bruxelles, Belgique

Internal members

Prof. RODRIGUEZ VILLALOBOS Hector
Chef du laboratoire de microbiologie
Cliniques Universitaires Saint Luc
Pôle de recherche en microbiologie médicale
Université catholique de Louvain, Bruxelles, Belgique

Prof. KABAMBA Benoit
Chef du Pôle de recherche en microbiologie médicale,
Institut de Recherche Expérimentale et Clinique (IREC)
Université catholique de Louvain Bruxelles, Belgique

SCIENTIFIC CURRICULUM VITAE

Publications

- Jorge Avilés, Jean Cyr Yombi, Carlos Erostegui, Maricruz Torrico, Marcelo Rojas, Héctor Rodríguez, Daniel Mercado2, Daniel Illanes & and Annie Robert. Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba, Bolivia **Infect Dis Diag. Treat 4: 136. DOI: 10.29011/2577-1515.100136.**

- Jorge Avilés, Jean Cyr Yombi, Carlos Erostegui, Maricruz Torrico, Marcelo Rojas, Héctor Rodríguez, Daniel Mercado, Daniel Illanes & and Annie Robert. Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia, Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

- Marcelo Rojas Mattos, Daniel Illanes, Jorge Avilés, Wilson Trujillo, Annie Robert. Spatial Distribution of Tuberculosis and Socioeconomic Inequalities in Cochabamba, Bolivia DOI: 10.29011/2577-2228/100065

- Mattos MR, Illanes D, Avilés J, Trujillo W, Martin A, et al. (2021) Economic Burden of Tuberculosis for Patients and their Households in Cochabamba, Bolivia. J Community Med Public Health 5: 227. DOI: https://doi.org/10.29011/2577-2228.100227

- Juan Pablo Escalera-Antezana, Roberto Torrez-Fernandez, Dagner Montalvan-Plata, Claudia Marcela Montenegro-Narváez, Jorge Avilés-Sarmiento, Lucia Elena Alvarado-Arneza, D. Katterine Bonilla-Aldana, Alfonso J. Rodríguez-Moralesa. Orthohantavirus pulmonary syndrome in Santa Cruz and Tarija, Bolivia, 2018

- E Valdez, J Avilés, A Saudan / Gaceta Médica Boliviana 35 (1), 22-26
Conocimientos, actitudes y práctica sobre el VIH/sida en docentes de la Facultad de Medicina, Universidad Mayor de San Simón

- VALDEZ, Edgar; AVILES, Jorge y SAUDAN, Anne. Knowledge, attitudes and practice on HIV/AIDS in teachers of the Faculty of Medicine, Universidad Mayor de San Simon. Gac Med Bol [online]. 2012, vol. 35, n. 1, pp. 22-26. ISSN 1012-2966.

Communications

- Conférence internationale annuelle sur les progrès du VIH (Bolivie/Argentine), Tarija-Bolivia, September 15-2017 : Oral presentation: Frailty and HIV (Lecturer) / **Avilés Jorge**

- Conférence internationale annuelle sur les progrès du VIH (Bolivie/Argentine) Tarija-Bolivia, September 15-2017 : Oral presentation: Microbioma and HIV (Lecturer) / **Avilés Jorge**

- 3rd international congress of infectology / 2nd international symposium of Zoonosis (Córdoba - Argentina): November 30-2019 Oral presentation: Update on Arenavirus (Chapare Virus) and Bolivian hemorrhagic fever / description of the Chapare virus outbreak in La Paz-Bolivia (Lecturer) / **Avilés Jorge**

- 1st international international symposium of Epidemiology (Cochabamba-Bolivie): July 2019 Oral presentation: Instetinal parasites in PLWHA adult Population in Cochabamba – Bolivia. **Avilés Jorge**, Jean Cyr Yomby , Carlos Erostegui, Robert A.

- 4th international congress of infectology, Ist international symposium of infection management in neutropenic patient (La Paz- Bolivie): August 2019 La Paz, Bolivia: Oral presentation: Instetinal parasites in VHI adult Population in Cochabamba - Bolivia (Retrospective Analisys) **Avilés Jorge**, Jean Cyr Yomby , Carlos Erostegui, Robert A.

- 2nd international symposium of Zoonosis (Tarija- Bolivie) September 2019: Oral presentation: Instetinal parasites in VHI adult Population in Cochabamba - Bolivia (prospective Analisys) **Avilés Jorge**, Jean Cyr Yomby , Carlos Erostegui, Robert A.

- 3rd international congress of infectology / 2nd international symposium of Zoonosis (Córdoba - Argentina) November 2019: Oral presentation: Intestinal Parasitic Infections in HIV /AIDS patients: epidemiological, nutritional and inmunological aspects in Cochabamba, Bolivia - **Avilés Jorge,** Jean Cyr Yomby, Carlos Erostegui, Robert A.

Communications as co-author

• 2nd international symposium of Zoonosis (Tarija-Bolivie) September 20-2019: Oral presentation: Orthohantavirus pulmonary syndrome in Santa Cruz and Tarija, Bolivia (Lecturer) / Avilés Jorge

Posters

• Avilés Jorge, Jean Cyr Yombi, Carlos Erostegui, Robert A. <<Intestinal parasites in HIV adult Population in Cochabamba - Bolivia>> Conférence internationale annuelle sur les progrès du VIH (Bolivie / Argentine) 2017. Tarija, Bolivia.

To my parents, for their guidance and their values,

To my wife, Wendy, and daughters who have had the patience and strength to sacrifice our precious time together...

For those friends and teachers who are no longer here because of COVID-19

To people living with HIV in Bolivia.

This research is for you, beyond this work, I undertake to contribute in my own way to improving your Quality of life. (IMPACT)

Acknowledgements

To the people who have made this possible since the coordination of the scholarship with the Belgian cooperation, Prof. Annie Robert and Dr. Daniel Illanes, Dr. Carlos Erostegui.

Special thanks to the personnel of the laboratory of parasitology of the LABIMED, the molecular biology laboratory of the faculty of biochemistry of the UMSS and to the personnel that implemented the Immunochromatography technique and the survey. **Carol Torrico, Claudia Torrico, Amílcar Lazcano.**

For the grant supported by AI-UMSS 2014-2019 from ARES-CCD, Belgian Government

The study was funded by the Belgian Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au Développement (ARES-CCD), through an Institutional support to UMSS in Bolivia (AI 2014-2023).

https://www.ares-ac.be/fr/cooperation-au-developpement/pays projets/projets-dans-le-monde/item/92-programme-d-appui-institutionnel-avec-la-universidad-mayor-de-san-simon.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Summary

Although some infectious diseases have declined in the last decade as part of the epidemiological transition in developing countries, gastrointestinal parasitic infections and HIV have a high impact due to socioeconomic factors. In addition, people with HIV are vulnerable to opportunistic parasitic infections, which perpetuate a cycle of malnutrition and immunosuppression, a context favored by diagnostic methods that fail to accurately identify certain infections such as coccidia.

Our proposal aimed to determine the prevalence of intestinal parasites in adults with HIV, including identifying the most burdensome species and associated factors, testing the validity and effectiveness of routinely applied diagnostic methods for detecting these infections in people living with HIV/AIDS, and mapping the distribution of parasitic infections in this population.

To determine the prevalence of the disease, we conducted a retrospective study using historical data from the regional reference laboratory (LABIMED) between 2011 and 2015, finding high rates of prevalence. However, the limited number of variables collected by this entity on the clinical monitoring of patients places some limitations on the study's conclusions. Using the findings from this study, we adapted the variables for a prospective cross-sectional design, which also examined the relationship between the disease and other factors.

The retrospective analysis found a clear predominance of protozoa in fecal samples processed in the reference laboratory, with a 33.2% infection rate in 2011, the

highest prevalence observed, and a 24.6% rate in 2014, the lowest during the period. The most prevalent pathogen species were *Giardia lamblia* and *Entamoeba histolytica/dispar*, with non-pathogenic species like *Blastocystis hominis* and *Entamoeba coli* also identified. One of the limitations of the study was that data on immune status were not available since they were not collected at LABIMED and were not linked to the parasitology laboratory.

In the cross-sectional study, 57.9% of recruited patients had undetectable viral loads, and 98% currently received ART, with most being administered Tenofovir/Lamivudin/Efavirenz (TDF/3TC/EFV). The study found a 33.0% prevalence of intestinal parasites, more evident in rural areas (37%), with *Giardia lamblia* and *Entamoeba histolytica/dispar* being the most common pathogenic species identified. Multiparasitism was reported in 7.8% of the cases, and the study found 55.4% of cases with parasitic infection attributed to pathogenic agents.

The study concludes by highlighting the need for tailored interventions to address the prevalence of intestinal parasitic infections among HIV-positive individuals in developing countries, where various factors such as poor sanitation and insufficient health system coverage contribute to the epidemiological context.

In addition, the low performance of the methods currently used has been established, generating an important sub-diagnosis that conditions in the deterioration of the patient, generating greater immunosuppression in the long term.

However, common protozoa such as *Blastocystis* spp. are more frequently associated with asymptomatic infections or the condition of a healthy carrier. It has

the potential to cause diseases classified as opportunistic, especially in immunosuppressed people, and there is controversy regarding its pathogenicity.

In turn, using more specific methods we found species such as *Microsporidium* spp. (8.9%), *I. Belli* (3.4%) and C*ryptosporidium* spp. (0.8%) that were not previously reported to generate subdiagnosis.

The prevalence of intestinal parasitic infections found in our study provides compelling evidence for the need to reduce underdiagnoses and implement additional laboratory tests with higher diagnostic accuracy for adult patients with HIV to address these infections in Bolivia.

The comparison of different staining methods allowed us to evaluate the comparative performance that later, together with additional methods such as Immunochromatography and concentration techniques, allowed us to develop a protocol for the detection of intestinal parasites in people with HIV to be applied in the national context.

Due to the various factors identified and the differences in disease distribution in different regions, tailored actions must be developed for each location to control the disease.

The epidemiological surveillance system should be adapted, using a monitoring strategy that incorporates complementary detection methods and ensures accurate diagnosis while limiting the damage.

These interventions can be developed as part of health promotion within the framework of tertiary prevention to limit harm.

Our results are currently being used to generate interventions that promote self-care in people with HIV, as well as diagnostic and therapeutic protocols. Non-governmental organizations and the Ministry of Health, through the national HIV control program, are collaborating to develop policies that will have a significant impact in developing countries, where the epidemiological context is influenced by various factors, such as poor sanitation, and low levels of education, malnutrition, inadequate coverage of the health system, and other comorbidities.

Résumé

Bien que certaines maladies infectieuses aient reculé au cours de la dernière décennie dans le cadre de la transition épidémiologique dans les pays en développement, les infections parasitaires gastro-intestinales et le VIH ont un impact élevé en raison de facteurs socio-économiques. De plus, les personnes séropositives sont vulnérables aux infections parasitaires opportunistes qui perpétuent un cycle de malnutrition et d'immunosuppression, un contexte favorisé par des méthodes de diagnostic qui n'identifient pas avec précision certaines infections comme les coccidies.

Notre travail de thèses visait à déterminer la prévalence des parasites intestinaux chez les adultes séropositifs, notamment en identifiant les espèces les plus nuisibles et les facteurs associés, en testant la validité et l'efficacité des méthodes diagnostiques appliquées en routine pour détecter ces infections chez les personnes vivant avec le VIH/SIDA et en cartographiant la distribution des infections parasitaires dans cette population.

Pour déterminer la prévalence de la maladie, nous avons mené une étude rétrospective en utilisant les données historiques du laboratoire régional de référence (LABIMED) entre 2011 et 2015, et nous avons constaté des taux élevés de prévalence. Cependant, le nombre limité de variables collectées par cette entité sur le suivi clinique des patients impose certaines limites aux conclusions de l'étude. À partir des résultats de cette étude, nous avons adapté les variables pour une

étude transversale prospective, qui a également examiné la relation entre la maladie et d'autres facteurs.

L'analyse rétrospective a révélé une nette prédominance des protozoaires dans les échantillons fécaux traités par le laboratoire de référence, avec un taux d'infection de 33,2 % en 2011, la prévalence la plus élevée observée, et un taux de 24,6 % en 2014, le plus bas de la période. Les espèces pathogènes les plus répandues étaient *Giardia lamblia* et *Entamoeba histolytica/dispar*, des espèces non pathogènes comme *Blastocystis hominis* et *Entamoeba coli* ayant également été identifiées. Dans l'étude transversale, 57,9 % des patients recrutés avaient une charge virale indétectable et 98 % recevaient un traitement antirétroviral, la plupart d'entre eux étant traités au Tenofovir/Lamivudin/Efavirenz (TDF/3TC/EFV). L'étude a révélé une prévalence de 33 % de parasites intestinaux, plus évidente dans les zones rurales (37 %), *Giardia lamblia* et *Entamoeba histolytica/dispar* étant les espèces pathogènes les plus fréquemment identifiées. Le multiparasitisme a été signalé dans 7,8 % des cas, et l'étude a permis d'attribuer 55,4 % des cas d'infection parasitaire à des agents pathogènes. L'étude conclut en soulignant la nécessité d'interventions adaptées pour lutter contre la prévalence des infections parasitaires intestinales chez les personnes séropositives dans les pays en développement, où divers facteurs tels qu'un mauvais assainissement et une couverture insuffisante du système de santé contribuent au contexte épidémiologique.

En outre, la faible performance des méthodes actuellement utilisées a été établie, générant un sous-diagnostic important qui conditionne la détérioration du patient, générant une plus grande immunosuppression à long terme.

Cependant, les protozoaires communs tels que *Blastocystis* spp. sont plus fréquemment associés à des infections asymptomatiques ou à l'état de porteur sain. Ils ont le potentiel de provoquer des maladies classées comme opportunistes, en particulier chez les personnes immunodéprimées, et leur pathogénicité fait l'objet d'une controverse.

Par ailleurs, en utilisant des méthodes plus spécifiques, nous avons trouvé des espèces telles que *Microsporidium* spp. (8,9 %), *I. belli* (3,4 %) et *Cryptosporidium* spp. (0,8 %) qui n'avaient pas été signalées auparavant et qui généraient des sous-diagnostics.

La prévalence des infections parasitaires intestinales constatée dans notre étude fournit des preuves irréfutables de la nécessité de réduire les sous-diagnostics et de mettre en œuvre des tests de laboratoire supplémentaires avec une plus grande précision diagnostique pour les patients adultes séropositifs afin de lutter contre ces infections en Bolivie.

La comparaison de différentes méthodes de coloration nous a permis d'évaluer les performances comparatives qui, plus tard, avec d'autres méthodes telles que l'immunochromatographie et les techniques de concentration, nous ont permis de développer un protocole pour la détection des parasites intestinaux chez les personnes séropositives qui peut être applique dans le contexte national.

En raison des divers facteurs identifiés et des différences de distribution de la maladie dans les différentes régions, des actions adaptées doivent être développées pour chaque région afin de contrôler la maladie.

Le système de surveillance épidémiologique doit être adapté, en utilisant une stratégie de suivi qui incorpore des méthodes de détection complémentaires et assure un diagnostic précis tout en limitant les dommages.

Ces interventions peuvent être développées dans le cadre de la promotion de la santé et de la prévention tertiaire pour limiter les dommages.

Nos résultats sont actuellement utilisés pour générer des interventions visant à promouvoir l'autosoin chez les personnes séropositives, ainsi que des protocoles diagnostiques et thérapeutiques. Des organisations non gouvernementales et le Ministère de la Santé, par le biais du programme national de lutte contre le VIH, collaborent pour développer des politiques qui auront un impact significatif dans les pays en développement, où le contexte épidémiologique est influencé par divers facteurs, tels que le manque d'hygiène et le manque de salubrité.

Tables of Content

List of Figures

List of Tables

List of abbreviations

ATCC	American Type Culture Collection
ART	Anti-Retroviral Therapy
CDC	Center for Disease Control
DALYs	Disability-Adjusted Life Years
CDVIR	Reference Center for HIV control and prevention in Cochabamba
CI	Confidence Intervals
CEHF l'UCLouvain.	Commission d'éthique biomédicale hospitalo-facultaire de Cliniques Universitaires Saint-Luc, Bruxelles, Belgium
CPG	Clinical Practice Guidelines (CPG)
CRF	Circulating Recombinant Form of HIV
CSW	Commercial Sex Workers
HAART	Highly Active Antiretroviral Therapy
DTV	Dolutegravir
EFV	Efavirenz
HIV/AIDS	Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome
INE	Bolivia National institute of Statistic / Instituto Nacional de Estadística
IIBISMED	Institute of Biomedical Research / UMSS
IPIs	Intestinal Parasitic Infections
JMP	Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene
LABIMED	Reference laboratory in the city of Cochabamba (UMSS)
3TC	Lamivudine /Lamivudina

MHC	Major Histocompatibility Complex
MSD	Ministry of Health and Sports (Bolivia) / Ministerio de Salud y Deportes, Bolivia
MSM	Men who have sex with men
MTS	Modified trichrome staining
NGOs	Non-Governmental Organizations (Non-profit organizations)
PAHO	Pan-American Health Organization
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PLWHA	People living with HIV/AIDS
PNC/VS	National HIV/AIDS Control Program Bolivia
PrEP	Pre-exposure prophylaxis
RNA	Ribonucleic acid
UMSS	Universidad Mayor de San Simón
UNAIDS	Joint United Nations Program on HIV/AIDS
SEDES	Servicio departamental de Salud / Departamental Health Service
SPSS	Statistical Package for Social Science
STHs	Soil-transmitted Helminths
STI	Sexual transmitted infections
TDF	Tenofovir
WBD	Water Borne Diseases
WHO	World Health Organization

CHAPTER 1: GENERAL INTRODUCTION

Chapter 1 Introduction

1.1 Intestinal Parasitic disease burden

The global burden of intestinal parasitic infections is a significant public health problem. These infections affect more than two billion people worldwide. [1]

More than four billion people are at risk of soil-transmitted helminthiasis (STH), those are the most common infections worldwide. Soil-transmitted helminths have multiple negative impacts on the nutritional status impairing nutrient absorption in the intestines and can compete with the host for the intake of vital nutrients. Additionally, some of these parasitic infections lead to reduced appetite and overall nutritional intake, which negatively impacts physical well-being, causing diarrhea and dysentery. [1-3]

Intestinal protozoa are also considered a public health problem, *E. histolytica* and *G. lamblia* represent a high burden of disease globally, particularly *E. histolytica/dispar*, which has a high prevalence, affecting 10% of the world's population and, in some countries, this percentage can be as high as 50%. [4]

The Conference on the Control of Communicable Diseases has reported that enteric pathogens, mainly protozoa, were isolated in 40.9% of diarrhea cases on average in developed countries. [5]

In addition, the global burden of intestinal parasitosis is increasing due to a lack of basic sanitation, lack of access to safe drinking water, and lack of knowledge about methods of prevention and treatment of these infections. These infections are

preventable and treatable but require prevention and treatment measures to reduce their impact significantly through mass deworming, promotion of healthy habits, hygiene, environmental sanitation, and adequate provision of drinking water. The burden of intestinal parasitosis varies based on the geographic location and age of the population and tends to affect more people living in rural or low-income areas. [3]

Different indicators should be used to measure the impact of parasitic infections, such as disability-adjusted life years (DALYs), as a measure of disease burden and provide a comparative indication of the public health importance of the disease. Intestinal parasitic infections are associated with a DALY of 5 266 000 worldwide.[6]

In various settings, the incidence of these diseases in the general population is much lower than in the immunocompromised population, in some studies even double that of immunocompetent individuals (42.06% vs. 17.79%). [7] With a tendency to chronicity and greater severity. Therefore, early and timely diagnosis is necessary.

1.1.1 Amoebiasis

Almost six species within the genus *Entamoeba* have been described in humans, including *E. histolytica*, *dispar*, *moshkovskii*, *poleki, coli,* and *hartmanii*. Among these, *E. histolytica* is the only species that has typically been described as pathogenic, but cases have been documented associating other species with diseases produced in immunocompromised individuals, reporting gastrointestinal

disorders in patients infected with *E. polecki* and *E. dispar*, and even with mixed *E. dispar-E. moshkovskii* infection. [8]

Even with these considerations, *Entamoeba histolytica* infections are responsible for millions of symptomatic infections and about 55,000 deaths annually worldwide. [9-11]

In addition, these species represent an obvious diagnostic problem as a confounding factor using light microscopy-based methods, due to their similar morphology.

Diagnosis is difficult because the pathogenic species *E. histolytica* is morphologically identical to the nonpathogenic species *E. dispar* and *E. moshkovskii*; therefore, microscopy is generally considered insufficient for the differentiation of these species, requiring sophisticate tools (antigen detection or molecular technology). However, especially in developing countries, where molecular detection methods are unavailable or accessible only for research purposes, their availability is limited in clinical care settings, where technical difficulties and costs make these detection methods inaccessible. [12]

The CDC recommends the use of methods that maximize cyst recovery, formalin fixatives of stool samples, or others that concentrate the sample before microscopic examination. [13]

1.1.2 Giardiasis

Giardia intestinalis is a common cause of parasitic diarrhea, with prevalences ranging from 2 to 7% in developed countries and from 20 to 30% in most developing countries worldwide according to WHO targets for 2030. [14]

The symptoms of giardiasis can be variable, but they mainly present as acute or chronic diarrhea associated with abdominal pain, nausea, and poor absorption of nutrients resulting in steatorrhea and weight loss.

The standard method for diagnosing giardiasis has historically consisted of identifying cysts in fecal samples by microscopy. Although this method is rapid and affordable, it is not sensitive due to the occasional excretion of *Giardia lamblia* cysts in feces and/or the presence of small numbers of cysts, thus generating a subsequent sub-diagnostic. [15]

Feces can be examined directly as fresh smears, methods that can optimize their detection are preferred by keeping the samples preserved in formalin or polyvinyl alcohol and stained with iodine, trichrome, hematoxylin, or after formalin-ethyl acetate concentration.

PCR detection is increasingly used for *Giardia* identification in developed countries as it offers improved sensitivity and specificity compared to microscopy and immunology-based detection methods. [16]

In Bolivia, immunochromatography is available but with limited accessibility due to cost and limited disposition.

1.1.3 Cryptosporidiosis

Cryptosporidium is a pathogenic protozoan, a major cause of diarrheal diseases in humans worldwide and is included in the World Health Organization's Neglected Diseases Initiative. [17,18]

Cryptosporidium species infecting humans is classified as two species, *C. parvum* (infecting mammals, including humans) and *Cryptosporidium hominis* (infecting primarily humans) however the differentiation of these two species is not possible using routine stains.

The impact of *Cryptosporidium* parasite on causing diarrhea around the globe is not fully established, particularly in individuals with weakened immune systems. In this population, intestinal cryptosporidiosis can lead to a persistent and severe condition with long-term health problems and high costs. Unfortunately, there is no clear evidence of effective treatments for this disease. [17] This infection is of particular concern as it is one of the leading causes of chronic diarrhea and death in patients with HIV/AIDS. Additionally, the presence of cryptosporidiosis can also indicate a weakened immune system. Microscopy, the current diagnostic mainstay, is considered suboptimal. Immunoassays has proven to be more sensitive methods of detecting these organisms in stool samples. Some of these commercial tests can be used to identify quickly both *Giardia* cysts and *Cryptosporidium* oocysts in the same test but are not currently available in Bolivia.

Molecular methods, particularly PCR, are gaining ground in terms of their diagnostic capacity but are not available in low-resource settings. [19]

Among the common *Cryptosporidium* species in humans, *C. parvum* is responsible for the majority of zoonotic infections in industrialized countries, but the role of zoonotic transmission in the epidemiology of cryptosporidiosis in developing countries remains unclear. [20]

1.1.4 Cyclosporiasis

Cyclospora cayetanensis is a type of coccidian parasite that can be transmitted directly through fecal-oral contact in humans. This parasite is found worldwide and is a significant cause of food-related outbreaks of gastrointestinal illness in several developed nations. The most vulnerable populations include children, individuals from foreign countries, and immunocompromised patients in areas where the parasite is common. However, in industrialized nations, *Cyclospora cayetanensis* has been known to affect people of all ages. [21]

C. cayetanensis is host specific and is the only species of this genus found in humans. The clinic is characterized by persistent diarrhea, more than 2 weeks duration, flatulence, abdominal cramps, constipation, and fatigue.

Detection of this parasite is often difficult by standard laboratory procedures, because the pathogen is very small, and measurement of oocysts is important to differentiate this species from *Cryptosporidium* oocysts; therefore, the laboratory should be notified when this diagnosis is suspected. [21,22]

Various techniques more precise than microscopy for the identification of Cyclospora have been developed. These include detection based on spectrophotometry, reverse transcriptase PCR in combination with agarose gel

electrophoresis, and other molecular biology tests, such as nested PCR. Again, in low-income settings, it is difficult to standardize laboratory techniques for the identification of this organism. Modern tools such PCR are expensive and subjects of contamination due to peccary laboratory conditions limiting their use in the clinical area. This underline the necessity to develop highly sensitive, faster and economic tests for the detection of this protozoan. [22,23]

1.1.5 Blastocystosis

Those are the most common enteric protozoa isolated from patients with diarrhea in most developed countries. *Blastocystis hominis* is considered a saprophyte, and refers to approximately 10 different genetically different populations that are all morphologically identical and cannot be distinguished by microscopy alone.

The diagnosis of infection with *Blastocystis* spp. it is generally based on the detection by microscopy of the vacuolar, granular, amebic, or cystic form in stool samples, using wet mount smears, iodine staining, trichrome staining, or iron-hematoxylin staining.

Various molecular techniques with increased sensitivity (PCR) have been developed. Advances in sequence analysis of *Blastocystis*-specific PCR products and subtype-specific PCR primers have led to progress in identifying various subtypes. [24]

The role in causing gastrointestinal symptoms and pathology remains uncertain, and the clinical characteristics of the disease that have been attributed to Blastocystis spp. include nausea, anorexia, abdominal pain, flatulence, and acute or

chronic diarrhea. Its clinical implication and role in immunosuppressed patients are under discussion. [25,26]

Metronidazole is the suggested drug of choice for these infectious agents. Cotrimoxazole, nitazoxanide, and a combination of paromomycin and metronidazole have also been used. These treatments are used for their broad spectrum of action and their adequate safety profile. [27]

1.1.6 Strongyloidiasis

Strongyloides stercoralis, a soil-transmitted nematode, is also a neglected tropical helminthiasis endemic in tropical and subtropical settings, where sanitary and hygiene conditions are poor. However, the worldwide prevalence of *S. stercoralis* is heterogeneously distributed and the current estimation of infection remains underestimated due to the use of the inadequate diagnostic method. Strongyloidiasis has been estimated to affect over 600 million people worldwide. [28,29]

Strongyloides stercoralis is transmitted when larvae present in the soil penetrate a person's skin, usually by direct contact. These larvae can also penetrate the intestinal wall and re-enter the host's bloodstream, or be excreted in human feces and become new larvae in the soil, which can perpetuate the cycle of infection.

In addition to transmission by direct contact with soil, other methods of transmission have been described, such as mother-to-child transmission during childbirth, transmission by organ transplantation, and sexual transmission in men who have sex with men.

Autoinfection, in which larvae generated in the host re-infect the host, leads to a state of chronic asymptomatic infection often with eosinophilia, while hyperinfection syndrome may develop when patients develop immunosuppression, due to drugs such as corticosteroids or following solid organ transplantation.

Strongyloidiasis can be diagnosed by direct visualization but is made difficult by the scant presence of the parasite in feces, larvae in feces or other body fluids, or by serology. [29]

1.1.7 Microsporidiosis

Microsporidia are a type of intracellular pathogens. Approximately 220 different types or genera and about 1,700 species of microsporidia are known, the classification of which is based mainly on their unique ultrastructural features, developmental process, host-parasite connection and molecular analysis. [30]

Intestinal infection by microsporidia includes species such as *Enterocytozoon bieneusi, Encephalitozoon intestinalis, E. hellem,* and E. c*uniculi*; of these *E. bieneusi* and *E. intestinalis* are species of microsporidia that cause more frequently enteric disease. Parasites, historically classified as spore-forming protozoa, are now classified as fungi. [30,31]

Intestinal microsporidiosis was first identified in AIDS patients, with *E. bieneusi* being the most common species, they are an important cause of opportunistic infections in HIV / AIDS and immunosuppressed patients.

The diagnosis of these agents is particularly difficult and is frequently based on the microscopic detection of spores in stool samples, which detects special fluorescent or trichrome stains to detect spores. However, detection and species determination can be difficult due to their small size, which requires reliable immunological diagnoses to complement the findings and increase the performance of diagnostic tests using molecular methods such as PCR or histochemistry when sporadic shedding is suspected.

Modified trichrome staining (MTS) and nested polymerase chain reaction (nested PCR) methods were used for the diagnosis of common intestinal microsporidia in fecal samples of patients with HIV/AIDS. [31]

1.1.8 Soil-transmitted helminths (STHs)

The three primary types of parasitic worms that infect humans through the soil are roundworms, whipworms, and hookworms. These infections remain one of the most significant neglected tropical diseases due to their impact on health, with nearly one billion people worldwide still infected with at least one species.[32]

The most common soil-transmitted helminths (STHs), *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm, affect particularly poor populations and developing countries, causing impaired childhood growth, cognitive damage, and malnutrition. [14,32,33]

Global estimates report reveals that more than 800 million people are affected by STHs. In Latin America, a total of 234 million people are affected by STHs, although

this number may be an underestimate as some countries such as Bolivia have not been included in the analysis owing to a lack of data. [33]

Currently, it is estimated that 800 million people worldwide are likely infected with *A. lumbricoides*, 451 million with hookworm and 435 million with *T. trichiura*. It has long been recognized that helminths polyparasitism is a common feature of human infections in helminths-endemic regions. These regions are also known to have a higher prevalence of intestinal protozoan infections, malaria, tuberculosis (TB), and HIV. [32]

Helminths polyparasitism and co-infection with major diseases are common in tropical regions. There are many factors that contribute to this phenomenon, including interactions between different species at the parasite community level within the host, and environmental factors at the host community level.

These factors can help explain why there is a positive relationship between different pathogens. However, there is still a need for new frameworks that can take into account these different factors at multiple levels to better understand how the parasite communities are structured and how they can be controlled.

1.2 Transmission modes of intestinal parasitic diseases

Examination of host-parasite interactions is crucial to understanding parasitic infections and how they adapt to their host, their study can help identify solutions for the prevention and treatment of parasitic diseases while exploring host-parasite communication.

Water-borne diseases (WBDs) are infectious illnesses, including those caused by bacterial, viral, and parasitic organisms, and are transmitted to humans through exposure to contaminated water in areas with inadequate sanitation, hygiene, and limited access to safe drinking water. [5,34]

The impact of water resources on the spread of enteric protozoa is significant, particularly when there is a risk of contamination of the water supply. Despite continued efforts to improve sanitation infrastructure, water quality, and environmental protection laws, leading to reduced pathogen levels in public water supplies, waterborne disease outbreaks have become a major concern for public health in developing countries.

The transmission of enteric protozoa through food is also possible but not as prevalent in developed areas. *Cryptosporidium, C. cayetanensis*, and *Giardia* are the leading enteric protozoa associated with foodborne infections, with approximately 10% of *Cryptosporidium* infections thought to be caused by this mode of transmission. [34,35]

Among the soil-transmitted helminths (STH) requires a passage through the soil to be infective. The modes of transmission can be divided into those that infect by the fecal-oral route, *Ascaris lumbricoides*, *Trichuris trichiura*, and those that infect by cutaneous penetration, hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis*. In Bolivia, recent surveys documented a dramatic decrease in the prevalence of STH infections as compared with the 1980s after thirty years of preventive chemotherapy, the World Health Organization (WHO) has now recommended discontinuing this intervention. [36]

Several studies have reported cases of parasitic, bacterial, and viral outbreaks worldwide related to sexual transmission, particularly among men who have sex with men (MSM). This has led to the recognition of several intestinal protozoa infections previously limited to tropical regions as sexually transmitted infections (STIs). [37,38]

1.3 Situation in emerging countries

These conditions cause a significant proportion of morbidity and mortality, primarily affecting people in developing countries. Additionally, they can also lead to major outbreaks in these regions. Notably, the WHO disclosed that diarrheal disease still affected more individuals than any other health condition, even in areas with high-income countries. [14]

Changes in the frequency, amount, duration, and intensity of precipitation may be the result of the impact of climate change on environmental conditions. This effect could be especially significant in emerging countries, where there is a prevalent risk of water borne diseases. As a consequence, the transmission and emergence of such illnesses could be increased. [5]

However, a vast number of diarrheal pathologies in developing countries, especially in children, are still attributed to an unknown etiology, constantly because the detection procedures, such as microscopy are applied despite their low sensitivity, specificity, and predictive values.

The most prevalent parasitic infections in low-resource settings correspond mostly to protozoan infections, among the most frequent are *Giardia intestinalis*, *Entamoeba* spp., *Cryptosporidium* spp., and *Cyclospora cayetanensis*. [14,38]

However common protozoa such as *Blastocystis* spp., and *Dientamoeba fragilis* are more frequently associated with asymptomatic infections or the healthy carrier condition. [39-40] Both have the potential to cause diseases classified as opportunistic in immunosuppressed, *Blastocystis* spp. has a relatively high prevalence in developed settings, but there is controversy regarding its pathogenicity. [40]

The persistence of gastrointestinal symptoms should be taken into account when identifying these carriers and assessing the need for treatment, especially in the pediatric population, in which growth retardation in weight and height may be associated with these infections, and special consideration should be given to the immunocompromised population. [41,42]

In emerging countries, opportunistic infections continue to be common causes of morbidity and mortality in PLWHA, which include those caused by parasitic infections and are frequently linked to chronic illnesses, poor adherence to ART, malnutrition, and the "wasting syndrome." Enteric infections are a major cause of diarrheal disease mainly in HIV/AIDS-infected patients. Among these, intestinal parasitic infections are common cause of morbidity and mortality in this population worldwide. [43,44]

1.4 HIV Issue

1.4.1 Characterization of the epidemic

Molecular epidemiology and phylogenetic analysis have become standard techniques to characterize HIV-1 transmission networks and reconstruct their origin and spread.

These approaches have the potential to identify population subgroups at risk, identify sub-epidemics, and inform prevention and intervention strategies aimed at mitigating the spread of the virus. [45,46]

PAHO and UNAIDS have categorized the HIV/AIDS epidemic into three levels: incipient or low-level, concentrated, and generalized. This classification is based on the prevalence of HIV in specific populations, such as pregnant women in urban areas and groups with higher-risk behaviors or those considered vulnerable, including commercial sex workers (CSWs), men who have sex with men (MSM), and injection drug users. [47]

1.4.2 Global situation and trends

The epidemiological and programmatic data of the HIV pandemic across nearly four decades show that over 80 million people have been affected by HIV-related causes and more than 40 million have died, with estimates ranging between 33.6 and 48.6 million. In 2021 alone, approximately 650,000 people died due to HIV, demonstrating a clear reduction in the public health response against the infection and disruption of HIV services caused by the COVID-19 pandemic. [47] (Figure 1)

The influence of this pandemic has caused an increase in the number of cases worldwide, especially in Latin America, where almost 4,000 people become infected every day. If this situation persists, an estimated 1.2 million new infections will occur by 2025, surpassing the 95-95-95 goals.

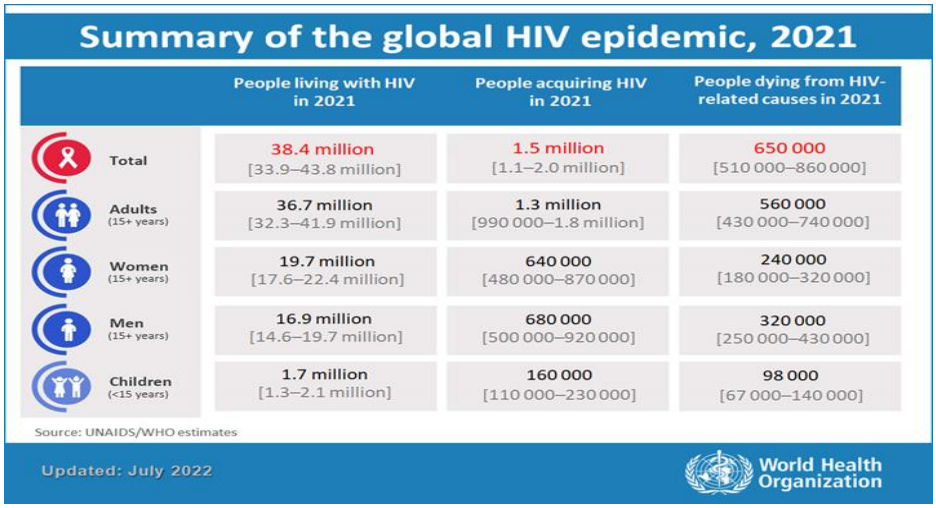


Figure 1: Summary of the Global HIV epidemic, 2021
Source: World Health Organization (WHO updated July 2022).

https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics

1.4.3 Global response to HIV, AIDS control
UNAIDS Strategy / 95-95-95

Globally, HIV/AIDS control policies are being pursued as per the UNAIDS strategy for 2030, which is commonly referred to as 95-95-95. The strategy's objective is to

ensure that 95% of all individuals who have contracted HIV will know their serology status that 95% of diagnosed HIV-infected individuals will receive effective antiretroviral therapy (ART) and that 95% of those undergoing ART treatment will have suppressed viral loads. [48]

The treatment cascade, designed to monitor the progress of the HIV epidemic and evaluate the outcomes of policies applied to struggle with the epidemic, will be critical to assess the achievement of the objectives set forth by UNAIDS as part of the global strategy. [49] (Figure 2)

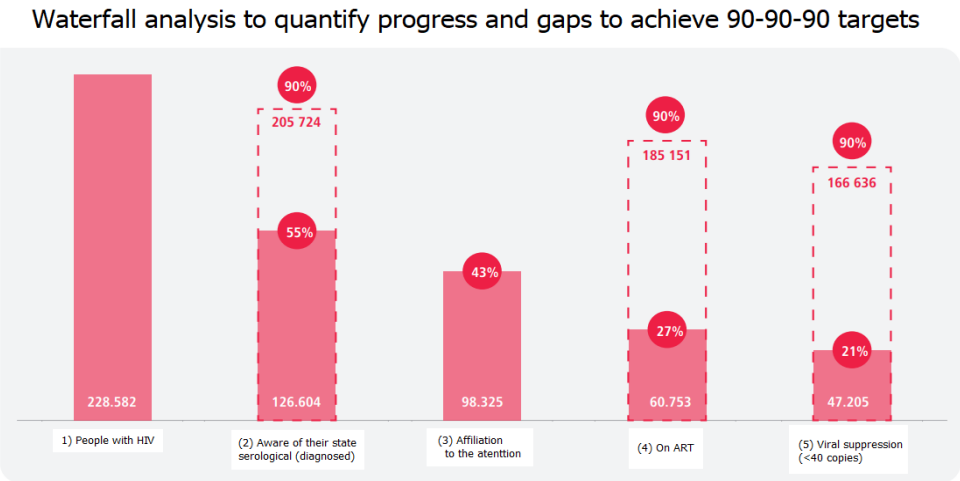


Figure 2: Continuum of Care Cascade / Analysis to Quantify Progress and Gaps to Achieve 90-90-90 Goals (hypothetical data)
Source: World Health Organization 2019

Various methods and sources of information were utilized to determine each aspect of the treatment cascade. For instance, the World Health Organization

(WHO) has introduced a guide for utilizing the data in the cascade to identify gaps in HIV and medical services and enhance programs. [49]

The report of UNAIDS in 2021 shows remarkable gains across the HIV testing and treatment cascade globally, founding that 84% of people living with HIV knew their HIV status, 73% were on antiretroviral therapy and 66% are virally suppressed. In Latin America and the Caribbean treatment, coverage reached 69%. [50] (Figure 3)

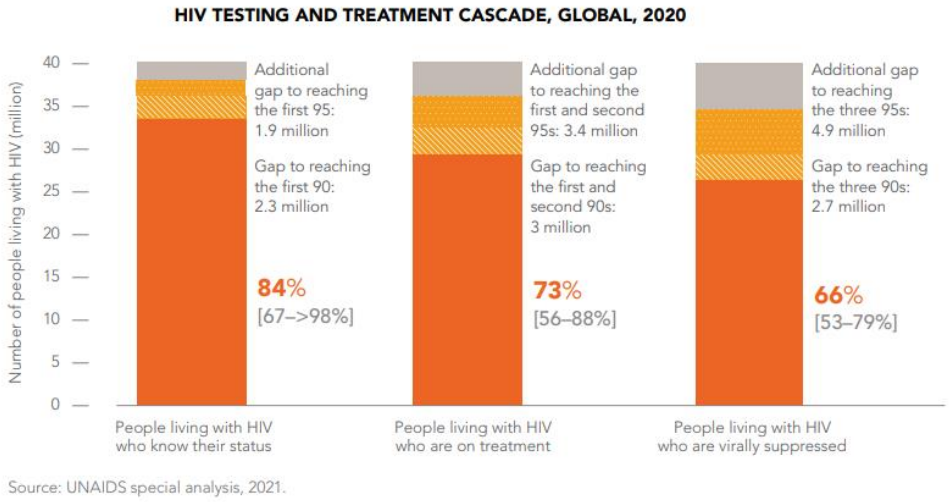


Figure 3: GLOBAL DATA UNAIDS / Continuum of Care Cascade, Progress and Gaps to Achieve 90-90-90 Goals
Source: UNAIDS 2021 DATA REFERENCE 2021

To conduct this analysis, the coverage and quality of healthcare services offered should be taken into account, which is deficient in various emerging countries. In Latin American countries, healthcare is predominantly provided in urban areas,

which has resulted in numerous HIV-positive individuals being uninformed of their status and having opportunistic infections as their initial symptom of the disease.

