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Diamantina Moreno-Gutierrez est médecin, spécialisée en maladies infectieuses tropicales et en épidémiologie. Elle travaille en tant que médecin et assistante de recherche dans des projets de lutte contre le paludisme et d'autres maladies infectieuses. Ses recherches portent sur l'évaluation des stratégies de détection et de traitement des infections paludéennes asymptomatiques en Amazonie péruvienne.

Le paludisme est un problème de santé publique au Pérou. Des observations parasitologiques, entomologiques et environnementales ont été combinées pour comprendre la transmission du paludisme. Nos résultats ont montré une forte hétérogénéité entre les communautés et une prévalence élevée d'infections palustres submicroscopiques principalement dues au *Plasmodium vivax*. Ensuite, deux études transversales ont été menées pour estimer les coûts économiques associés à la détection passive des cas et à la prise en charge du paludisme non compliqué pour déterminer si les comportements de recherche de soins influencent ces coûts. De plus, une étude de cohorte basée sur la population a été menée pour évaluer son efficacité. La plupart des infections palustres étaient asymptomatiques et submicroscopiques. Les parties prenantes devraient envisager de mettre en œuvre des stratégies visant à éliminer les infections palustres symptomatiques et asymptomatiques.

Malaria is a public health problem in Peru. Parasitological, entomological, and environmental observations were combined to understand malaria transmission. We found high heterogeneity across communities and high prevalence of submicroscopic malaria infections mainly due to Plasmodium vivax. Then, two cross-sectional studies were conducted to estimate the economic costs associated with passive case detection and management of uncomplicated malaria and explore whether healthcare-seeking behaviours influence those costs. There was a delay in seeking care, and the costs of uncomplicated malaria were mainly borne by the families. Since active case detection was added to routine malaria surveillance, a population-based cohort study was conducted to assess its effectiveness. Most malaria infections were asymptomatic and submicroscopic. Stakeholders should consider implementing strategies to eliminate both symptomatic and asymptomatic malaria infections.

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Evaluation of strategies for the detection and treatment of asymptomatic malaria



Evaluation of optimized strategies for the detection and treatment of asymptomatic malaria infections in the Peruvian Amazon

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docteur en sciences médicales

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LIST OF ABBREVIATIONS

ACD	Active case detection
API	Annual parasite index
ACT	Artemisinin-based combination therapy
<i>An.</i>	<i>Anopheles</i>
AS	Artesunate
BH	Bello Horizonte
CHW	Community health worker
CJ	Catorce de Julio
CQ	Chloroquine
EsSalud	Social health insurance system
FFAA	Armed Forces
GDP	Gross Domestic Product
GNI	Gross national income
G6PD	Glucose-6-phosphate dehydrogenase
GTS	Global Technical Strategy
HF	Health Facility
HRP-II	Histidine-rich protein II
HU	Huaman Urco
IHIS	Integrated Health Insurance scheme
KAP	Knowledge, attitudes, and practices
LAMP	Loop-mediated isothermal amplification
LI	Libertad
LM	Light microscopy
MA	Mangua
MDA	Mass drug administration
MDGs	Millennium Development Goals
MoH	Ministry of Health
MQ	Mefloquine

MT	Mazan town
NMCP	National Malaria Control Program
ODK	Open Data Kit
<i>P.</i>	<i>Plasmodium</i>
PA	Puerto Alegre
PAB	Puerto Abeja
PCD	Passive case detection
pLDH	<i>Plasmodium</i> lactate dehydrogenase
PQ	Primaquine
PE	Primero de Enero
PEN	Peruvian Nuevos Soles
PO	Puerto Obrero
POC	Point of care
PU	Puinahua
PNP	National Police
qPCR	Quantitative real-time polymerase chain reaction
RDTs	Rapid diagnostic tests
RHDL	Regional Health Direction of Loreto
RDT	Rapid diagnostic test
SA	Salvador
SC	Santa Cruz
SF	San Francisco de Buen Paso
SPR	Slide Positivity Rate
SDGs	Sustainable Development Goals
SU	Sucusari
SZ	San Antonio de Zambrano
TA	Tamanco
US\$	United States Dollars
UM	Urco Miraflores
VB	Visto Bueno
WTP	Willingness to Pay
WHO	World Health Organization