Certain individuals infected with HIV know about their illness but are unable to obtain antiretroviral therapy (ART) due to cultural, psychosocial, and financial constraints. Certain HIV patients are recommended ART but do not respond well to the treatment due to reasons such as non-adherence, unfavorable pharmacokinetics, and idiosyncratic biological factors that make them predisposed to drug-induced toxicity, causing adverse drug reactions. However, the cause of why only certain individuals are affected by drug-induced toxicity is still unclear, and many cases of drug-induced hepatotoxicity are eventually labeled as compound specific and idiosyncratic. [51]

Between 2010 and 2019, new infections increased by 21% in Latin America but declined by 29% in the Caribbean. The COVID-19 pandemic has accentuated the weaknesses in health systems, including financial, technical, and human resources. The socio-economic impact has been severe for key populations and threatens the sustainability of national responses. [52]

The goal of ending the AIDS epidemic is ambitious and requires global health and development efforts, as well as adequate resources. Without these measures, it may be difficult to attain the desired outcomes.

Three specific goals have been identified to ensure that 95% of all people living with HIV know their serological status, receive antiretroviral therapy, and achieve viral suppression. Furthermore, strategies must be developed to improve treatment

adherence to overcome the challenges imposed by the COVID-19 pandemic and safeguard progress toward the desired objective. [47,53]

Providing HIV treatment to all those in need is crucial for making tangible progress in combating the epidemic. To advance with a clear direction and accountability, a final goal is necessary to unite diverse stakeholders in an effort toward ending the HIV epidemic. [48,50,52]

The COVID-19 pandemic, is an opportune moment to implement a new approach and establish new goals for HIV treatment.

1.4.4 Status report on HIV treatment scale-up

The World Health Organization (WHO) reported that in low- and middle-income countries, almost 25 million individuals were receiving antiretroviral treatment (ART) by the end of 2020. The number of individuals receiving treatment grew on average by 1.6 million per year between 2017 and 2020, which indicates the effectiveness of the test and start initiative. The global target is to have 34 million people on treatment by 2025. [47]

There have been considerable obstacles to expanding treatment and meeting the UNAIDS objective of eradicating the AIDS epidemic. Although treatment coverage for adults and children has been increasing, there are significant gaps in coverage across different regions. It is estimated that more than 22 million people worldwide who are living with HIV are not receiving treatment, and in some countries, the individuals who do receive treatment tend to be the most accessible. [47,53]

A careful analysis shows significant disparities in access to HIV treatment between different regions or districts in the same country, indicating unequal treatment for people in some parts of the country.

Treatment coverage for HIV in 2021 on the African continent varied from 41% in Eastern and Southern Africa to 19% in North Africa. In regions outside Africa, limited or no progress has been made since 2020 in reducing AIDS-related deaths in countries across the Middle East, Eastern Europe, Central Asia, and parts of Asia.

This is primarily due to persistently insufficient treatment coverage, underscoring the need for more significant and sustained efforts to expand treatment coverage and address the obstacles that constrain access to treatment. [47,53]

1.4.5 Access to treatment and access to usual care

A status report on HIV treatment

To reach the global target of 34 million people on treatment by 2025, the number of people receiving antiretroviral therapy must increase by at least 1.3 million each year. [48,50]

Continued gains were made in 2021 across the 95–95–95 targets. In 2021, 85% of people living with HIV globally knew their HIV status, 75% of people living with HIV received antiretroviral therapy (representing 88% of those who knew their HIV-positive status), and 68% of people living with HIV achieved HIV viral suppression (representing 92% of people on treatment). Progress towards the 95–95–95 targets is apparent across all regions. In most regions, the largest gaps in the service

cascade are in the first two 95s: knowledge of HIV status and being on treatment. Continued gains were made in 2021 across the 95–95–95 targets.[41]

Latin America has made little progress in reducing new HIV infections in the region, with the number increasing by 5% in 2021. Reporting 2.2 million people in the region (1.5 million–2.8 million) were living with HIV. Among those living with HIV in 2021, 82% knew their HIV status, 69% were accessing treatment (85% of those who knew their HIV status) 63% were virally suppressed (91% of those on treatment). [50]

1.4.6 HIV Natural History

Based on genetic homology, HIV is classified into two predominant strains, HIV-1 and HIV-2, along with circulating recombinant forms (CRFs). HIV-1 is the most common and pathogenic strain, classified into three main groups: M (main or principal), O (outlier), and N (not M not O). Group M has the highest prevalence, with at least nine subtypes. HIV infection results in a wide range of initial clinical manifestations, making it challenging to diagnose early. [54-56]

The clinical spectrum includes primary infection as an acute retroviral syndrome, infection with early symptoms, later asymptomatic chronic infection, and advanced immunodeficiency, which can lead to opportunistic infections and pathologies associated with chronic HIV infection. [43]

Acute retroviral syndrome, the initial clinical manifestation of HIV infection, is challenging to detect due to its non-specific symptoms. This often leads to a

prolonged latency period during which many patients are unaware that are infected and can transmit the disease.

Asymptomatic HIV-infected persons suffer from a chronic, progressive disease, and viral replication continues in the body of untreated individuals, resulting in a gradual decline in cell-mediated immunity. This decrease is related to an increased susceptibility to opportunistic diseases and AIDS-related illnesses. [57]

HIV depresses the immune system, mainly by infecting CD4+ T lymphocytes, cells that organize the primary immune response. Without treatment, the natural clinical course of HIV infection varies widely, with some HIV-positive people having a high CD4 cell count and suppressed viral load, while others present with very low CD4 counts and a high viral load, even when asymptomatic. [57]

The mechanism behind the decline of CD4+ T cells in HIV infection has been extensively researched, and the role of virus replication in this process is becoming increasingly evident, among other factors that contribute to HIV-mediated immune activation. Patients with lower RNA viral loads often exhibit higher CD4+ T-cell counts, suggesting the involvement of viral replication. HIV-2 infection is characterized by lower and often undetectable viral loads, leading to an attenuated infection that progresses more slowly to AIDS. [58]

The advanced stage of HIV infection is called Acquired Immunodeficiency Syndrome (AIDS). [41,42] The progression of HIV infection is accompanied by great complexity due to the intricate interplay between the pathogen and the host, leading to varying clinical manifestations and ultimately, the onset of AIDS. [57]

According to the time of progression, people with HIV can be classified into different groups:

- Slow progressors, representing at least 5-10%, take 10 years or more to develop AIDS, and in some cases may even remain asymptomatic for more than 20 years. However, gradual progression occurs, with the loss of CD4+ T lymphocytes, leading to the development of AIDS eventually.
- Typical progressors, which make up to 80% of HIV-infected people, develop a latency period of 7 to 10 years.
- Rapid progressors, accounting for approximately 10-15%, progress to AIDS in 3 years or less.

Elite controllers make up a small group of HIV-infected patients who show an undetectable HIV viral load and normal blood CD4+ counts without progression. A multifactorial hypothesis suggests that host defense response, viral characteristics, and environmental factors may contribute to the variation observed in the natural course of HIV infection. [58]

Despite this evidence, there is still uncertainty about the factors that determine this variation, factors that contribute to the absence of long-term progression and viral control in HIV-infected individuals may differ between the virus and the host.

According to the World Health Organization (WHO), early initiation of ART reduces the risk of HIV transmission, AIDS-related illnesses, and death. Furthermore, evidence has demonstrated the clinical benefits of early ART, including improved survival rates, immune function, and quality of life. [59]

Ongoing research efforts should prioritize identifying the host factors causally related to these phenotypes. A comprehensive understanding of viral, host, and environmental factors in the natural course of HIV infection is essential in developing effective interventions that are tailored to the needs of HIV-infected individuals. [60]

The idiosyncratic characteristics of the host actively participate in the established natural selection for viral variants, through the expression of various receptors and co-receptors where the target cells and the virus interact. [61]

1.5 Risk factors for HIV infection

1.5.1 Host factors

Certain behaviors and factors can increase the risk of infection, such as engaging in unprotected sexual intercourse, having another Sexual transmitted infection (STI), receiving blood transfusions, or experiencing accidents with contaminated needles, particularly among healthcare personnel. Intravenous drug use is also a significant risk factor in certain contexts. [62,63]

In Latin America, sexual transmission remains the most common way of transmission of HIV.

1.5.2 Genetic factors

Host factors, such as polymorphisms in genes encoding chemokines and cell receptors that prevent viral entry into cells, have been extensively studied.

Evidence suggests that the presence of certain polymorphisms can decrease the risk of AIDS progression in many HIV carriers.

Heterozygous polymorphisms exhibit a significant reduction in the risk of progressing to AIDS, with a delay in progression ranging from two to four years. These effects may be associated with race, as some of the described polymorphisms are common in Caucasians, while others appear to be protective in African women. One significant factor is a series of polymorphisms in the chemokine receptors and co-receptors of the CD4 molecule. [58]

Specific deletions in the encoding genes result in an incomplete protein that cannot reach the cell surface, thus limiting the potential for infection by preventing the entry of the virus into the cell. These deletions are not uniformly distributed among the world population. [58,64,65]

Allelic variation in cytokine genes that inhibit co-receptor expression has been identified as important in the progression of AIDS. In multicenter studies that focus on the natural history of AIDS, heterozygous receptor expression is associated with reduced CD4+ cell counts and progression to AIDS. However, this effect disappears when baseline viral load is taken into account. [64]

Other genetic factors associated with the Major Histocompatibility Complex (MHC) are also linked to variability in the progression to AIDS, with some increasing the risk of a rapid progression.

Large cohort studies have shown that there is an association with slower progression when the major histocompatibility complex (class I) alleles are heterozygous. On the other hand, evidence suggests that having a higher number of homozygous alleles is associated with a worse prognosis. [66]

1.5.3 Viral factors

Viral aspects, including quasi-species development from numerous mutations and evading the immune system, contribute to variability in tropism and pathogenicity. In HIV-infected patients, differences in progression have been observed in those with viruses containing deleted genes. [67]

Studies have identified an association between recombinant strains of HIV and rapid progression to AIDS, particularly in Africa. [68] However, despite this finding, global and regional HIV infection rates do not seem to be significantly impacted, and bodies such as the WHO and UNAIDS do not issue alerts regarding these variants. When subjected to antiretroviral treatment, these variants have a similar efficacy profile.

1.6 Pathological process

While there have been continuous advances in our understanding of the pathophysiology of HIV infection, the specific mechanisms of virulence and pathogenicity of this disease require further investigation. [69,70] Therefore, a broader understanding of the pathogenesis of this infection is essential to establish control strategies and comprehend relevant manifestations such as primary infection, variations in progression to AIDS, and especially viral determinants in pathogenicity.

Viral replication is the cardinal event in the disease progression. HIV infection results in the high and constant production of new virions and the subsequent

destruction of CD4+ lymphocytes, known as the cytopathic effect. This effect is compensated for several years until the reserves are depleted, resulting in acquired immunodeficiency. Although there are several postulates on the specific mechanisms that trigger this effect, more research is needed to shed light on the topic. [70]

An explanation of the viral cytopathic effect exposes the loss of osmotic regulation and mechanical effect destroying the host cell, this could be explained by the massive exocytosis of thousands of virions. Another postulate describes the accumulation of harmful molecules, such as non-integrated viral RNA. [67]

Apoptosis or programmed cell death is also postulated, caused, or stimulated by viral replication and consequent susceptibility to certain mediators resulting in autoimmune cell destruction. [70]

1.7 Preclinical stage

Acute HIV infection, in general, manifests itself within 2 to 4 weeks after contact with an infected person, due to the non-specificity of its clinical presentation the vast majority of patients in this phase results unapparent. During this phase, most of those infected have non-specific flu-like symptoms such as fever, headache, and rash. Therefore, in most cases, HIV is not considered a differential diagnosis, a frequent situation in emerging countries. Establishing early diagnostic interventions is important, as a person can experience significant health benefits starting ART during this phase. [71,72]

1.8 Chronic HIV infection

Currently, HIV infection is considered a chronic pathology that persists despite ART, making it desirable to find ways to reduce the burden of chronic infection. Even though periods of remission have been described with therapeutics such as stem-cell transplantation. Therapeutic strategies in this regard, such as latency reversal agents and immunomodulatory agents, and other immunotherapies, should be evaluated based on the evidence. [69]

During this stage, viral replication continues, and the immune system gradually weakens. While certain individuals can progress several years without showing symptoms, others may present symptoms and worsening of the immune function only a few years after primary infection.

Chronic HIV infection generally progresses to AIDS in a period that can vary from 2 to 10 years or more. Currently, individuals receiving ART can remain in this stage for several decades. Even though transmission during this stage remains possible, the risk is considerably reduced when receiving ART as prescribed and maintaining an undetectable viral load while engaging in sexual intercourse. [71-73]

CHAPTER 2: INTESTINAL PARASITIC INFECTIONS AND HIV

CHAPTER 2:

2.1 Overview of parasitic infections and HIV

The specific burden of parasitic diseases in people with HIV is significant and has become a major public health problem worldwide, especially in developing countries with high rates of HIV and limited healthcare infrastructure.

Some of the parasites that can cause infections in people with HIV include:

- Toxoplasmosis, this parasite can cause an infection that can affect the brain and other organs in people with HIV. Cerebral toxoplasmosis is a common complication in HIV patients with a low CD4 cell count and may be one of the first opportunistic infections to appear in these patients. [74,75]
- Leishmaniasis, transmitted by mosquito bites, is an infection that can affect the skin, mucous membranes, and other organs. In people with HIV, may result severe and require prolonged treatment, is a common complication in HIV patients with a low CD4 cell count, frequency of the disease varies according to the geographic region and the immune status of the patient. [76]
- Chagas disease, transmitted by the bite of *Triatomas* insects, can cause a multisystem disease. In people with HIV, Chagas disease can be more severe and can affect the heart and digestive system. HIV-Chagas co-infection is an endemic problem in Latin America, and more effort is needed to detect and treat Chagas disease in HIV patients.[77]

- Malaria, transmitted by mosquito bites, and people with HIV are at increased risk of developing severe malaria. HIV-malaria co-infection remains a major problem.[78]

The specific burden of parasitic diseases in people with HIV is higher than in the general population due to the suppression of the immune system that occurs in people with HIV. Co-infection with parasitic diseases can also worsen the health of people with HIV and increase the risk of complications. [74-78]

Prevention of parasitic diseases in people with HIV includes maintaining good hygiene, education about safe food and water practices, treatment of parasitic infections with appropriate medications, and antiretroviral therapy including Pre-exposure prophylaxis (PrEP) to prevent HIV infections. In addition, early detection and treatment of parasitic diseases in people with HIV are essential to improve their quality of life and reduce mortality.[79-81]

Intestinal parasitic infections (IPIs) are a major public health concern worldwide, particularly in developing countries with poor sanitation and hygiene practices. These infections are caused by a variety of parasites, including protozoa and helminths, and can lead to a range of symptoms such as diarrhea, abdominal pain, and malnutrition. [82]

The co-infection of IPIs and HIV has become a significant issue, particularly in areas with a high prevalence of both conditions. HIV-positive individuals are more susceptible to IPIs and may experience more severe symptoms, leading to increased morbidity and mortality. This is because HIV weakens the immune system, making individuals more vulnerable to infections. [11-23]

Some of the common IPIs that affect HIV-positive individuals include Cryptosporidium, Giardia, Strongyloides, and Entamoeba histolytica. These parasites can cause chronic diarrhea, weight loss, malabsorption, and other gastrointestinal symptoms, which can lead to malnutrition and wasting syndrome.

To manage and prevent co-infection of IPIs and HIV, it is essential to implement strategies that improve sanitation and hygiene practices, provide appropriate treatment and care for individuals with HIV and IPIs, and increase awareness and education about these conditions. This includes regular screening and monitoring for IPIs in HIV-positive individuals, as well as providing access to antiretroviral therapy and medications to treat IPIs.

Despite the substantial shift in the global epidemiological profile in recent decades, known as the epidemiological transition. Parasitic diseases continue to contribute considerably to the infectious disease burden worldwide.

The incidence and severity of these diseases may be higher in immunosuppressed patients and their pathogenicity may be uncertain. Many self-limiting and sporadic intestinal parasites have now become opportunistic parasites causing uncontrollable life-threatening diarrhea among people living with HIV/AIDS. [11-32]

Various enteric protozoa cause zoonotic diseases transmitted from animals to humans (anthropozoonosis) and are associated with poor sanitary conditions, becoming an emerging issue of public health. As humans move further into wildlife domains, parasitic diseases can pose a serious threat to wildlife, which in turn can act as reservoirs and/or amplifiers of emerging and re-emerging diseases. [9,83]

Enteric protozoan species are associated with gastrointestinal and diarrheal pathologies in humans, and some of these agents cause serious illness, especially in immunosuppressed populations. [35,84]

Intestinal parasites, especially coccidian parasites, cause gastrointestinal symptoms such as severe diarrhea which increases morbidity and mortality rates in PLWHA.

Co-infection of IPIs and HIV is a significant health issue that requires a multifaceted approach to prevent and manage. Improved sanitation and hygiene practices, regular screening and monitoring, and appropriate treatment and care can help reduce the burden of these conditions on individuals and communities affected by HIV.

2.2. Intestinal parasitic infections in PLWHA

The advanced and severe phase of HIV infection, known as acquired immunodeficiency syndrome (AIDS), results in a weakened immune system that is unable to combat opportunistic infections, as well as HIV-related diseases. Currently, individuals with HIV are diagnosed with AIDS when their CD4+ cell count is less than 200/mm3, or when certain opportunistic infections are detected.[43,84]

These situations increase the risk of infection by intestinal parasites, which are considered to play an important role, especially with the progression of immunosuppression, establishing chronic and relapsing diseases that are underdiagnosed and subsequently generating a cycle that leads to malnutrition, higher levels of immunosuppression, and new opportunistic infections. [43,44]

Immunosuppression facilitates opportunistic parasitic infections, leading to more severe clinical symptoms and a higher prevalence of parasites. Both opportunistic and non-opportunistic are commonly found in HIV-infected patients. However, opportunistic intestinal parasites, such as *Cryptosporidium parvum*, *Isospora belli*, *Microsporidia* spp., and *Strongyloides stercoralis*, are significantly more frequent in HIV patients with low immunity and diarrheal symptoms. Also, *Blastocystis hominis* and some of the non-pathogenic *Entamoeba* can cause OI in HIV-infected patients with immunosuppression. [85]. (Figure 4)

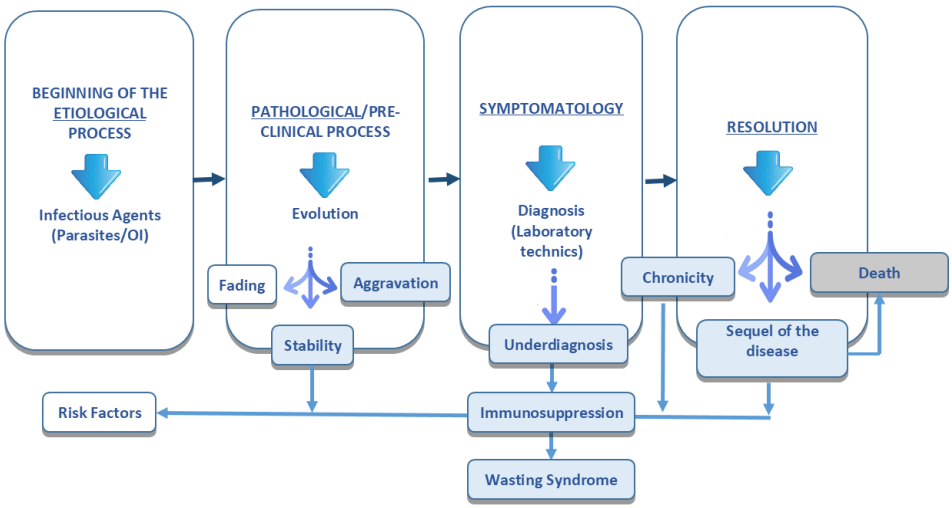


Figure 4: Conceptual framework of parasitic infections (IO) in PLWHA
Source: Own elaboration

Diarrhea is characterized by the abnormal passage of unformed or liquid stools at an increased frequency. It is classified as acute if it lasts for less than 2 weeks, persistent if it continues for 2-4 weeks, and chronic if it lasts for more than 4 weeks. Intestinal parasites are a common cause of severe chronic diarrhea in HIV-positive

patients, and some types of parasites are more prevalent in this group of patients. [86,87]

Common types of intestinal parasites that infect HIV patients are *Entamoeba histolytica, Giardia lamblia*, and *Cryptosporidium parvum* [86]. In addition, *Microsporidium* spp., *Cystoisospora (Isospora) belli*, and *Strongyloides stercoralis*, among other parasites, are also known to infect HIV patients. The rate of infection with these parasites is higher in HIV patients with a CD4 count below 200 cells/µL [88].

Opportunistic parasites, such as *Cryptosporidium parvum*, *Isospora belli*, and *Microsporidium* spp. are often associated with persistent diarrhea in PLWHA. However, these parasites can also frequently present asymptomatically. [84,88]

Coccidia parasites cause gastrointestinal symptoms such as severe chronic diarrhea which increases morbidity and mortality rates in PLWHA. Many self-limiting and sporadic intestinal parasites have now become opportunistic parasites causing uncontrollable life-threatening diarrhea among the population with AIDS. [43,86]

The etiological agents vary from one patient to another according to the immunological status of the patients, the geographical distribution from one country to another, and in addition, the endemicity and seasonal variation of enteric pathogens must be considered.[87,88]

CHAPTER 3: HIV AND PARASITIC INFECTIONS IN BOLIVIA

Chapter 3. HIV and parasitic infections in Bolivia:

3.1 General context of Plurinational state of Bolivia

The Plurinational State of Bolivia, located in the central region of South America, is administratively organized into nine autonomous departments, but with some dependence on the central government, which is located in the city of La Paz. Its constitutional capital is Sucre, the headquarters of the judicial body; its total area is 1,098,581 km2 with 12,186,079 inhabitants (estimation for 2023), and the density of 10.36 inhabitants/km² estimated for 2019, reaching 26.2 in the Cochabamba department, geographical area of study location.

The country is divided into four geographic regions:

The Andean Region covers a third of the territory and is home to approximately 40% of the Bolivian population (2023 est.), occupies an area of 142,815.53 km², and is comprised of the Andes Mountains. It has a cold and dry climate during most of the year. The highest peaks in the country are found in these regions.

The Amazon Region is one of the largest continuous forest ecosystems in the world. The region is home to numerous native ecosystems and cultures. The Bolivian Amazon is considered one of the most pristine and well-preserved areas in South America. It constitutes 40% of the national territory, 1 comprises the departments of Pando, Beni, and the north of the departments of La Paz, Cochabamba, and Santa Cruz. (Figure 5)

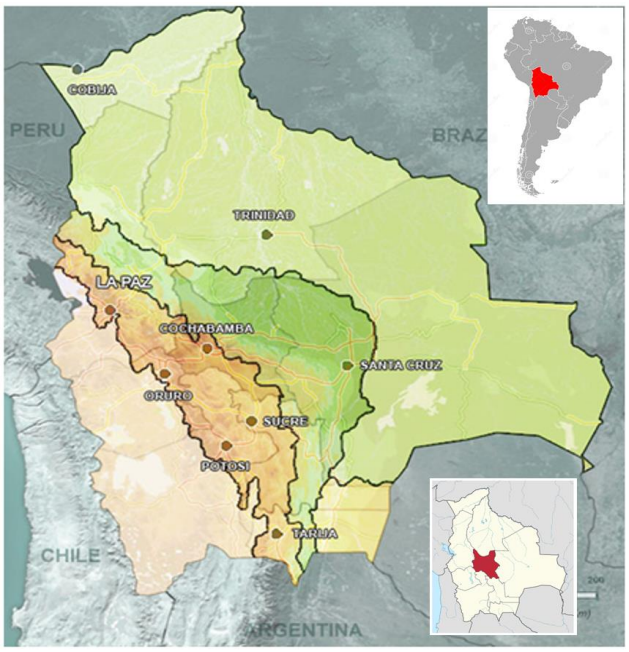


Figure 5: Geographic regions of Bolivia / Cochabamba
Source: Bolivia Country Document - Disaster Preparation Program of the European Commission (2017)

The urban population represents 84.1% of the total population, according to INE estimates for 2023. Most of the country's population is concentrated in the departments of Santa Cruz, Cochabamba, and La Paz, which gather more than 70% of the Bolivian population. Women represent 54%. The population pyramid of 2020 showed a predominantly young population with a median age of 24.6 years. (Figure

6.)

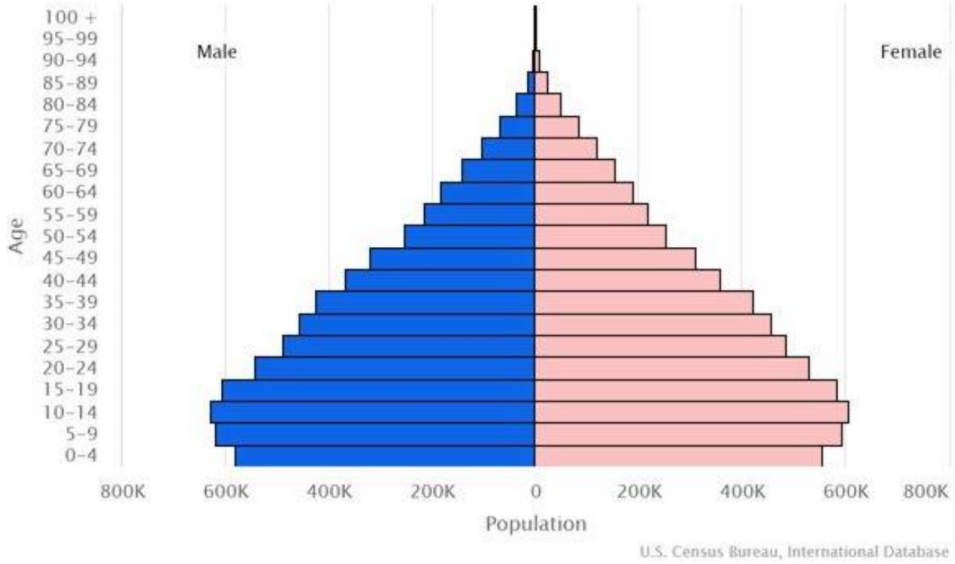


Figure 6: Bolivia: distribution of the population by age groups, 2020
Source: www.CIA.gov

Bolivia ranks at or near the bottom among Latin American countries in several areas of health and development, including poverty, education, fertility, malnutrition, mortality, and life expectancy. Bolivia income inequality is the highest in Latin America and one of the highest in the world. Public education is of poor quality, and educational opportunities are among the most unevenly distributed in Latin America.

Cochabamba is one of the nine departments that make up the Plurinational State of Bolivia and is the third most economically important in Bolivia. (Figure 7)

Figure 7: Political division of Bolivia / Geographic regions of Bolivia (Nine Departments)

Source: Bolivia Country Document - Disaster Preparation Program of the European Commission (2017)

Capital of the homonymous department and the Cercado province. It has a population of 2,117.112 inhabitants in the metropolitan area according to the estimation of the National Institute of Statistics (INE) by 2023.

3.2 Sanitary conditions, water and food security

The provision of safe water and sanitation services is vital to reducing the burden of disease, and investment in water and sanitation interventions has numerous benefits. Despite this, specific data on the hygienic-sanitary quality of food in Bolivia is not available, government and Bolivian organizations have made efforts

to improve food safety and hygiene standards in the country. In addition, initiatives regarding food safety and hygiene best practices have been implemented.

In Bolivia, it must be emphasized that the appropriate conditions for services such as drinking water and alimentary security are distributed unequally among urban and rural areas, in the latter these conditions practically do not exist, and geographical conditions make access to these services greatly difficult. (Figure 8)

Figure 8: People using at least basic drinking water services (% of the population) - South America / Distribution of water, Sanitation, and Hygiene- 2017

Source: WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene (washdata.org).

The previous figure shows how People using at least basic drinking water services (% of the population) varies by country in South America. The shade of the country corresponds to the magnitude of the indicator. The darker the shade, the higher the value. The country with the highest value in the region is Chile, with a value of

100.00. The country with the lowest value in the region is Peru, with a value of 93.14. Bolivia reaches 93.9, according to Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene (washdata.org).

3.2.1 Health system organization in Bolivia

The Bolivian health system is organized into two large sectors: public and private but long, and short-term social security, NGOs, the church as well as traditional medicine are also described. The public sector includes the Ministry of Health and Sports (MSD) and the social security subsector. The MSD establishes four management areas: (Figure 9).

1) National, corresponding to the MSD itself.

2) Departmental, corresponding to the Departmental Health Service (SEDES), dependent on the government

3) Municipal, corresponding to the Local Health Directory (DILOS), and

4) local, corresponding to the health establishment in its area of influence at an operational level. The private sector is made up of insurance companies and private health service providers for and without profit.

According to the Constitution of Bolivia, all persons have the right to free public health insurance. Bolivia is one of the few countries in the region implementing a universal health coverage model, focusing on the right to health care. The Bolivian government set out to provide free universal coverage to 50% of the population, doubling previous coverage rates. However, by 2020 only 30.6% of the population

had access to the benefits of this insurance through the social subsector, according to WHO only four out of ten people in Bolivia access health services.

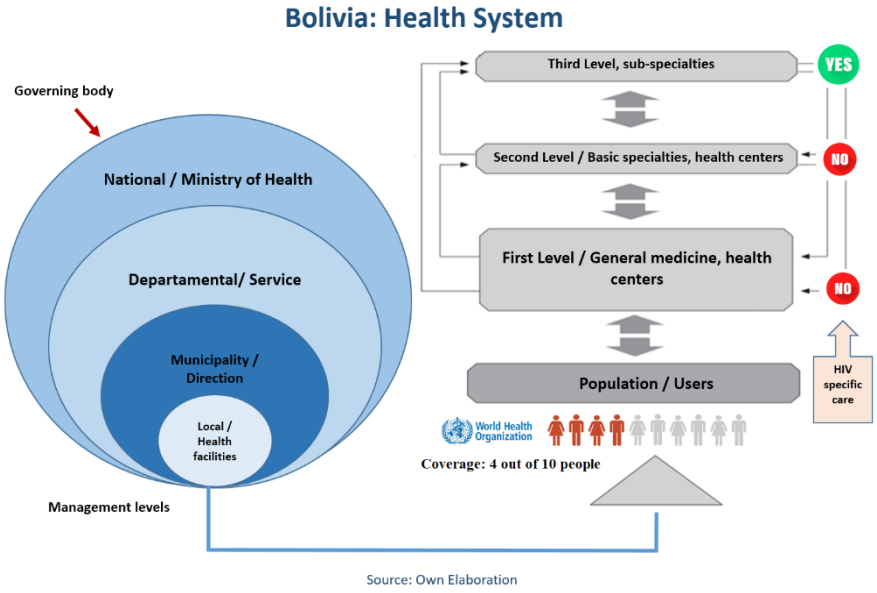


Figure 9: Health System Organization in Bolivia
(Source: Own elaboration)

The Ministry of Health and Sports (MSD) serves less than half of the total population through the public subsector. The social security subsector is made up of Compulsory Social Security for people belonging to the formal economy sector, short-term (health services), and long-term insurance (which is the responsibility of the Pension Fund Administrators). The private sector offers services for 10% of the population and functions primarily based on direct pocket payments. Around 30% of the population does not have more access to health care services than that offered by traditional medicine, directly charged to their income.

The financing of the health sector uses its resources for current expenditure and depends on external resources (donations, credits) for health investment projects. The public subsector is financed based on public funds allocated to municipalities in terms of per capita allocations (20% of the central government's tax revenues) and uses the infrastructure of the MSD.

The social security subsector is financed with contributions provided by employers and workers in the formal sector and with state re-courses when the latter functions as an employer (education, health, public companies, decentralized institutions, and ministries) and has its establishments and personnel.

The private sector is divided into for-profit organizations (private insurance and services, clinics) and non-profit organizations (NGOs and services provided by the Church).

Bolivia has feeble health indicators. Although its infant mortality has declined in recent years - from 46 per 1000 live births in 2003 it decreased to 22.3 in 2023 - it is still high in the region and is above the Latin American average.

The same applies to mortality in children under five, which in 2021 reached a figure of 27 per 1000 live births. Deaths in children under five are mostly due to preventable diseases. One of the most serious problems is chronic malnutrition, which affects a quarter of the preschool population. The prevalence of this problem is even greater in children living in rural areas (37%).

The maternal mortality ratio amounted to 161 deaths/100.000 live births in 2020, which is, the second-highest rate in the Latin American region. There are still large

differences in access to this type of service between different socioeconomic groups and different ethnicities.

Life expectancy at birth has been increasing. According to INE estimates, in 2008 it was 72.5 years (71 years for men and 74 years for women). These facts, however, are much lower than those of the other Andean countries.

However, non-communicable diseases concentrate an increasing percentage of deaths in the country. Among the latter, neurological disorders and digestive illnesses stand out, and infectious diseases such as HIV and enteric infections continue to represent a high burden.

3.2.2 Special Health system organization in the context of HIV

Regarding HIV care, the health system is concentrated in the public sector and urban areas, despite the existence of patient care and follow-up services, the lack of specialized laboratory tests both for confirming the infection and for monitoring the immune status and detecting opportunistic diseases.

These services refer patients to the public health system, which is also the only instance responsible for the distribution of antiretroviral treatment, in this way the private health system is forced to register their patients and perform their laboratories and diagnostic tests in the public system limiting their attention to patient monitoring and comprehensive services such as physiotherapy, dentistry, psychological and nutritional counseling, thus complementing services that are not offered by the public sector. (Figure 10)

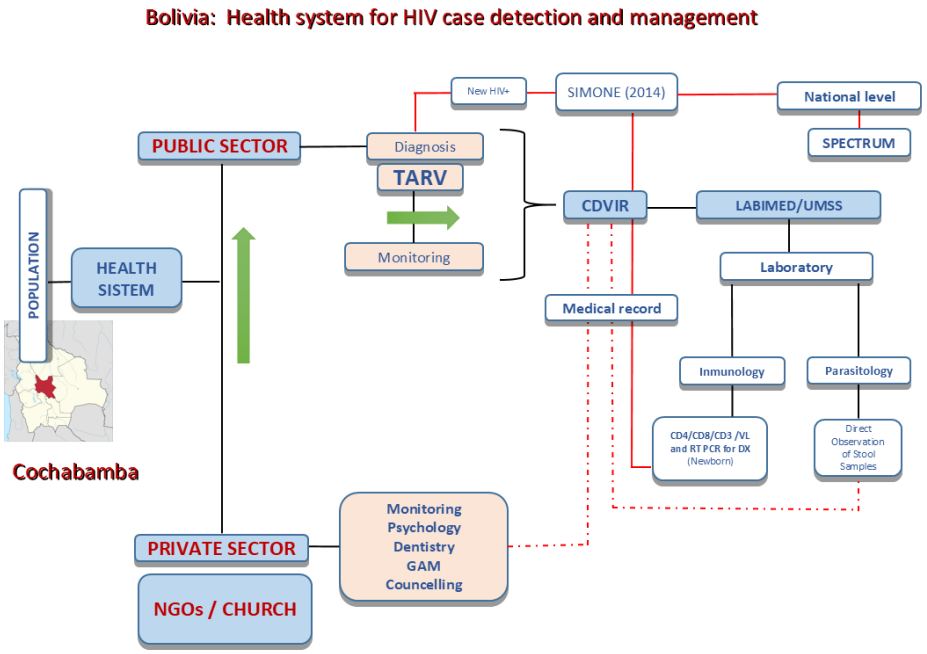


Figure 10: HIV-related health system organization in Bolivia
(Source: Own elaboration)

3.3 Characteristics of the Intestinal parasitic infections in Bolivia

Parasitic infections, including intestinal parasitosis, are considered endemic in Bolivia and affect both children and adults. There is a lack of national data on the prevalence of parasitic infections in Bolivia, and there is no national data available. The statistical information available is limited due to insufficient research and surveillance in this area.

This situation has prompted the World Health Organization (WHO) to recommend further research in this area.

In Bolivia, preventive chemotherapy (PC) based on the biannual administration of a single dose of mebendazole to the school-age children was applied since 1985 and has achieved results documenting a dramatic decrease in the prevalence of hookworm, *A. lumbricoides* and *T. trichiura*, Based on these findings, and following WHO recommendations, in 2016, they discontinued the delivery of PC and started a surveillance program through annual cross-sectional parasitological surveys in sentinel sites. [36,90]

The prevalence of infection by common soil-transmitted helminths (STH), *Ascaris lumbricoides, Trichuris trichiura,* and *hookworm* infection is among the highest in Latin America. However, in Bolivia, the spatial distribution and burden of soil-transmitted helminths infections are poorly documented.[90,93,95]

It is estimated that almost 38.0% of the Bolivian population is infected with some generous *A. lumbricoides, T. trichiura*, and *hookworm*, respectively. Assuming independence from the three infections, 48.4% of the population is infected with any soil-transmitted helminths. [92]

Empirical-based estimates, according to treatment recommendations by the WHO, suggest a total of 2.9 million annualized treatments for the control of STH in Bolivia. [92-94]

A study carried out with the general population in Cochabamba in 2015 identified the most common parasites as *Blastocystis hominis*, with a rate of 44.5%; *Giardia*

lamblia, with a rate of 10.6%; and *Entamoeba histolytica/dispar* at 8,1 %, and were classified as pathogenic intestinal protozoa. [95]

Previous studies' findings demonstrate intestinal parasitic infection rates are higher in lowland Bolivia than in high-altitude areas. Protozoan infection was shown to be high in both lowlands and high-altitude areas. Therefore, we should pay the same attention to helminths and protozoan infections. [90]

3. 4 Availability and accessibility of diagnostic

Although complex laboratory techniques have been developed to allow better identification of these infections, such as immunological and molecular tests, availability and accessibility in the context of emerging countries should be evaluated.

Bolivia's overall health infrastructure and resources are limited, which directly affects the availability and accessibility of diagnostic tools and treatments for parasitic infections.

In the context of PLWHA care in Bolivia and through a global fund grant, only direct microscopic examinations of stool samples for the presence of intestinal parasites are available. Performed through a protocol of care and should be performed at least once a year during routine follow-up controls. Access to other diagnostic methods is limited by cost and availability and can only be accessed in private laboratories or research processes.

Microscopic examination is a frequently used diagnostic method for detecting intestinal parasites, but it has limitations that must be carefully considered to

achieve accurate diagnosis and treatment of infections. These limitations include low sensitivity, and low specificity.

Additionally, technical expertise is required for the application of microscopic examination, and well-trained and experienced laboratory technicians are necessary to correctly interpret and identify the results.

Precise diagnosis by microscopic examination requires appropriate collection, processing, and storage of specimens. Unfortunately, the substandard laboratory conditions in Bolivia restrict this aspect.

This technique is limited in detecting certain agents such as Coccidia and *Cryptosporidium* sp. due to low sensitivity and specificity. Thus, various other techniques, including immunochromatography, special staining, and molecular biology, are needed to provide an accurate diagnosis and avoid sub-diagnosis. These techniques cannot be performed in low-resource settings, so their availability is limited. [89]

3.5 Characteristics of the Intestinal parasitic infections in PLWHA in Bolivia

There are no studies in Bolivia on intestinal parasite infection in people with HIV. We highlight the lack of effective surveillance systems related to the care and monitoring of HIV patients in Bolivia, and more specifically on opportunistic infections such intestinal parasites, these are some of the reasons why it may be

difficult to find updated information on these infections in some geographical areas of the Latin American context.

3.6 Characteristics of HIV infections in Bolivia

In the region of the Americas, and in Bolivia antiretroviral therapy is widely available in the context of patients with a confirmed diagnosis recruited by the public health system. [52-53] In addition, these patients receive medical services that are available in a complementary manner in the public and private sectors, some benefits not available in the public domain can be found at the private level in Non-Governmental Organizations (NGOs).

This complementarity seeks comprehensive health care. Despite the existence of a private care system for PLWHA, in Bolivia by law, all patients diagnosed with HIV must confirm their diagnosis, be monitored, and receive ART in the public system.

The data referring to the cascade of the continuum of care in Bolivia for 2022 consider an estimated 28,000 patients, 26.245 (93%) patients with confirmed diagnoses, 16.411 (59%) with ART, and 12.156 (43%) with suppressed viral load. (Figure 11)

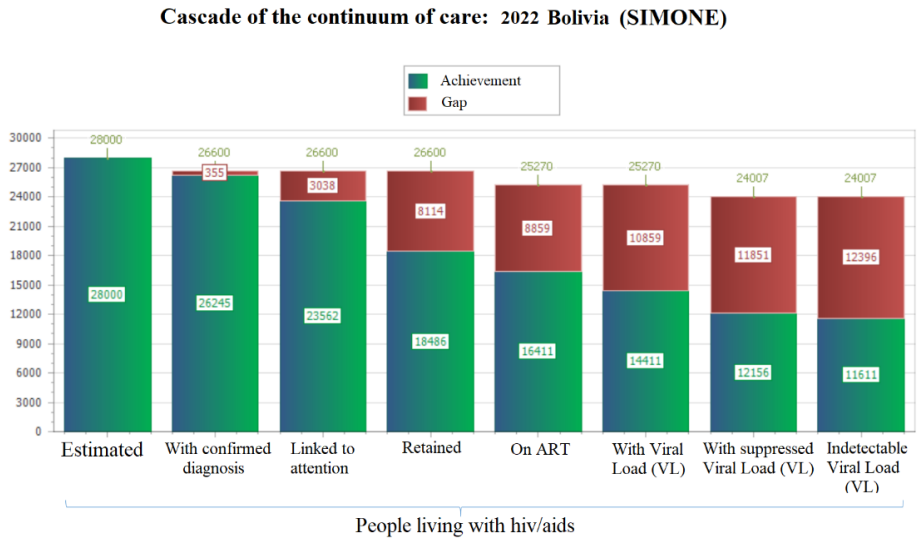


Figure 11: Continuum of Care Cascade, Progress and Gaps to Achieve 90-90-90 Goals – Bolivia 2022

Source: HIV program monitoring and evaluation system (SIMONE)

http://simone10.vih.org.bo:8082/ContinuoAtencion.aspx

The data corresponding to Cochabamba, considering the periods in which the interventions described in our research were carried out estimated 3,928 patients with confirmed diagnoses 1.847 (47%) with ART, and 554 (14%) with suppressed viral load, the update for 2022 is also shown below. (Figure 12)

Although there is an estimate of the PLWHA population of approximately 3535 patients, about 500 patients performed periodic controls every 3 months in 2017, this was a criterion to limit the sample size because after this period the same patients were received again saturating the sampling process.

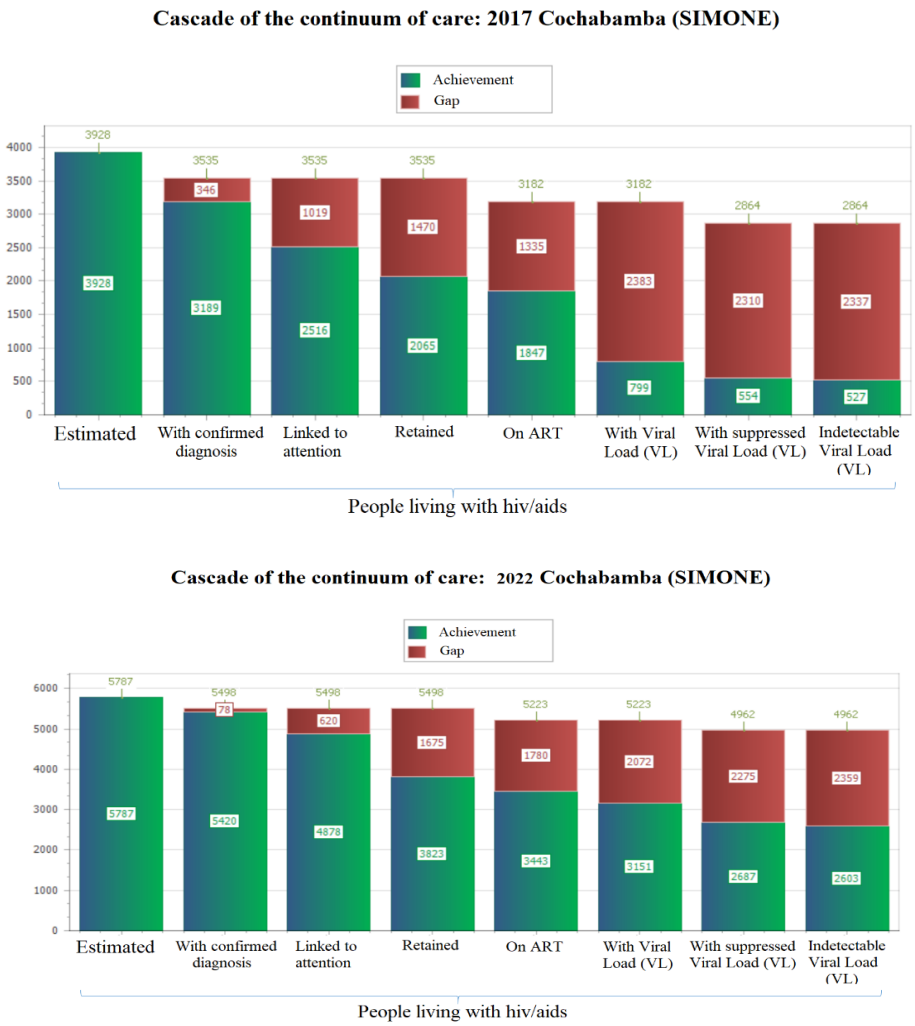


Figure 12: Continuum of Care Cascade, Progress and Gaps to Achieve 90-90-90 Goals – Cochabamba 2017 and 2022.

Source: HIV program monitoring and evaluation system (SIMONE)

http://simone10.vih.org.bo:8082/ContinuoAtencion.aspx

3.7 Epidemiology of HIV in Bolivia

By the end of 2020, UNAIDS had estimated that 25,000 people in Bolivia would be living with HIV/AIDS. The departments most affected and with the highest notification of cases in the country, especially at the expense of their capital cities, are Santa Cruz, Cochabamba and La Paz. The predominant route of transmission is sexual with more than 90%. [47]

The prevalence of HIV is low in Bolivia, with only 0.3% (2021 est.) concentrated in the population between 15 and 49 years of age. HIV/AIDS is currently a public health problem due to the constant and continuous spread of the disease. Early detection of infection is a priority public health strategy for targeting and distributing prevention messages, and early and opportune treatment of the disease. In Bolivia, about 12% of the total number of people notified at the time of diagnosis are in the AIDS phase, with late detection of the disease.

In Bolivia the HIV / AIDS epidemic is of a concentrated type, with prevalences above 5% in vulnerable populations, mainly in MSM population groups. The cumulative cases of HIV / AIDS reported in the country between January 1984 and 2021 amount to 29.577, with an estimated national underreporting greater than 10%.

The ratio of reported HIV/AIDS cases for men to women is greater than 2. Although a feminization of the epidemic can be assumed, since the male to female ratio has decreased considerably in recent years, at the beginning of the epidemic it was 9: 1. It is important to highlight that the offer of rapid HIV tests to women pregnant women and CSW is higher than that made for men. [96]

The prevalence of HIV in the MSM population reported in Bolivia by 2021 is close to 15%, and the highest prevalence in MSM is observed in the Department of Santa Cruz. The prevalence in CSW reported through the Departmental Centers for Surveillance and Reference of STI/AIDS (CDVIR) and other epidemiological surveillance studies carried out in the country is less than 1%, according to the National Program report in 2021. (Figure 13)

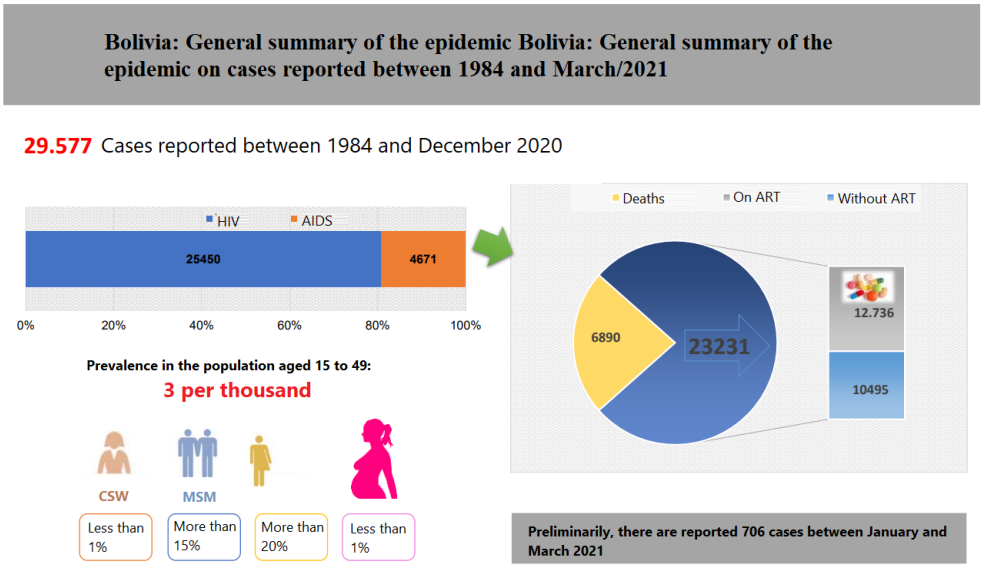


Figure 13: General summary of the epidemic on cases reported between 1984 and March 2021 (Bolivia)
Source: National HIV / AIDS / STI Control Program

Short information is available on the impact of HIV/AIDS on certain population groups, but it is estimated that HIV is reaching groups that have not been taken into account by national policy, such as the street population, clients of the CSW, and the rural population (estimated prevalence: 0.2%, 3.5%, and 0.05%, respectively),

and has reached all socioeconomic levels, covers all ages, and has speared the rural environment. [96].

Over the course of 2011 to 2021, there has been a perceptible increase in the number of new asymptomatic HIV and AIDS cases. (Figure 14) This trend contrasts with reports from other countries in the region, which have recorded a decline in the number of new cases.

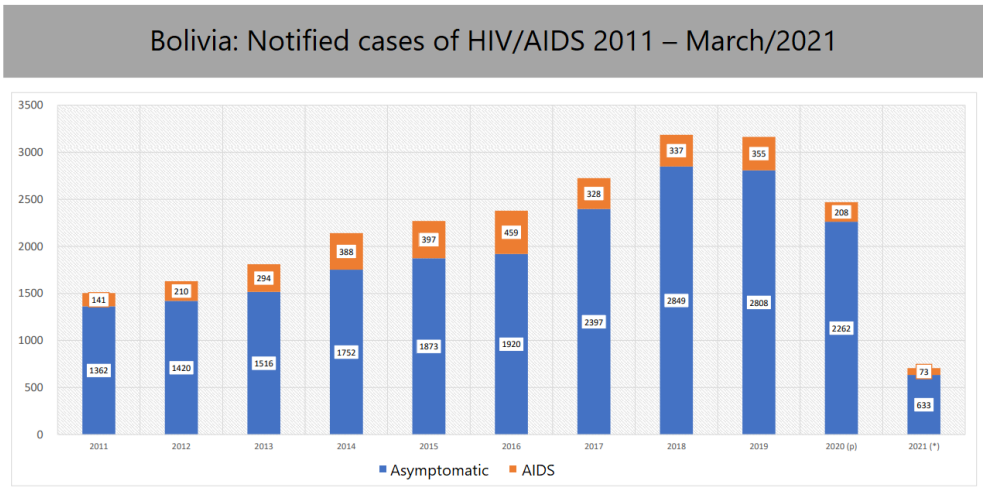


Figure 14: Notified cases of HIV/AIDS 2011 – March2021, incidence to 2021 (Bolivia)
Source: National HIV / AIDS / STI Control Program

The rate of AIDS infections increased in Bolivia by 22%, according to the global annual report in 2021 by the Joint United Nations Program on HIV / AIDS (UNAIDS). Positioning the country as the second with the highest rate of infections after Chile (34%). [47]

Despite this, according to the National Program report in 2021, life expectancy in Bolivia for PLWHA increased from 5 to 20 years in the last decade, due to anti-retroviral therapy (ART).

HIV prevalence is concentrated in this main axis of the country, with a higher incidence in Santa Cruz (45.4%), followed by La Paz (21.5%) and Cochabamba (19.3%), the graph to the right shows the concentration in the urban area. (Figure 15)

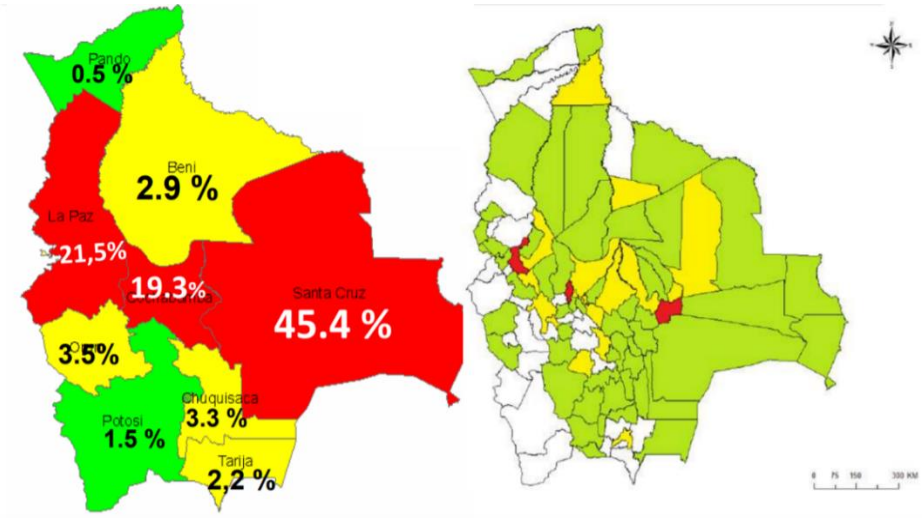


Figure 15: Distribution of reported cases of HIV / AIDS by Department (%), accumulated to 2018 (Bolivia)
Source: National HIV / AIDS / STI Control Program

Healthcare resources for managing all aspects of HIV, including prevention, epidemiological surveillance, diagnosis, healthcare, and therapy, are concentrated

in the urban areas of Bolivia's "Trunk axis," highlighted in red in the graph, where the highest prevalence rates are reported. These resources are largely funded by international cooperation, with up to 90% subsidization from the resources.

3.7.2 Sociocultural context in connection with HIV in Bolivia

Bolivia is a multiethnic and multicultural country with scarce resources. Migratory processes, combined with social factors such as excessive alcohol consumption, a lack of awareness and knowledge regarding HIV/AIDS prevention and transmission, high rates of violence and prejudice, and false beliefs on the subject, facilitate the rapid transmission of STIs and HIV in the country.

There is limited information on the processes and dynamics of sexuality and its determinants among a certain vulnerable population in rural migrant areas, which will make it possible to design appropriate prevention and care strategies aimed at reducing the rate of STIs and HIV/AIDS. Studies conducted in migrant communities and Quechua-speaking municipalities in the department of Chuquisaca show that a significant segment of the population is unaware of HIV/AIDS/STIs and how to prevent them. Condom use is low and the population frequently engages in risky sexual practices. [97]

3.7.3 Antiretroviral therapy (ART) in Bolivia.

Latin American governments have committed to ending the AIDS epidemic by 2030. This commitment is in line with the Political Declaration on HIV and AIDS approved by the United Nations General Assembly in June 2016 and, more recently, specific goals have been set in Latin America for 2030. [53,62]

During the period 2000 to 2020, the number of people accessing antiretroviral therapy in Bolivia has increased significantly in response to UNAIDS global strategy, reaching 12,438 patients by March/2021. [96] (Figure 16)

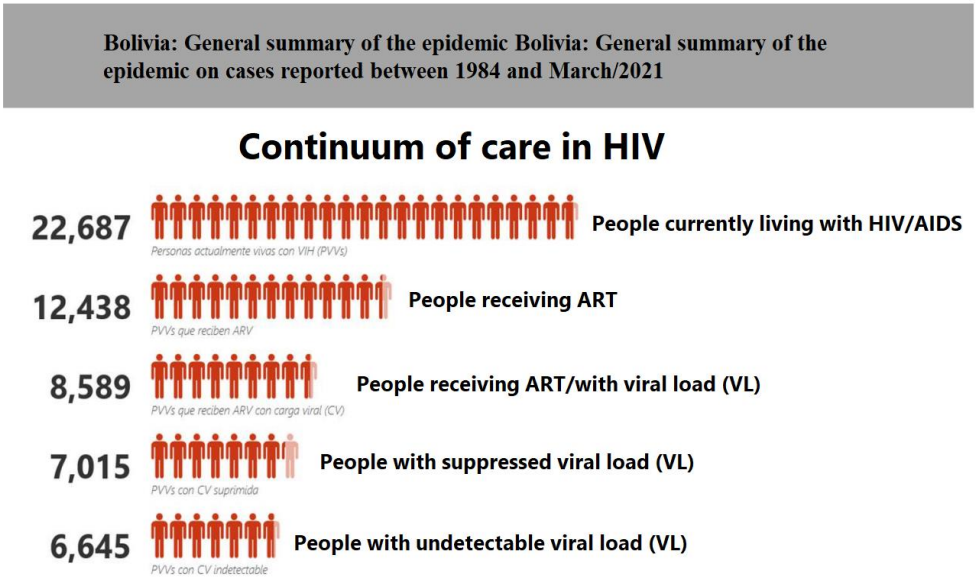


Figure 16: Continuum of care in HIV, General summary of the epidemic on cases reported between 1984 and March 2021 (Bolivia)
Source: National HIV / AIDS / STI Control Program

For 2021 Bolivia is one of the Countries whose national standard recommends: TDF+3TC as the basis of the first line scheme, with the addition of dolutegravir (DTV) as the first recommendation and Efavirenz (EFV) as an alternative. (Figure 17)

Recommended first-line ART regimen in Bolivia (2021)

Basis of the 1st Line scheme	1st Line recommended	1st Line alternative
TDF + 3TC	+ DOLUTEGRAVIR (DTG)	Or EFAVIRENZ (EFV 400 mg)

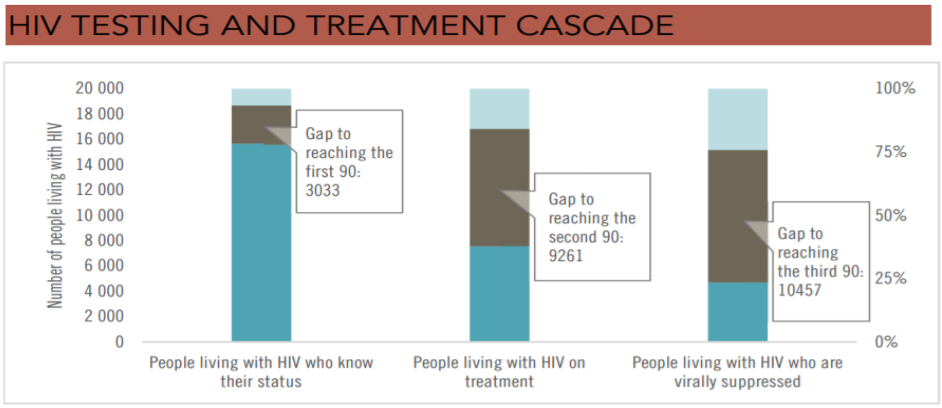
Figure 17: Recommended First-line regimen for ART in Bolivia (2021). Source: Own elaboration based on the ANTIRRETROVIRAL THERAPY GUIDE FOR ADULTS (UPDATE 2020 /2021)

Access to PrEP through public health services is almost nil in the region. Only Brazil has it available in some health centers. In other countries, for now, it is available through phase four clinical studies or feasibility studies. In some, it can be accessed by buying it in private pharmacies. Such is the case of Bolivia, its approximate cost is 100 Euros, which represents a clear limitation to access.

The main barrier currently facing PrEP in Latin America is the regulation of its prescription. Both tenofovir and emtricitabine are authorized in the Latin American markets for the treatment of HIV infection -and many countries have included them in their first-line regimens-, but they still do not have authorization for their prophylactic use.

3.7.4 Testing and treatment

Although it is understood that at the level of Latin America, the information is deficient and the national reports overestimate some data for political purposes Bolivia is not the exception. The cascade of HIV testing and treatment in Bolivia is reported in the progress report of the 90-90-90 strategy with an advance of 75% in the first 90, with a gap that estimates consider that it could cover only 50% of the cases nationwide, the second 90 reaches 36% coverage, and the third goal of this strategy would reach 23% by 2018. (Figure 18).



	People living with HIV who know their status	People living with HIV on treatment	People living with HIV who are virally suppressed
All ages	75% [49– >95%]	36% [24–55%]	23% [15–34%]
Children (0–14)	>95% [74– >95%]	62% [42–88%]	56% [38–79%]
Women (15+)	83% [56– >95%]	41% [28–61%]	25% [17–37%]
Men (15+)	71% [45– >95%]	34% [21–51%]	21% [13–32%]

Figure 18: Scope of compliance with the 90-90-90 UNAIDS strategy by Bolivia by 2018
Source: UNAIDS DATA 2018

CHAPTER 4: GUT IMMUNOLOGY

Chapter 4: Gut Immunology

4.1. Immunologic control in gut

The gastrointestinal tract serves various functions: including nutrient digestion and absorption, immune-hormonal response, and acting as a protective barrier excluding potentially harmful agents.

To preserve health, the immune system must maintain a fragile balance that allows the elimination of foreign pathogens without triggering immune disorders, such as allergies or autoimmune diseases. [100]

The intestinal tract is inhabited by a variety of microorganisms that undertake essential functions for the host's physiology. The human gastrointestinal tract contains a diverse range of microorganisms such as bacteria, fungi, viruses, and parasites that interact with each other and with the immune system.

The relationship between the host and these microorganisms is one of interdependence and is regulated by the host's immune system. The intestinal tract is rich in immune cells, and lymphoid structures known as Peyer's patches maintain the microbial load, while also offering protection against microbial invasions.

The intestinal epithelium and antigen-presenting cells facilitate communication between the microbiota and the immune system, resulting in the activation of innate and adaptive immune responses. This interaction between the host and the microbial community is pivotal in regulating the physiology of the intestinal mucosa. [101]

Numerous studies support the notion that the microbiome plays a vital role in regulating the immune system, specifically by influencing neurons that are responsible for gut function. As a result, this can modulate the activity of CD4+ helper T cells, called intestinal regulatory T cells, whose primary role is to prevent immune activation that could cause peripheral autoimmunity. [102]

Adaptive immunity generates B lymphocytes that develop antibodies, cytotoxic T lymphocytes that destroy infected host cells, and helper T lymphocytes that result in producing memory B and T lymphocytes, providing long-term protection against future infections caused by the same antigen-carrying pathogen.

The gut microbiota has a critical role in maintaining the host's immune balance, especially by regulating immune homeostasis. Several factors such as age and gender impact the gut microbiota, which can then impact the development of autoimmune diseases. It has also been observed that the gut microbiota has a role in initiating extraintestinal diseases by causing the movement of immune cells from the gut. [101]

4.2 Microbiota relationship with diseases

The microbiota has been extensively investigated for its direct impact on various systems such as pulmonary, mucosal, and gastrointestinal. With the discovery of the direct impact of the lung microbiota on lung disease, there has been a surge of interest in exploring the role of the intestinal microbiota in lung disease and its association with other conditions such as autoimmune and degenerative diseases.

Altered microbial composition and function, known as "dysbiosis," has been linked to autoimmune disorders like rheumatoid arthritis, multiple sclerosis, and autoimmune liver disease. In animal models, the impact of certain changes to the microbiome on host autoimmunity has been investigated. One way in which the microbiota can affect the host is by inducing epigenetic changes in host cells. [101-104]

Associations of host disease with dysbiosis have been described, including reduced microbial diversity. Innate immunity is involved in microbiota-dependent autoimmunity, adaptive immunity, and autoimmune diseases; several studies have described the crucial role of microbial metabolites in the host immune response.

The cellular response to infection has long been linked to the occurrence of autoimmune diseases, key innate cellular components in the line of defense against infectious diseases are recognized as integral players in the interaction between the host immune system and the intestinal microbiota, immunoglobulin A constitutes a regulated link between the intestinal microbiota and host immunity. [100]

Dysbiosis induced by parasitic infections can have an impact on the host's susceptibility to infection and inflammatory response. The intestinal microbiota can affect the pathogenicity of some parasites through bacteria-parasite interactions and by producing necessary metabolites and creating an environment that supports parasite proliferation and growth. Additionally, immunoregulatory factors can indirectly affect changes in the gut microbiota composition.

In parasitic infections, the intestinal microbiota can undergo significant changes and may impact the magnitude of the inflammatory response within the central nervous system by altering bacterial metabolite production. [103]

The therapeutic effectiveness of probiotics in combating dysbiosis associated with certain diseases may rely on specific microbiota composition. Research suggests that an increase in particular bacterial phyla within the gut microbiota can lessen the severity of diseases related to fungal, helminths, protozoan, or bacterial infections.. [105]

In the therapeutic context, antiparasitic medications, probiotics, and fecal transplants have been assessed for their effectiveness against various infections caused by protozoa like *Giardia Lamblia, Cryptosporidium parvum*, and nematodes like *Toxocara canis* and *Strongyloides*. [106-107]

Gaining a deeper understanding of the interaction between hosts and microbes, as well as identifying the underlying molecules produced by the microbiota that impact the immune system and disease progression, could lead to more personalized interventions and therapies for immune disorders based on microbial molecules.

4.3 Gut Microbiome Alterations during HIV Infection

Changes in the composition of the microbiome during HIV infection may be caused by several factors, such as the loss of effective innate and adaptive immune responses and a significant decrease in the CD4+ T cell subsets that regulate intestinal bacteria and restrict microbial translocation. HIV infection-related damage to the intestinal mucosa is characterized by early disruption of the epithelial barrier and depletion of CD4+ T-cells, leading to the translocation of microbial products throughout the body. Gut-associated lymphoid tissues (GALT) constitute the primary site of HIV transmission, replication, seeding, and viral CD4+ T-cell depletion, resulting in chronic and persistent immune activation. [106]

Microbial products that enter the body through the intestinal barrier can disrupt the proper cellular response to fight viral infections and lead to inflammation in the gut. To promote intestinal healing and maintain gut health in patients undergoing antiretroviral therapy, it is critical to understand how the gut microbiota interacts

with HIV infection. Even with antiretroviral treatment, the translocation of microbial products from the gut to the rest of the body can lead to ongoing immune activation.

4.4 Gut microbiota and malnutrition

The intestinal microbiota provides essential capabilities for the fermentation of non-digestible substrates, such as dietary fibers and endogenous intestinal mucus. This fermentation favors the growth of specialized microbes that produce short-chain fatty acids. In addition, other specific products of the gut microbiota have been directly implicated in human health outcomes, we are entering an era where we can increasingly modify health through food and measure the effects through our microbes or metabolites.

The disrupted healthy, mature intestinal anaerobic microbiota, can cause malabsorption and make the intestine vulnerable to pathogenic invasion. Even with therapy and a special diet to combat infection, it may still be difficult to fully restore this microbiota. Future research should pay attention to the specific microorganisms and nutrients needed to restore healthy and balanced microbiota.

Food and water security and breastfeeding, critical factors for the maturation of healthy gut microbiota, have been identified as protective factors against malnutrition.

Metagenomic research has exposed distinct modifications in the gut microbiota which are related to ineffective treatment, and the overall changes in the gut microbiota are related to inadequate response to treatment, despite proper nourishment. [107]

4.5 Interactions between parasitic infections and HIV on Gut microbiota

Infections by various pathogens, such as intestinal parasites, may alter the diversity of the intestinal microbiota and influence the structure and metabolism of intestinal epithelial cells generating an interaction of effects in case of coexistence of these pathologies. However, the interaction between intestinal microbiota and intestinal epithelial cells during infection remains unclear. [108]

Human immunodeficiency virus (HIV) infection can cause changes in the intestinal microbiota, associated with persistent inflammation during HIV infection, which in turn can trigger immune activation and chronic inflammation. Since the gastrointestinal tract is a major site of viral replication, HIV-induced loss of T-helper cells in the gut can damage intestinal barriers and weaken intestinal immunity, resulting in alterations known as intestinal dysbiosis. [110]

Additionally, antiretroviral therapy affects the diversity of the gut microbiota, and current research has yielded mixed results on this topic. In a recent study, the active fraction of microbiota richness was found to be reduced in patients receiving ART compared to those not receiving treatment, as well as in healthy controls. [109,110]

CHAPTER 5: THESIS OBJECTIVES

Chapter 5: Thesis objectives

5.1 Research questions

a) Establish the magnitude of intestinal parasitism and its risk factors in PLWHA at Cochabamba - Bolivia.
b) Identify the intestinal parasitic species with the highest disease burden in PLWHA in in this geographical context.
c) Determine the factors associated with intestinal parasitic infections in PLWHA.

Objectives and methods of the research

5.2 Overall objective

Determine the magnitude and characteristics of intestinal parasitic infections in PLWHA, establishing the factors associated with these infection, to develop therapeutic strategies focused on the prevention and control of this type of infections at different levels, determining guidelines to implement control programs and increase the quality of life of people living with HIV (PLWHA) in Cochabamba-Bolivia.

5.3 Specific objectives

1. To establish the magnitude and type of intestinal parasitic infections in PLWHA, through the identification of the species with the highest disease burden.
2. Establish the validity and performance of the diagnostic methods routinely used to detect these infections in Bolivia.
3. Ascertain the associated factors related with intestinal parasitic infections in PLWHA in Bolivia.

CHAPTER 6: MATERIALS AND METHODS

Chapter 6: Materials and methods

6. 1 Methods

6.1.1 Approximation to the incidence and prevalence

To establish the magnitude and characteristics of intestinal parasitosis in PLWHA, and adequately describe the associated factors related to these infections, different methodologies have been developed to address the object of study.

In the first instance, an approximation of the prevalence has been made using the databases of the Reference laboratory in the city of Cochabamba LABIMED, The percentage of the target population that can be founded in LABIMED vary according the number of patients that realize at least one routine control by year in CDVIR, an estimation based on the database of the System for Monitoring and evaluation in the National Program for HIV control (SIMONE) established a range from 60 to 70%.

These public nature centers serve as key care facilities that provide regular laboratory monitoring for patients in both the public and private sectors, particularly for those living with HIV/AIDS (PLWHA) who undergo monthly clinical evaluations.

However, given the challenges associated with geographic access in some regions of Cochabamba, patients may be evaluated no more than once every three months. As a standard practice, health professionals at these centers maintain regular contact with patients to schedule laboratory monitoring tests, which may include

direct microbiological tests for the detection of intestinal parasites, as well as blood tests to assess kidney and liver function, immunological tests (such as the CD3/CD4 ratio, CD4 and CD8 T lymphocyte cell counts), and viral load testing.

Generally, funding from the Global Fund for Malaria, tuberculosis, and HIV has subsidized these procedures. However, over recent years, the subsidy from this organization has decreased, resulting in a reduction in the frequency of laboratory monitoring tests for patients. For example, coproparasitological examinations were previously conducted every three months, but now they are only conducted upon the request of the treating physician and not as a routine practice. Similarly, the frequency of T/CD4 lymphocyte count and viral load tests has also decreased to only once a year.

In this context, the database of the reference laboratory for PLWHA in Cochabamba (LABIMED) has been used, which consists of two laboratories located in different infrastructures and with independent databases. There have been many problems collecting this information, and the reports of the patients were not in digital format, resorting to the paper records of each patient in the medical history.

6.1.2 Materials and methods study 1 (Retrospective data)

The official data from the National HIV/AIDS Program (SIMONE) reports an estimated 3,535 notified cases of accumulated HIV in 2016 that have been retained in Cochabamba, understanding that LABIMED as a reference laboratory concentrates an estimated percentage of at least 60% of this population.

In the context of the control and monitoring of PLWHA in Bolivia, all patients registered in the National HIV/AIDS Control Program (PNC/VS) are regularly

monitored by laboratory tests that are routinely performed by different laboratories within LABIMED.

The parasitology laboratory only performs direct microscopy tests, while the immunology laboratory focuses on flow cytometry counts and viral load tests. As these databases were not linked, immunological variables were not taken into account in the analysis. However, this was addressed in subsequent interventions, and modifications were introduced.

We will emphasize that methods such as specific staining for the detection of parasites such as *Cryptosporidium*, and *Microsporidia* are not routinely performed, since the National Program does not subsidize them.

The study was conducted in Cochabamba according to an analytical design carried out based on the reports of the reference laboratory in the city of Cochabamba (LABIMED) on HIV-infected outpatients followed in the CDVIR, who performed routine laboratory tests from January 2011 to December 2015, considering only one sample per patient-year, and reporting pathogens preferentially in polyparasitism cases.

A retrospective analysis was carried out founded on the macroscopic and microscopic identification of parasitic elements present in the stool, using direct parasitological methods.

The lack of an information management system was the main limitation of this process. Selection criteria were to be a patient with confirmed HIV infection, who is followed and treated at the HIV reference center, and to be at least 18 years of age.

Records that did not have complete data were excluded; but the numbers were low: 6 (1.9%) in 2011, 5 (1%) in 2012, 10 (1.9%) in 2013, 8 (1.4%) in 2014, and 10 (1.6%) in 2015.

Once these difficulties were resolved due to the limited information with few clinical variables reported in the laboratories, it was possible to carry out a retrospective study covering 5 years evaluating fecal samples corresponding to 2510 patients with confirmed HIV in the period described.

Polyparasitism was defined as at least two different species identified in one stool sample.

Data were collected on a specially designed spreadsheet, based on the paperback reports of the parasitology laboratory, which was transferred for statistical analysis to SPSS 24.0.

Characteristics of study participants were reported as mean, range, and percentage as appropriate. The $\chi 2$ test or Fisher's exact p-value was used for all categorical variables. The frequency of parasitic infections was computed for grouped data according to sex, age, and the consistency of the stool sample.

Logistic regression was proposed for the analysis. Subsequently in this process, collinearity and possible interactions between study variables were evaluated, eliminating variables that did not contribute to the model. A multivariate model was established using the likelihood ratio test, with variables showing an association $p<0.05$ being considered significant, and a 95% confidence interval (CI) was applied. As patients could be evaluated over several years, separate models were developed for each year, and the process was also carried out for the entire period (2011 to 2015), eliminating collinearity. (Figure 19)

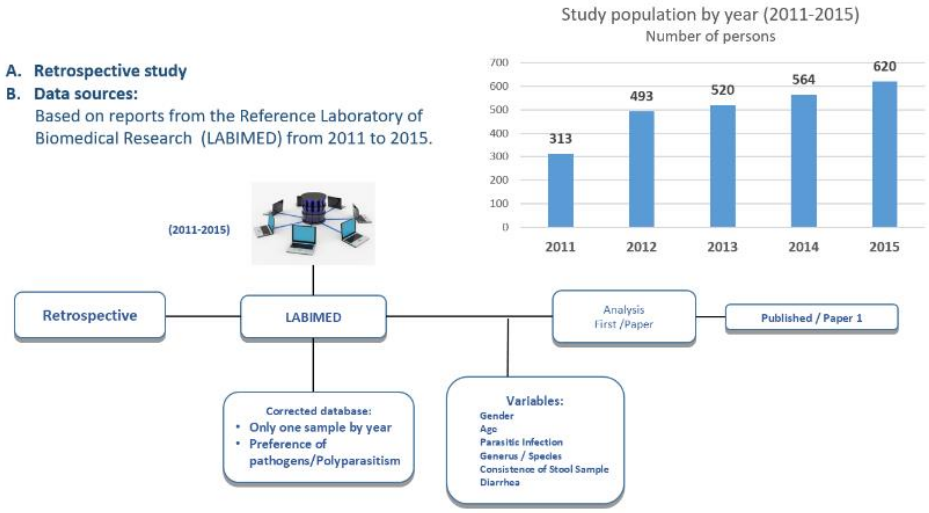


Figure 19: Retrospective study design / Intestinal parasitic infections in HIV/AIDS Patients.
Source: Own elaboration

Ethical approval was obtained prior to the beginning of this study from the Ethics Committees of the Department of Cochabamba-Bolivia and the Universidad Mayor de San Simón, Institute of Biomedical Research (IIBISMED/UMSS). Code: CB-2016-015.

6.1.3 Materials and methods 2 (Cross-sectional study)

The approach to the patients was established within the framework of routine controls in the CDVIR, with the consensus of the departmental health service (SEDES) to involve the medical staff in the study and also organized meetings with the civilian population of PLWHA in associations and mutual aid groups.

The CDVIR conducts clinical and laboratory checks on patients at minimum intervals every three months, with more frequent checks as necessary depending on the patient's clinical state. Our cross-sectional study considered this periodicity in establishing an outreach strategy for patients undergoing checks at the CDVIR.

The sampling process considered all consecutive patients who attended the CDVIR and met the criteria to be admitted to the study. Excluding patients with recent gastrointestinal processes or in current treatment with antibiotics, in addition to suffering from any chronic decompensated health problem.

After approximately 3 months (From December 2017 to March 2018), the saturation of this process was reached and no more potential patients were found to be recruited.

From December 2017 to March 2018, all consecutive patients with confirmed HIV infection who consulted at CDVIR were enrolled in the cross-sectional study designed based on the findings of the previous retrospective study. In the recruitment process of the study, all consecutive patients were enrolled and responded to the nutritional and immunological survey, reaching 510 patients in this period, a significant sample of patients concerning the total number of PLWHA estimated in Cochabamba (N=3,535 PLWHA).