TABLE OF CONTENTS

SUMMARY	viii
SAMENVATTING	x
1. INTRODUCTION	1
1.1. Rationale.....	1
1.2. Objectives	3
1.3. Thesis outline	4
2. BACKGROUND	5
2.1. Malaria global burden	5
2.2. Malaria in the Peruvian Amazon	10
2.3. Malaria surveillance in the Peruvian Amazon	12
2.4. Asymptomatic malaria	15
2.4.1. Heterogeneity of the asymptomatic malaria reservoir	15
2.5. Malaria case management in the Peruvian Amazon	17
2.5.1. Diagnostics and asymptomatic malaria.....	18
2.5.2. Treatment for uncomplicated malaria	20
2.6. The economic burden of malaria.....	22
3. INTEGRATING PARASITOLOGICAL AND ENTOMOLOGICAL OBSERVATIONS TO UNDERSTAND MALARIA TRANSMISSION IN RIVERINE VILLAGES IN THE PERUVIAN AMAZON	25
3.1. Abstract.....	25
3.2. Background	26
3.3. Material and methods	27
3.4. Results	32
3.5. Discussion	42
4. ECONOMIC COSTS ANALYSIS OF UNCOMPLICATED MALARIA CASE MANAGEMENT IN THE PERUVIAN AMAZON	49
4.1. Abstract.....	49
4.2. Background	50
4.3. Material and methods	52
4.4. Results	64

4.3. Discussion	78
4.4. Conclusions	84
5. EFFECTIVENESS OF A MALARIA SURVEILLANCE STRATEGY BASED ON ACTIVE CASE DETECTION DURING HIGH TRANSMISSION SEASON IN THE PERUVIAN AMAZON	85
5.2. Abstract.....	85
5.2. Background	86
5.3. Material and methods	88
5.4. Results	97
5.5. Discussion	109
5.1. Conclusions	115
6. GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES...	117
6.1. General discussion.....	117
6.2. Limitations and strengths	125
6.3. Conclusions and perspectives.....	127
7. REFERENCES	130
8. ANNEXES	149
SCIENTIFIC CURRICULUM VITAE.....	165

SUMMARY

Malaria remains a public health problem in the Peruvian Amazon. To understand malaria transmission in riverine communities of Mazan district in the Peruvian Amazon, we combined parasitological, entomological, and environmental observations. Despite heterogeneity across communities, the prevalence of submicroscopic malaria infections was high and mainly due to *Plasmodium vivax*. Furthermore, housing with incomplete walls was the main risk factor for malaria, and communities in Mazan basin had higher entomological indicators, prevalence, and incidence rates than communities in the Napo basin. In that scenario and considering that routine malaria surveillance is based on passive case detection (PCD) using light microscopy (LM) for malaria diagnosis, we conducted two cross-sectional studies to estimate the economic costs associated with passive case detection (PCD) and management of uncomplicated malaria episodes and explore whether healthcare-seeking behaviours significantly influence those costs. Although malaria case management was free, there was a delay in seeking care, and the costs of uncomplicated malaria incurred by families were mainly indirect costs due to loss of working days.

Since the Peruvian Ministry of Health (MoH) improved routine malaria surveillance by the addition of an optimized strategy recommended by the World Health Organization (WHO) known as active case detection (ACD) to overcome the limitations of the routine malaria surveillance based on PCD using LM for detecting both asymptomatic infected individuals and symptomatic individuals that do not attend the health services, a population-based cohort study was conducted to assess the effectiveness of this optimized strategy based on ACD using light

microscopy (LM) compared with quantitative real-time polymerase chain reaction (qPCR). The optimized ACD-strategy using LM had less effectiveness than qPCR. Furthermore, qPCR results confirmed that most malaria infections were asymptomatic, submicroscopic, and mainly due to *P. vivax*.

As asymptomatic and submicroscopic malaria infections are common, undetected, and untreated using a standard diagnostic test such as light microscopy due to low effectiveness, optimized strategies based on ACD using molecular tests added to PCD could reduce the malaria burden in the Peruvian Amazon. However, to ensure optimal allocation of resources for malaria elimination, Peruvian stakeholders should consider implementing mass drug administration (MDA) with a single treatment using artemisinin-based combination therapy (ACT) plus primaquine or tafenoquine for uncomplicated malaria by both *P. falciparum* and *P. vivax* infections. Furthermore, high heterogeneity in malaria transmission creates opportunities for targeted control interventions such as MDA in clusters of high malaria prevalence.

SAMENVATTING

Malaria blijft een probleem voor de gezondheidszorg in het Peruaanse Amazonegebied. Om de malaria transmissie binnen de rivierstammen van het Mazan district in het Peruaanse Amazonegebied beter te begrijpen hebben wij een gecombineerde studie van parasitologie, entomologie en het milieu gedaan.