Stool samples have been analyzed by several methods, including direct observation methods, specific staining; immunochromatography, and molecular techniques such as Polymerase Chain Reaction (PCR). (Figure 20)

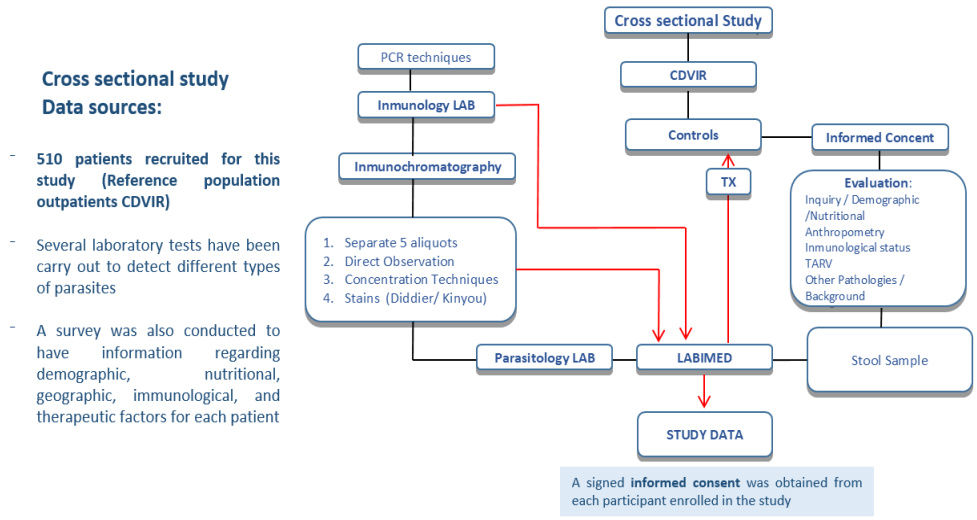


Figure 20: Cross sectional study design / intestinal parasitic infections in HIV/AIDS Patients (n=510)
Source: Own elaboration

Only 503 recruited patients provided fecal specimens under appropriate conditions.

Once the sample was obtained was treated in suitable preservation media and distributed in four aliquots, the first of which was examined by the parasitology laboratory, and direct microscopic observation was carried out.

Routinely, another aliquot was processed for concentration techniques, and specific stains (modified Ziehl Neelsen, Kinyou, trichromic Diddier modified and Giemsa).

Microbiological tests were performed by two observers (Parasitologists) separately, as standard procedures in this reference laboratory, and in two other laboratories with a subsequent reading of the plates to establish adequate quality control.

Another aliquot was analyzed by the Immunochromatography technique, only available for *Cryptosporidium spp*., *Giardia intestinalis*, and *amoeba spp*. carrying out quality control by establishing a second reading with a different brand of diagnostic kit, and also performing a concordance analysis.

Finally, the fourth aliquot was sent to storage under appropriate conditions (-20) degrees Celsius while waiting for the home molecular biology methods, reserved for the identification of the most prevalent strains of microsporidium (*E. Bieneusi, E. Intestinalis, E. Cuniculi and E. Hellem*).

Molecular diagnostic methods considered only the application of conventional PCR, which was evaluated with 50 random samples, including 30 patient samples that tested positive for microsporidium spp. by Diddier staining and modified Kinyou staining.

All samples processed by PCR were negative in this process driving us to evaluate the application of the method. Quality controls between staining techniques showed an adequate concordance, with a Kappa index of 99%.

Therefore, the PCR technique was not available until after the publication of the results of this study. Due to the lack of resources and the impossibility of obtaining control strains for these microsporidium species. Only control strains of *E. Bieneusi* and *E. Intestinalis* were obtained.

Data were analyzed by the t-test (student), chi-square, one-way ANOVA, and the Fisher's exact test when appropriate. Multivariate logistic analysis was used to evaluate the association between parasitic infection and clinical and socio-epidemiological characteristics. The magnitude of association between variables was estimated by the odds ratio (OR) with a 95% confidence interval (95% CI). The level of significance was set at $p < 0.05$.

A signed informed consent was obtained from each participant enrolled in the study, the study protocol was approved by the Commission d'éthique biomédicale hospitalo-facultaire de l'UCLouvain. (CEHF) Cliniques Universitaires Saint-Luc, Bruxelles, Belgium. In addition to the UMSS Ethics and Research Committee in Bolivia, being approved by both instances before its implementation.

A computerized information system based on CsPro software was developed to unify the databases of the parasitology and immunology laboratories at LABIMED. BMI was used to assess nutritional status, and unintentional weight loss during the last year was also evaluated. Both situations were considered for analysis because in individuals living with HIV/AIDS, any progressive and unintentional weight loss is considered a wasting syndrome.

In order to establish the appropriate therapy for each patient with a pathogenic parasitic infection identified during the study at the CDVIR, a laboratory follow-up protocol was established for 2 weeks. Free treatment was prescribed based on broad-spectrum antiparasitic drugs that were dosed by the treating physician at the CDVIR.

6.1.4 Recruitment of study population

During the study period, all consecutive patients monitored at the CDVIR were enrolled in the cross-sectional study. After their consent, a questionnaire was administered to each participant by the research team who had been specifically trained for this task and who also clarified all the study objectives and the patients doubts.

At the time of clinical controls in the reference center (CDVIR), patients were referred to the LABIMED for collecting the stool sample and to maintain optimal conditions for laboratory analysis.

The study participants were adult patients aged over 18 years old of both genders, with HIV-confirmed infection, who had not used anthelmintic in the previous three months and were followed up at the center for reference of HIV/AIDS (CDVIR) where the study was conducted.

Professional counseling was offered to all participants before parasitological testing, and a stool sample was collected from each participant and used for four different intestinal parasite testing. All diagnostic results were kept strictly confidential and deworming treatments were proposed to all participants found to be infected with any pathogen parasite. (Albendazole, Nitazoxanide).

6.1.5 Laboratory procedures

Parasitological diagnostic tests were performed on each fresh stool sample and processed in parallel using the following methods:

a) Direct observation of fresh feces

b) Concentration method (modified Ritchie)

c) Immunochromatography

d) Specific stains (Modified Ziehl–Neelsen/Kinyoun, modified Diddier and Giemsa)

e) Molecular biology methods, for technical feasibility reasons, these techniques could not be performed in the study.

Stool samples were analyzed by direct microscopy with physiological saline and iodine, and after that concentration methods were applied (i.e., formalin ether or formalin-ethyl acetate), the spontaneous sedimentation allowed the detection of helminths eggs and larvae, as well as protozoan cysts and also *S. stercoralis* and *hookworm* larvae.

The Ritchie technique was used on concentrate samples to prepare fecal smears, coupled with 3 different stains. Modified Ziehl Neelsen technique (Kinyoun), modified Diddier technique and Giemsa were used to identify *Cryptosporidium* spp., *I. belli*, and *C. cayetanensis*. (Coccidian and microsporidium sp.).

Three slides of each sample were prepared and examined by two analysts. When no parasites and/ or commensals were found in the sample, two additional slides were prepared to confirm the result.

A colored chromatographic immunoassay was developed for the simultaneous qualitative detection of Cryptosporidium, Giardia (α1-giardin and/or CWP1), Entamoeba (histolytica and/or dispar) in stool samples with the ability to detect Giardia trophozoites and cysts.

CHAPTER 7: MAIN RESULTS

Chapter 7: Main Results

7.1 Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba, Bolivia: Retrospective Analysis

The CDVIR laboratory received a total of 2,510 fecal samples between the years 2011 and 2015, from patients with a confirmed HIV diagnosis who underwent laboratory tests. The number of samples assessed each year during this period varied between 313 and 620. (Figure 21)

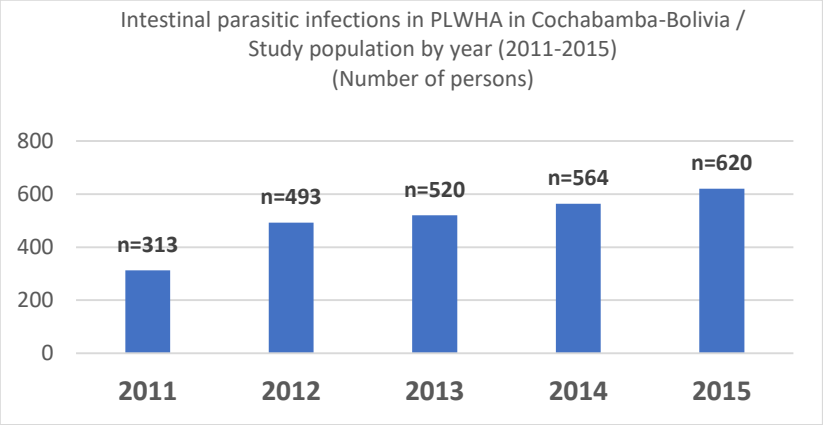


Figure 21: Intestinal parasitic infections in PLWHA in Cochabamba-Bolivia study population in retrospective analysis.
Source: Own elaboration

The analysis of the data was performed each year separately for several reasons. The first reason was the variability of the population, which increased with new patients diagnosed and recruited each year and that decreased due to loss to follow up and death. The second reason was that some patients were considered for several years in the follow-up framework.

The global distribution of patients attending LABIMED was predominantly male, with a men/women ratio close to the 1.7. This ratio does not vary during the entire study period. The age distributions showed a greater proportion of people in age over 40 years and was homogeneous across the years. Liquid feces occurred at a frequency of up to 48% in 2011 and remained close to 40% across the years. (Table 1)

	2011	**2012**	**2013**	**2014**	**2015**	
	(n=313)	**(n=493)**	**(n=520)**	**(n=564)**	**(n=620)**	**P Value**
Characteristic						
sex						0.37
Man –no. (%)	197 (62.9)	316 (64.1)	329 (63.3)	334 (59.2)	400 (64.5)	
Men/Women Ratio	1.69	1.78	1.72	1.45	1.82	
Age-yr-no. (%)	36±12	37±12	37±12	38±13	37±13	0.67*
<30	116 (37.1)	160 (32.5)	162 (31.2)	157 (27.8)	193 (31.1)	
30 – 39	89 (28.4)	155 (31.4)	163 (31.3)	180 (31.9)	196 (31.6)	
≥40	108 (34.5)	178 (36.1)	195 (37.5)	227 (40.2)	231 (37.3)	
Aspect of feces (Liquid)-no. (%)						<0.001
Yes	151 (48.2)	210 (42.6)	188 (36.2)	222 (39.4)	292 (47.1)	
Lienteric feces-no. (%)						0.002
yes	28(8.9)	24(4.9)	37(7.1)	44(7.8)	70(11.3)	
a*ANOVA F test, no.: number						

Table 1. Characteristics of all participants by year

Twelve types of parasite genera were identified. The proportion of parasitic infection, defined as positive for at least one intestinal parasite, was 33.2%, the highest in 2011, and the lowest in 2014, with a prevalence of 24.5%. The highest polyparasitism rate was detected in 2011, at 9.9%, and the lowest in 2015 (7.3%). (Table 2)

Taking into account the entire study period, polyparasitism was present in 60% of patients with an identified pathogen (OR=7.13 CI 95% 4.96-10.23).

Outcomes /Year	**2011**	**2012**	**2013**	**2014**	**2015**
Parasite Infection – n (%)	**(n=313)**	**(n=493)**	**(n=520)**	**(n=564)**	**(n=620)**
Any	104 (33.2)	142 (28.8)	158 (30.4)	139 (24.6)	168 (27.1)
Without infection	209(66.8)	351(71.2)	362(69.6)	425(75.4)	452(72.9)
Pathogens	27(26.0%)	32(22.5%)	43(27.2%)	59(42.4%)	50(29.8%)
Polyparasitism	31 (9.9)	41 (8.3)	40 (7.7)	48 (8.5)	45 (7.3)
Parasites species – n (%)					
Pathogens					
Giardia lamblia	15 (4.8)	15 (3.0)	26 (5.0)	37 (6.6)	30 (4.8)
Entamoeba histolytica/dispar	9 (2.9)	11 (2.2)	13 (2.5)	17 (3.0)	16 (2.6)
Strongyloides stercoralis	1 (0.3)	4 (0.8)	1 (0.2)	3 (0.5)	4 (0.6)
Trychomona hominis	1 (0.3)	1 (0.2)	3 (0.6)	2 (0.4)	0
Isospora belli	1 (0.3)	0	0	0	0
Taenia spp.	0	1 (0.2)	0	0	0
Non-pathogens					
Blastocystis hominis	32 (10.2)	47 (9.5)	66 (12.7)	56 (9.9)	44 (7.1)
Entamoeba coli	38 (12.1)	47 (9.5)	38 (7.3)	21 (3.7)	59 (9.5)
Hymenolepis nana	3 (1.0)	9 (1.8)	4 (0.8)	2 (0.4)	8 (1.3)
Chilomastix mesnili	4 (1.3)	5 (1.0)	4 (0.8)	1 (0.2)	4 (0.6)
Entamoeba hartmanni	0	2 (0.4)	3 (0.6)	0 (0.0)	1 (0.2)
Iodoameba butschili	0	0	0	0	2 (0.3)

Table 2. Prevalence of Intestinal parasitic infections by year in adult patients of a reference laboratory (LABIMED), Cochabamba-Bolivia

Throughout the course of the study period, there was a clear predominance of parasites classified as protozoa in fecal samples processed in the reference laboratory. The most prevalent pathogen species in order of frequency were *Giardia Lamblia* and *Entamoeba histolytica/dispar*. Among non-pathogenic species *Blastocystis hominis*, *Entamoeba coli.* The most frequently detected parasite was *B.*

hominis, reaching 12.7%, the highest rates in 2013 and decreasing to 7.1% in 2015. (Table 2)

The prevalence of parasite infections, and polyparasitism according to age, sex and stool consistence is presented in (Table 3, part 1-5), within each year.

			PARASITE INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
	Sex				0.53
		F	36/116(31.0)	1	
		M	**68/197**(34.5)	1.17(0.71-1.91)	
	Age				0.11
		<30	46/116(39.7)	1	
2011		30 – 39	34/89(38.2)	0.94(0.53-1.65)	
(n=313)		≥40	24/108(22.2)	0.44(0.42-0.78)	
	Liquid feces				0.16
		Yes	56/151(37.1)	1.40 (0.87-2.24)	
		No	48/162(29.6)	1	
	Lienteric feces				0.90
		Yes	9/28(32.1)	0.95(0.41-2.17)	
		No	95/285(33.3)	1	
			POLYPARASITIC INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
	Sex				0.56
		F	13/116(11.2)	1	
		M	18/197(9.1)	0.80(0.38-1.69)	
	Age				**0.03**
		<30	16/116(13.8)	1	
2011		30 – 39	11/89(12.4)	0.88(0.39-2.01)	
(n=313)		≥40	4/108(3.7)	0.24(0.08-0.74)	
	Liquid feces				0.69
		Yes	16/151(10.6)	1.16 (0.55-2.44)	
		No	15/162(9.3)	1	
	Lienteric feces				0.42
		Yes	4/28(14.3)	1.59(0.51-4.93)	
		No	27/285(9.5)	1	

Table 3. Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 1 / 2011)

			PARASITE INFECTION		
Year / Characteristic			Yes no. /N. (%)	OR (95% IC)	P value
2012 (n=493)	**Sex**				**0.09**
		F	59/177(33.3)	1	
		M	83/316(26.3)	0.70(0.47-1.05)	
	Age				**<0.001**
		<30	65/160(40.6)	1	
		30 – 39	43/155(27.7)	0.58(0.36-0.92)	
		≥40	34/178(19.1)	0.35(0.22-0.58)	
	Liquid feces				**<0.001**
		Yes	78/210(37.1)	2.02(1.36-3.00)	
		No	64/283(22.3)	1	
	Lienteric feces				**0.001**
		Yes	15/25(60.0)	3.66(1.63-8.18)	
		No	127/468(27.1)	1	

			POLYPARASITIC INFECTION		
Year / Characteristic			Yes no. /N. (%)	OR (95% IC)	P value
2012 (n=493)	**Sex**				**0.005**
		F	23/177(13.0)	1	
		M	18/316(5.7)	0.40(0.21-0.77)	
	Age				**0.001**
		<30	21/160(13.1)	1	
		30 – 39	16/155(10.3)	0.76(0.38-1.52)	
		≥40	4/178(2.2)	0.15(0.05-0.45)	
	Liquid feces				**0.03**
		Yes	24/210(11.4)	2.02(1.05-3.86)	
		No	17/283(6.0)	1	
	Lienteric feces				**0.004**
		Yes	6/25(24.0)	3.91(1.46-10.41)	
		No	35/468(7.5)	1	

Table 3: Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 2 / 2012)

			PARASITE INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2013 (n=520)	**Sex**				0.24
		F	64/191(33.5)	1	
		M	94/329(28.6)	0.79(0.54-1.17)	
	Age				**0.003**
		<30	60/162(37.0)	1	
		30 – 39	56/163(34.4)	0.89(0.57-1.40)	
		≥40	42/195(21.5)	0.47(0.29-0.75)	
	Liquid feces				**0.002**
		Yes	73/188(38.8)	1.85(1.26-2.71)	
		No	85/332(25.6)	1	
	Lienteric feces				0.16
		Yes	15/37(40.5)	1.62(0.82-3.22)	
		No	143/483(29.6)	1	
			POLYPARASITIC INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2013 (n=520)	**Sex**				0.14
		F	19/191(9.9)	1	
		M	21/329(6.4)	0.62(0.32-1.18)	
	Age				0.41
		<30	16/162(9.9)	1	
		30 – 39	12/163(7.4)	0.73(0.33-1.59)	
		≥40	12/195(6.2)	0.60(0.27-1.30)	
	Liquid feces				**0.001**
		Yes	24/188(12.8)	2.89(1.49-5.59)	
		No	16/332(4.8)	1	
	Lienteric feces				**0.008**
		Yes	7/37(18.9)	3.18(1.30-7.79)	
		No	33/483(6.8)	1	

Table 3: Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 3 / 2013)

			PARASITE INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2014 (n=564)	Sex				0.18
		F	64/230(27.4)	1	
		M	75/334(22.5)	0.77(0.52-1.13)	
	Age				**<0.001**
		<30	53/157(33.8)	1	
		30 – 39	49/180(27.2)	0.73(0.46-1.17)	
		≥40	37/227(15.9)	0.38(0.24-0.62)	
	Liquid feces				**0.02**
		Yes	66/222(29.7)	1.56(1.06-2.30)	
		No	73/342(21.1)	1	
	Lienteric feces				**<0.001**
		Yes	22/44(50.0)	3.44(1.84-6.44)	
		No	117/520(22.3)	1	
			POLYPARASITIC INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2014 (n=564)	Sex				0.01
		F	28/230(12.2)	1	
		M	20/334(6.0)	0.46(0.25-0.84)	
	Age				**0.04**
		<30	18/157(11.5)	1	
		30 – 39	19/180(10.6)	0.91(0.46-1.80)	
		≥40	11/227(4.8)	0.39(0.18-0.86)	
	Liquid feces				**0.005**
		Yes	28/222(12.6)	2.32(1.27-4.24)	
		No	20/342(5.8)	1	
	Lienteric feces				**0.003**
		Yes	9/44(20.5)	3.17(1.42-7.07)	
		No	39/520(7.5)	1	

Table 3: Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 4 / 2014)

			PARASITE INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2015 (n=620)	Sex				0.41
		F	64/220(29.1)	1	
		M	104/400(26.0)	0.86(0.59-1.24)	
	Age				**<0.001**
		<30	76/193(39.4)	1	
		30 – 39	48/196(24.5)	0.50(0.32-0.77)	
		≥40	44/231(19.0)	0.36(0.23-0.56)	
	Liquid feces				**0.002**
		Yes	96/292(32.9)	1.74(1.22-2.50)	
		No	72/328(22.0)	1	
	Lienteric feces				0.77
		Yes	20/70(28.6)	1.09(0.63-1.89)	
		No	148/550(26.9)	1	
			POLYPARASITIC INFECTION		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2015 (n=620)	Sex				0.99
		F	16/220(7.3)	1	
		M	29/400(7.3)	1.00(0.53-1.88)	
	Age				**0.003**
		<30	24/193(12.4)	1	
		30 – 39	12/196(6.1)	0.46(0.22-0.95)	
		≥40	9/231(3.9)	0.29(0.13-0.63)	
	Liquid feces				**0.02**
		Yes	29/292(9.9)	2.15(1.14-4.05)	
		No	16/328(4.9)	1	
	Lienteric feces				0.15
		Yes	8/70(11.4)	1.79(0.80-4.02)	
		No	37/550(6.7)	1	

Table 3: Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 5 / 2015)

Parasitic infections

Association with age and stool consistency was consistently significant, each year, there was a predominant proportion of parasite infections in ages under 30, and this association was significant, except in 2011, with also higher frequencies of polyparasitism in this age group. The liquid stool was significantly associated with an increased risk of parasites detected by direct microscopic examination in all years except 2011.

Although the proportion of women with parasitic infections was higher in all years except 2011, the difference was not statistically significant. Similarly, women were observed to have a higher prevalence of polyparasitism than men, but statistical significance was only observed in 2012 (p=0.005). (Table 3, part 1-5)

Polyparasitism

Statistical analysis of polyparasitism revealed a consistent association with age throughout the study period, except in 2013, a higher prevalence of multiple infections among individuals younger than 30 years. Similarly, a sustained association between liquid stool consistency and polyparasitic infections was identified in all years except 2011. (Table 3, part 1-5)

Pathogen infection

Based on the analysis, it was found that the association between pathogenic agents and sex was significant only in 2012 (p=0.02), with a high proportion in women. Age showed a significant association with pathogen infection only in 2013 (p=0.02), showing a higher frequency in those over 40 years old. The presence of liquid stool during sample collection showed consistent significance and was associated with the presence of pathogens throughout the study period, except in 2012. On the

other hand Lienteric stool, was only associated with pathogens in 2011 and 2015. (Table 4, part 1-5).

			Pathogen Infection		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2011 (n=313)	Sex				0.87
		F	9/36(25.0)	1	
		M	18/68(26.5)	1.08 (0.43-2.73)	
	Age				0.85
		<30	11/46(23.9)	1	
		30 – 39	10/34(29.4)	1.32(0.49-3.61)	
		≥40	6/24(25.0)	1.06(0.34-3.34)	
	Liquid feces				**0.004**
		Yes	21/56(37.5)	4.20 (1.53-11.56)	
		No	6/48(12.5)	1	
	Lienteric feces				**0.004**
		Yes	6/9(66.7)	7.05(1.62-30.6)	
		No	21/95(22.1)	1	

Table 4. Pathogen parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 1/2011)

			Pathogen Infection		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2012 (n=493)	Sex				**0.02**
		F	19/59(32.2)	1	
		M	13/83(15.7)	0.39(0.18-0.88)	
	Age				0.21
		<30	16/65(24.6)	1	
		30 – 39	12/43(27.9)	1.18(0.49-2.84)	
		≥40	4/34(11.8)	0.41(0.13-1.34)	
	Liquid feces				0.12
		Yes	24/79(30.4)	3.00(1.24-7.26)	
		No	8/63(12.7)	1	
	Lienteric feces				0.10
		Yes	7/14(50.0)	4.12(1.32-12.82)	
		No	25/128(27.1)	1	

Table 4: Pathogen parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 2/2012)

			Pathogen Infection		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2013 (n=520)	Sex				0.88
		F	17/64(26.6)	1	
		M	26/94(27.7)	1.06(0.52-2.16)	
	Age				**0.02**
		<30	19/60(31.7)	1	
		30 – 39	8/56(14.3)	0.36(0.14-0.91)	
		≥40	16/42(38.1)	1.33(0.58-3.04)	
	Liquid feces				**<0.001**
		Yes	32/73(43.8)	5.25(2.40-11.50)	
		No	11/85(12.9)	1	
	Lienteric feces				0.24
		Yes	6/15(40.0)	1.91(0.64-5.73)	
		No	37/143(25.9)	1	

Table 4: Pathogen parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 3/2013)

			Pathogen Infection		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2014 (n=564)	Sex				0.10
		F	32/64(50.0)	1	
		M	27/75(36.0)	0.56(0.28-1.11)	
	Age				0.98
		<30	22/53(41.5)	1	
		30 – 39	21/49(42.9)	1.06(0.48-2.32)	
		≥40	16/37(43.2)	1.07(0.46-2.51)	
	Liquid feces				**<0.001**
		Yes	41/66(62.1)	5.01(2.42-10.38)	
		No	18/73(24.7)	1	
	Lienteric feces				0.09
		Yes	13/22(59.1)	2.23(0.88-5.63)	
		No	46/117(39.3)	1	

Table 4: Pathogen parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 4/2014)

			Pathogen Infection		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2015 (n=620)	Sex				0.49
		F	21/64(32.8)	1	
		M	29/104(27.9)	0.79(0.40-1.56)	
	Age				0.91
		<30	23/76(30.3)	1	
		30 – 39	15/48(31.3)	1.05(0.48-2.30)	
		≥40	12/44(27.3)	0.86(0.38-1.97)	
	Liquid feces				**<0.001**
		Yes	44/96(45.8)	9.31(3.68-23.50)	
		No	6/72(8.3)	1	
	Lienteric feces				**0.03**
		Yes	10/20(50.0)	2.70(1.05-6.97)	
		No	40/148(27.0)	1	

Table 4: Pathogen parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year (Part 5/2015)

Multivariate Analysis

Initially, the assumptions for the binary logistic regression were evaluated, such as the linearity in the logarithm of the odds ratio, the absence of multicollinearity, and the independence between the data.

To assess collinearity in the proposed binary logistic regression analysis, correlations and interactions between predictor variables were analyzed. The correlation matrix was calculated for the overall data set and then separately for each year to thoroughly explore the relationship between the variables. As a result, collinearity was identified. Therefore, it was determined that the logistic regression model was inadequate for the entire study period (2011 to 2015).

Performing an analysis for each year independently, it was found that neither variable was significant in 2011, and a multivariate analysis could not be applied. In 2012, although all the variables were significant, the collinearity between lientery and the presence of liquid stool again has disabled to perform this analysis. In 2013, the situation was similar, with age and the presence of liquid stool being significant. In 2014, the same occurred despite the addition of age as a significant variable. Finally, in 2015, only age and the presence of liquid stool were significant but correlated. (Table 3, part 1-5)

The binary logistic regression model in these circumstances is limited in explaining the variability in the outcome variable. Several possible solutions were considered to address the problem of collinearity in the logistic regression analysis, such as eliminating highly correlated predictor variables, transforming variables, and weighting variables. However, due to the limited number of variables available in the LABIMED database, it was decided that regression was not necessary, and a bivariate analysis was performed instead to conclude the study.

7.2 Pathogenic and nonpathogenic intestinal parasites in patients with HIV/AIDS in Cochabamba, Bolivia: a cross-sectional study

According to the study design, data from all participants who provided stool samples and completed the questionnaires were included. However, only 503 out of the 510 collected samples were appropriate for analysis.

In this analysis, a total of 503 patients were evaluated, with 63.2% (318) being male, resulting in a male-to-female ratio of 1.72. The age of the participants ranged from 17 to 88 years old, with an average age of 36 years and a standard deviation of 13.0. Of the total patients enrolled, 57.9% (291) had undetectable viral loads, 98% (493/503) received ART, of which 96.6% received the Tenofovir/Lamivudin/Efavirenz (TDF/3TC/EFV) scheme.

The median number of controls in the CDVIR during the last year was 4. Concerning the CD4 cell count 50.5% do not reach 350 cells/mm3, 21.9% are between 350-500 cells/mm3 and 27.6% have counts> 500 cells/mm3. The BMI average was 23.8 SD ± 5.2, and 40.8% reported unintentional weight loss during the last year. The monthly income average was 263(USD) SD ± 145, with a median of 288. (Table 5).

The prevalence of intestinal parasites was 33.0%, with 166 out of 503 samples showing parasitic infections; multiparasitism was reported in 7.8% of the cases, and 18.3% (92/503) were attributed to pathogenic agents.

The pathogenic species found were: *Entamoeba histolytica/dispar* (6.6%), *Giardia lamblia* (4.0%), *Strongyloides stercoralis* (1.2%), *Ascaris lumbricoides* (0.6%), *Isospora belli* (0.6%), and *Trichuris trichiura* (0.2%).

Blastocystis hominis (10.1%), *Chilomastix mesnili* (1.6%), *Trichomonas hominis* (0.4%), *Hymenolepis nana* (0.4%), *Entamoeba coli* (0.2%), and *Endolimax nana*

(0.2%) were identified as non-pathogenic parasites, considering direct observation techniques. (Table 6)

Features	All recruited patients (N=503)
Sex-no. (%)	
Male	318(63.2%)
Men/Women Ratio	1.72
Age-yrs	
Median (Range)	34(17;88)
Average ±SD	36±13
Scholarship No. yrs	
Median (Range)	11(0;22)
Average ±SD	10±4
Therapy time (ART)-yrs	
Median (Range)	2.6(0;14.4)
Average ±SD	2.9±2.3
Time since the last control-days	
Median (Range)	0131(0;1675)
Average ±SD	142±133
Number of controls-no	
Median (Range)	4(0;9)
Average ±SD	4±2
Actual CD4 count-no.(cells/mm³)	
<350	254(50.5)
350-500	110(21.9)
>500	139(27.6)
Average ±SD	356±267
ART yes-no.(%)	493(98.0)
Undetectable Viral Load yes-no.(%)	291(57.9)
Actual Scheme of ART	
AZT/3TC/EFV	17(3.4)
TDF/3TC/EFV	476(96.6)
Income / month (USD)	
Median (IQ Range)	288(144;360)
Average ±SD	263±145
BMI - kg/m²	
Median (IQ Range)	23.7(22.0-26.0)
Average ±SD	23.8±5.2
Unintentional weight loss yes-no.(%)	205(40.8)
Average ±SD	1.3±2.1

Table 5. Characteristics of patients with HIV/AIDS recruited in the cross-sectional study

Outcomes	VIH (+) (n=503)
Parasite Infection -no(%)	
Any	166(33.0)
Polyparasitism	39(7.8)
Pathogen infection	92(18.3)
Parasites species-no(%)	
Direct Observation	
Pathogens species-no(%)	
Entamoeba histolytica/dispar	33(6.6)
Giardia lamblia	10(4.0)
Strongyloides stercolaris	6(1.2)
Ascaris lumbricoides	3(0.6)
Isospora belli	3(0.6)
Non-Pathogens species-no(%)	
Blastocystis hominis	51(10.1)
Chilomastix mesnili	8(1.6)
Hymenolepis nana	2(0.4)
Trichomonas hominis	2(0.4)
Trichuris trichura	1(0.2)
Entamoeba coli	1(0,2)
Endolimax nana	1(0.2)

Table 6. Prevalence of Intestinal parasitic infections by species in adult patients with HIV, Cochabamba-Bolivia

For reasons of technical availability, the Immunochromatography method was established only for the detection of *Giardia lamblia, Entamoeba histolytica/dispar*, and *Cryptosporidium*. The prevalence of *Giardia lamblia* was 5.2% highly concordant with the direct observation method (Kappa = 0.77 P <0.001). *Entamoeba histolytica/dispar* was 0.4% and had a low concordance with direct observation (Kappa = 0.11 P <0.001). *Cryptosporidium* spp. was 0.4% and had low concordance with direct observation Kappa = 0.005, P = 0.92. (Table 7)

Outcomes	VIH (+) (n=503)
Parasite Infection -no(%)	
Immunochromatography	
Giardia lamblia	26(5.2)
Ameba SP.	2(0.4)
Cryptosporidium sp.	2(0.4)
Stains	
(1) Kinyou (Modified Ziehl Neelsen)*	
Microsporidium sp.	45(8.9)
Isospora belli	18(3.6)
Strongyloides stercolaris	7(1.4)
Cryptosporidium sp.	4(0.8)
(2) Diddier / Modified***	
Microsporidium sp.	45(8.9)
Isospora belli	17(3.4)
Strongyloides stercolaris	7(1.4)
Cryptosporidium sp.	4(0.8)
(3) Giemsa**	
Isospora belli	13(2.6)
Microsporidium sp.	8(1.6)

*Kappa for Stain 1 y 2 =0.99 P<0.001
**Kappa for Stain 1 y 3 =0.40 P<0.001
***Kappa for Stain 2 y 3 =0.41 P<0.001

Table 7. Prevalence of Intestinal parasitic infections by species in adult patients with HIV, Cochabamba-Bolivia, Results of immunochromatography and staining studies.

In addition, staining methods have been implemented in this study specifically for the detection of species that cannot be identified by direct methods such as *Coccidia, Microsporidium,* and nematodes. The results for Kinyou staining (Modified Ziehl Neelsen) showed a prevalence of *Microsporidium.* spp. of (8.9%), *I.*

belli (3.6%), *S. stercoralis* (1.4%) and *Cryptosporidium* sp. (0.8%). Modified Diddier staining showed a prevalence of *Microsporidium sp.* at (8.9%), *I. belli* (3.4%), *S. stercoralis* (1.4%), and *Cryptosporidium* spp. (0.8%), these two methods showed a high concordance (Kappa = 0.99, P <0.001). Giemsa stain showed *I. belli* (2.6%) and *Microsporidium* spp*,* (1.6%), but showed a low concordance with the other two stains (Kappa= 0.41, P <0.001). (Table 7)

Concordance analysis between the different stains showed a high concordance between the Kinyoun and modified Diddier stains (Kappa 0.99), while the Giemsa stain indicated a low concordance compared to Kinyoun and Giemsa, respectively (Kappa 0.41 and 0.40).

Both genders were affected by intestinal parasitic infections, 30.5% (97/318) in men, and 37.3% (69/185) in women. Showing a higher risk of infection in woman but without statistical significance (OR=1.35 CI 95% 0.92 to 1.98 p=0.12). (Table 8)

Age did not show a significant association with intestinal parasite infection in this study (p=0.34). But consistently found a higher frequency of infections in people under 29 years of age.

Likewise, current CD4 cell count (p=0.17), body mass index (p=0.93), income level (p=0.36) and receiving ART (OR=0.32 CI 95% 0.89-1.15 p=0.07) did not show significance. On the other hand, a significant association was found respect to parasitic infection and the presence of liquid consistence of feces in the enrolled patients (OR = 2.02 IC 95% 1.12-3.64 p=0.02). (Table 8)

Features	Parasites Infection		
	Yes	**OR IC (95%)**	**aOR IC (95%)**
Sex			
Female	69/185(37.3)	1.35(0.92-1.98)	1.46(0.96 to 2.23)
Male	97/318(30.5)	1	1
Age			
≤29 Yr	67/187(35.8)	1	1
30-39 yr	52/152(34.2)	0.93(0.59-1.45)	0.87(0.54-1.40)
≥40 yr	47/164(28.7)	0.72(0.45-1.13)	0.71(0.45-1.13)
Consistence Liquid (Feces)			
Yes	24/50(48.0)	2.02(1.12-3.64)	2.26(1.29 to 4.25)
No	142/453(31.3)	1	1
CD4 T cells count			
< 350 per mm3	93/254(36.6)	1	1
350-500 per mm3	35/110(31.8)	0.80(0.50-1.30)	0.81(0.50-1.32)
>500 per mm3	38/139(27.3)	0.65(0.41-1.02)	0.63(0.39-1.00)
ART			
No	6/10(60.0)	1	1
Yes	160/493(32.5)	0.32(0.89-1.15)	0.25(0.07-0.94)
BMI- Kg/M^2			
Under weight (<20)	16/51(31.4)	1.05(0.54-2.05)	1.07(0.53-2.15)
Normal (20-25)	91/270(33.7)	1.11(0.59-2.12)	1.09(0.56-2.11)
Over weight (>25)	59/182(32.4)	1	1
Income			
< Minimum wage	91/261(34.9)	1	1
> Minimum wage	75/242(31.0)	1.19(0.82-1.73)	1.19(0.82-1.73)
unintentional weight loss			
Yes	73/205(35.6)	1.34(0.92-1.96)	1.12(1.01-1.24)
No	87/298(29.2)	1	1

Table 8. Characteristics Association between the prevalence of intestinal parasites and socio-epidemiological factors in patients with HIV/AIDS

Multivariate analysis revealed statistically significant associations showing a lower probability of parasitic infections in those receiving antiretroviral treatment (OR=0.25; CI, 0.07 to 0.94; P = 0.04). While the liquid consistency of the stool during the direct examination was associated with the presence of parasitic infection (OR=2.26; CI, 1.29 to 4.25; P = 0.01), involuntary weight loss showed a higher risk of infections (OR = 1.12; CI, 1.01 to 1.24; P = 0.03).

The analysis conducted on pathogen infections showed a statistically significant association between liquid stool consistency and the probability of being infected, Individuals who exhibit this characteristic have a higher likelihood of being infected by a pathogen (OR = 3.59, IC 95% = 1.27-10.20, p = 0.01). Furthermore, the study showed that individuals who were receiving antiretroviral therapy (ART) had a lower predisposition to pathogenic infection. (RR = 0.53, IC 95% = 0.46-0.62, p = 0.025). (Table 9)

There was no statistically significant association between unintentional weight loss and pathogen infection (OR = 1.34, 95% CI 0.70-2.55, p = 0.37).

The study also examined the probability of developing an intestinal parasitic infection caused by a pathogen when the CD4 count was lower than 150 /mL; while the results were not statistically significant (OR=1.88, CI=0.94-3.74, p=0.072), the study found that the group with CD4 counts below this threshold had a higher incidence of pathogenic parasitic infections, with 66.0% (33/50) of individuals displaying such infections compared to 50.9% (59/116) in the group with CD4 counts above this cutoff.

The probability of pathogenic infection in individuals with CD4 counts below 150/mL was assessed for the most common pathogens. However, the study did not yield statistically significant results. Specifically, the group with counts below this threshold had a *G. Lamblia* infection rate of 38.7% (12/31), with an OR of 1.61(0.71-

3.63) and a p-value of 0.25. As for *E. histolytica/dispar*, the frequency of infection in this group was 22.7% (10/44) and the OR was 0.60(0.27-1.34) with a p-value of 0.21.

Features	Pathogenic Parasite Infection		
	Yes	**OR IC (95%)**	**aOR IC (95%)**
Sex			
Female	36/69(52.2%)	0.80(0.43-1.49)	0.98(0.50-1.95)
Male	56/97(57.7%)	1	1
Age			
≤29 Yr	35/67(52.2)	1	1
30-39 yr	32/52(61.5)	0.93(0.59-1.45)	1.27(0.55-2.96)
≥40 yr	25/47(53.2)	0.72(0.45-1.13)	0.91(0.41-2.06)
Consistence Liquid (Feces)			
Yes	19/24(79.2%)	3.59(1.27-10.20)	3.83(1.26-11.66)
No	73/142(51.4%)	1	1
CD4 T cells count			
< 350 per mm3	51/93(54.8%)	1	1
350-500 per mm3	19/35(54.3%)	0.98(0.45-2.13)	0.92(0.40-2.12)
>500 per mm3	22/38(57.9%)	1.13(0.53-2.42)	1.13(0.42-3.01)
ART			
No	6/6(100%)	1	
Yes	86/160(53.8%)	0.53*(0.46-0.62)	
BMI- Kg/M^2			
Under weight (<20)	12/16(75%)	1	1
Normal (20-25)	48/91(52.7%)	0.80(0.62-1.07)	0.44(0.12-1.64)
Over weight (>25)	32/59(54.2%)	0.81(0.62-1.04)	0.39(0.11-1.36)
Income			
< Minimum wage	41/75(54.7%)	1	1
> Minimum wage	51/91(56.0%)	1.06(0.57-1.96)	1.16(0.58-2.31)
Unintentional weight loss			
Yes	36/60(60.0%)	1.34(0.70-2.55)	1.37(0.68-2.78)
No	56/106(52.8%)	1	1
*Relative Risk			

Table 9. Characteristics of patients with HIV/AIDS and their association with prevalence of Pathogenic intestinal parasites

The analysis was also performed for the most frequent parasite, *B. hominis*, although it was not considered in the group of pathogens, and showed a frequency of 23.5% (12/51) and an OR of 0.62 (0.29-1.33), with a p-value of 0.22.

At the time of developing assumptions and conducting exploratory analysis to establish binary logistic regression within the context of multivariate analysis, taking into account the infection by any intestinal parasite, and additionally, for the pathogenic infection, correlations and interactions between variables were identified. These were eliminated from the process through exploratory analysis using the likelihood method. As a result, in both cases, it was found that the only variable that significantly contributed to the model was the liquid consistency of the stool. Therefore, it was deemed unnecessary to perform a refreshment (Table 9).

CHAPTER 8: DISCUSSION AND KEY ACTIONS

Chapter 8: Discussion and Key actions

Using the information currently available in Bolivia for 2022, it is possible to conclude that approximately 56% of people living with HIV in Cochabamba are receiving antiretroviral therapy. However, if we take into account new notifications and new treatment initiations, this percentage does not exceed 37%. The optimistic forecast is that this disparity will decrease by 2030. [111]

The regional reference center for HIV control (CDVIR) in Cochabamba, concentrates patients diagnosed with HIV from the entire health system who attend regular check-ups. The socio-epidemiological profile of the patients studied corroborates the national data reported by the Ministry of Health regarding the general prevalence, but the comorbidities referring to parasitic infections had not been studied in detail until the development of this study.

Parasitic infections that could be linked to systemic inflammatory processes associated to alterations in the intestinal microbiome have been detected in many cases. [103-105] These infections have been identified as significant factors in immunosenecence and premature aging in individuals living with HIV, although symptoms may not appear in most cases, as evidenced in this study.

Another important aspect is related to the diagnostic capacity of these infectious agents in the context of developing countries and especially Bolivia, where molecular detection methods are not accessible due to the lack of adequate trained personnel, technical support, equipment and costs. These have been carried out within the framework of research but are not applicable to the clinical context.

This report also identifies shortcomings in the reference laboratories for the identification of intestinal parasites such as coccidia and *Cryptosporidium* that show low concordance in the framework of the second reading of the staining plates as part of the quality control in the studies carried out and contrasted by molecular biology.

The quality control process of the laboratory procedures established in the cross-sectional study includes a second reading of the staining plates that was carried out for the detection of coccidia and parasites that are difficult to detect by light microscopy, with special emphasis on *Microsporidium* and *Cryptosporidium*.

The staining plates were sent to two independent laboratories in the same geographical context and to another one abroad. The concordance rate with the laboratory abroad was 99%, while the local laboratories showed concordance rates of less than 10% for parasites such as *Cryptosporidium* and less than 5% for *Microsporidium*.

These data allow us to determine the existence of a significant underdiagnoses of these etiological agents currently in the local context, due to the deficiency of laboratories to detect these parasitic forms, even considering patients with a long history of chronic diarrheal diseases. Based on the results of these studies, the LABIMED laboratory has implemented a registration system for direct corproparasitological studies carried out, including those carried in general population.

A follow-up process was provided to patients who tested positive for any parasitic etiologic agent, and a laboratory control was established during the 2 weeks

following participation in the study, which was carried out by the CDVIR, and the drugs for treatment were subsidized by the study.

CDVIR physicians prescribed treatment for each case were pathogenic species was identified. The decision for treatment was made based on the spectrum of sensitivity of each parasite in cases where a pathogenic parasite was detected. In these cases coproparasitological control was performed to verify the efficacy of the treatment. For those cases in which treatment efficacy was not evident, a longer follow-up process was established for 6 weeks.

Albendazole is the drug of choice for intestinal and disseminated microsporidiosis, it reduces gastrointestinal symptoms and has extensive histopathological evidence of elimination in the intestine and weight gain in patients with *E. intestinalis* infection. In addition, as part of the treatment, PLWHA must take special care when consuming drinking water. Additional recommendations include increasing attention to hand washing and personal hygiene, avoiding eating undercooked meat or seafood.

To establish a comparison of our study population, a methodology similar to that of the first study described in our intervention has been considered, analyzing the LABIMED database corresponding to the 2016 period, adding both the PLWHA population and the general population referred to this instance, considering that this group comes to the reference laboratory with a high diagnostic suspicion and evident clinical manifestations, which conditions a higher probability of obtaining positive results in the screening for intestinal parasites.

This information showed a similar distribution of parasitic diseases in PLWHA to that reported in our studies, with a higher prevalence of *B. hominis* (30.4%), *G. lamblia* (11.6%), *E. coli* (9.2%), *E. histolytica/dispar* (8%) and *H. nana* (1.8%).

In the general population *B. hominis* reaches 28.6%, *E. coli* 7.4% and *Chilomastix mesnili* 1%, being the only parasitic forms reported. It is important to highlight that PLWHA carries out routine controls in this laboratory, while the general population comes to the laboratory sent under high diagnostic suspicion and generally with symptoms. The discrepancy between the motives for seeking consultation and the laboratory testing results may suggest a greater occurrence of parasitic diseases in PLWHA.

The study conducted by Zurita-Céspedes et al. at LABIMED Cochabamba in 2015 showed that among the general population, Blastocystis hominis was the most frequent parasite, affecting 44.5%, followed by Giardia lamblia with 10.6%, and Entamoeba histolytica/dispar with 8.1%. Higher frequencies were reported in pasty and semi-pasty samples (53.69% and 38.33%, respectively). [95]

However, it is important to note that, as in the LABIMED data analysis we performed, the study population was more likely to have positive results because of the high diagnostic suspicion at this regional referral center. Therefore, this control group was not considered at the time of our study.

8.1 Major results compared to literature

Our study established the epidemiological situation of HIV-intestinal parasite coinfection in a defined geographical context and evaluated the factors associated with this coinfection.

The global prevalence rate of intestinal parasite infections in the cross-sectional study reached 33.0%, and polyparasitism 7.8%, consistent data with retrospective analysis. That is to say, one out of every 3 recruited patients was identified for parasitic infection. Our data showed that the main predisposing factor to identify a patient with intestinal parasitic infections was the presence of liquid feces (Diarrhea) at the time of sample collection. Also, unintentional weight loss, low current CD4+ cell counts and not receiving ART showed association with the probability of having a parasitic infection.

We must emphasize that despite UNAIDS strategies and that in the study more than 98% received ART at least 3 months before recruitment, more than 50.5% of the participants had a CD4 T lymphocyte level lower than 350 (cells/mm3) and only 57.9% had an undetectable viral load.

The sample of patients accessed during the cross-sectional study consisted mostly of individuals who regularly attended routine check-ups, as indicated by the fact that 32.0% attended from one to four check-ups and 34.8% attended more than five controls.

Other factors, such as seasonality (the study was conducted at the beginning of the year) and the use of different laboratory technicians, may have contributed to the differences in the results between the comparison periods. This may explain why the prevalence of *E. coli* was significantly higher in 2011 (12.1%) compared to the cross-sectional study (0.2%). In addition, the prevalence of *E. histolytica/Dispar* in the cross-sectional study (6.6%) was higher than the maximum reported in 2014 (3.0%).

The most prevalent parasite found in both the retrospective and cross-sectional studies was Blastocystis hominis, which is generally considered non-pathogenic. Nonetheless, there is an ongoing controversy about its pathogenicity, even in patients with severe immunosuppression resulting from other causes. Some studies have linked it to diarrhea and non-specific digestive symptoms in individuals living with HIV. [112] In our study no clinical manifestations were associated with this particular parasite infection.

The prevalence found is different from other studies found in the South American region. In the cross-sectional study developed in Peru by Omayra Chincha L. et. Al., the 39.8% positive parasitological tests were found in a population of HIV-positive patients; *B. hominis* was the most prevalent species with 24.6%, *I. belli* 8.4%, *Cryptosporidium* spp. (4.5%), *G. lamblia* (4.2%), *Cyclospora cayetanensis* (3.3%), *E. histolytica* and *H. nana* (1.2%). [113] (Figure 19)

In the study of Omayra Chincha L. (Perú), different techniques were implemented sequentially, which were the basis for designing our cross-sectional study, taking into account the findings in a wide literature review.

The findings in that study do not correspond with those described in LABIMED/UMSS (Cochabamba), where the most common species were *G. lamblia* and *E. histolytica/dispar*. This difference may be attributed in part to variations in the epidemiological profile of the region and in part to differences in the detection methods used to identify intestinal parasites. LABIMED used direct observation methods in its retrospective data, which limited the identification of other parasitic species present in other regions, such as Coccidia, *Microsporidium* spp., and *Cryptosporidium* spp.

Our study applied several techniques, including direct observation, sedimentation, modified Ziehl-Neelsen (Kinyoun), and Diddier stains, to mainly detect coccidian oocysts. These techniques are part of the LABIMED protocol, which is currently considered as the reference laboratory for identifying these agents.

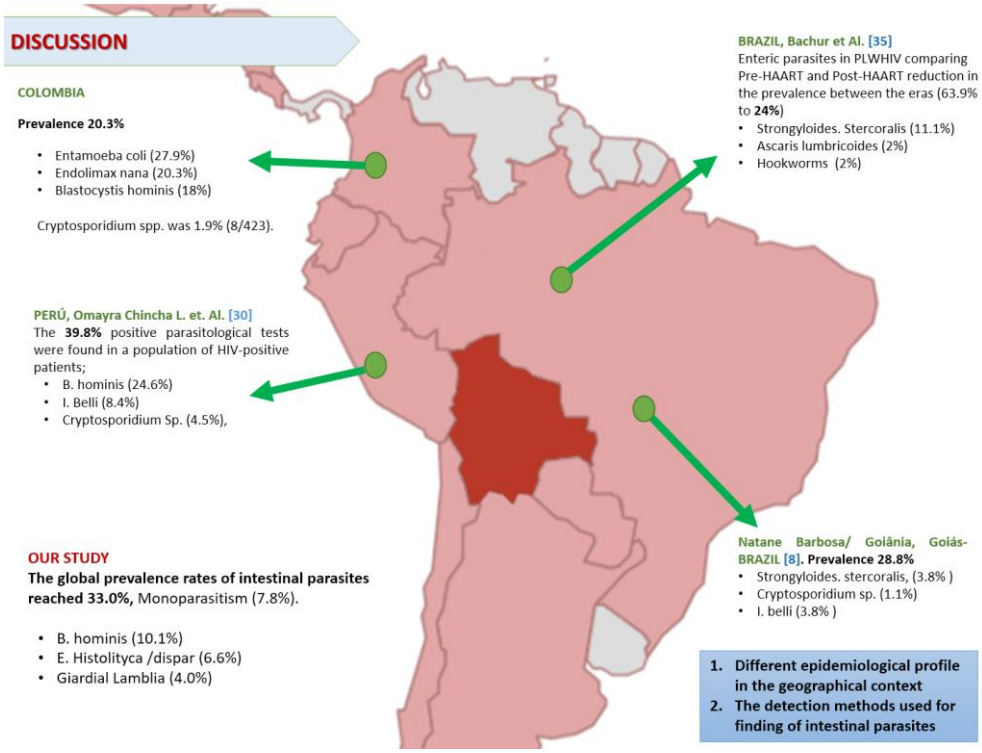


Figure 22: Results related to literature of intestinal parasitic infections in PLWHA in Cochabamba-Bolivia
Source: Own elaboration

In our series, there is a significant increase in the number of direct microbiological tests performed in the LABIMED service between 2011 and 2015. In the absence of statistics related to the prevalence of intestinal parasites in PLWHA in Bolivia, it is

difficult to establish whether this represents a real increase in the burden of these diseases or if other factors intervene, such as the increase in the number of newly diagnosed patients each year admitted and monitored by the National HIV/AIDS Control Program or if these present in chronic stages with low CD4 levels, which would condition a greater risk of infections. The symptoms for which the parasitological tests were requested have not been recorded as laboratory routine in the CDVIR control protocol, being a limitation of the retrospective study.

The presence of opportunistic agents (*Cryptosporidium* spp., M*icrosporidium*, and *Isospora belli*) is an infrequent finding among HIV/AIDS patients attending the CDVIR of Cochabamba, this is attributable to the screening methods since there was no systematic detection of these infections in the period 2011-2015. There is evidence that direct microbiological tests for the detection of intestinal parasites are insufficient, especially for agents reported as highly prevalent in people with AIDS, such as *Cryptosporidium* and *Microsporidium.*

Most studies were performed to identify *Cryptosporidium* spp. tend to focus on clinical settings due to the strong association of this infection with immunosuppression. Detection of *Cryptosporidium* spp. in stool samples is usually performed by multiple techniques such as acid-fast staining, direct fluorescent antibodies (DFA), and enzyme immunoassays, while molecular methods such as polymerase chain reaction (PCR) are increasingly used in reference diagnostic laboratories to identify *Cryptosporidium* spp. to species level. [114] However, in the context of clinical outpatient monitoring of PLWHA in Bolivia, these methods are not currently applicable. Our study used a microscopic examination of stool samples using several techniques to detect *Cryptosporidium* spp. and found these to be effective for the purpose of the research.

Tests for *Cryptosporidium* spp., *Microsporidium* spp., and *Isospora belli* are not routinely performed; therefore, healthcare professionals should specifically request testing for these infections based in a protocol, and especially in HIV patients with a CD4 cell count below 100 cells/µl.

For *Microsporidium* spp., the most common diagnostic method is to stain samples by using fluorescence, such as Calcofluor White M2R, Uvitex 2B, or Fungi Fluor, and by using Weber's modified trichrome staining**.** [115]

The prevalence of intestinal parasites can vary according to geographical characteristics of the region, socioeconomic characteristics, health system, or nutritional aspects of the individuals studied. As referred by Bachur et al. in 2008 in Brazil, who compared enteric parasites in PLWHA in Pre-HAART and Post-HAART period using the direct, Lutz, Baermann-Moraes and modified Ziehl-Neelsen methods for fecal parasitological examinations. They found a difference for *Strongyloides stercoralis* ($p<0.001$), *Ascaris lumbricoides* ($p<0.001$), Hookworms ($p<0.001$), *Trichuris trichiura* ($p<0.001$), *Giardia duodenalis* ($p = 0.008$), *I. belli* ($p = 0.010$), and *Cryptosporidium* sp. ($p = 0.001$), between the two periods respectively. Also finding a significant reduction in the prevalence of enteroparasites between the eras (63.9% to 24%; $p<0.0001$). Although the prevalence of opportunistic parasites was higher than in our study. [116] The results of Bachur et al. showed a significant difference between these two periods in terms of a reduction in prevalence as the prevalence of enteric parasites decreased.

In Bolivia, the absence of statistics related to the prevalence of intestinal parasites in PLWHA considering these periods makes it impossible to perform a comparative analysis.

Before ART was distributed free to HIV patients, intestinal parasites were common in HIV patients. Even after the availability of free ART, intestinal parasites remain a major cause of morbidity [117]. HIV induces an immunodeficiency that favors opportunistic infection, contributes to worsening the clinical picture of the patient by causing malnutrition, weight loss and chronic diarrhea, wasting syndrome [43,44].

Antiretroviral therapy has spread in the region, the high adherence of patients to antiretroviral therapy markedly improves the immune response, slows the progression of the disease and reduces susceptibility to infections, as a result, 98% of recruited patients in our prospective study they received ART. The effect of this intervention has gradually reduced the incidence of opportunistic infections in patients with HIV / AIDS. However, poor adherence to treatment negatively affects the prognosis of patients.

Therefore, if ART coverage is high with a small number of people in AIDS phase or immunosuppression, the prevalence of opportunistic parasites will be significantly reduced. Similarly, in the results of our cross-sectional study, the presence of parasitic infections was lower in patients with ART ($p = 0.04$, multivariate analysis).

ART is currently administered upon confirmation of HIV infection, so immunosuppression levels should not be low. Despite this, more than 50% of PLWHA in our study had CD4+ cell counts below 350 (cells/mm3).

Malnutrition was studied in the context of a frequent event in the advanced stages of HIV infection. Within the pathogenesis of this condition associated with HIV, the main factors responsible considered was the absorption deficit, metabolic alterations, and the atrophy of the intestinal villi due to a cytopathic effect of HIV,

the Hypercatabolic states associated with aggregate processes are responsible wasting syndrome.[44]

The absorption deficit is caused by parasites such as *G. lamblia*, which is also associated with villus atrophy and metabolic alterations, as well as conditioning poor intake. In our study, *G. lamblia* reports one of the highest prevalence (4%), concerning the pathogens identified by direct methods and immunochromatography, also associated with feces with a lienteric appearance, evidencing a malabsorption syndrome. [118]

Poor sanitary conditions, the management of water and sewerage services, associated with the lack of regulation and public policies, represent a serious problem evidenced in the scope of this study. Although governments have made notable progress in the last decade in providing good quality water to the population, these are still insufficient, especially in rural areas.

The high prevalence rate of commensal parasites observed in the present study can be considered as an indicator of poor hygiene and sanitation, as well as the consumption of contaminated food and water by these patients. The waterborne diseases, especially those caused by intestinal protozoa, are among the most relevant pathogens described in PLWHA [119].

Geographic context, weather, and sanitary conditions, including the provision of drinking water and excreta disposal, largely determine the epidemiological profile related to intestinal parasitic infections. Despite the fact that there are few studies related to the prevalence of intestinal parasites in this geographic context, differences between ecologic regions are evident. In this regard, the findings showed that the detection of *S. stercoralis*, *Cryptosporidium* sp. and *I. belli* was

lower than the prevalence reported by Barcelos et al. in a similar study carried out in Goiânia, Goiás-Brazil. [120]

The Coccidia life cycles are suited to waterborne and foodborne transmission and their oocysts are resistant to conventional water treatment protocols**.**

The presence of diarrhea showed significant correlation with infection by intestinal parasites, corroborating the findings of Barcelos et al, and Bachur et al. [116,120] who reported a positive relationship between diarrhea and intestinal parasites positive samples, Cardoso et al. [121]

Diarrhea accompanied by wasting is a major problem among HIV-infected persons, particularly in developing countries. A number of opportunistic disorders affect the gastro intestinal system of persons with HIV/AIDS, including parasitic infections such as *Cryptosporidium*, *Giardia*, and *Isospora*. [123]

In line with a global systematic review and meta-analysis performed by Ze-Dong Wang et al., where prevalence of *Isospora belli* was reported at 2.5%, a similar prevalence was observed in our study using appropriate detection methods based on Kinyoun staining (3.6%). [122]

Cryptosporidiosis is known to cause severe and prolonged diarrhea among immune-compromised individuals. Non-specific mechanisms such as villi atrophy and inflammatory reactions could play a role in the disease's progression. [123,124]

Molecular biology methods are useful in identifying parasitic infections that cannot be detected by other means of diagnosis. These methods have been standardized and can be employed if the necessary conditions are present. Unfortunately, their

clinical use is often limited in areas with limited resources due to technical availability and cost.

During our study, a home PCR focused on the detection of microsporidium was implemented, due to the difficulties involved in the identification and adequate treatment of these agents specifically in patients with chronic diarrhea refractory to treatment. In the short term, we propose to evaluate the implementation of this technique and validate it adequately.

This implies great importance for clinical practice in terms of microbiological studies, in Bolivia, the subsidy for laboratory studies for PLWHA only allows direct microscopic studies in the reference laboratory (LABIMED), performing controls on PLWHA only through direct microscopy methods, molecular biology techniques are not available in this context and have only been feasible in the context of this study.

8.2 Public health actions related to results

Opportunistic infections (OIs) represent a cause of morbidity and mortality in PLWHA, although there is a high degree of uncertainty due to the poor coverage of the health system referring specifically to HIV, caused by the centralization of services at the urban level, despite the information that evidence an extension and increase of transmission in rural areas.

In the geographic context of Cochabamba/Bolivia, it is necessary to increase surveillance and control of intestinal parasitic infections. Routine diagnostic methods used until the time of our intervention were unable to identify etiologic agents associated with the clinical deterioration of the patient, resulting in a significant number of underdiagnoses.

Within the framework of disease control, taking into account prevention interventions, and also considering the long length and short latency of the exposed health problem, the following actions were proposed in search of contributing to the control of the problem:

A. Our studies have facilitated the training of personnel in various diagnostic techniques utilized in the LABIMED reference laboratory. Unfortunately, personnel trained under these programs are not guaranteed adequate job stability, which poses a risk to the availability of trained personnel in the reference center. To mitigate this risk, it is essential to adhere to current labor legislation when hiring laboratory personnel to ensure their job security and the continuity of capacity-building interventions. Additionally, necessary materials and supplies are allocated annually within the UMSS budget to guarantee adequate supply levels.
B. Thus, based on the evidence generated, we suggest the inclusion of concentration, staining, immunochromatography methods, in addition to standardizing and achieving the availability of molecular biology methods, especially for the identification of microsporidium species, due to their implications and usefulness in clinical practice.

   These tests should be used to reduce the prevalence of these infections and their consequences, such as wasting syndrome and intestinal microbiome alterations. Therefore, better detection capacity together with health interventions within the framework of prevention will contribute to reduce the prevalence of these pathologies in PLWHA.
C. Deficiencies have been noted in the documentation of microbiological and immunological diagnoses in LABIMED and CDVIR. A new registry system has

been implemented; this system integrates and digitizes the Parasitology and Immunology databases, making the information easily accessible to be systematized for epidemiological surveillance purposes.

The proposed interventions are framed in the etiological research, determination of risk factors, the description of the transmission dynamics, applying monitoring of the evolution of the problem, improving the diagnostic and treatment processes, interfering the cycle that perpetuates the disease producing immunosuppression resulting from underdiagnosis, the consequent chronicity, sequels and greater immunosuppression.

Appropriate initiation of antiretroviral therapy before the patient's immune system is severely compromised can prevent opportunistic infections. It is uncertain whether specific chemoprophylactic regimens are effective in preventing microsporidiosis, and the prevalent species identified in this particular population in our study remain unknown. Therefore, antiretroviral therapy should be part of the initial treatment for microsporidia infections, and emphasis should be placed on strategies for improving adherence.

Currently, UMSS (LABIMED), CDVIR, and the National HIV Control Program collaborated on a pilot diagnostic algorithm for PLWHA presenting to the reference laboratory. That includes, for all newly diagnosed HIV patients, the collection of a stool sample initially examined by light microscopy, with an aliquot subjected to different stains (modified Kinyou and Diddier).

Patients with high suspicion based on the clinical status, presenting steatorrhea or diarrhea, undergo stains (Kinyoun/Diddier modified) immunochromatography and homemade molecular biology techniques for the detection of *Microsporidium* spp.,

and *Cryptosporidium* spp., based on a history of chronic diarrhea and recent involuntary weight loss.

8.3 General Conclusions and perspectives

Resources have been focused to detect and confirm the health problem regarding opportunistic infections, specifically those caused by parasitic agents. In Bolivia there was a considerable and important underdiagnosis.

The dynamics of the disease have been determined, a small percentage of PLWHA in Bolivia had self-limited and slight symptomatic parasitism, the vast majority had a stable infection that contributing to immunological deterioration, chronic inflammatory processes that progress in the natural course of the disease to cases serious and the death of the patient.

These process is favored by a deficient diagnostic process determined mainly by the limitations of the diagnostic tests implemented by the National Program for HIV Control in Bolivia (Direct observation). A diagnostic algorithm that allows the identification of parasitic agents has been proposed to reduce the consequent underdiagnosis. In addition this must be sustainable through the transfer of knowledge and technology generated through the doctoral program.

The evidence collected in the framework of this thesis, exhibits the high rates of prevalence of intestinal parasitic infections, generates evidence on the need to implement additional interventions in the context of surveillance of HIV patients, deploy laboratory tests that must have a higher performance in the monitoring and treatment of patients with HIV/AIDS.

It is possible that there is an increase in the actual number of cases of intestinal parasites in the country, which require adequate management to avoid the morbidity and mortality complications of PLWHA in Bolivia. Currently it is crucial to strengthen surveillance of HIV patients.

Our results underscores the meaning of conducting regular testing for opportunistic infections in individuals living with HIV in the country and emphasizes the importance of using different parasitological methods to determine diagnoses and avoid subdiagnosis. In addition, this study identifies the factors associated with enteric parasite infections based on the epidemiological situation in Cochabamba, highlighting the need for preventative measures to reduce infection rates and prevent their occurrence.

Timely and appropriate treatment is essential to address the clinical conditions of patients with parasitic infections, and its implementation will prevent complications of morbidity and mortality in people living with HIV in Bolivia

The immediate focus is developing interventions to establish care protocols for treating parasitic infections and implementing a proper diagnostic algorithm to avoid incorrect diagnoses. Additionally, information systems for epidemiological surveillance need improvement to monitor and evaluate the activities of the National HIV Control Program in Bolivia.

In the context of the research, the cross-sectional study database contains anthropometric, geographic, and immunological information that will be used for future publications. Among the proposed studies, an evaluation of the laboratory methods used and their comparative performance, including the sensitivity and specificity of the PCR technique for detecting coccidia, is proposed.

CDVIR physicians in clinical practice face challenges in treating and detecting *Cryptosporidium* spp. and *Microsporidium* spp. infections. Therefore, there is a need to optimize the PCR technique and manage the availability of drugs to improve the therapeutic response at a national level.

There are limitations in this study on cultural aspects of care of the Aymara and Quechua populations in Cochabamba. Ethnocultural issues can play an important role in influencing the medical care for these communities. In many cases, these communities have specific cultural beliefs and practices related to health and illness that may differ from Western medicine and practices. These factors can influence how these communities seek medical care and the trust they have in medical providers.

The language barrier may also limit their access to adequate medical care. In addition, transportation problems, lack of medical services, and poor healthcare infrastructure are additional challenges that may affect these communities.

The community's cultural beliefs and practices regarding sexuality may influence their level of participation in the study. Some community members may be less likely to talk about sexual issues, which limits the generalizability of the study results. Due to the sensitive nature of the subject matter, aspects of sexual orientation are difficult to probe in these populations.

Generally, members of Quechua and Aymara indigenous groups who choose to participate in a study may have different attitudes and beliefs than those who do not, resulting in self-selection bias. This limits the generalizability of the results to the community at large.

8.4 Health policies and guidelines

Identification of parasitic comorbidities in PLWHA has been achieved, showing an underdiagnosis, a therapeutic intervention, and protocols for the identification of parasitic infections have been proposed. This will allow a timely and adequate treatment, even though this intervention has only been in force during the study implementation period, thanks to the subsidy for treatment, administering broad-spectrum treatments to eliminate infections in all persons identified with pathogenic intestinal parasitic infection, all in coordination with the CDVIR clinicians.

Endemic areas with high rates of parasitic disease often implement large-scale treatment programs to lessen the impact on public health. The goal is to reduce the burden of disease throughout the community, but effective and ethical treatment with proper planning must be ensured. Proper coordination between all parties involved is essential, and evaluating the effectiveness of the program and its impact on the population is essential. A comprehensive approach is required to address the root causes of parasitosis, as a mass treatment alone may not be sufficient. It must include preventive measures and individualized follow-up medical treatment and may involve other forms of specialized care for patients.

There is a problem related to adherence to antiretroviral treatment, as evidenced by the low percentage of patients currently achieving undetectable viral loads in our cross-sectional study. Despite the 98% ART coverage, only 57.9% of patients achieved viral suppression. In countries such as Belgium, data shows that 98% of patients at the reference center receive ART and 96-97% achieve undetectable viral

loads. While adherence is not the only factor to consider in this issue, toxicity with current regimens is very infrequent. We suggest conducting a qualitative study to identify the factors related to poor adherence to antiretroviral treatment, exploring accessibility from various cultural, economic, geographic, and social factors.

9. References

1. Alemu, Y., Degefa, T., Bajiro, M., & Teshome, G. (2022). Prevalence and intensity of soil-transmitted helminths infection among individuals in model and non-model households, South West Ethiopia: A comparative cross-sectional community based study. PLOS ONE, 17(10), e0276137. https://doi.org/10.1371/journal.pone.0276137
2. Chammartin, F., Scholte, R. G., Guimarães, L. H., Tanner, M., Utzinger, J., & Vounatsou, P. (2013). Soil-transmitted helminth infection in South America: A systematic review and geostatistical meta-analysis. The Lancet Infectious Diseases, 13(6), 507-518. https://doi.org/10.1016/S1473-3099(13)70071-9
3. Montresor A, Mwinzi P, Mupfasoni D, Garba A (2022) Reduction in DALYs lost due to soil-transmitted helminthiases and schistosomiasis from 2000 to 2019 is parallel to the increase in coverage of the global control programmes. PLoS Negl Trop Dis 16(7): e0010575. https://doi.org/10.1371/journal.pntd.0010575
4. Wale M, Gedefaw S. Prevalence of Intestinal Protozoa and Soil Transmitted Helminths Infections among School Children in Jaragedo Town, South Gondar Zone of Ethiopia. J Trop Med. 2022 Mar 12;2022:5747978. doi: 10.1155/2022/5747978. PMID: 35313547; PMCID: PMC8934235.
5. Cissé G. Food-borne and water borne diseases under climate change in low- and middle-income countries: Further efforts needed for reducing environmental health exposure risks. Acta Trop. 2019 Jun;194:181-188. doi: 10.1016/j.actatropica.2019.03.012. Epub 2019 Apr 1. PMID: 30946811; PMCID: PMC7172250.
6. Gizaw, Z., Addisu, A., & Gebrehiwot, M. (2019). Socioeconomic Predictors of Intestinal Parasitic Infections Among Under-Five Children in Rural Dembiya, Northwest Ethiopia: A Community-Based Cross-sectional Study. Environmental Health Insights, 13. https://doi.org/10.1177/1178630219896804
7. Sepahvand, F., Mamaghani, A. J., Ezatpour, B., Badparva, E., Zebardast, N., & Fallahi, S. (2022). Gastrointestinal parasites in immunocompromised patients; A comparative cross-sectional

study. Acta Tropica, 231, 106464. https://doi.org/10.1016/j.actatropica.2022.106464

8. Sard BG, Navarro RT, Esteban Sanchis JG. Amebas intestinales no patógenas: una visión clinicoanalítica [Non-pathogenic intestinal amoebae: a clinical-analytical overview]. Enferm Infecc Microbiol Clin. 2011 Mar;29 Suppl 3:20-8. Spanish. doi: 10.1016/S0213-005X(11)70023-4. PMID: 21458707.
9. Li J, Cui Z, Li X, Zhang L. Review of zoonotic amebiasis: Epidemiology, clinical signs, diagnosis, treatment, prevention and control. Res Vet Sci. 2021 May;136:174-181. doi: 10.1016/j.rvsc.2021.02.021. Epub 2021 Feb 27. PMID: 33676155.
10. Shirley DT, Farr L, Watanabe K, Moonah S. A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. Open Forum Infect Dis. 2018 Jul 5;5(7):ofy161. doi: 10.1093/ofid/ofy161. PMID: 30046644; PMCID: PMC6055529.
11. Carrero JC, Reyes-López M, Serrano-Luna J, Shibayama M, Unzueta J, León-Sicairos N, de la Garza M. Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. Int J Med Microbiol. 2020 Jan;310(1):151358. doi: 10.1016/j.ijmm.2019.151358. Epub 2019 Sep 19. PMID: 31587966.
12. Zulfiqar H, Mathew G, Horrall S. Amebiasis. 2023 Jan 31. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30137820.
13. Uddin MJ, Leslie JL, Petri WA Jr. Host Protective Mechanisms to Intestinal Amebiasis. Trends Parasitol. 2021 Feb;37(2):165-175. doi: 10.1016/j.pt.2020.09.015. Epub 2020 Oct 23. PMID: 33502317; PMCID: PMC7840892.
14. Abuseir S. A systematic review of frequency and geographic distribution of water borne parasites in the Middle East and North Africa. East
15. Mediterr Health J. 2023;29(2):151–161. https://doi.org/10.26719/emhj.23.016Ryan U, Hijjawi N, Feng Y, Xiao L. Giardia: an under-reported foodborne parasite. Int J Parasitol. 2019 Jan;49(1):1-11. doi: 10.1016/j.ijpara.2018.07.003. Epub 2018 Nov 1. PMID: 30391227.
16. Leung AKC, Leung AAM, Wong AHC, Sergi CM, Kam JKM. Giardiasis: An Overview. Recent Pat Inflamm Allergy Drug Discov. 2019;13(2):134-143. doi: 10.2174/1872213X13666190618124901. PMID: 31210116.

17. Diptyanusa A, Sari IP. Treatment of human intestinal cryptosporidiosis: A review of published clinical trials. Int J Parasitol Drugs Drug Resist. 2021 Dec;17:128-138. doi: 10.1016/j.ijpddr.2021.09.001. Epub 2021 Sep 21. PMID: 34562754; PMCID: PMC8473663.
18. Hassan EM, Örmeci B, DeRosa MC, Dixon BR, Sattar SA, Iqbal A. A review of Cryptosporidium spp. and their detection in water. Water Sci Technol. 2021 Jan;83(1):1-25. doi: 10.2166/wst.2020.515. PMID: 33460403.
19. O'Leary JK, Sleator RD, Lucey B. Cryptosporidium spp. diagnosis and research in the 21st century. Food Waterborne Parasitol. 2021 Aug 20;24:e00131. doi: 10.1016/j.fawpar.2021.e00131. PMID: 34471706; PMCID: PMC8390533.
20. Widmer G, Carmena D, Kváč M, Chalmers RM, Kissinger JC, Xiao L, Sateriale A, Striepen B, Laurent F, Lacroix-Lamandé S, Gargala G, Favennec L. Update on Cryptosporidium spp.: highlights from the Seventh International Giardia and Cryptosporidium Conference. Parasite. 2020;27:14. doi: 10.1051/parasite/2020011. Epub 2020 Mar 13. PMID: 32167464; PMCID: PMC7069357.
21. Almeria S, Cinar HN, Dubey JP. Cyclospora cayetanensis and Cyclosporiasis: An Update. Microorganisms. 2019 Sep 4;7(9):317. doi: 10.3390/microorganisms7090317. PMID: 31487898; PMCID: PMC6780905.
22. Li J, Zhang K, Zhang L. Cyclospora cayetanensis. Trends Parasitol. 2022 May;38(5):419-420. doi: 10.1016/j.pt.2021.11.003. Epub 2021 Dec 3. PMID: 34865971.
23. Li J, Wang R, Chen Y, Xiao L, Zhang L. Cyclospora cayetanensis infection in humans: biological characteristics, clinical features, epidemiology, detection method and treatment. Parasitology. 2020 Feb;147(2):160-170. doi: 10.1017/S0031182019001471. Epub 2019 Nov 8. PMID: 31699163.
24. Rojas-Velázquez L, Morán P, Serrano-Vázquez A, Portillo-Bobadilla T, González E, Pérez-Juárez H, Hernández E, Partida-Rodríguez O, Nieves-Ramírez M, Padilla A, Zaragoza M, Ximénez C. The regulatory function of Blastocystis spp. on the immune inflammatory response in the gut microbiome. Front Cell Infect Microbiol. 2022 Aug 31;12:967724. doi: 10.3389/fcimb.2022.967724. PMID: 36118018; PMCID: PMC9470931.
25. Ismail MH, Abbas SK, Molan AL. Novel association between blastocystosis and some hemogram parameters in Iraqi people

with and without irritable bowel syndrome. Ann Parasitol. 2022;68(3):491-500. doi: 10.17420/ap6803.455. PMID: 36584332.
26. Stensvold CR, Clark CG. Pre-empting Pandora's Box: Blastocystis Subtypes Revisited. Trends Parasitol. 2020 Mar;36(3):229-232. doi: 10.1016/j.pt.2019.12.009. Epub 2020 Jan 27. PMID: 32001133.
27. Rajamanikam A, Hooi HS, Kudva M, Samudi C, Kumar S. Resistance towards metronidazole in Blastocystis sp.: A pathogenic consequence. PLoS One. 2019 Feb 22;14(2):e0212542. doi: 10.1371/journal.pone.0212542. PMID: 30794628; PMCID: PMC6386359.
28. Krolewiecki A, Nutman TB. Strongyloidiasis: A Neglected Tropical Disease. Infect Dis Clin North Am. 2019 Mar;33(1):135-151. doi: 10.1016/j.idc.2018.10.006. PMID: 30712758; PMCID: PMC6367705.
29. Yeung S, Bharwada Y, Bhasker S, Boggild A. Strongyloidiasis: what every gastroenterologist needs to know. Ther Adv Chronic Dis. 2022 Nov 15;13:20406223221137499. doi: 10.1177/20406223221137499. PMID: 36407019; PMCID: PMC9666874.
30. Han B, Pan G, Weiss LM. Microsporidiosis in Humans. Clin Microbiol Rev. 2021 Dec 15;34(4):e0001020. doi: 10.1128/CMR.00010-20. Epub 2021 Jun 30. PMID: 34190570; PMCID: PMC8404701.
31. Khabisi SA, Najafi AN, Khorashad AS. Molecular diagnosis of intestinal microsporidia infection in HIV/AIDS-patients in Zahedan city, Southeast of Iran. Ann Parasitol. 2022;68(1):87-92. doi: 10.17420/ap6801.412. PMID: 35491745.
32. Loukas A, Maizels RM, Hotez PJ. The yin and yang of human soil-transmitted helminth infections. Int J Parasitol. 2021 Dec;51(13-14):1243-1253. doi: 10.1016/j.ijpara.2021.11.001. Epub 2021 Nov 10. PMID: 34774540; PMCID: PMC9145206.
33. Donohue RE, Cross ZK, Michael E. The extent, nature, and pathogenic consequences of helminth polyparasitism in humans: A meta-analysis. PLoS Negl Trop Dis. 2019 Jun 18;13(6):e0007455. doi: 10.1371/journal.pntd.0007455. PMID: 31211774; PMCID: PMC6599140.
34. Chan EYY, Tong KHY, Dubois C, Mc Donnell K, Kim JH, Hung KKC, Kwok KO. Narrative Review of Primary Preventive Interventions against Water-Borne Diseases: Scientific Evidence of Health-EDRM in Contexts with Inadequate Safe Drinking Water. Int J Environ Res Public Health. 2021 Nov 23;18(23):12268. doi:

10.3390/ijerph182312268. PMID: 34885995; PMCID: PMC8656607.
35. Li J, Wang Z, Karim MR, Zhang L. Detection of human intestinal protozoan parasites in vegetables and fruits: a review. Parasit Vectors. 2020 Jul 29;13(1):380. doi: 10.1186/s13071-020-04255-3. PMID: 32727529; PMCID: PMC7392835.
36. Della Bella C, Spinicci M, Rojo D, Grassi A, Gamboa H, Benagiano M, Torrez R, Tapinassi S, Gabrielli S, Cancrini G, Macchioni F, Alnwaisri H, Azzurri A, Monasterio J, Montresor A, Olliaro P, D'Elios MM, Bartoloni A. Decline in Total Serum IgE and Soluble CD30 in the Context of Soil-Transmitted Helminth Decline in Bolivia. Am J Trop Med Hyg. 2020 Apr;102(4):847-850. doi: 10.4269/ajtmh.19-0180. PMID: 31989919; PMCID: PMC7124912.
37. Fernández-Huerta M, Zarzuela F, Barberá MJ, Arando M, Esperalba J, Rodríguez V, Vall M, Falcó V, García-Pérez JN, Pumarola T, Espasa M, Sulleiro E. Sexual Transmission of Intestinal Parasites and Other Enteric Pathogens among Men Who Have Sex with Men Presenting Gastrointestinal Symptoms in an STI Unit in Barcelona, Spain: A Cross-Sectional Study. Am J Trop Med Hyg. 2019 Dec;101(6):1388-1391. doi: 10.4269/ajtmh.19-0312. PMID: 31549611; PMCID: PMC6896874.
38. Dagne N, Alelign A. Prevalence of Intestinal Protozoan Parasites and Associated Risk Factors among School Children in Merhabete District, Central Ethiopia. J Parasitol Res. 2021 Nov 25;2021:9916456. doi: 10.1155/2021/9916456. PMID: 34868670; PMCID: PMC8639259.
39. Popruk S, Adao DEV, Rivera WL. Epidemiology and subtype distribution of Blastocystis in humans: A review. Infect Genet Evol. 2021 Nov;95:105085. doi: 10.1016/j.meegid.2021.105085. Epub 2021 Sep 14. PMID: 34530156.
40. Van Gestel RS, Kusters JG, Monkelbaan JF. A clinical guideline on Dientamoeba fragilis infections. Parasitology. 2019 Aug;146(9):1131-1139. doi: 10.1017/S0031182018001385. Epub 2018 Aug 31. PMID: 30165915.
41. Ramezanzadeh S, Beloukas A, Pagheh AS, Rahimi MT, Hosseini SA, Oliveira SMR, de Lourdes Pereira M, Ahmadpour E. Global Burden of Cyclospora cayetanensis Infection and Associated Risk Factors in People Living with HIV and/or AIDS. Viruses. 2022 Jun 12;14(6):1279. doi: 10.3390/v14061279. PMID: 35746750; PMCID: PMC9228463.
42. Khan W, Rahman H, Rafiq N, Kabir M, Ahmed MS, Escalante PLR. Risk factors associated with intestinal pathogenic parasites in

schoolchildren. Saudi J Biol Sci. 2022 Apr;29(4):2782-2786. doi: 10.1016/j.sjbs.2021.12.055. Epub 2022 Jan 3. PMID: 35531160; PMCID: PMC9072892.