Ondanks de diversiteit binnen deze rivierstammen is de prevalentie van de submicroscopische malaria hoog en voornamelijk veroorzaakt door *Plasmodium vivax*.

Bovendien zijn de huizen met onvolledig gebouwde muren de algemene risicofactor voor Malaria. Het Mazan-bekken heeft hogere entomologische indicatoren en een hogere prevalentie en incidentenratio dan het Napo-bekken. In dit scenario en rekening houdend dat de routine malaria surveillance gebaseerd is op de passieve case detectie (PCD) en lichtmicroscopie (LM) voor de diagnose van Malaria hebben we 2 cross-sectionele studies uitgevoerd om de economische kost te schatten die geassocieerd is met de passieve case detectie (PCD) en het beleid van niet-gecompliceerde malaria episodes en of de invloed van het wel/niet op doktersbezoek gaan op deze economische kost. Alhoewel de malariagezondheidszorg gratis is, is er toch een vertraging van de medische hulpvraag door de patiënt. Voornamelijk omdat de normale medische kosten die door de families gedragen worden zich vertalen in onrechtstreekse kosten door het verlies van aantal werkdagen en dus inkomsten.

Sinds het Peruaanse ministerie van volksgezondheid de routine malaria surveillance verbeterd heeft door het implementeren van een geoptimaliseerde strategie door de Wereldgezondheidsorganisatie als de actieve case detectie om de beperkingen van de routine malariasurveillance gebaseerd op de PCD die LM gebruikt voor de detectie van zowel asymptomatische geïnfecteerde individuen die door de gezondheidszorg niet bereikt worden werd een cohortstudie opgezet om de

effectiviteit van deze geoptimaliseerde strategie gebaseerd op de ACD die lichtmicroscopie (LM) vergelijkt met kwantitatieve real-time polymerase ketting reactie(qPCR). De geoptimaliseerde ACDstrategie met LM is minder effectief dan deze met qPCR. Bovendien bevestigen de qPCR resultaten dat de meeste malaria infecties asymptomatisch en submicroscopisch veroorzaakt door *P. vivax* zijn. Aangezien asymptomatische en submicroscopische malaria infecties gewoon zijn en dus niet gedetecteerd en dus ook niet behandeld door het gebruik van een standaard diagnostische test als lichtmicroscopie met een lage effectiviteit kan de implementatie van geoptimaliseerde strategieën gebaseerd op ACD die moleculaire tests gebruiken gecombineerd met de PCD een reductie van het Malariaprobleem in het Peruaanse Amazonegebied betekenen. Hoewel, om een optimaal gebruik van de budgetten voor de uitroeiing van malaria zouden de Peruaanse stakeholders rekening moeten houden met het implementeren van een beleid gebaseerd op toediening van preventieve anti-malaria medicatie op grote schaal die slechts 1 behandeling vergt gebruikmakend van artemisinin-based combination therapy (MDA) met primaquine of tafenoquine voor ongecompliceerde malaria van zowel *P. falciparum* en *P. vivax* infecties. Bovendien zorgt een hoge heterogeniteit in malariatransmissies voor opportuniteiten om targeted control interventies zoals de MDA in clusters met een hoge malaria prevalentie in te zetten.

1. INTRODUCTION

1.1. Rationale

Malaria remains a global public health problem, with an estimated 241 million cases and 627,000 deaths globally in 2020 [1]. Most cases (about 85%) in the Peruvian Amazon are concentrated in the Loreto department, caused by *Plasmodium vivax* and *P. falciparum* (ratio of *P. vivax*/*P. falciparum*: 5/1) and mainly transmitted by the mosquito *Anopheles darlingi* [2,3]. Riverine villages, characterized by poverty and limited access to health facilities, concentrate an important proportion of reported malaria cases in Loreto; however, transmission dynamics in these villages remain understudied [4,5].

According to World Health Organization (WHO), malaria treatment should begin with a parasitological diagnosis. Furthermore, every malaria case should be tracked in a surveillance system [6]. In line with this, routine malaria surveillance in the Peruvian Amazon is based on passive case detection (PCD) using light microscopy (LM) for malaria diagnosis. Individuals with malaria-like symptoms are systematically tested by LM at health facilities and treated following national guidelines. However, PCD using LM has several limitations for detecting asymptomatic and symptomatic infections with limited access to health services, poor quality and limited access for diagnosis by LM, and incomplete treatment. Hence, a high proportion of patients are undetected, untreated and contribute to malaria transmission [7].