43. Justiz Vaillant AA, Naik R. HIV-1 Associated Opportunistic Infections. 2023 Jan 27. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30969609.
44. SeyedAlinaghi S, Ghayomzadeh M, Mirzapour P, Maroufi SF, Pashaei Z, Ali Z, Tantuoyir MM, Aghaie N, Vahedi F, Salmani R, MohsseniPour M, Qaderi K, Shahidi R, Peyman A, Varshochi S, Afzalian A, Maroufi SP, Mehraeen E, Dadras O, Hackett D. A systematic review of sarcopenia prevalence and associated factors in people living with human immunodeficiency virus. J Cachexia Sarcopenia Muscle. 2023 Mar 16. doi: 10.1002/jcsm.13212. Epub ahead of print. PMID: 36929581.
45. Sivay, M. V., Totmenin, A. V., Zyryanova, D. P., Osipova, I. P., Nalimova, T. M., Gashnikova, M. P., Ivlev, V. V., Meshkov, I. O., Chokmorova, U. Z., Narmatova, E., Motorov, U., Akmatova, Z., Asybalieva, N., Bekbolotov, A. A., Kadyrbekov, U. K., Maksutov, R. A., & Gashnikova, N. M. (2021). Characterization of HIV-1 Epidemic in Kyrgyzstan. Frontiers in Microbiology, 12. https://www.frontiersin.org/articles/10.3389/fmicb.2021.753675/full
46. Fujimoto, K., Bahl, J., Wertheim, J. O., Vecchio, N. D., Hicks, J. T., Damodaran, L., Hallmark, C. J., Lavingia, R., Mora, R., Carr, M., Yang, B., Schneider, J. A., Hwang, Y., & McNeese, M. (2021). Methodological synthesis of Bayesian phylodynamics, HIV-TRACE, and GEE: HIV-1 transmission epidemiology in a racially/ethnically diverse Southern U.S. context. Scientific Reports, 11. https://doi.org/10.1038/s41598-021-82673-8
47. Monitoreo Global del SIDA 2023 / Indicadores y preguntas para el seguimiento de los progresos de la Declaración Política sobre el VIH y el SIDA de 2021. https://www.unaids.org/sites/default/files/media_asset/global-aids-monitoring_es.pdf
48. UNAIDS / JC2684 An ambitious treatment target 90-90-90 to help end the AIDS epidemic. https://www.unaids.org/sites/default/files/media_asset/90-90-90_en.pdf
49. Organización Mundial de la Salud; 2019. Licencia: CC BY-NC-SA 3.0 IGO.Manual de uso de datos en cascada para detectar deficiencias en los servicios de VIH y de salud y mejorar los

programas [Cascade data use manual: to identify gaps in HIV and health services for programme improvement].
50. UNAIDS 2021 DATA REFERENCE 2021 / Joint United Nations Programme on HIV/AIDS-Geneva-Switzerland. https://www.unaids.org/sites/default/files/media_asset/JC3032_AIDS_Data_book_2021_En.pdf
51. García-Blanco D, Gravier-Hernández R, Rabeiro-Martínez CL, Gil Del Valle L, Pérez-Ávila J. Pharmacogenetic Markers: A Path toward Individualized HIV Therapy. MEDICC Rev. 2019 Apr-Jul;21(2-3):59-68. doi: 10.37757/MR2019.V21.N2-3.11. PMID: 31401638.
52. Programa Conjunto de las Naciones Unidas Sobre el VIH/SIDA (ONUSIDA 2021).ESTRATEGIA MUNDIAL CONTRA EL SIDA 2021-2026 ACABAR CON LAS DESIGUALDADES. ACABAR CON EL SIDA. https://onusidalac.org/1/images/global-AIDS-strategy-2021-2026_es.pdf
53. IN DANGER: UNAIDS Global AIDS Update 2022. Geneva: Joint United Nations Programme on HIV/AIDS; 2022. Licence: CC BY-NC-SA 3.0 IGO.
54. Li J, Gao Q, Zhang M, Liu J, Jia Y, Feng Y, Xia X, Dong X. A newly emerging HIV-1 circulating recombinant form (CRF110_BC) comprising subtype B and C among intravenous drug users in Yunnan, China. J Infect. 2021 Mar;82(3):e8-e10. doi: 10.1016/j.jinf.2020.12.015. Epub 2020 Dec 19. PMID: 33352212.Sabin CA, Lundgren JD. The natural history of HIV infection. Curr Opin HIV AIDS. 2013 Jul;8(4):311-7. doi: 10.1097/COH.0b013e328361fa66. PMID: 23698562; PMCID: PMC4196796.
55. Ye M, Chen X, Duo L, Ma J, Cao L, Zhang C, Zheng YT. Identification of two novel HIV-1 circulating recombinant forms of CRF111_01C and CRF116_0108 in southwestern Yunnan, China. Virulence. 2022 Dec;13(1):19-29. doi: 10.1080/21505594.2021.2010399. PMID: 34951549; PMCID: PMC9794008.
56. Hemelaar J, Elangovan R, Yun J, et al. Global and regional molecular epidemiology of HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. Lancet Infect Dis. 2019;19:143–155.
57. Lv T, Cao W, Li T. HIV-Related Immune Activation and Inflammation: Current Understanding and Strategies. J Immunol Res. 2021 Sep 29;2021:7316456. doi: 10.1155/2021/7316456. PMID: 34631899; PMCID: PMC8494587.

58. Hartana CA, Yu XG. Immunological effector mechanisms in HIV-1 elite controllers. Curr Opin HIV AIDS. 2021 Sep 1;16(5):243-248. doi: 10.1097/COH.0000000000000693. PMID: 34270465; PMCID: PMC8373669.
59. Massanella M, Bender Ignacio RA, Lama JR, Pagliuzza A, Dasgupta S, Alfaro R, Rios J, Ganoza C, Pinto-Santini D, Gilada T, Duerr A, Chomont N; MERLIN Study Group. Long-term effects of early antiretroviral initiation on HIV reservoir markers: a longitudinal analysis of the MERLIN clinical study. Lancet Microbe. 2021 May;2(5):e198-e209. doi: 10.1016/S2666-5247(21)00010-0. Epub 2021 Mar 23. PMID: 35544209.
60. Fenwick C, Joo V, Jacquier P, Noto A, Banga R, Perreau M, Pantaleo G. T-cell exhaustion in HIV infection. Immunol Rev. 2019 Nov;292(1):149-163. doi: 10.1111/imr.12823. PMID: 31883174; PMCID: PMC7003858.
61. Margolis DM, Archin NM, Cohen MS, Eron JJ, Ferrari G, Garcia JV, Gay CL, Goonetilleke N, Joseph SB, Swanstrom R, Turner AW, Wahl A. Curing HIV: Seeking to Target and Clear Persistent Infection. Cell. 2020 Apr 2;181(1):189-206. doi: 10.1016/j.cell.2020.03.005. Epub 2020 Mar 26. PMID: 32220311; PMCID: PMC7896558.
62. ONUSIDA Oficina Regional Para América Latina y el Caribe. (2021). Estrategias diferenciales de presentación de servicios de VIH en América Latina en tiempos de COVID-19. http://onusidalac.org/1/images/DSD_MMD_LAC_Spanish.pdf
63. Panamerican Health Organization -PAHO. Informe final sobre los Objetivos de Desarrollo del Milenio relacionados con la salud en la Región de las Américas. Washington D.C.:OPS,2017. https://iris.paho.org/bitstream/handle/10665.2/34114/9789275118782_spa.pdf?sequence=5&isAllowed=y
64. Visseaux B, Le Hingrat Q, Damond F, Charpentier C, Descamps D. Physiopathologie de l'infection par le VIH-2 [Physiopathology of HIV-2 infection]. Virologie (Montrouge). 2019 Oct 1;23(5):277-291. French. doi: 10.1684/vir.2019.0789. PMID: 31826849.
65. Hossain SMM, Khatun L, Ray S, Mukhopadhyay A. Identification of key immune regulatory genes in HIV-1 progression. Gene. 2021 Aug 5;792:145735. doi: 10.1016/j.gene.2021.145735. Epub 2021 May 26. PMID: 34048875.
66. Yang Y, Liu W, Hu D, Su R, Ji M, Huang Y, Shereen MA, Xu X, Luo Z, Zhang Q, Liu F, Wu K, Liu Y, Wu J. HIV-1 Nef Interacts with LMP7 To Attenuate Immunoproteasome Formation and Major Histocompatibility Complex Class I Antigen Presentation. mBio.

2020 Oct 27;11(5):e02221-19. doi: 10.1128/mBio.02221-19. PMID: 33109760; PMCID: PMC7593969.
67. Joshi A, Punke EB, Mehmetoglu-Gurbuz T, Peralta DP, Garg H. TLR9 polymorphism correlates with immune activation, CD4 decline and plasma IP10 levels in HIV patients. BMC Infect Dis. 2019 Jan 16;19(1):56. doi: 10.1186/s12879-019-3697-9. PMID: 30651082; PMCID: PMC6335820.
68. Ge, Y., Liu, Y., Fu, G., Lu, J., Li, X., Du, G., Fei, G., Wang, Z., Li, H., Li, W., & Wei, P. (2022). The Molecular Epidemiological and Immunological Characteristics of HIV-1 CRF01_AE/B Recombinants in Nanjing, China. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.936502
69. Lee MJ, Fidler S, Frater J. Immunotherapeutic approaches to HIV cure and remission. Curr Opin Infect Dis. 2022 Feb 1;35(1):31-41. doi: 10.1097/QCO.0000000000000803. PMID: 34873077.
70. Zila V, Müller TG, Müller B, Kräusslich HG. HIV-1 capsid is the key orchestrator of early viral replication. PLoS Pathog. 2021 Dec 30;17(12):e1010109. doi: 10.1371/journal.ppat.1010109. PMID: 34968390; PMCID: PMC8717999.
71. Lange UC, Verdikt R, Ait-Ammar A, Van Lint C. Epigenetic crosstalk in chronic infection with HIV-1. Semin Immunopathol. 2020 Apr;42(2):187-200. doi: 10.1007/s00281-020-00783-3. Epub 2020 Feb 11. PMID: 32047948; PMCID: PMC7174272.
72. Desormais I, Harimenshi D, Niyongabo T, Lacroix P, Aboyans V, Preux PM. HIV clinical stages and lower extremity arterial disease among HIV infected outpatients in Burundi. Sci Rep. 2021 Apr 15;11(1):8296. doi: 10.1038/s41598-021-87862-z. PMID: 33859308; PMCID: PMC8050048.
73. Parekh BS, Ou CY, Fonjungo PN, Kalou MB, Rottinghaus E, Puren A, Alexander H, Hurlston Cox M, Nkengasong JN. Diagnosis of Human Immunodeficiency Virus Infection. Clin Microbiol Rev. 2018 Nov 28;32(1):e00064-18. doi: 10.1128/CMR.00064-18. PMID: 30487166; PMCID: PMC6302353.
74. Feleke DG, Gebreweld A, Zewde G. Toxoplasmosis in Pregnant Women and HIV/AIDS Patients in Ethiopia: A Systematic Review and Meta-Analysis. J Parasitol Res. 2019 Oct 1;2019:4670397. doi: 10.1155/2019/4670397. PMID: 31662891; PMCID: PMC6791202.
75. Ayoade F, Joel Chandranesan AS. HIV-1 Associated Toxoplasmosis. 2022 Sep 20. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 28722907.

76. Guedes DL, Justo AM, Barbosa Júnior WL, Silva EDD, Aquino SR, Lima Junior MSDC, Montarroyos U, Bezerra GSN, Vieira AVB, Pereira VRA, Medeiros ZM. Asymptomatic Leishmania infection in HIV-positive outpatients on antiretroviral therapy in Pernambuco, Brazil. PLoS Negl Trop Dis. 2021 Jan 21;15(1):e0009067. doi: 10.1371/journal.pntd.0009067. PMID: 33476331; PMCID: PMC7853496.
77. Clark EH, Bern C. Chagas Disease in People with HIV: A Narrative Review. Trop Med Infect Dis. 2021 Nov 9;6(4):198. doi: 10.3390/tropicalmed6040198. PMID: 34842854; PMCID: PMC8628961.
78. Roberds A, Ferraro E, Luckhart S, Stewart VA. HIV-1 Impact on Malaria Transmission: A Complex and Relevant Global Health Concern. Front Cell Infect Microbiol. 2021 Apr 12;11:656938. doi: 10.3389/fcimb.2021.656938. PMID: 33912477; PMCID: PMC8071860.
79. Antela A, Azcoaga A, Sampedro E, Poveda T. Primary prevention. Enferm Infecc Microbiol Clin (Engl Ed). 2018 Sep;36 Suppl 1:31-34. English, Spanish. doi: 10.1016/S0213-005X(18)30244-1. PMID: 30115406.
80. Ambrosioni J, Petit E, Liegeon G, Laguno M, Miró JM. Primary HIV-1 infection in users of pre-exposure prophylaxis. Lancet HIV. 2021 Mar;8(3):e166-e174. doi: 10.1016/S2352-3018(20)30271-X. Epub 2020 Dec 11. PMID: 33316212.
81. Bavinton, B. R., & Grulich, A. E. (2021). HIV pre-exposure prophylaxis: Scaling up for impact now and in the future. The Lancet Public Health, 6(7), e528-e533. https://www.sciencedirect.com/science/article/pii/S2468266721001122?via%3Dihub
82. Deku JG, Botchway KA, Kinanyok S, Gedzeah CK, Duneeh RV, Duedu KO. Intestinal Parasitic Infection and Associated Risk Factors among HIV-Infected Patients Seeking Healthcare in a Rural Hospital in Ghana. J Pathog. 2022 Aug 22;2022:5652637. doi: 10.1155/2022/5652637. PMID: 36046216; PMCID: PMC9424049.
83. Fregonesi BM, Zagui GS, de Abreu Tonani KA, Machado CS, Gomes-Silva G, Padula JA, Martone-Rocha S, Razzolini MTP, Plath M, Segura-Muñoz S. Human health risk assessment for (re)emerging protozoan parasites in surface water used for public supply and recreational activities. Environ Monit Assess. 2022 May 7;194(6):407. doi: 10.1007/s10661-022-10058-z. PMID: 35524884.

84. Lengongo JVK, Ngondza BP, Ditombi BM, M'Bondoukwé NP, Ngomo JMN, Delis AM, Lekounga PB, Bouyou-Akotet M, Mawili-Mboumba DP. Prevalence and associated factors of intestinal parasite infection by HIV infection status among asymptomatic adults in rural Gabon. Afr Health Sci. 2020 Sep;20(3):1024-1034. doi: 10.4314/ahs.v20i3.5. PMID: 33402948; PMCID: PMC7751509.
85. Gedle, D., Kumera, G., Eshete, T. et al. Intestinal parasitic infections and its association with undernutrition and CD4 T cell levels among HIV/AIDS patients on HAART in Butajira, Ethiopia. J Health Popul Nutr 36, 15 (2017). https://doi.org/10.1186/s41043-017-0092-2
86. Amoo JK, Akindele AA, Amoo AOJ, Efunshile AM, Ojurongbe TA, Fayemiwo SA, Thomas BN, Ojurongbe O. Prevalence of enteric parasitic infections among people living with HIV in Abeokuta, Nigeria. Pan Afr Med J. 2018 May 28;30:66. doi: 10.11604/pamj.2018.30.66.13160. PMID: 30344850; PMCID: PMC6191249.
87. Lengongo JVK, Ngondza BP, Ditombi BM, M'Bondoukwé NP, Ngomo JMN, Delis AM, Lekounga PB, Bouyou-Akotet M, Mawili-Mboumba DP. Prevalence and associated factors of intestinal parasite infection by HIV infection status among asymptomatic adults in rural Gabon. Afr Health Sci. 2020 Sep;20(3):1024-1034. doi: 10.4314/ahs.v20i3.5. PMID: 33402948; PMCID: PMC7751509.
88. Wondmieneh A, Gedefaw G, Alemnew B, Getie A, Bimerew M, Demis A. Intestinal parasitic infections and associated factors among people living with HIV/AIDS in Ethiopia: A systematic review and meta-analysis. PLoS One. 2020 Dec 31;15(12):e0244887. doi: 10.1371/journal.pone.0244887. PMID: 33382867; PMCID: PMC7775096.
89. Establishment of PCR laboratory in developing countries (2nd ed.). World Health Organization (2016). https://apps.who.int/iris/handle/10665/249549
90. Asai T, Cordova Vidal C, Strauss W, Ikoma T, Endoh K, Yamamoto M. Effect of Mass Stool Examination and Mass Treatment For Decreasing Intestinal Helminth and Protozoan Infection Rates in Bolivian Children: A Cross-Sectional Study. PLoS neglected tropical diseases. 2016;10(12):e0005147.
91. Alvarez Di Fino EM, Rubio J, Abril MC, Porcasi X, Periago MV. Risk map development for soil-transmitted helminth infections in Argentina. PLoS Negl Trop Dis. 2020 Feb 10;14(2):e0008000. doi: 10.1371/journal.pntd.0008000. PMID: 32040473; PMCID: PMC7010237.

92. Chammartin F, Scholte RG, Malone JB, Bavia ME, Nieto P, Utzinger J, Vounatsou P. Modelling the geographical distribution of soil-transmitted helminth infections in Bolivia. Parasit Vectors. 2013 May 25;6:152. doi: 10.1186/1756-3305-6-152. PMID: 23705798; PMCID: PMC3681678.
93. Camacho-Alvarez I, Goyens P, Luizaga-López JM, Jacobs F. Geographic differences in the distribution of parasitic infections in children of Bolivia. Parasite Epidemiol Control. 2021 Jun 10;14:e00217. doi: 10.1016/j.parepi.2021.e00217. PMID: 34189287; PMCID: PMC8219986.
94. Asai T, Cordova Vidal C, Strauss W, Ikoma T, Endoh K, Yamamoto M. Effect of Mass Stool Examination and Mass Treatment For Decreasing Intestinal Helminth and Protozoan Infection Rates in Bolivian Children: A Cross-Sectional Study. PLoS neglected tropical diseases. 2016;10(12):e0005147.
95. Zurita Céspedes, Brian Iván, Moya Álvarez, Rusberth Rodrigo, Moya Álvarez, Kattya Lineth, Tellez León, Tatiana Margarita, & Torrico Rojas, Mary Cruz. (2018). FRECUENCIA DE PARÁSITOS INTESTINALES EN EXÁMENES COPROPARASITOLÓGICOS DIRECTOS PROCESADOS EN EL LABORATORIO DE INVESTIGACIÓN MÉDICA, 2011-2015. Revista Científica Ciencia Médica, 21(2), 6-12. Recuperado en 06 de abril de 2023, de http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S1817-74332018000200002&lng=es&tlng=es.
96. Situación del VIH/SIDA en Bolivia al 31 de marzo de 2021 / ESTADO PLURINACIONAL DE BOLIVIA MINISTERIO DE SALUD Y DEPORTES PROGRAMA NACIONAL ITS/VIH/SIDA-HV. https://www.idhbolivia.org/images/Estadisticas/ResEpidVIH-Abril2021.pdf
97. López Dorado Carola Claudia, Pozo Torres Natalia Alejandra, Pozo Uribe Juan Manuel. INFORMACIÓN EN LA PREVENCIÓN DE LA INFECCIÓN DE VIH-SIDA EN LA POBLACIÓN SEXUALMENTE ACTIVA DE LA COMUNIDAD CHUQUI - CHUQUÍ, PROVINCIA OROPEZA DEL DEPARTAMENTO DE CHUQUISACA JULIO A SEPTIEMBRE 2012. Arch.Boliv.Med. [revista en la Internet]. 2012 [citado 2023 Mayo 03] ; 18(86): 31-36. Disponible en: http://www.revistasbolivianas.ciencia.bo/scielo.php?script=sci_arttext&pid=S0004-05252012000200004&lng=es.
98. Czeresnia JM, Weiss LM. Strongyloides stercoralis. Lung. 2022 Apr;200(2):141-148. doi: 10.1007/s00408-022-00528-z. Epub 2022 Apr 9. PMID: 35396957; PMCID: PMC8994069.

99. Chu C, Rotondo-Trivette S, Michail S. Chronic diarrhea. Curr Probl Pediatr Adolesc Health Care. 2020 Aug;50(8):100841. doi: 10.1016/j.cppeds.2020.100841. Epub 2020 Aug 27. PMID: 32863166.
100. Felix KM, Tahsin S, Wu HJ. Host-microbiota interplay in mediating immune disorders. Ann N Y Acad Sci. 2018 Apr;1417(1):57-70. doi: 10.1111/nyas.13508. Epub 2017 Oct 6. PMID: 28984367; PMCID: PMC5889363.
101. Belkaid Y, Harrison OJ. Homeostatic Immunity and the Microbiota. Immunity. 2017 Apr 18;46(4):562-576. doi: 10.1016/j.immuni.2017.04.008. PMID: 28423337; PMCID: PMC5604871.
102. Choi, J., Kim, B., Akuzum, B., Chang, L., Lee, J., & Kwon, H. (2022). TREGking From Gut to Brain: The Control of Regulatory T Cells Along the Gut-Brain Axis. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.916066
103. Alloo J, Leleu I, Grangette C, Pied S. Parasite infections, neuroinflammation, and potential contributions of gut microbiota. Front Immunol. 2022 Dec 8;13:1024998. doi: 10.3389/fimmu.2022.1024998. PMID: 36569929; PMCID: PMC9772015.
104. Li B, Selmi C, Tang R, Gershwin ME, Ma X. The microbiome and autoimmunity: a paradigm from the gut-liver axis. Cell Mol Immunol. 2018 Jun;15(6):595-609. doi: 10.1038/cmi.2018.7. Epub 2018 Apr 30. PMID: 29706647; PMCID: PMC6079090.
105. Burgess SL, Gilchrist CA, Lynn TC, Petri WA Jr. Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. Infect Immun. 2017 Jul 19;85(8):e00101-17. doi: 10.1128/IAI.00101-17. PMID: 28584161; PMCID: PMC5520446.
106. Crakes KR, Jiang G. Gut Microbiome Alterations During HIV/SIV Infection: Implications for HIV Cure. Front Microbiol. 2019 May 22;10:1104. doi: 10.3389/fmicb.2019.01104. PMID: 31191468; PMCID: PMC6539195.
107. Million, M., Diallo, A., & Raoult, D. (2017). Gut microbiota and malnutrition. Microbial Pathogenesis, 106, 127-138. https://doi.org/10.1016/j.micpath.2016.02.003
108. Zhou, A., Yuan, Y., Yang, M., Huang, Y., Li, X., Li, S., Yang, S., & Tang, B. (2022). Crosstalk Between the Gut Microbiota and Epithelial Cells Under Physiological and Infectious Conditions. Frontiers in Cellular and Infection Microbiology, 12. https://doi.org/10.3389/fcimb.2022.832672

109. Ashuro, A. A., Lobie, T. A., Ye, Q., Leng, X., Li, Z., Pan, F., & Fan, G. (2020). Review on the Alteration of Gut Microbiota: The Role of HIV Infection and Old Age. AIDS Research and Human Retroviruses, 36(7), 556-565. https://doi.org/10.1089/aid.2019.0282
110. Deusch S, Serrano-Villar S, Rojo D, Martínez-Martínez M, Bargiela R, Vázquez-Castellanos JF, Sainz T, Barbas C, Moya A, Moreno S, Gosalbes MJ, Estrada V, Seifert J, Ferrer M. Effects of HIV, antiretroviral therapy and prebiotics on the active fraction of the gut microbiota. AIDS. 2018 Jun 19;32(10):1229-1237. doi: 10.1097/QAD.0000000000001831. PMID: 29683848.
111. UNAIDS DATA 2022. Geneva: Joint United Nations Programme on HIV/AIDS; 2022. Licence: CC BY-NC-SA 3.0 IGO.
112. Sepahvand F, Mamaghani AJ, Ezatpour B, Badparva E, Zebardast N, Fallahi S. Gastrointestinal parasites in immunocompromised patients; A comparative cross-sectional study. Acta Trop. 2022 Jul;231:106464. doi: 10.1016/j.actatropica.2022.106464. Epub 2022 Apr 12. PMID: 35421383.
113. Chincha LO, Antonio Bernabé-Ortiz, Frine Samalvides C, Leslie Soto A, Eduardo Gotuzzo H, et al. (2009) Infecciones parasitarias intestinales y factores asociados a la infección por coccidias en pacientes adultos de un hospital público de Lima, Perú. Revista chilena de infectología. 26: 440-444.
114. Dacal E, Köster PC, Carmena D. Diagnóstico molecular de parasitosis intestinales. Enferm Infecc Microbiol Clin (Engl Ed). 2020 Jan;38 Suppl 1:24-31. English, Spanish. doi: 10.1016/j.eimc.2020.02.005. PMID: 32111362.
115. Pacheco FT, Silva RK, Martins AS, Oliveira RR, Alcantara-Neves NM, et al. (2013) Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. The Journal of parasitology 99: 1002-1008.
116. Bachur TP, Vale JM, Coelho IC, Queiroz TR, Chaves Cde S (2008) Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases 12:115-122.
117. John C. Hall BJH, Cockerell CJ (2011) HIV/AIDS in the Post-HAART Era: Manifestations, Treatment, and Epidemiology.
118. Trelis M, Taroncher-Ferrer S, Gozalbo M, Ortiz V, Soriano JM, Osuna A, Merino-Torres JF. Giardia intestinalis and Fructose Malabsorption: A Frequent Association. Nutrients. 2019 Dec

5;11(12):2973. doi: 10.3390/nu11122973. PMID: 31817420; PMCID: PMC6950212.

119. World Health Organization. ISBN 978 92 4 156482 3 (NLM classi-cation: WA 675) Preventing diarrhoea through better water, sanitation and hygiene - Exposures and impacts in low- and middle-income countries / WHO Library Cataloguing-in-Publication Data.
120. Barcelos NB, Silva LFE, Dias RFG, Menezes Filho HR, Rodrigues RM (2018) Opportunistic and non-opportunistic intestinal parasites in HIV/ AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goias, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 60: e13.
121. Cardoso LV, Galisteu KJ, Schiesari Junior A, Chahla LA, Canille RM, et al. (2011) Enteric parasites in HIV-1/AIDS-infected patients from a Northwestern Sao Paulo reference unit in the highly active antiretroviral therapy era. Revista da Sociedade Brasileira de Medicina Tropical 44:665-669.
122. Wang, ZD., Liu, Q., Liu, HH. et al. Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasites Vectors 11, 28 (2018). https://doi.org/10.1186/s13071-017-2558-x
123. Huppmann, A. R., & Orenstein, J. M. (2010). Opportunistic disorders of the gastrointestinal tract in the age of highly active antiretroviral therapy. Human Pathology, 41(12), 1777-1787. https://www.sciencedirect.com/science/article/pii/S0046817710002133
124. Papadia, C., Kelly, P., Caini, S., Roberto Corazza, G., Shawa, T., Franzè, A., Forbes, A., & Di Sabatino, A. (2010). Plasma citrulline as a quantitative biomarker of HIV-associated villous atrophy in a tropical enteropathy population. Clinical Nutrition, 29(6), 795-800 https://doi.org/10.1016/j.clnu.2010.04.008

7.1 Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba, Bolivia: longitudinal study

OPEN ACCESS

GAVIN PUBLISHERS

Infectious Diseases Diagnosis & Treatment

Research Article

Aviles J, et al. Infect Dis Diag Treat 4: 136.
DOI: 10.29011/2577-1515.100136.

Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba, Bolivia

Jorge Aviles[1,2*], Jean Cyr Yombi[3], Carlos Erostegui[2], Maricruz Torrico[4], Rosemary Yanez[2], Marcelo Rojas[1], Daniel Illanes[2], Annie Robert[1]

[1]Institut de Recherche Expérimentale et Clinique (IREC) Pôle de recherche en Epidémiologie et Biostatistique, Université catholique de Louvain UCL, Brussels, Belgium

[2]Instituto de Investigaciones Biomédicas IIBISMED, Universidad Mayor de San Simón (UMSS), Cochabamba, Bolivia

[3]Service de médecine interne et pathologies infectieuses, Centre de Référence SIDA, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

[4]Laboratorio de Investigación Biomédica (LABIMED), Universidad Mayor de San Simón (UMSS), Cochabamba, Bolivia

***Corresponding author:** Jorge Aviles, Institut de Recherche Expérimentale et Clinique (IREC) Pôle de recherche en Epidémiologie et Biostatistique, Université catholique de Louvain UCL, Brussels, Belgium

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

Received Date: 21 January, 2020; **Accepted Date:** 13 February, 2020; Published Date: 18 February, 2020

Abstract

Background: Thanks to the widespread use of Antiretroviral Therapy (ART), Human Immunodeficiency Virus (HIV) infection is becoming a chronic manageable disease. In low resources settings where ART is available, but not widely, opportunistic infections such as parasites diseases remain common. These conditions represent a public health problem in the world due to the high prevalence in developing countries and particularly for patients with HIV/AIDS. To date there have been no systematic study on the prevalence of intestinal parasites in people living with HIV/AIDS (PLWHIV) in Bolivia. The study aimed to evaluate the occurrence of intestinal parasitic infections in PLWHIV who attended routine controls at the reference center for HIV control and prevention in Cochabamba (CDVIR) between January 2011 and December 2015.

Methods: Ethical approval was obtained prior to the commencement of this study from the Ethics Committees of the Department of Cochabamba-Bolivia and the Universidad Mayor de San Simón, Institute of Biomedical Research (IIBISMED/UMSS). Code: CB-2016-015.

A Retrospective Analysis was carried out Based on the reports of the reference laboratory in the city of Cochabamba (LABIMED) using direct parasitological methods founded on the macroscopic and microscopic identification of parasitic elements present in the stool. Data was collected in an Excel spreadsheet, based on reports from the parasitology laboratory, which was transferred for statistical analysis to SPSS 24.0 (SPSS Inc., Chicago IL, USA). The characteristics of the study participants are reported as mean, range and percentage, as appropriate. The test of X^2 or Fisher's exact test was used for all categorical variables. Logistic regression analysis was used to test the associations. A given statistical test was reported significant if it resulted in a p-value <0.05.

Results: During the 5-years of the study, a number varying between 313 and 620 patients were assessed each year, and 12 parasitic species were identified. The highest prevalence was 33.2% in 2011 and there was 9.9% of polyparasitism. In 2012, the prevalence was 28.6%, with 8.3% of polyparasitism, in 2013 it reached 30.4% with a polyparasitosis of 7.7%, while in 2014 it was 24. 5%, the lowest in the period, with a polyparasitism rate of 8.5%. Finally, in 2015 the prevalence reached 27.1% with a polyparasitism rate of 7.3%. The most prevalent species in order of frequency were *Blastocystis Hominis, Entamoeba coli* (non-Pathogen), *Giardia Lamblia* and *Entamoeba histolytica/dispar,* respectively. Regression analysis showed no significant association with sex or with consistence of stool sample but prevalence was higher in people under 30 years of age.

Conclusion: Taking into account the epidemiological and geographical context, the frequency of presentation of these infections reach practically one third of these population and thus remains a high problem in Bolivia. So, further studies are required to clarify the epidemiology of these infectious diseases in this endemic region.

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

Keywords: HIV; Parasites; Prevalence; Reference laboratory for HIV

List of abbreviations

ART	:	Anti-Retroviral Therapy
CDVIR	:	Reference center for HIV control and prevention in Cochabamba
CI	:	Confidence intervals
HAART	:	Highly active antiretroviral therapy
HIV/AIDS	:	Human Immunodeficiency Virus
IIBISMED	:	Institute of Biomedical Research
LABIMED	:	Biomedical Research Laboratory / Reference laboratory for HIV
PLWHIV	:	People living with HIV/AIDS
PNC/VS	:	National HIV/AIDS Control Program
UMSS	:	Universidad Mayor de San Simón
UNAIDS	:	Joint United Nations Program on HIV/AIDS
SPSS	:	Statistical Package for Social Science

Background

In December 2013, the Joint United Nations Program on HIV/AIDS (UNAIDS) supported the efforts led by countries and regions to set new targets for increasing HIV treatment beyond 2015. A powerful momentum is now being built towards a new narrative on the treatment of HIV and AIDS. A new, final, ambitious but achievable goal: by 2020, 90% of all people living with HIV will know their HIV status, 90% of all people diagnosed will receive antiretroviral therapy, and 90% of all people receiving antiretroviral therapy will have viral suppression [1].

At the end of 2015, there were 2.1 million [1.8 million–2.4 million] new HIV infections worldwide, adding up to a total of 36.7 million [34.0 million–39.8 million] PLWHIV. Global coverage of ART reached 46% [43–50%], and in Latin America and the Caribbean, treatment coverage reached 55% [47-64%] [1].

Because rates of new HIV infections in adults are relatively static, epidemics in Latin America are generally stable even with a steady decrease in the number of people newly infected with HIV since early 2000s. The total number of people living with HIV in this region continued to grow and reach 1.8 million [1.5 million-2.1 million] in 2010, up from 2.0 million [1.7 million-2.3 million] in 2015 [1].

Advances in ART allow considering infection by HIV as a disease of chronic course compatible with a good quality of life when adequately monitored, and therapy has been instituted. Thanks to ART, HIV infection is now a chronic manageable disease but the management of comorbidities such as cardiovascular, renal, liver, co-infections with hepatitis B or C are now the greatest challenges. In low resources settings where ART is now available, but not widely, Opportunistic Infections (OIs) such as parasites diseases remain common and continue to be a leading cause of morbidity and mortality in PLWHIV, because of a severe immunosuppression caused by either a lack of knowledge of serological status in patients presenting late with an opportunistic infection as an or late manifestation of AIDS, a lack of adherence to antiretroviral treatment, or a therapeutic failure due to resistance to ART [2].

Parasitic diseases represent a medical, economic and social problem in the world due to its high prevalence in developing countries and particularly for PLWHIV. It is estimated that worldwide, about two billion people are affected by soil transmitted helminthes such as *Ascaris lumbricoides*, *Ancylostoma/Necator* spp. and *Trichuris trichiura*, 50 million by *Entamoeba histolytica* and 2.8 million by *Giardia duodenalis* (synonymous *G. lamblia* and *G. intestinalis*) [3]. The clinical importance of these infections continues to be debated, especially in the case of Blastocystis Hominis.

Parasitic infections are common among patients with HIV/AIDS. These infections are responsible of frequent complications in this population such as chronic diarrhea that contributes to the development of malabsorption and malnutrition in AIDS patient; its lifetime incidence among HIV-infected patients is estimated to be 30-70% [4]. Intestinal parasites are therefore an important cause of morbidity and mortality in PLWHIV. Frequent pathogenic parasites who infect HIV patients are rarely involved agents before the epidemic of HIV/AIDS with prevalence varying considerably according to the geographic context and the epidemiological profile of each country.

In developing countries, rates as high as 60 to 80% are reported [5-7], with concomitant infections by multiple parasites. Several diagnostic studies in AIDS patients without antiretroviral therapy indicate that opportunistic infections account for a large proportion of cases of chronic diarrhea (75-80%) [1]. The most common pathogens are usually enteric parasites including some that are unusual and have not been implicated in human disease until the advent of AIDS [8], such as *Cryptosporidium* spp., *Microsporidium* or *Isospora belli*, for which there are no effective primary prophylaxis guidelines. There is no specific treatment for cryptosporidiosis, whose cure depends on the immunological recovery related to ART [9]. The treatment of microsporidiosis also depends on ART, but there are effective complementary drugs. In intestinal or disseminated infections produced by *Enterocytozoon bieneusi*, the treatment of choice is fumagillin. If other species are involved, albendazole is recommended and the treatment of choice for isosporosis is cotrimoxazole [5,9,10].

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

There are currently no epidemiological estimates the prevalence of intestinal parasites in PLWHIV in Bolivia. The data provided comes from a national HIV treatment program and, therefore, can be expected to be representative. This study aimed to evaluate the occurrence of intestinal parasitic infections that occur most frequently in patients who attended in routine checkups for HIV infection (PLWHIV) at the reference center for HIV control and prevention in Cochabamba (CDVIR) between January 2011 and December 2015, and thus provides elements for building strategies for prevention and control.

Methods

Settings

In Bolivia 16.640 confirmed cases of HIV were reported since June 2016 in the health care system and 1.015 deaths related to AIDS diseases. Official data indicate that the estimated HIV prevalence is 0.15% with an annual incidence of approximately 0.01% (1000 new cases per year), meaning that approximately 10 out of every 10,000 people in the country would have been notified as HIV cases, of which about 2.400 would be on ART in the public health care system. In Cochabamba, the HIV / AIDS program of the Department Health Service (SEDES) registered 408 new infections in 2015. The surveillance system shows a predominantly young epidemic, with 67% of cases being aged less than 40 years [2].

In this context, all patients confirmed to be HIV infected are registered and treated free of charge in the National HIV/AIDS Control Program (PNC/VS) and periodically monitored at the CDVIR in every department of Bolivia. In Cochabamba until 2016, approximately 1.400 PLWHIV were cared for at the center were clinical checkups and routine laboratories are performed in the Medical Research Laboratory (LABIMED) of the Universidad Mayor de San Simón (UMSS). These checkups are subsidized and include direct corproparasitological test, determination of viral load and flow cytometry to measure CD4, CD8 and the ratio CD4/CD8, once a year.

The CDVIR has limitations regarding infrastructure and personnel, infections are empirically handled due to a lack of dedicated laboratories and also because these additional test represent a cost for the patient who often cannot pay. Therefore, there is a lack of etiologic diagnoses and patients are treated based on therapeutic tests. Many patients do not respond adequately to these therapies generating complications such as chronic diarrhea, malnutrition, or wasting syndrome.

Study population

The present study was conducted in Cochabamba according to an analytical design carried out retrospectively with the reports of the LABIMED on HIV-infected outpatients followed in the CDVIR, from January 2011 to December 2015. Considering only one sample per patient-year, all the results of the participants were obtained from the parasitology laboratory registry, correspond to routine stool tests performed on all fecal samples using simple wet/saline and iodine mounts, in addition to concentration technique by the formol-ether sedimentation method performed simultaneously on all stool samples and the presence of parasites was analyzed.

Methods such as specific staining for detection of parasites like as *cryptosporidium* or microsporidium are not routinely performed and the patient has to pay for other tests if required, since the national program does not subsidize them. Polyparasitism was defined as at least two different species identified in one stool sample.

Selection criteria were to be a patient with confirmed HIV infection, who is followed and treated at the HIV reference center, and to be at least 18 years of age. Records that did not have complete data were excluded; but the numbers were low: 6 (1.9%) in 2011, 5 (1%) in 2012, 10 (1.9%) in 2013, 8 (1.4%) in 2014, and 10 (1.6%) in 2015.

Statistical analysis

Data were collected on a specially designed Excel spreadsheet, based on the paperback reports of the parasitology laboratory, which was transferred for statistical analysis to SPSS 24.0 (SPSS Inc., Chicago IL, USA). Characteristics of study participants are reported as mean, range and percentage as appropriate. The $\chi 2$ test or Fisher's exact p-value was used for all categorical variables. Frequency of parasitic infections were computed for grouped data according to sex, age and the consistence of the stool sample. Logistic regression analysis was used for testing associations, subsequently, with variables that showed a significant association, a multivariate model was built to estimate adjusted odds ratios. A p-value <0.05 was considered as significant and 95% Confidence Intervals (CI) were computed. We developed separate models per year because a single patient can be in several years.

Results

A total of 2510 fecal samples were received between 2011 and 2015 from patients with a confirmed diagnosis of HIV infection and who attended laboratory tests in the CDVIR. During this period a number varying between 313 and 620 was assessed each year. The global distribution of patients attending LABIMED was predominantly male (Table 1), with a men/women ratio close to the 1.7 masculinity index stated in the report on progress in the HIV response in Bolivia 2014 by UNAIDS [4]. This ratio does not vary during the entire study period. The age distributions showed a greater proportion of people in age over 40 years and was homogeneous across years. Liquid feces occurred at a frequency of up to 48% in 2011 and remained close to 40% across years.

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

	2011	2012	2013	2014	2015	
	(n=313)	(n=493)	(n=520)	(n=564)	(n=620)	P Value
Characteristic						
sex						0.37
Man –no. (%)	197 (62.9)	316 (64.1)	329 (63.3)	334 (59.2)	400 (64.5)	
Men/Women Ratio	1.69	1.78	1.72	1.45	1.82	
Age-yr-no. (%)	36±12	37±12	37±12	38±13	37±13	0.67*
<30	116 (37.1)	160 (32.5)	162 (31.2)	157 (27.8)	193 (31.1)	
30 – 39	89 (28.4)	155 (31.4)	163 (31.3)	180 (31.9)	196 (31.6)	
≥40	108 (34.5)	178 (36.1)	195 (37.5)	227 (40.2)	231 (37.3)	
Aspect of feces (Liquid)-no. (%)						<0.001
Yes	151 (48.2)	210 (42.6)	188 (36.2)	222 (39.4)	292 (47.1)	
Lienteric feces-no. (%)						0.002
yes	28(8.9)	24(4.9)	37(7.1)	44(7.8)	70(11.3)	
a*ANOVA F test, no.: number						

Table 1: Characteristics of all participants by year.

During the review of laboratory reports of stool samples, twelve types of parasites genera were identified. The proportion of parasitic infection, defined as positive for at least one intestinal parasite, was 33.2%, the highest in 2011 (Table 2), and it was the lowest in 2014 with a prevalence of 24.5%. The highest polyparasitism rate was detected in 2011 with 9.9% and the lowest in 2015 (7.3%).

Outcomes /Year	2011	2012	2013	2014	2015
Parasite Infection – n (%)	**(n=313)**	**(n=493)**	**(n=520)**	**(n=564)**	**(n=620)**
Any	104 (33.2)	141 (28.6)	158 (30.4)	138 (24.5)	168 (27.1)
Polyparasitism	31 (9.9)	41 (8.3)	40 (7.7)	48 (8.5)	45 (7.3)
Parasites species – n (%)					
Blastocystis hominis	32 (10.2)	47 (9.5)	66 (12.7)	56 (9.9)	44 (7.1)
Entamoeba coli	38 (12.1)	47 (9.5)	38 (7.3)	21 (3.7)	59 (9.5)
Giardia lamblia	15 (4.8)	15 (3.0)	26 (5.0)	37 (6.6)	30 (4.8)
Entamoeba histolytica/dispar	9 (2.9)	11 (2.2)	13 (2.5)	17 (3.0)	16 (2.6)
Hymenolepis nana	3 (1.0)	9 (1.8)	4 (0.8)	2 (0.4)	8 (1.3)
Chilomastix mesnili	4 (1.3)	5 (1.0)	4 (0.8)	1 (0.2)	4 (0.6)
Strongyloides stercoralis	1 (0.3)	4 (0.8)	1 (0.2)	3 (0.5)	4 (0.6)
Entamoeba hartmanni	0	2 (0.4)	3 (0.6)	0 (0.0)	1 (0.2)
Trychomona hominis	1 (0.3)	1 (0.2)	3 (0.6)	2 (0.4)	0
Iodoameba butschili	0	0	0	0	2 (0.3)
Isospora belli	1 (0.3)	0	0	0	0
Taenia sp	0	1 (0.2)	0	0	0

Table 2: Prevalence of Intestinal parasitic infections by year in adult patients of a reference laboratory (LABIMED), Cochabamba-Bolivia.

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

Throughout the course of the study period, there was a clear predominance of parasites classified as protozoa in fecal samples processed in the reference laboratory. In 2011, the infection rate was 33.2%, the highest prevalence observed in the study, while in 2014 it was 24. 5%, the lowest in the period. The parasitic species mostly found were *Blastocystis homminis, Entamoeba coli* (No pathogen), *Giardia lamblia and Entamoeba histolytica/dispar* (Table 2). *B. homminis* was the most frequently detected parasitic specie, reaching 12.7%, the highest rates in 2013 and decreasing to 7.1% in 2015. The prevalence of parasite infections and polyparasitism according to age, sex and stool consistence is presented in (Table 3), within each year.

Association with age, sex and stool consistence was not significant, but each year, there was a predominant proportion of parasite infections in age under 30. In all years but 2011, there was a greater proportion of women with parasitic infections. Similarly, polyparasitism was also higher in women than in man. The age group mostly affected was under 30 years of age, with also higher frequencies of polyparasitism in this age group. This association was significant, except in 2011. Liquid feces were significantly associated with a higher presence of parasites in direct microscopic examination each year (Table 3).

			Parasite Infection			Polyparasitism		
Year / Characteristic			**Yes** no. /N. (%)	**OR (95% IC)**	**P value**	**Yes** no. /N. (%)	**OR (95% IC)**	**P value**
2011 (n=313)	Sex				0.53			0.31
		F	36/116(31.0)	1		13/36(36.1)	1	
		M	68/197(34.5)	1.17(0.71-1.91)		18/68(26.5)	0.64(0.27-1.52)	
	Age				0.11			0.27
		<30	46/116(39.7)	2.30(1.28-4.14)		16/46(34.8)	2.67(0.78-9.15)	
		30 – 39	34/89(38.2)	2.16(1.16-4.00)		11/34(32.4)	2.39(0.66-8.70)	
		≥40	24/108(22.2)	1		4/24(16.7)	1	
	Liquid feces				0.16			0.77
		Yes	56/151(37.1)	1.4 (0.87-2.24)		15/48(31.3)	1	
		No	48/162(29.6)	1		16/56(28.6)	0.88(0.38-2.04)	
	Lienteric feces				0.9			0.32
		Yes	9/28(32.1)	0.95(0.41-2.17)		4/9(44.4)	2.02(0.50-8.08)	
		No	95/285(33.3)	1		27/95(28.4)	1	

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

2012 (n=493)	Sex				0.08			0.03
		F	59/177(33.3)	1		23/59(39.0)	1	
		M	82/316(25.9)	0.70 (0.47-1.05)		18/83(21.7)	0.43(0.20-0.91)	
	Age				<0.001			0.04
		<30	64/160(40.0)	2.82(1.73-4.60)		21/65(32.3)	3.58(1.12-11.48)	
		30 – 39	43/155(27.7)	1.62(0.97-2.71)		16/43(37.2)	4.44(1.32-14.94)	
		≥40	34/178(19.1)	1		4/34(11.8)	1	
	Liquid feces				<0.001			0.66
		Yes	78/210(37.1)	2.06(1.39-3.07)		17/63(27.0)	1	
		No	63/283(22.3)	1		24/79(30.4)	1.18(0.57-2.46)	
	Lienteric feces				0.001			0.22
		Yes	14/24(58.3)	3.7(1.6-8.70)		6/14(42.9)	1.99(0.65-6.15)	
		No	127/469(27.1)	1		35/128(27.3)	1	
2013 (n=520)	Sex				0.24			0.3
		F	64/191(33.5)	1		19/64(29.7)	1	
		M	94/329(28.6)	0.79(0.54-1.17)		21/94(22.3)	0.68(0.33-1.40)	
	Age				0.003			0.69
		<30	60/162(37.0)	2.14(1.34-3.41)		16/60(26.7)	0.91(0.38-2.19)	
		30 – 39	56/163(34.4)	1.90(1.91-3.05)		12/56(21.4)	0.68(0.27-1.71)	
		≥40	42/195(21.5)	1		12/42(28.6)	1	
	Liquid feces				0.002			0.04
		Yes	73/188(38.8)	1.85 (1.26-2.71)		16/85(18.8)	1	
		No	85/332(25.6)	1		24/73(32.9)	2.11(1.02-4.39)	
	Lienteric feces				0.16			0.05
		Yes	15/37(40.5)	1		7/15(46.7)	2.91(0.98-8.64)	
		No	143/483(29.6)	1.62(0.82-3.22)		33/143(23.1)	1	

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

2014 (n=564)	Sex				0.18			0.04
		F	63/230(27.4)	1		28/64(43.8)	1	
		M	75/334(22.5)	0.77(0,52-1.13)		20/75(26.7)	0.47(0.23-0.95)	
	Age				<0.001			0.68
		<30	53/157(33.8)	2.70(1.66-4.40)		18/53(34.0)	1.21(0.49-3.00)	
		30 – 39	49/180(27.2)	1.99(1.22-3.22)		19/49(38.8)	1.49(0.60-3.71)	
		≥40	36/227(15.9)	1		11/37(29.7)	1	
	Liquid feces				0.02			0.06
		Yes	66/222(29.7)	1.59(1.08-2.34)		20/73(27.4)	1	
		No	72/342(21.1)	1		28/66(42.4)	1.95(0.96-3.97)	
	Lienteric feces				<0.001			0.49
		Yes	22/44(50.0)	3.48(1.86-6.51)		9/22(40.9)	1.39(0.55-3.52)	
		No	116/520(22.3)	1		39/117(33.3)	1	
2015 (n=620)	Sex				0.41			0.68
		F	64/220(29.1)	1		16/64(25.0)	1	
		M	104/400(26.0)	0.86(0.59-1.24)		29/104(27.9)	1.16(0.57-2.36)	
	Age				<0.001			0.39
		<30	76/193(39.4)	2.76(1.78-4.27)		24/76(31.6)	1.80(0.75-4.32)	
		30 – 39	48/196(24.5)	1.38(0.86-2.19)		12/48(25.0)	1.30(0.49-3.46)	
		≥40	44/231(19.0)	1		9/44(20.5)	1	
	Liquid feces				0.002			0.25
		Yes	96/292(32.9)	1.74(1.22-2.50)		16/72(22.2)	1	
		No	72/328(22.0)	1		29/96(30.2)	1.51(0.75-3.07)	
	Lienteric feces				0.77			0.16
		Yes	20/70(28.6)	1.09(0.63-1.89)		8/20(40.0)	2.00(0.76-5.27)	
		No	148/550(26.9)	1		37/148(25.0)	1	

Table 3: Parasitic infections according to characteristics of adult patients in a reference laboratory (LABIMED), by year.

In these multivariate analyses, liquid feces remained significantly associated with higher prevalence of parasitic infections each year but in 2011(Table 4). There was a non-significant association of parasitic infection with gender, but a significant decrease with age; infections remained significantly higher in patients under 30 years. The association of parasitic infection with stool consistence and lientery were not significant each year, but adjusted OR were similar.

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

Year / Characteristic	OR (95% IC).	P value
2011		
Men (yes=1)	1.22(0.74-2.00)	0.44
Age		0.38
<30 yrs (yes=1)	2.51(1.38-4.60)	
30 - 39 yrs (yes=1)	2.34(1.24-4.41)	
≥40 yrs (yes=1)	1	
Liquid feces (yes =1)	1.46(0.90-2.38)	0.13
Lienteria (yes=1)	0.78(0.33-1.84)	0.57
2012		
Men (yes=1)	0.70(0.47-1.06)	0.09
Age		0.2
<30 yrs (yes=1)	2.75(1.66-4.54)	
30 - 39 yrs (yes=1)	1.53(0.90-2.60)	
≥40 yrs (yes=1)	1	
Liquid feces (yes =1)	1.90(1.27-2.86)	0.002
Lienteria (yes=1)	3.25(1.36-7.77)	0.008
2013		
Men (yes=1)	0.81(0.55-1.20)	0.29
Age		0.03
<30 yrs (yes=1)	2.12(1.32-3.42)	
30 - 39 yrs (yes=1)	2.02(1.25-3.25)	
≥40 yrs (yes=1)	1	
Liquid feces (yes =1)	1.84(1.25-2.73)	0.002
Lienteria (yes=1)	1.37(0.68-2.77)	0.38
2014		
Men (yes=1)	0.84(0.56-1.25)	0.39
Age		0.18
<30 yrs (yes=1)	2.63(1.60-4.33)	
30 - 39 yrs (yes=1)	1.89(1.15-3.09)	
≥40 yrs (yes=1)	1	
Liquid feces (yes =1)	1.34(0.89-2.02)	0.15
Lienteria (yes=1)	2.84(1.48-5.46)	0.002
2015		
Men (yes=1)	0.91(0.63-1.33)	0.64
Age		0.01

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

<30 yrs (yes=1)	2.65(1.70-4.12)	
30 - 39 yrs (yes=1)	1.32(0.83-2.11)	
≥40 yrs (yes=1)	1	
Liquid feces (yes =1)	1.70(1.18-2.47)	0.004
Lienteria (yes=1)	0.89(0.50-1.57)	0.69

Table 4: Multivariate logistic regression analysis of factors associated with intestinal parasitic infections in adult HIV patients.

Discussion

The countries in Latin America are adopting the continuous model of HIV care, and are monitoring the milestones of the so-called cascade of attention and treatment of HIV infection. One of the most important barriers identified is the retention of attention. During the study period, percentages of linkage and retention towards CDVIR services can be estimated around 40%. Ministry of Health in Bolivia reported in 2017 that only 42% of the PLWHIV in Cochabamba are linked and retained in the health system [11,12]. It is however difficult to know the number of PLWHIV and it is usually an estimate.

HIV / AIDS is currently a public health problem in Bolivia due to the constant and continuous spread of the disease. The available information for the year 2016 allows us to determine that approximately 1 in 4 PLWHIV would be receiving antiretroviral treatment, even if new notifications and new initiations are considered, the percentage does not exceed 37% (6128/16640). OIs represent a cause of morbidity and mortality in PLWHIV, although there is a high degree of uncertainty due to coverage of the mortality system in Bolivia [2]. According to the 2014 report of UNAIDS, the knowledge of serologic status among all people living with HIV reached 79%, 82% of people living with HIV and who know their status are on treatment, and 77% on treatment who are virally suppressed [2].

Considering the rates of retention, the proportion of patients attending the CDVIR can be estimated to: 64% in 2011, 82% in 2012, 71% in 2013, 65% in 2014, and 60% in 2015. In the present series, diagnosis of new cases and deaths or migrations can explain the increase in the number of PLWHIV tested for parasitic infections in the study period.

Variations in the prevalence of intestinal parasites can be attributed to geographical characteristics of the region, socioeconomic characteristics, health system, or nutritional aspects of persons enrolled.

The rationale for repeated analysis in successive years responds to fluctuations related to new diagnoses of PLWHIV who enter and perform controls at the reference center and patients who for various reasons are lost to the service that cannot be analyzed in time series.

The results in our series show that *Blastocystis hominis* is the more frequent parasite in patients attending CDVIR, as in most studies conducted in PLWHIV. In Peru, it was reported with 24.6% [13]. In patients with HIV, as in other patients as well, there is a great controversy regarding its pathogenicity [14]. Some studies did not find a correlation between diarrhea or other nonspecific digestive symptoms, and *B. Hominis*, but in Brazil cases associated with symptomatology have been reported [5,14].

In our study, the highest prevalence of intestinal parasites infection in our series was reached in 2011 (33.2%), lower than in published studies. In the study of Omayra Chincha conducted in Peru, a prevalence of 39.8% was found in PLWHIV and, *B. Hominis* was the most prevalent specie with 24.6%.

Species reported in Peru include: *I. belli* was 8.4%, *Cryptosporidium Sp.* was 4.5%, *Cyclospora cayetanensis* was 3.3%, and, *E. histolytica and H. nana* were 1.2%. [13] But, all these parasites were not found in Bolivia, where species such as *Entamoeba coli, Giardia lamblia* and *E. histolytica/dispar* are the most prevalent.

Opportunistic agents such as *Cryptosporidium spp., microsporidium, and Isospora belli* were less frequent among patients with HIV in Cochabamba [14].

The reasons may be the geographical context and may also be the methods used for the detection of intestinal parasites. There are differences between studies in detection methods [16]. It is obvious that the use of direct microbiological tests for detection of intestinal parasites is insufficient especially for detection of agents such as cryptosporidium and microsporidium reported as highly prevalent in people with AIDS. Within a clinical setting, the search for Cryptosporidium in stool specimens relies on the use of different techniques: acid-fast staining, Direct Fluorescent Antibody (DFA), and/or enzyme immunoassays for detection of Cryptosporidium sp. antigens. Molecular methods (e.g., polymerase chain reaction - PCR) are increasingly used in reference diagnostic labs, since they can identify Cryptosporidium at the species level [16], and PCR are the reference method.

For *microsporidium*, the most common diagnostic methods are based on stains, such as Calcofluor White M2R, Uvitex 2B, or Fungi Fluor, and by using Weber's modified trichrome staining or other modifications [16]. Tests for *Cryptosporidium, Microsporidium*, and *Isospora belli* are not routinely done in most reference laboratories for routine controls; they are performed

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

only when; healthcare providers should specifically request testing for such parasites.

Identify the different species of microsporidium is important because some of them do not respond adequately to the specific treatment. Low efficacy of albendazole has been described against *Enterocytozoon bieneusi,* since it yields only to decline in the parasite load and degenerative alterations of the spores. Clinically, stool frequency and volume decrease in infected patients having a stable body weight [17-21].

In the study conducted in Peru, authors used four different techniques in addition to direct examination, to detect a broad spectrum of parasites, including coccidia oocysts. Direct corproparasitological methods do also not allow to detect coccidia as *Cryptosporidium* and *I. belli.*

Bachur et al in 2008 in Brazil studied enteric parasites in PLWHIV by comparing before HAART and after HAART, using two different techniques, Baermann-Moraes and modified Ziehl-Neelsen methods for fecal parasitological examinations. They found a significant reduction of the infection rates of *S. stercoralis* from 30.1% to 11%, of *Ascaris lumbricoides* from 15.6% to 2%, of *Hookworms* from 13.7% to 2%, of *Trichuris trichiura* from 13.1% to 1%, *of Giardia duodenalis* from 7.9% to 1%, of *I. belli* from 4.8% to 1%, of *Cryptosporidium sp.* from 8.1% to 0, and no significant reduction of *E. histolytica/ dispar* from 3.3% to 1%, [13]. The overall prevalence of enteric parasites decreased from 63.9% to 24%. When the coverage with HAART is high with a reduced number of people with AIDS related diseases, the prevalence of opportunistic parasites is low [8].

Bachur et al performed a study in Brazil comparing the period before and after introduction of Highly Active Antiretroviral Therapy (HAART). The findings showed a significant difference between these two periods in terms of a reduction of the prevalence of Strongyloides *stercoralis – Ascaris lumbricoides, hookworms, Trichuris trichiura, Hymenolepis nana, Giardia duodenalis, Entamoeba histolytica/dispar, Isospora belli, Cryptosporidium* sp, and non-pathogenic protozoans as well; thus the prevalence of enteric parasites decreased from 63.9% to 24% ($p<0.001$) [8].

In the absence of statistics related to the prevalence of intestinal parasites in PLHIV in Bolivia, taking into account the growth in the number of newly diagnosed patients each year who are admitted and retained by the national HIV/AIDS control program and the reported number of deaths, considering the prevalence reported by Bachur in Brazil before and after HAART [8].

The symptomatology of the patient and the CD4 count at the time of the study were not reported to the CDVIR and it did not appear in the records of the LABIMED. Also we had no information about the treatment.

Within the limitations of the study, the demographic and clinical variables included in the analysis are very abridged due to limitations in the registration system and lack of linkage between the databases of the different laboratories, making it difficult to link the information on the count of CD4, virologic status, staging / performance status, along with sociodemographic[Rodriguez-Perez, 2019 #38] data (e.g. rural versus urban, area of residence), for this reason are not available or included in this study. However, our results point that there is still a huge number of cases of intestinal parasites in Bolivia and therefore.

Conclusions

There is a need for an adequate management to avoid the morbidity and mortality complications in PLWHIV in Bolivia. The present results highlights that it is crucial to strengthen surveillance of HIV patients. The diagnostic methods currently available in the LABIMED do not allow for the diagnosis of a wide spectrum of parasites for which direct methods are not sensitive such as cryptosporidium, microsporidium and isospores. This study highlights the importance of reinforcing the routine testing for opportunistic infections in people living with HIV in the country, the diagnoses must be determined by the use of appropriate parasitological methods. The HIV control strategies should consider routinely investigate the presence of intestinal parasites and generate detection protocols to facilitate the diagnosis and treatment of patients with HIV.

One aspect that should be highlighted is that in the absence of Bolivian national or sub-national estimates regarding the prevalence of intestinal parasites in PLWHIV, it is difficult to relate the prevalence of enteric parasites with the background population.

Although multivariable regression has been used to control confusion, the very limited number of variables included in the reference laboratory records limits the analysis of the many possible confounding factors not measured.

Absolute prevalence estimates in the context of care, follow-up and monitoring performed at the referral center show a high prevalence of parasitic infection. It is reasonable to consider that the patient monitoring and report system should be evaluated.

There is also a need for low-cost diagnostic tools to identify intestinal parasites and monitor HIV, especially in patients without ART who have a deteriorated general health status and function (fragility), with an increased risk of morbidity and mortality. In this population, it is necessary to establish diagnostic protocols for the detection of intestinal parasites that cannot be diagnosed with the currently available methods.

Finally, it is necessary to establish a data collection system and network that allows concentrate the data of the PLWHIV at the departmental and national levels, since the reports of hospitalization, laboratory of parasitology and immunology (CD4 and viral load) are not linked in the reference laboratories.

Trial registration

Ethical approval for the study was obtained from the Ethics Committees of the Department of Cochabamba-Bolivia and the

Citation: Aviles J, Yombi JC, Erostegui C, Torrico M, Yanez R, et al. (2020) Intestinal Parasitic Infections in Adult Living with HIV in Cochabamba Bolivia. Infect Dis Diag Treat 4: 136. DOI: 10.29011/2577-1515.100136.

Universidad Mayor de San Simón, Institute of Biomedical Research (IIBISMED/UMSS). Code: CB-2016-015 and the Commission d'éthique biomédicale hospitalo-facultaire de l'UCLouvain. (CEHF) Cliniques universitaries Saint-Luc Bruxelles.

Funding

Jorge Aviles is supported by grant AI-UMSS 2014-2019 from ARES-CCD, Belgian Government

The study was funded by the Belgian Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au Développement (ARES-CCD), through an Institutional support to UMSS in Bolivia (AI 2014-2019). More Information: https://www.ares-ac.be/fr/cooperation-au-developpement/pays-projets/projets-dans-le-monde/item/92-programme-d-appui-institutionnel-avec-la-universidad-mayor-de-san-simon.The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

JA, CE, MR, DI, AR, analyzed and interpreted the patient data regarding the parasitological disease and the epidemiological factors. MT and RY performed the microbiological and molecular examination of the stool samples; JA, JY and AR realized statistical analysis and were a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Render thanks for the active participation of the Institute of Biomedical Research, (IIBISMED) and LABIMED staff of the Universidad Mayor de San Simon (UMSS), as well as the Departmental Health Service (SEDES) and CDVIR in Cochabamba for their contribution to the realization of this study.

References

1. (UNAIDS) (2017) JUNPoHA. 90-90-90 An ambitious treatment target to help end the AIDS epidemic
2. (UNAIDS) JUNPoHA. UNAIDS 2017 | REFERENCE - UNAIDS DATA 2017. UNAIDS/JC2910E 2017:248.
3. Wilcox CM (2000) Etiology and evaluation of diarrhea in AIDS: a global perspective at the millennium. World J Gastroenterol 6: 177-186.
4. WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases, Second WHO report on neglected tropical diseases. WHO reference number: WHO/HTM/NTD/2013.1; January 2013.
5. John C. Hall BJH, Clay J. Cockerell (2011) HIV/AIDS in the Post-HAART Era: Manifestations, Treatment, and Epidemiology 2011.
6. Marcos LA, Gotuzzo E (2013) Intestinal protozoan infections in the immunocompromised host. Current opinion in infectious diseases 26: 295-301.
7. Rodriguez-Perez EG, Arce-Mendoza AY, Montes-Zapata ÉI, Limón A, Rodríguez LÉ, et al., (2019) Opportunistic intestinal parasites in immunocompromised patients from a tertiary hospital in Monterrey, Mexico. Infez Med 27: 168-174.
8. Bachur TP, Vale JM, Coelho IC, Queiroz TR, Chaves Cde S (2008) Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases 12:115-122.
9. Barcelos NB, Silva LFE, Dias RFG, Menezes Filho HR, Rodrigues RM (2018) Opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goias, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 60: e13.
10. Buchacz K, Baker RK, Palella FJ Jr, Chmiel JS, Lichtenstein KA, et al. (2010) AIDS-defining opportunistic illnesses in US patients, 1994-2007: a cohort study. AIDS (London, England) 24: 1549-1559.
11. Zorbozan O, Quliyeva G, Tunali V, Ozbilgin A, Turgay N, et al. (2018) Intestinal Protozoa in Hiv-Infected Patients: A Retrospective Analysis. Turkiye parazitolojii dergisi 42:187-190.
12. Bolivia MdS (2014) Informe Nacional De Progresos En La Respuesta Al Vih/Sida, 2014 Seguimiento a la Declaración Política sobre el VIH/sida 2011. 2014:73.
13. Chincha LO, Antonio Bernabé-Ortiz, Frine Samalvides C, Leslie Soto A, Eduardo Gotuzzo H, et al. (2009) Infecciones parasitarias intestinales y factores asociados a la infección por coccidias en pacientes adultos de un hospital público de Lima, Perú. Revista chilena de infectología. 26: 440-444.
14. Di Cristanziano V, D'Alfonso R, Berrilli F, Sarfo FS, Santoro M, et al. (2019) Lower prevalence of Blastocystis sp. infections in HIV positive compared to HIV negative adults in Ghana. PLoS One. 14: e0221968
15. Gentilini M, Chieze F (1990) Socioeconomic aspects of human immunodeficiency virus (HIV) infection in developing countries. Bulletin de l'Academie nationale de medecine 174: 1209-1219; discussion 19-21.
16. Wang ZD, Liu Q, Liu HH, Li S, Zhang L, et al. (2018) Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasites & vectors 11: 28.
17. Willemot P, Klein MB (2004) Prevention of HIV-associated opportunistic infections and diseases in the age of highly active antiretroviral therapy. Expert review of anti-infective therapy 2: 521-532.
18. Ford N, Shubber Z, Meintjes G, Grinsztejn B, Eholie S, et al. (2015) Causes of hospital admission among people living with HIV worldwide: a systematic review and meta-analysis. The lancet HIV 2: e438-44.
19. Cama VA, Mathison BA (2015) Infections by Intestinal Coccidia and Giardia duodenalis. Clinics in laboratory medicine 35:423-444.
20. Cardoso LV, Galisteu KJ, Schiesari Junior A, Chahla LA, Canille RM, et al. (2011) Enteric parasites in HIV-1/AIDS-infected patients from a Northwestern Sao Paulo reference unit in the highly active antiretroviral therapy era. Revista da Sociedade Brasileira de Medicina Tropical 44:665-669.
21. Silva CV, Ferreira MS, Borges AS, Costa-Cruz JM (2005) Intestinal parasitic infections in HIV/AIDS patients: experience at a teaching hospital in central Brazil. Scandinavian journal of infectious diseases 37:211-215.

7.2 Prevalence of opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in Cochabamba, Bolivia: longitudinal study

GAVIN PUBLISHERS

OPEN ACCESS

Current Research on HIV/AIDS

Avilés J, et al. Curr Res HIV: CRHA-120.
DOI: 10.29011/2575-7105.100120

Research Article

Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia

Jorge Avilés [1,2§*], Jean Cyr Yombi 3§, Carlos Erostegui [2]&, Maricruz Torrico[4]&, Marcelo Rojas[1]&, Héctor Rodriguez[5]&, Daniel Mercado[2]&, Daniel Illanes[2]& and Annie Robert[1§]

[1]Institut de Recherche Expérimentale et Clinique (IREC) Pôle de recherche en Epidémiologie et Biostatistique, Université catholique de Louvain UCLouvain, Brussels, Belgium

[2]Instituto de Investigaciones Biomédicas IIBISMED, Universidad Mayor de San Simón (UMSS), Cochabamba, Bolivia

[3]Service de médecine interne et pathologies infectieuses, Centre de Référence SIDA, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

[4]Laboratorio de Investigación Biomédica (LABIMED), Universidad Mayor de San Simón (UMSS), Cochabamba, Bolivia

[5]Institut de Recherche Expérimentale et Clinique (IREC) Pôle de recherche en microbiologie médicale, Université catholique de Louvain UCLouvain et Cliniques universitarires St-Luc, Brussels, Belgium

[*]**Corresponding author:** Jorge Avilés, Institut de Recherche Expérimentale et Clinique (IREC) Pôle de recherche en Epidémiologie et Biostatistique, Université catholique de Louvain UCLouvain, Brussels, Belgium

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

Received Date: 30 March, 2020; **Accepted Date:** 09 April, 2020; **Published Date:** 14 April, 2020

Abstract

Background: People living with HIV frequently have opportunistic infections; intestinal parasites are the most common infections that impair their health status. This study examined prospectively the prevalence of intestinal parasites in patients with the Human Immunodeficiency Virus (HIV) who were treated at the Reference Assistance Service (CDVIR) of Cochabamba-Bolivia, and analyzed its correlation with clinical, laboratory, and socio-epidemiological parameters.

Methods: A total of 503 patients were enrolled. Stool sample for each has been analyzed by several methods, including direct observation methods, and specifics staining; Kinyoun's or the Diddier modified method.

Results: The prevalence of intestinal parasites was 30.0% including the following species, *Giardia lamblia*(4.0%), *Chilomastix mesnili*(1.6%), *Strongyloides stercoralis*(1.2%), *Isospora belli*(0.6%), *Ascaris lumbricoides*(0.6%), *Hymenolepis nana* (0.4%), *Trichuris trichiura*(0.2%) as pathogens. *Blastocystis Homminis*(10.1%), *Entamoeba histolytica/dispar*(6.6%), *Entamoeba coli*(0.2%), *Trychomona homminis*(0.4%), *Endolimax nana*(0.2%) as non-pathogens.

Multivariate analysis, showed statistical significance in relation to a parasitic infection with the fact of being on antiretroviral therapy (OR = 3.82% CI, 1.01 to 14.46, P = 0.05). The current CD4 cell count (OR = 1.11% CI, 1.00 to 1.23, P = 0.05). The liquid appearance of the faeces at the time of direct examination (OR = 0.44% CI, 0.24 to 0.83, P = 0.01).

Conclusion: In conclusion, health assistance and the extensive use of antiretroviral therapy allows improving immunological states. The prevalence of parasitic infections found in this study generates substantial evidence of a need to implement additional laboratory tests in the monitoring and treatment of patients in order to reduce the risk of infections in this population.

Keywords: Opportunistic ; Infections; Parasites; HIV; Prevalence; Reference laboratory

Abbreviations: ART-Anti-Retroviral Therapy; CDVIR-Reference center for HIV control and prevention in Cochabamba; CI-Confidence intervals; HAART-Highly active antiretroviral therapy; HIV/AIDS-Human Immunodeficiency Virus; IIBISMED-Institute of Biomedical Research; LABIMED-Reference laboratory

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

in the city of Cochabamba; PLWHIV-People living with HIV/AIDS; PNC/VS-National HIV/AIDS Control Program; UMSS-Universidad Mayor de San Simón; UNAIDS-Joint United Nations Program on HIV/AIDS; SPSS -Statistical Package for Social Science

Introduction

The global HIV/AIDS control strategies promoted by UNAIDS consider that ending the AIDS epidemic is more than a historic obligation to the 39 million people who have died from the disease. It also represents a momentous opportunity to lay the foundation for a healthier, more just and equitable world for future generations. Ending the AIDS epidemic will inspire broader global health and development efforts, demonstrating what can be achieved through global solidarity, evidence-based action and multi-sectorial partnerships[(UNAIDS)., 2017 #1;(UNAIDS)., 2017 #1]. Although many strategies will be needed to close the book on the AIDS epidemic, one thing is certain: it will be impossible to end the epidemic without bringing HIV treatment to all who need it.

According to this strategy by 2020, 90% of all people living with HIV will know their HIV status. 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of all people receiving antiretroviral therapy will have viral suppression [1].

The Latin American reports on the HIV epidemic in 2017 show 1.8 million [1.4 million-2.1 million] people living with HIV (PLWHIV) in this region. In 2016, approximately 97,000 [79,000-120,000] new HIV infections occurred in the region. The number of new HIV infections did not vary from 2010 to 2016. Over this period, the number of AIDS-related deaths in the region experienced a decrease of 12% [2].

The human immunodeficiency virus (HIV) and the consequent acquired immunodeficiency syndrome (AIDS) in the world, has modified the epidemiological behavior of intestinal parasite infections. Severe episodes of diarrhea and malnutrition are now a prognostic marker, since it is an evolutionary predictor of AIDS [3].

Parasitic infections are an important cause of morbidity and mortality, especially with the emergence of immunosuppressive diseases [4]. The magnitude of intestinal parasitic infection in acquired immunodeficiency syndrome patients requires careful consideration in developing countries where poor nutrition is associated with poor hygiene and several tropical diseases. In Bolivia, the climatic conditions favor the spread of parasites.

The introduction of highly active anti-retroviral therapy (HAART) has improved the immune status and viral load. Additionally, secondary prophylaxis has reduced opportunistic infections and mortality rates associated with HIV.

Despite the availability of ART, Opportunistic Infections (OIs) continue to cause considerable morbidity and mortality in the world. Because many HIV-infected persons are unaware of their HIV infection and present with an OI as the initial indicator of their disease. Some individuals are aware of their HIV infection, but do not take ART due to psychosocial or economic factors; and some patients are enrolled in HIV care and prescribed ART, but do not attain an adequate virologic and immunologic response due to inconsistent retention in care, poor adherence, unfavorable pharmacokinetics, or unexplained biologic factors [5,6].

HIV-infected people have a high prevalence of *Cryptosporidium sp., Microsporidium sp*, and *Isospora* infection in low-income countries and patients with diarrhea, reinforcing the importance of routine surveillance for opportunistic intestinal protozoa in HIV-infected people [7].

Several protozoan species belonging to *Cryptosporidium, Microsporidia* and *Isospora*, are common causative pathogens responsible of significant morbidity and mortality in HIV patients [7,8].

With a worldwide distribution of *Cryptosporidium, C. parvum and C. hominis* are the most common species detected in humans, though other species [7]. Despite the use of ART in many countries, the infection rates of *Cryptosporidium* in HIV patients are still high, accounting for up to a third of diarrhea cases in HIV patients [9].

Cryptosporidium is the most common parasite encountered in the immunocompromised host, followed by *Cyclospora* and *Isospora*. In recent years, *Microsporidia* and *Blastocystis* have also emerged as important players, due in part to improved molecular diagnostic assays [10].

Bolivia is a low income country, HIV / AIDS is currently a public health problem in Bolivia due to the constant and continuous spread of the disease. The available information for the year 2016 allows us to determine that approximately 1 in 4 PLWHA would be receiving antiretroviral treatment, even if official new notifications and new initiations mentions not exceeding 37% [2].

Official data indicate that the estimated HIV prevalence is 0,15% with an annual incidence of approximately 0.01% (1000 new cases per year), meaning that approximately 10 out of every 10,000 people in the country would have been notified as HIV cases, of which about 2,400 were on antiretroviral treatment in the public health system [11]. In this context, 16.640 confirmed cases of HIV were reported since June 2016 in the health system and 1.015 deaths for causes related to AIDS.

According to the 2017 global AIDS update [2], the Knowledge of status among all people living with HIV has reached 79%, 82%

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

of people living with HIV know their status and are on treatment, and 77% on treatment are virally suppressed. In Cochabamba, the HIV / AIDS program of the Departmental Health Service (SEDES), registered a total of 408 new infections in 2015 [11].

Investment in health is low and does not reach 6% of the general budget of the state [11] this results in an evident lack of infrastructure and equipment, for this reason despite having technical experts, advanced diagnostic methods for the detection of diseases are routinely not available. This is evident in HIV care, investment in this aspect comes from almost 95% of the global fund and international cooperation grant.

The reference laboratories are limited to monitoring indicators such as CD4 leukocyte count, viral load and routine laboratory tests, including a direct corproparasitological examination to investigate intestinal parasitic infections with great limitations that make it difficult using this technique for detection of HIV marker diseases such as Cryptosporidium and Microsporidium (Coccidias). Physicians must resort to specific tests that are not subsidized and the patient assumes cost of diagnosis and treatment.

Although molecular biology methods can identify parasitic infections when they are standardized and there are adequate conditions, their clinical application in limited resource settings is restricted by feasibility, technical availability and cost. The reference laboratory for HIV program in Cochabamba (LABIMED) does not currently perform the detection of parasitic infections by molecular biology methods and these diagnostic methods are not available in the country.

Publications referring to the presentation of parasitic diseases in PLWHA at the national level are not available in Bolivia, but there is research that shows a high prevalence of helminths in populations living in high altitudes [12].

To date there have been no systemic study on the prevalence of intestinal parasites in PLWHA in Bolivia. Our aim was to assess the prevalence and evaluate the epidemiologic factors related to HIV-intestinal parasites co-infection in Cochabamba.

Methods

Settings

Cochabamba is a city in central Region of Bolivia. It is the third most populated department with 1.758.143 population in 2012, according to official data from the National Institute of Statistics of Bolivia. The regional prevalence of HIV of 0.16% in 2014, making Cochabamba the second city of HIV prevalence in Bolivia [11].

The increasing use of antiretroviral therapy (ART) means that HIV is becoming a chronic disease, but many health services in Bolivia do not currently count with personnel or equipment for the management of complex health conditions.

The health system in Bolivia centralizes the care of HIV patients in regional referral centers (CDVIR), where we recruited all the patients and the study was implemented in Cochabamba.

The study participants were adult patients (aged over 18 years old) of both genders, with HIV confirmed infection, who had not used anthelmintic in the previous three months and were followed up at the center for reference of HIV/AIDS and Sexual transmitted diseases (CDVIR) where the study was conducted in the city of Cochabamba, Bolivia.

From December 2017 through March 2018 all consecutive patients with confirmed HIV infection consulting at the CDVIR were enrolled in the study if they provided written informed consent.