Faced with malaria resurgence in the Loreto department, malaria surveillance was improved by the addition of an optimized malaria surveillance strategy known as active case detection (ACD) using light microscopy (LM) as a complementary strategy for malaria control in communities with an increased number of cases previously reported by PCD [8,9]. This optimized ACD-strategy is based on household visits and screening of population for malaria using LM and the treatment of confirmed malaria infections irrespective of symptoms. However, there is a lack evidence on its effectiveness in different epidemiological settings, specially in endemic areas where most infected individuals have low parasitemia levels [10–13]. This thesis aimed to characterize malaria transmission in the Peruvian Amazon, to estimate the economic costs associated with the PCD and case management of uncomplicated malaria episodes in riverine communities in the Peruvian Amazon from both the provider's and patient's perspectives, explore whether healthcare-seeking behaviours significantly influence those costs and to assess the short-term effectiveness of a malaria surveillance strategy based on ACD during high transmission season in the Peruvian Amazon.

1.2. Objectives

The main objective of the thesis is:

- To assess optimized strategies for the detection and treatment of asymptomatic malaria infections in the Peruvian Amazon.

The specific objectives of the thesis are:

- To characterize malaria transmission and identify risk factors in the Peruvian Amazon
- To estimate the economic costs associated with the passive case detection (PCD) and case management of uncomplicated malaria episodes in the Peruvian Amazon
- To assess the effectiveness of an optimized malaria surveillance strategy based on active case detection (ACD) for the detection and treatment of asymptomatic malaria infections in the Peruvian Amazon.

1.3. Thesis outline

The thesis is organised in seven chapters.

Chapter 1 presents the rationale and the objectives of the thesis

Chapter 2 describes the global and Peruvian malaria burden. Next, it provides a background on malaria surveillance. After that, it focuses on asymptomatic malaria, malaria case management and economic burden.

Chapter 3 characterizes the malaria transmission in four communities, two on each of the Mazan and Napo river basins in the Peruvian Amazon and identifies malaria risk factors.

Chapter 4 estimates the economic costs of uncomplicated malaria case management.

Chapter 5 estimates the effectiveness of a malaria surveillance strategy based on active case detection during high transmission season.

Chapter 6 discusses the main finding of the thesis research, their implications for malaria control in Peru, and provides perspectives for future research.

Chapter 7 lists the bibliographic references

Chapter 8 includes the annexes.

2. BACKGROUND

2.1. Malaria global burden

From 1955 to 2021, 40 countries and territories have achieved at least three years of zero indigenous cases and have been declared malaria-free by World Health Organization (WHO) [14]. However, the Global Technical Strategy for malaria (GTS) 2020 milestones for a reduction in malaria case incidence and mortality rate of at least 40% from a 2015 baseline were not achieved globally. In 2020, of the 93 countries that were malaria endemic globally in 2015, only 30 (32%) achieved 40% or more reduction in case incidence or reported zero malaria cases, and 40 countries (43%) achieved 40% or more reduction in mortality rate (Figure 1 and Figure 2) [15]. Despite the considerable efforts to control and eliminate malaria, this disease remains a significant problem in global public health with an estimated 241 million malaria cases in 2020 increasing from 224 million in 2015.

In 2020, malaria cases were concentrated in African Region with an estimated 228 million cases (about 95% of cases) while the Americas Region contributed with an estimated 0.65 million cases. In this Region, malaria cases increased by 8% (from 602,000 in 2015 to 653,000 in 2020) while malaria deaths decreased by 3% (from 414 in 2015 to 402 in 2020). More than 77% of malaria cases in 2020 were from three countries (Venezuela-Bolivarian Republic of, Brazil and Colombia) and the majority of deaths (67%) in 2020 occurred in Brazil and Venezuela (Bolivarian Republic of) [15].

On the other hand, in the Americas Region, three countries have achieved malaria-free status; El Salvador in 2021, Argentina in 2019 and Paraguay in 2018. Additionally, Belize, French Guiana, Guatemala, Haiti, Honduras and Peru were all on target for the 2020 malaria morbidity GTS milestone of a reduction of at least 40% in case incidence (Figure 1).

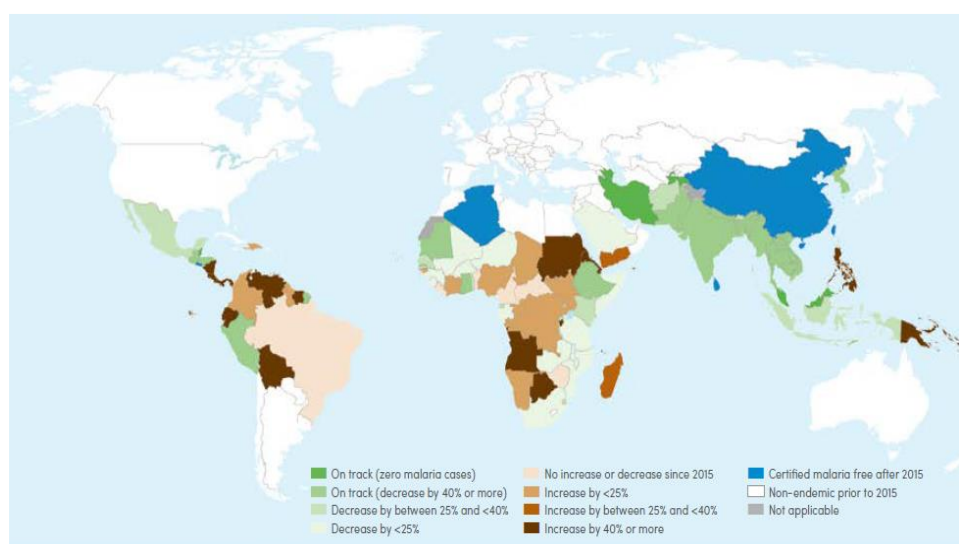


Figure 1. Malaria endemic countries showing progress towards the GTS 2020 malaria case incidence milestone of at least 40% reduction from a 2015 base [13].

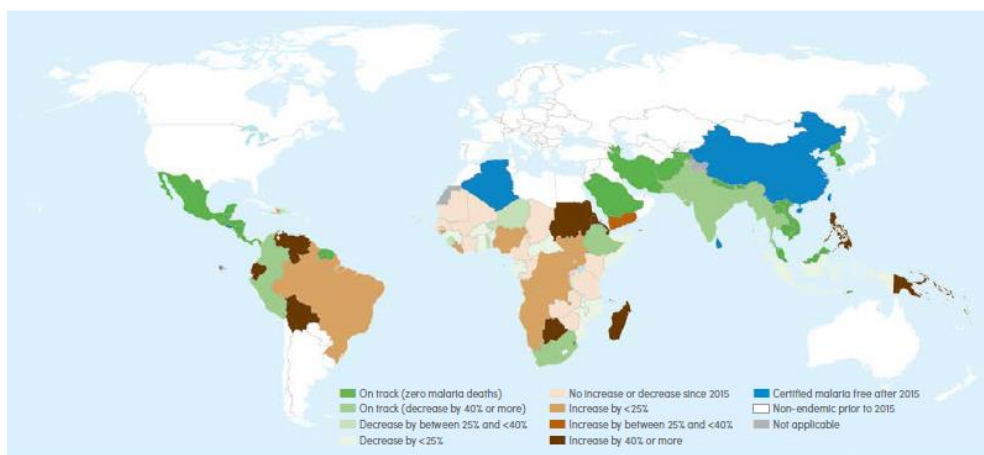


Figure 2. Malaria endemic countries showing progress towards the GTS 2020 malaria mortality rate milestone of at least 40% reduction from a 2015 baseline [13].

According to the WHO, the path to malaria elimination is characterized by different phases: control, pre-elimination, elimination, prevention of reintroduction (Table 1) [9,16] .

Table 1. Definition of phases towards malaria elimination [9,16]

Malaria control is the reduction of disease incidence, prevalence, morbidity, or mortality to a level that it is no longer a public health problem. Continued intervention is required to sustain control.
Pre-elimination programmes are implemented in countries to reorient their National Malaria Control Program (NMCP) between the sustained control and elimination stages. During this period, high coverage with good-quality laboratory and clinical services should be reached, reporting and surveillance should be reinforced, followed by other programme adjustments to halt transmission nationwide.
Malaria elimination is the interruption of local transmission (that is, reducing the rate of malaria cases to zero) of a specified parasite in a defined geographic area. Continued measures are required to prevent the reestablishment of transmission.
WHO certification of elimination is the official recognition of malaria-free status granted by WHO after it has been proven beyond reasonable doubt that the chain of local human malaria transmission by Anopheles mosquitoes has been fully interrupted in an entire country for at least 3 consecutive years.
Re-introduction of transmission is the renewed presence of a constant measurable incidence of cases and mosquito-borne transmission in an area over a succession of years. An indication of the possible re-establishment of transmission would be the occurrence of three or more introduced and/or indigenous malaria infections in the same geographical focus, for 2 consecutive years for <i>P. falciparum</i> and for 3 consecutive years for <i>P. vivax</i>.
Malaria eradication is a permanent reduction to zero of the worldwide incidence of infection caused by human malaria parasites as a result of deliberate efforts. Once eradication has been achieved, intervention measures would no longer be needed.

Globally the most predominant species are *Plasmodium. falciparum* and *Plasmodium vivax*. *P. falciparum* is the most prevalent malaria parasite in the African Region, the Eastern Mediterranean Region, the Western Pacific Region and the South-East Asia Region while *P. vivax* is the most prevalent in the Americas Region (Table 2)

Table 2. Species distribution of *Plasmodium* by Region [15].

Region	Species of <i>Plasmodium</i>
Central Africa	<i>P. falciparum</i> (100%)
West Africa	<i>P. falciparum</i> (almost 100%), other (<1%)
East and Southern Africa	
High transmission	<i>P. falciparum</i> (almost 100%), <i>P. vivax</i> (<1%), other (<1%)
Low transmission	<i>P. falciparum</i> (91%), <i>P. vivax</i> (9%), other (<1%)
South-East Asia	<i>P. falciparum</i> and mixed (60%), <i>P. vivax</i> (39%), other (<1%)
Americas	<i>P. falciparum</i> and mixed (25%), <i>P. vivax</i> (75%), other (<1%)
Eastern Mediterranean	<i>P. falciparum</i> and mixed (74%), <i>P. vivax</i> (26%), other (<1%)
Western Pacific	<i>P. falciparum</i> and mixed (70%), <i>P. vivax</i> (30%), other (<1%)

2.2. Malaria in the Peruvian Amazon

Peru is located in South America and has a population of about 32 million inhabitants. It is divided in three geographic regions: Coast, Andes, and Amazon [17].

The majority of cases occurred in the Peruvian Amazon, mainly in Loreto region, where malaria transmission is permanent throughout the year, seasonal with high transmission season (HTS) between January and July, and low transmission season (LTS) between August and December [18]. This region has a tropical climate with a population estimated at risk of about 1 million, poverty in rural areas is higher (44.4%) than in urban areas (15.1%) [19], where poorer individuals are more likely to be at risk of malaria due to occupational hazards such as subsistence farming, fishing, and hunting. Two species are of primary concern, *P. falciparum* and *P. vivax* (ratio of *P.vivax/P.falciparum*: 5/1), and the primary vector is *Nyssorhynchus darlingi* (previously *Anopheles darlingi*) [20–22].

Intensified control efforts supported by the Global Fund (GF) PAMAFRO project resulted in a significant decrease in malaria burden in the Peruvian Amazon from 87,805 cases in 2005 to 23,060 cases in 2011 (about 70% reduction). Unfortunately, this achievement disappeared after the end of the GF project, reaching 63,189 reported cases in 2015 [2].

In 2017, the Peruvian Ministry of Health (MoH) approved the Malaria Zero Plan (MZP), which aimed to eliminate malaria in the Peruvian Amazon by 2030; as a result, in the first phase (from 2017 to 2021), symptomatic malaria cases detected through malaria surveillance using light microscopy (LM) or rapid diagnostic test (RDT) have been reduced by over 60%, decreasing from 63,189 cases in 2015 in Peru (95.4% of the total cases occurred in Loreto region) to 17,658 cases in 2021 (85.4% of the total cases occurred in Loreto region) [2] (Figure 3).

However, over 70% of malaria cases in the Peruvian Amazon are asymptomatic and a high proportion of infections are submicroscopic, only detectable by molecular methods [4,23,24]; thus, a large proportion of these infections are undetected and untreated timely, and these represent important foci for malaria transmission in the Peruvian Amazon. This concern is included in the second phase of the MZP.

Noteworthy, the achievement of MZP occurred during the COVID-19 pandemic with a markedly reduced number of reported malaria cases due to disruptions in essential health services, and reduced control activities carried out by MZP, which could lead to variability in the regional malaria database and malaria resurgence due to lack in malaria diagnosis and anti-malarial treatment [25].

According to the MoH, there were 10,939 malaria cases (80.2% of the total cases occurred in Loreto region) in the first six months of 2022 compared to 6,532 malaria cases in 2021 during the same period [26].