At the time of carrying out the clinical controls in the reference center (CDVIR), patients were referred to the laboratory (LABIMED) for collecting the stool sample and to maintain optimal conditions for their analysis. Microbiological tests were performed by two observers (Parasitologists) separately as a standard procedure in this reference laboratory.

After their consent, a questionnaire was administered to each participant by the team who had been specifically trained for this task and who also clarified all the study objectives and the patients doubts. A stool sample was collected from each of the participants and used for four different intestinal parasites testing. All participants were offered professional counseling before parasitological testing. All diagnostic results were kept strictly confidential. Deworming treatments (Albendazole, Nitazoxanida) were proposed to all participants found to be infected.

Patients were instructed on how to collect their stool samples and received vials identified with codification; such system was used in the health system for the SPECTRUM reports in Bolivia. Fecal samples were stored, further processed and analyzed in the Parasitology Laboratory of the school of medicine at "Universidad Mayor de San Simon" (Cochabamba, Bolivia).

Laboratory Procedures

Techniques for parasitological diagnosis of fecal samples were performed for each fresh stool specimen and processed using the following methods: a) Direct observation of fresh feces, b) Concentration method (modified Ritchie) c) Immunochromatography d) Specific stains (Kinyou/Ziehl Modified Nielseen, modified Diddier and Giemsa) e) Molecular biology methods; for technical feasibility reasons, these last techniques could not be performed.

Stool samples were analyzed by direct microscopy with physiological saline and iodine. After concentration methods (i.e., formalin ether or formalin-ethyl acetate), the spontaneous sedimentation allowed the detection of helminth eggs and larvae,

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

as well as protozoan cysts and also *S. stercoralis* and *hookworm* larvae. The Ritchie technique was used on concentrate samples to prepare fecal smears, coupled with 3 different stains. Modified Ziehl Neelsen technique (Kinyoun), modified Diddier technique and Giemsa were used to identify *Cryptosporidium sp, I. belli*, and *C. cayetanensis*. (*Coccidias and microsporidium sp.*). Three slides of each sample were prepared and examined by two analysts. When no parasites and/ or commensals were found in the sample, two additional slides were prepared to confirm the result.

Data were registered in CsPro 7.0 and analyzed with Statistical Package for Social for the Science (SPSS) version 24.0 statistical software. Data were analyzed by the t-test (student), chi-square, one-way ANOVA and the Fisher's exact test when appropriate. Multivariate logistic analysis was used to evaluate the association between parasitic infection and clinical and socio-epidemiological characteristics. The magnitude of association between variables was estimated by the odds ratio (OR) with a 95% confidence interval (95% CI). The level of significance was set at $p < 0.05$.

Results

Complete data were collected from all participants who had provided stool samples as well as answered the questionnaires. The summary of these data are presented in Table 1.

Features		All recruited patients (N=503)
Sex-no. (%)		
	Male	318(63.2%)
	Men/Women Ratio	1.72
Age-yrs		
	Median (Range)	34(17;88)
	Average ±SD	36±13
Scholarship No. yrs		
	Median (Range)	11(0;22)
	Average ±SD	10±4
Therapy time (ART)-yrs		
	Median (Range)	2.6(0;14.4)
	Average ±SD	2.9±2.3
Time since the last control-days		
	Median (Range)	0131(0;1675)
	Average ±SD	142±133
Number of controls-no		
	Median (Range)	4(0;9)
	Average ±SD	4±2
Actual CD4 count-no.(cells/mm^3)		
	<350	254(50.5)
	350-500	110(21.9)
	>500	139(27.6)
	Average ±SD	356(267)
ART yes-no.(%)		493(98.0)
Undetectable Viral Load yes-no.(%)		291(57.9)
Actual Scheme of ART		
	AZT/3TC/EFV	17(2.2)
	TDF/3TC/EFV	476(95.8)
Income / month (USD)		
	Median (IQ Range)	288(144;360)
	Average (SD)	263±145
BMI		
	Median (IQ Range)	23.7(4.1)
	Average (SD)	23.8±5.2

Table1: Characteristics of patients with HIV/AIDS recruited in the study.

Fecal samples were collected from each one of the 503 patients, 318 (63.2%) participants were male, with a ratio male/ female of 1.72, their age ranged from 17 to 88 years old, with mean 36.1 ± SD 13.0.

Of the total of patients recruited 57.9% (291) have undetectable viral loads, 98% (493) currently receive ART, 96.3% of this patients receiving the Tenofovir/Lamivudin/Efavirenz (TDF/3TC/EFV) scheme. The median number of controls attended in the CDVIR during the last year was 4. Respect to the CD4 cell count 50.5% does not reach 350 cells/mm^3, 21.9% are between 350-500 cells/mm^3 and 27.6% have counts> 500 cells/mm^3, the BMI average was 23.7 SD +/- 13.6, the average monthly income was 263(USD) with a median of 288 and SD +/- 144 Table1.

HIV-infected patients of both genders were positive for intestinal parasites: 97/318(30.5%) infected men and 69/185(37.3%) infected women, showing a lower risk of infection in male gender but without statistical significance OR=0.74 IC 95% (0.50 to1.08 P=0.12). Age was not a determinant of infection by intestinal parasites P=0.34. Likewise, the current CD4 cell count (P=0.17), antiretroviral therapy (P = 0.07), body mass index

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

(P=0.46) and income level (P = 0. 36) did not show a significant association with intestinal parasite infection in this study. On the other hand, a significant association was found respect to parasitic infection and the absence of clinical symptoms or diarrhea in the recruited patients OR = 0.49 IC 95% (0.27-0.89 P=0.017) Table2.

Features		Parasites			
		Yes	OR	IC (95%)	P value
Sex					0.12
	Female	69/185(37.3)	1		
	Male	97/318(30.5)	0.74	(0.50-1.08)	
Age					0.34
	≤29 Yr	67/187(35.8)	1		
	30-39 yr	52/152(34.2)	0.93	(0.59-1.45)	
	≥40 yr	47/117(28.7)	0.72	(0.46-1.13)	
Consistence Liquid (Feces)					0.02
	Yes	24/50(48.0)	1		
	No	142/453(31.3)	0.49	(0.27-0.89)	
CD4 T cells count					0.17
	< 350 per mm3	93/254(36.6)	1		
	350-500 per mm3	35/110(31.8)	0.80	(0.50-1.30)	
	>500 per mm3	38/139(27.3)	0.65	(0.41-1.02)	
ART					0.07
	No	6/10(60.0)	1		
	Yes	160/493(32.5)	0.32	(0.89-1.15)	
BMI- Kg/M^2					0.46
	Under weight (<20)	10/22(44.5)	0.58	(0.24-1.39)	
	Normal (20-25)	97/298(32.6)	1		
	Over weight (>25)	59/181(32.6)	1.00	(0.68-1.49)	
Income					0. 36
	< Minimum wage	75/2242(31.0)	1		
	> Minimum wage	53/186(28.5)	0.84	(0.58-1.22)	

Table 2: Characteristics of patients with HIV/AIDS and their association with prevalence of intestinal parasites.

*Fisher exact test

Multi-parasitism was rare in our population. In the HIV infected group, most participants were infected by only one parasite (33.0% of the patients), while 7.8% were infected with two or more species.

The study shows a general prevalence of intestinal parasites in the PLWHIV population of 33.0%. A total of 12 species were identified during the study period, using the method of direct observation of stool. For parasites considered as pathogenic species, we detected *Giardia lamblia* (4.0%), *Chilomastix mesnili* (1.6%), *Strongyloides stercoralis* (1.2%), *Isospora belli* (0.6%), *Ascaris lumbricoides*

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

(0.6%), *Hymenolepis nana* (0.4%), *Trichuris trichiura* (0.2%). *Blastocystis Homminis* (10.1%), *Entamoeba histolytica/dispar* (6.6%), *Trychomona Homminis* (0.4%), *Entamoeba coli* (0.2%), *Endolimax nana* (0.2%) as non-pathogens. The parasitic species identified in the study are shown in Table 3.

		VIH (+) (n=503)
Outcomes		
Parasite Infection -no(%)		
	Any	166(33.0)
	Polyparasitism	39(7.8)
Parasites species-no(%)		
Direct Observation		
	Blastocystis homminis	51(10.1)
	Entamoeba histolytica/dispar	33(6.6)
	Giardia lamblia	10(4.0)
	Chilomastix mesnili	8(1.6)
	Strongyloides stercolaris	6(1.2)
	Isospora belli	3(0.6)
	Ascaris lumbricoides	3(0.6)
	Hymenolepis nana	2(0.4)
	Entamoeba coli	1(0,2)
	Trychomona homminis	2(0.4)
	Trichuris trichura	1(0.2)
	Endolimax nana	1(0.2)
Immunochromatography		
	Giardia lamblia	26(5.8)
	Ameba SP.	2(0.4)
	Cryptosporidium sp.	2(0.4)
Stains		
	(1) Kinyou (Modified Ziehl Neelsen)*	
	Microsporidium sp.	45(8.9)
	Isospora belli	18(3.6)
	Strongyloides stercolaris	7(1.4)
	CRIPTOSPOR	4(0.8)
	(2) Diddier / Modified***	
	Microsporidium sp.	45(8.9)
	Isospora belli	17(3.4)
	Strongyloides stercolaris	7(1.4)
	Cryptosporidium sp.	4(0.8)
	(3) Giemsa**	
	Isospora belli	13(2.6)
	Microsporidium sp.	8(1.6)

Table 3: Prevalence of Intestinal parasitic infections by species in adult patients with HIV, Cochabamba-Bolivia.

*Kappa for Stain 1 y 2 =0.99 P<0.001

**Kappa for Stain 1 y 3 =0.40 P<0.001

***Kappa for Stain 2 y 3 =0.41 P<0.001

For reasons of technical availability, the Immunochromatography method was established only for the detection of *Giardia lamblia, Entamoeba histolytica/dispar* and *Cryptosporidium*. Prevalence of *Giardia lamblia* was 5.8% highly concordant with the direct observation method (Kappa = 0.77 P <0.001). *Entamoeba histolytica/dispar* was 0.4% and had a low concordance with direct observation (Kappa = 0.11 P <0.001). *Cryptosporidium sp.* was 0.4% and had low concordance with direct observation Kappa = 0.005, P = 0.92. Table 3.

In addition, staining methods have been implemented in this study specifically for the detection of species that cannot be identified by direct methods such as *Coccidias, Microporidium* and *nematodes*. The results for Kinyou staining (Modified Ziehl Neelsen) showed a higher prevalence of *Microsporidium. sp.* (8.9%), *I. belli* (3.6%), *S. stercoralis* (1.4%) and *Cryptosporidium* sp. (0.8%). Modified Diddier staining showed a prevalence of *Microsporidium sp.* Of 8.9%, *I. belli* 3.4%, *S. stercoralis* 1.4% and *Cryptosporidium sp.* 0.8%, these two methods showed a high concordance (Kappa = 0.99, P <0.001). Giemsa stain showed *I. belli* (2.6%) and Mic*rosporidium sp.* (1.6%), but showing a low concordance with the other two stains (Kappa= 0.41, P <0.001), Table 3.

Associations between the incidence of different intestinal parasitic infections and socio-epidemiological factors in patients with HIV/ AIDS are presented in Table 4.

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

		Parasite Infection		
	β±SE	OR	(95% IC)	P value
Men (yes=1)	-0.38±0.22	1.50	0.96 to 2.23	0.08
Age (per 10 yrs)	-0.15±0.09	1.16	0.98 to 1.37	0.09
Scholarship No. yrs	-0.47±0.25	1.60	0.97 to 2.60	0.06
ART(yes=1)	-1.34±0.68	3.82	1.01 to14.46	0.05
Therapy time (ART) yrs (per 10 yrs)	0.02±0.05	0.99	0.90 to1.08	0.76
Time since the last control yrs (per yr)	0.55±0.30	0.58	0.32 to 1.04	0.07
Number of Controls- last year	0.38±0.22	1.04	0.90 to 1.19	0.51
>5		1		0.66
1-4	0,05±0.53	1.05	0.37 to 3.00	0.92
0	0,21±0.24	1.23	0.78 to 1.96	0.38
Initial Viral load (per million)	-0.11±0.11	1.11	0.90 to 1.38	0.33
Actual Viral load (per million)	0.10±0.16	0.90	0.66 to 1.23	0.52
INITIAL CD4 counts (per hundred)	0.04±0.06	0.96	0.86 to 1.07	0.47
ACTUAL CD4 counts (per hundred)	-0.11±0.05	1.11	1.00 to 1.23	0.05
Income ((per thousand USD)	0.09±0.07	0.92	0.80 to 1.06	0.24
BMI (per ten)	-0.12±0.20	1.12	0.76 to 1.65	0.55
Liquid faeces (yes =1)	0.81±0.32	0.44	0.24 to 0.83	0.01
unintentional weight loss- last yr.	-0.11±0.05	1.12	1.01 to 1.24	0.03

Table 4: Associations between the incidence of intestinal parasitic infections and socio-epidemiological factors.

Intestinal parasitic infections were frequent but not statistically significant in men (OR = 1.5% CI, 0.96 to 2.23, P = 0.08). Also the age expressed higher risk in populations under 29 years.

Whereas, in multivariate logistic regression analysis, the factors that showed statistical significance in relation to a parasitic infection were the fact of being on antiretroviral therapy (OR = 3.82% CI, 1.01 to 14.46, P = 0.05). The current CD4 cell count (OR = 1.11% CI, 1.00 to 1.23, P = 0.05). The liquid appearance of the faeces at the time of direct examination (OR = 0.44% CI, 0.24 to 0.83, P = 0.01).

Discussion

As the under 40 years of age group corresponds to active individuals with respect to their capacity to work, procreate, and have sexual intercourses, the prevalence of HIV-infected people in this age group can result in significant impairment to the population and the economic development of the country [13].

Opportunistic infections are common in patients with HIV/AIDS and are related to a high morbidity in this population. Before ART was freely distributed to HIV patients, intestinal parasites, opportunistic or not, were common in HIV patients. Even after free ART availability, intestinal parasites are still a big concern [14]. The HIV virus induces an immunodeficient state that favors infection by enteroparasites, which, in turn, contribute to worsen the patient clinical condition by causing malnutrition, weight loss, and chronic diarrhea [15].

Malnutrition is common in the later stages of HIV infection [16]. Hypercatabolic states generally associated with aggregate infectious processes should be considered among the main factors responsible for the malnutrition linked to HIV. Absorption deficit are caused by parasites such as *G. lamblia*. Villous atrophy and metabolic alterations in addition to a deficient intake. These factors contribute to generate the so-called wasting syndrome.

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

In this study, *G. lamblia* reports one of the highest prevalence (4%), in relation to the pathogens identified by direct methods and Immunochromatography.

Intestinal parasites are a diverse group of microorganisms that include single-cell protozoans and multi-cellular intestinal worms, capable of disrupting the absorption of nutrients. The waterborne diseases, especially those caused by intestinal protozoa, are among the most relevant pathogens described in PLWHIV [10].

The poor management of water and sewage services, associated with the lack of regulation and public policies represent a serious problem in Bolivia. The quality of drinking water has been a concern in the past and it is still one today. Although remarkable progress has been made in the last decade by governments in providing good quality water to population, these remain insufficient.

Good hygiene habits are the best way to avoid contamination and reinfection by intestinal parasites. Health security is necessary for the prevention of diseases transmitted by water and food. The high positivity rate for commensals observed in the present study may be considered as an indicator of poor hygiene and sanitation, as well as of consumption of contaminated water and food by these patients.

The present study examined the occurrence of intestinal parasites in patients with HIV/ AIDS who were treated at the CDVIR. The socio-epidemiological profile of the studied patients, corroborates the national data reported by the Ministry of health.

Our study aimed at finding the epidemiology of HIV-intestinal parasites co-infection in Cochabamba and to evaluate the factors associated to this co-infection. The overall prevalence of intestinal parasites was 33.0%. Our data showed that the major predisposing factor for intestinal parasitic infections was the number of years of schooling. ART revealed an association between the probability of having parasitic infection and antiretroviral therapy, in the same way the presence of diarrhea. Monoparasitism predominated among the study participants (25.2%). No significant correlations were found between parasite presence and CD4+ T cell counts or HIV RNA levels.

Detection of *S. stercoralis, Cryptosporidium sp.* and *I. belli* in fecal samples of patients with HIV/ AIDS was lower than the prevalence reported by Soúza Junior and Garcia-Zapata in a similar study conducted in Goiânia, Goiás-Brazil [8]. It is important to note that there are few studies on the prevalence of intestinal parasites, including S. stercoralis and intestinal coccidia, in patients with HIV/ AIDS in this geographical context.

The Coccidia life cycles are suited to waterborne and foodborne transmission and their oocysts are resistant to conventional water treatment protocols [17].

Antiretroviral therapy has gradually reduced the incidence of opportunistic infections in patients with HIV/ AIDS. However, poor adherence to treatment negatively affects the effectiveness of antiretroviral therapy and the prognosis of patients. In this study, the use of antiretroviral therapy provided protection against parasites (OR=3.83, p=0.05). Patients' high adherence to antiretroviral therapy markedly improves the immune response, slows down the progression of disease and reduces the susceptibility to opportunistic infections [8]. It must be taken into account that currently the ART is administered once the HIV infection is diagnosed in such a way that immuno-suppression levels are currently not as low.

The presence of diarrhea shown significant correlation with infection by intestinal parasites, corroborating the findings of Pavie et al In contrast Bachur et al. and Wanyiri et al. [19] who reported a positive relationship between diarrhea and intestinal parasites positive samples, Cardoso et al., [18].

Cryptosporidiosis can cause severe chronic diarrhea leading to electrolyte imbalance, mal-absorption of nutrients, and profound weight loss. The physiopathology of the infection is not well-understood. Non-specific mechanisms such as the atrophy of villi and inflammatory reaction could be contributing factors to this mal-absorption process, therefore, orally taken ARV treatment will not be an exception and thus will also suffer poor absorption leading to lower ART efficacy.

A low prevalence of *Isospora belli* reported was observed in our study, in line with the study done in Yaoundé [19] where only 1.9% prevalence was reported and in Congo by Wumba et al. in 2010 where a prevalence of 1.7% was reported [20]. In contrast this prevalence was as high as 4.5% in Thailand [21] and 7% in Brazil [22,23]. In two studies done in China, I belli was not reported [24,25].

Although molecular biology methods can identify parasitic infections when they are standardized and when there are adequate conditions, their clinical application is restricted by feasibility, technical availability and cost in limited resource settings. This implies a great importance for the clinical practice regarding the microbiological studies in this context.

In Bolivia, the subsidy of laboratory studies for PLWHA only allows direct microscopic studies in the reference laboratory (LABIMED), molecular biology techniques are not available in this context, performing the controls to PLWHA only through direct microscopy methods.

Conclusions

To conclude, the prevalence of intestinal parasitic infections found in this study generates substantial evidence on the need to implement additional laboratory tests in the monitoring and treatment of patients with HIV/AIDS, the additional tests must have greater performance.

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

On the other hand, this study shows which factors were related to infection by enteric parasites according to the epidemiological scenario in Cochabamba, highlighting the need to implement preventive measures to reduce infection rates and prevent the worsening of patients' clinical conditions.

From these results, we suggest the inclusion of methods of concentration, staining, Immunochromatography and immunofluorescence, in addition to standardizing and achieving the availability of molecular biology methods, especially for the identification of microsporidium species, for its clinical implications regarding its therapeutic. These tests should be prescribed appropriately to reduce the prevalence of these infections and their consequences, such as wasting syndrome and alterations of the intestinal microbiome.

Declarations

Ethical Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the "Comité de Bioética de la investigación de la Universidad Mayor de San Simón" (CBI) under the registration N° CBI-2016-015, and the Commission d'éthique biomédicale hospitalo-facultaire de l' UCLouvain. (CEHF) Cliniques universitaries Saint-Luc Bruxelles.

Informed consent: "Informed consent was obtained from all individual participants included in the study."

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

Jorge Aviles is supported by grant AI-UMSS 2014-2019 from ARES-CCD Belgian Government, Rue Royale 180, 1000 Bruxelles, Bélgium

The study was funded by the Belgian Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au Développement (ARES-CCD:), through an Institutional support to UMSS in Bolivia (AI 2014-2019). More Information: https://www.ares-ac.be/fr/cooperation-au-developpement/pays-projets/projets-dans-le-monde/item/92-programme-d-appui-institutionnel-avec-la-universidad-mayor-de-san-simon.The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' Contributions

Conceptualization: [Jorge Avilés]; Methodology, statistical analysis and were a major contributors in writing the manuscript: [Annie Robert, Jorge Avilés]; Formal analysis and investigation: [Annie Robert, Jean Cyr Yombi, Héctor Rodriguez, Jorge Avilés]; Writing - original draft preparation: [Jorge Avilés, Carlos Erostegui, Marcelo Rojas]; Writing - review and editing: [Daniel Mercado; Daniel Illanes]; Funding acquisition: [Annie Robert, Jorge Avilés]; Resources: [Académie De Recherche Et D'Enseignement Supérieur]; Supervision and performed the microbiological and molecular examination of the stool samples laboratory test: [Maricruz Torrico; Jorge Avilés]. All authors read and approved the final manuscript.

Acknowledgments

Special thanks to the personnel of the laboratory of parasitology of the LABIMED, the molecular biology laboratory of the faculty of biochemistry of the UMSS and to the personnel that implemented the Immunochromatography technique and the survey.

¶ Carol Torrico2, Claudia Torrico2, Amílcar Lazcano2

References

1. UNAIDS, JUNPoHA (2014) 90-90-90 An ambitious treatment target to help end the AIDS epidemic.
2. UNAIDS, JUNPoHA (2017) UNAIDS 2017 | REFERENCE - UNAIDS DATA 2017. UNAIDS/JC2910E 248.
3. Wilcox CM (2000) Etiology and evaluation of diarrhea in AIDS: a global perspective at the millennium. World J Gastroenterol 6: 177–186.
4. WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases, Second WHO report on neglected tropical diseases. WHO reference number: WHO/HTM/NTD/2013.1.
5. John C. Hall BJH, Cockerell CJ (2011) HIV/AIDS in the Post-HAART Era: Manifestations, Treatment, and Epidemiology.
6. Marcos LA, Gotuzzo E (2013) Intestinal protozoan infections in the immunocompromised host. Current opinion in infectious diseases 26: 295-301.
7. Wang ZD, Liu Q, Liu HH, Li S, Zhang L, et al. (2018) Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasites & vectors 11: 28.
8. Barcelos NB, Silva LFE, Dias RFG, Menezes Filho HR, Rodrigues RM (2018) Opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goias, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 60: e13.

Citation: Avilés J, Yombi JC, Erostegui C, Torrico M, Rojas M, et al. (2020) Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia. Curr Res HIV: CRHA-120. DOI: 10.29011/2575-7105.100120

9. Buchacz K, Baker RK, Palella FJ, Jr., Chmiel JS, Lichtenstein KA, et al. (2010) AIDS-defining opportunistic illnesses in US patients, 1994-2007: a cohort study. AIDS (London, England) 24: 1549-59.

10. Zorbozan O, Quliyeva G, Tunali V, Ozbilgin A, Turgay N, et al. (2018) Intestinal Protozoa in HIV-Infected Patients: A Retrospective Analysis. Turkiye parazitolojii dergisi 42: 187-190.

11. Bolivia MdS (2014) Informe Nacional De Progresos En La Respuesta Al VIH/SIDA, 2014 Seguimiento a la Declaración Política sobre el VIH/sida 2011. 73.

12. Asai T, Cordova Vidal C, Strauss W, Ikoma T, Endoh K, et al. (2016) Effect of Mass Stool Examination and Mass Treatment For Decreasing Intestinal Helminth and Protozoan Infection Rates in Bolivian Children: A Cross-Sectional Study. PLoS neglected tropical diseases 10: e0005147.

13. Gentilini M, Chieze F (1990) Socioeconomic aspects of human immunodeficiency virus (HIV) infection in developing countries. Bulletin de l'Academie nationale de medecine 174:1209-1219; discussion 19-21.

14. Bachur TP, Vale JM, Coelho IC, Queiroz TR, Chaves Cde S (2008) Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases 12: 115-122.

15. Willemot P, Klein MB (2004) Prevention of HIV-associated opportunistic infections and diseases in the age of highly active antiretroviral therapy. Expert review of anti-infective therapy 2: 521-532.

16. Ford N, Shubber Z, Meintjes G, Grinsztejn B, Eholie S, et al. (2015) Causes of hospital admission among people living with HIV worldwide: a systematic review and meta-analysis. The lancet HIV 2: e438-444.

17. Cama VA, Mathison BA (2015) Infections by Intestinal Coccidia and Giardia duodenalis. Clinics in laboratory medicine 35: 423-444.

18. Cardoso LV, Galisteu KJ, Schiesari Junior A, Chahla LA, Canille RM, et al. (2011) Enteric parasites in HIV-1/AIDS-infected patients from a Northwestern Sao Paulo reference unit in the highly active antiretroviral therapy era. Revista da Sociedade Brasileira de Medicina Tropical 44: 665-669.

19. Vouking MZ, Enoka P, Tamo CV, Tadenfok CN (2014) Prevalence of intestinal parasites among HIV patients at the Yaounde Central Hospital, Cameroon. The Pan African medical journal 18: 136.

20. Wumba R, Longo-Mbenza B, Mandina M, Odio WT, Biligui S, et al. (2010) Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, Democratic Republic of Congo. Parasite (Paris, France) 17: 321-328.

21. Punpoowong B, Viriyavejakul P, Riganti M, Pongponaratn E, Chaisri U, Maneerat Y (1998) Opportunistic protozoa in stool samples from HIV-infected patients. The Southeast Asian journal of tropical medicine and public health 29: 31-34.

22. Pacheco FT, Silva RK, Martins AS, Oliveira RR, Alcantara-Neves NM, et al. (2013) Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. The Journal of parasitology 99: 1002-1008.

23. Silva CV, Ferreira MS, Borges AS, Costa-Cruz JM (2005) Intestinal parasitic infections in HIV/AIDS patients: experience at a teaching hospital in central Brazil. Scandinavian journal of infectious diseases 37: 211-215.

24. Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, et al. (2012) Co-infection of HIV and intestinal parasites in rural area of China. **Parasites & vectors** 5: 36.

25. Tian LG, Cheng GJ, Chen JX, Cai YC, Guo J, Tong XM, et al. (2012) [Survey on co-infection with HIV and intestinal parasites in high prevalence areas of HIV/AIDS, China]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 24: 168-72.

Anex 1. UMSS ethics committee endorsement

UNIVERSIDAD MAYOR DE SAN SIMON
FACULTAD DE MEDICINA

Cochabamba, marzo 9 de 2018

Señor
Dr. Jorge Avilés
Presente:

Distinguido Dr. Avilés:

En respuesta a su solicitud en el Comité de Bioética de la Investigación de la Facultad de Medicina de la Universidad Mayor de San Simón consta que el protocolo de investigación

En 2016 no se asignaban códigos a los trabajos, sin embargo desde este año hemos implementado una codificación funcional. Al trabajo presentado por usted le corresponde el código: CBI-2016-015.

Este protocolo fue presentado el 28 de junio de 2016 y revisado el 7 de agosto de ese mismo año habiendo sido aprobado.

Sin otro particular y esperando cumplir su solicitud para los trámites que requiere, a nombre del Comité de Bioética de la Investigación de la Facultad de Medicina, me despido con las consideraciones más distinguidas.
Atte.

Dr. Daniel Mercado, SJ
Secretario de actas

c.c./Arch

Ciencia y Conocimiento DESDE 1832

Av. Aniceto Arce N° 0171 * Casilla 3119 Teléfonos: 4231508 4232206 * Fax: (591-4) 4231698
Email: medicina@umss.edu.bo - Cochabamba-Bolivia

Anex 2. INSTRUMENS

ANEXO 1. FORMULARIO

ESTUDIO DE PREVALENCIA DE PARASITOS INTESTINALES Y VIH

Cuestionario. Formulario de recolección de datos.

1. Código Nº — Adhiera aquí la etiqueta autoadhesiva

2. Sexo ____ (0=Masculino / 1=Femenino)

3. Servicio de Salud ____

¿Cuál es su fecha de nacimiento?

4. Fecha de nacimiento (Día/Mes/Año) ____ Edad ____ años

Lugar de Nacimiento ____

¿Cuántos años de estudio tiene completos y aprobados?

5. Escolaridad / Nivel ____ años ____

6. Dirección actual

Municipio ____

Zona ____

Barrio ____

Calle ____

¿Recuerda en qué fecha y año comenzó sus controles en el CDVIR?

7. Fecha del diagnóstico de confirmación de la Infección por VIH (Día/Mes/Año) ____

8. Fecha en que se realizó el primer control (Día/Mes/Año) ____

(Obtener este dato de la historia clínica si aparece consignado).

Lugar de Diagnóstico/ Ciudad de Bolivia ____ Otro País: ____

Fecha en que se realizó el último control (Día/Mes/Año) ____

9. ¿Cuántas veces concurrió a controlarse este año? ____ controles
Obtener este dato del carné perinatal o de la historia clínica si aparece consignado.

Carga Viral Inicial ☐ Carga Viral Actual ☐

CD4 Inicial ☐ CD4 Actual ☐

CD8 Inicial ☐ CD8 Actual ☐

Relación CD4 / CD8 Inicial ☐ Relación CD4 / CD8 Actual ☐

TAR SI ☐ NO ☐

Esquema de TAR Inicial ☐ Esquema de TAR Actual ☐

Fecha de Inicio ☐ (Día/Mes/Año)

Obtener estos datos de la historia clínica si aparece consignado.

¿Le han brindado información sobre cómo se contagia y se puede prevenir la infección por parásitos intestinales, y qué debería hacer usd. Si tuviera parásitos intestinales?

10. Consejería previa de Parásitos intestinales: ☐ SI ☐ NO No sabe/no contesta ☐

¿Le han realizado durante sus controles un estudio de parásitos intestinales?

11. Estudio previo de parásitos intestinales: SI ☐ NO ☐

¿Conoce el resultado?

12. Resultado Positivo ☐ Negativo ☐

¿Le realizaron algún tipo de tratamiento luego de conocer el resultado?

SI ☐ NO ☐

Por favor, dígame cual fue el tratamiento:

☐

Obtener corroboración de la historia clínica si aparecen consignados.

13. Valoración del tratamiento Adecuado ☐ Inadecuado ☐

En la variable 13, y en todas las siguientes vinculadas a valoración del tratamiento, la calificación de Adecuado o Inadecuado deberá realizarse confrontando los datos obtenidos con las pautas de tratamientos indicadas en la " **GUIA DE TRATAMIENTO ANTIRRETROVIRAL EN ADULTOS / Estado Plurinacional de Bolivia. Ministerio de Salud y Deportes. Dirección General de Servicios de Salud. Programa Nacional ITS/VIH/SIDA.**". El encuestador deberá contar con los algoritmos de tratamiento para realizar valoración.

Si el estudio previo de parásitos intestinales no se realizó o su resultado fue negativo pasar a las preguntas relacionadas con la variable 14.

Si el resultado del estudio previo de parásitos intestinales fue positivo continuar con las siguientes preguntas.

Finalmente le pediría que conteste una última pregunta. Sumando todos los ingresos de su hogar, ¿a cuánto asciende sus ingresos por mes?

Considerando que el salario mínimo es de 1600 Bs.

14. Ingreso mensual del hogar _____ Salarios mínimos

Se debe dividir el total de ingresos regulares del hogar sobre el monto correspondiente al salario mínimo de cada país. Se debe tratar, en la medida de lo posible, que la persona encuestada responda haciendo una estimación de su promedio mensual de ingresos.

Evaluación Nutricional / Antropometría

Peso _____

Talla _____

IMC: _____

Individual / Familiar (Marcar con X si está presente el antecedente)

Antecedentes de obesidad: _____ _____

Antecedentes de Diabetes: _____ _____

Antecedentes de hipertensión Arterial:

Antecedentes de Hipercolesterolemia: _____ _____

Antecedentes de SCA / IAM: _____ _____

Añadir algún antecedentes familiar de importancia _____

Añadir algún antecedentes personal de importancia _____

Tipo de alimentación * _____

*Obtener el dato de tipo de alimentación en base a la evaluación y criterios consignados en el documento del taller realizado para capacitación del equipo de encuestadores.

¡Gracias por su colaboración. (Fin de la entrevista)

Datos d el encuestador:

Código Nº _____ Firma: _____

ANEXO 2. CONSENTIMIENTO INFORMADO

ESTUDIO DE PREVALENCIA DE PARASITOS INTESTINALES y VIH

INFORMACION A LA PVVS[7]

La invitamos a participar como voluntario en un estudio de investigación. Por favor lea esta hoja informativa cuidadosamente, o permítame leérsela. Ud. tiene la libertad de preguntar sobre posibles riesgos y beneficios, sus derechos como voluntario, y sobre cualquier aspecto de la investigación que no sea claro. Cuando todas sus preguntas hayan sido contestadas, usted puede decidir si desea participar en el estudio o no. Este proceso se llama "consentimiento informado".

PROPÓSITO Y BENEFICIOS

El propósito de este estudio es conocer la frecuencia de las infecciones por parásitos intestinales y VIH en PVVS atendidos en el sistema público del país. Los beneficios que usted pudiera tener incluyen descartar la infección por parásitos intestinales y recibir tratamiento de ser necesario. El beneficio para la sociedad es que esta información nos ayudará a conocer que tan frecuentes es estas enfermedades y que acciones son necesarias para prevenirla.

PROCEDIMIENTOS

Si usted acepta participar en este estudio, responderá un cuestionario sobre información personal. Anónimamente (sin que se conozca su nombre) y en

_____

.

forma privada, un entrevistador capacitado lo entrevistará para completar el cuestionario. El cuestionario incluye preguntas acerca de sus controles por la infección por VIH. Cuando termine con el cuestionario, le realizaremos un test para parásitos intestinales. Estimamos que la entrevista y la obtención de muestras demoren aproximadamente 20 minutos.

Los resultados de las pruebas confirmatorias realizadas en el laboratorio del IIBISMED estarán disponibles después de algunos días. Si una infección por parásitos intestinales es confirmada, usted recibirá tratamiento adecuado, y se le brindará consejería acerca de cómo evitar una nueva infección.

RIESGOS O MOLESTIAS

Usted puede sentir que algunas preguntas invaden su privacidad. Si este es el caso, por favor usted es libre

de rehusarse a contestarlas. Usted deberá informar a su médico o al/los investigadores de cualquier complicación que usted crea está relacionada con los procedimientos del estudio.

Conocer el resultado de alguna prueba diagnóstica, puede causar tensión severa, ansiedad y depresión, a las personas examinadas.

INFORMACION ADICIONAL

Usted es libre de rehusarse a responder cualquier pregunta y a solicitar información en cualquier momento durante el estudio, y tiene el derecho de recibir respuestas que satisfagan sus inquietudes. Si tiene alguna pregunta respecto al estudio, puede preguntar al entrevistador ahora, o llamar a la persona señalada como contacto arriba.

Confidencialidad

Toda la información recogida en este estudio será manejada con rigurosa confidencialidad Los cuestionarios y las muestras serán identificados solamente con un código, sin su nombre. Solo los investigadores nombrados anteriormente tendrán acceso a esta investigación. La información será conservada hasta que los reportes del estudio sean publicados, y por un máximo de 3 años. No revelaremos su nombre en ningún informe o publicación resultante de este estudio.

Retiro del estudio

Su participación en este estudio es completamente voluntaria. Usted puede rehusarse a participar o puede retirarse del estudio en cualquier momento que lo desee sin ninguna pérdida de beneficios, y sin afectar su cuidado médico y tratamiento.

Nombre del entrevistador

Firma del entrevistador

Fecha

INFORMED CONSENT FORMULARIE

ANEXO 2. CONSENTIMIENTO INFORMADO

ESTUDIO DE PREVALENCIA DE PARASITOS INTESTINALES y VIH

INFORMACION A LA PVVS[7]

La invitamos a participar como voluntario en un estudio de investigación. Por favor lea esta hoja informativa cuidadosamente, o permítame leérsela. Ud. tiene la libertad de preguntar sobre posibles riesgos y beneficios, sus derechos como voluntario, y sobre cualquier aspecto de la investigación que no sea claro. Cuando todas sus preguntas hayan sido contestadas, usted puede decidir si desea participar en el estudio o no. Este proceso se llama "consentimiento informado".

PROPÓSITO Y BENEFICIOS

El propósito de este estudio es conocer la frecuencia de las infecciones por parásitos intestinales y VIH en PVVS atendidos en el sistema público del país. Los beneficios que usted pudiera tener incluyen descartar la infección por parásitos intestinales y recibir tratamiento de ser necesario. El beneficio para la sociedad es que esta información nos ayudará a conocer que tan frecuentes es estas enfermedades y que acciones son necesarias para prevenirla.

PROCEDIMIENTOS

Si usted acepta participar en este estudio, responderá un cuestionario sobre información personal. Anónimamente (sin que se conozca su nombre) y en

_____

forma privada, un entrevistador capacitado lo entrevistará para completar el cuestionario. El cuestionario incluye preguntas acerca de sus controles por la infección por VIH. Cuando termine con el cuestionario, le realizaremos un test para parásitos intestinales. Estimamos que la entrevista y la obtención de muestras demoren aproximadamente 20 minutos.

Los resultados de las pruebas confirmatorias realizadas en el laboratorio del IIBISMED estarán disponibles después de algunos días. Si una infección por parásitos intestinales es confirmada, usted recibirá tratamiento adecuado, y se le brindará consejería acerca de cómo evitar una nueva infección.

RIESGOS O MOLESTIAS

Usted puede sentir que algunas preguntas invaden su privacidad. Si este es el caso, por favor usted es libre

de rehusarse a contestarlas. Usted deberá informar a su médico o al/los investigadores de cualquier complicación que usted crea está relacionada con los procedimientos del estudio.

Conocer el resultado de alguna prueba diagnóstica, puede causar tensión severa, ansiedad y depresión, a las personas examinadas.

INFORMACION ADICIONAL

Usted es libre de rehusarse a responder cualquier pregunta y a solicitar información en cualquier momento durante el estudio, y tiene el derecho de recibir respuestas que satisfagan sus inquietudes. Si tiene alguna pregunta respecto al estudio, puede preguntar al entrevistador ahora, o llamar a la persona señalada como contacto arriba.

Confidencialidad

Toda la información recogida en este estudio será manejada con rigurosa confidencialidad Los cuestionarios y las muestras serán identificados solamente con un código, sin su nombre. Solo los investigadores nombrados anteriormente tendrán acceso a esta investigación. La información será conservada hasta que los reportes del estudio sean publicados, y por un máximo de 3 años. No revelaremos su nombre en ningún informe o publicación resultante de este estudio.

Retiro del estudio

Su participación en este estudio es completamente voluntaria. Usted puede rehusarse a participar o puede retirarse del estudio en cualquier momento que lo desee sin ninguna pérdida de beneficios, y sin afectar su cuidado médico y tratamiento.

Nombre del entrevistador

Firma del entrevistador

Fecha

CONSENTIMIENTO INFORMADO

Código Nº | Adhiera aquí la etiqueta autoadhesiva

Dejo constancia que he sido informada y he comprendido las características y los alcances del Proyecto **ESTUDIO DE SEROPREVALENCIA DE PARASITOS INTESTINALES Y VIH**, y manifiesto mi conformidad en participar en el mismo.

He sido informado que se me realizará procedimientos para obtener una muestra de heces para diagnosticar mi situación de salud respecto a la parasitos intestinales y al VIH.

Se me ha comunicado que si algún resultado es positivo, se realizaran un estudios confirmatorios de los resultado.

Se me ha puesto en conocimiento y he manifestado mi acuerdo respecto a que si el resultado del Test es positivo, recibiré el tratamiento apropiado para parasitos intestinales.

Firma de conformidad: Aclaración de firma:

Fecha: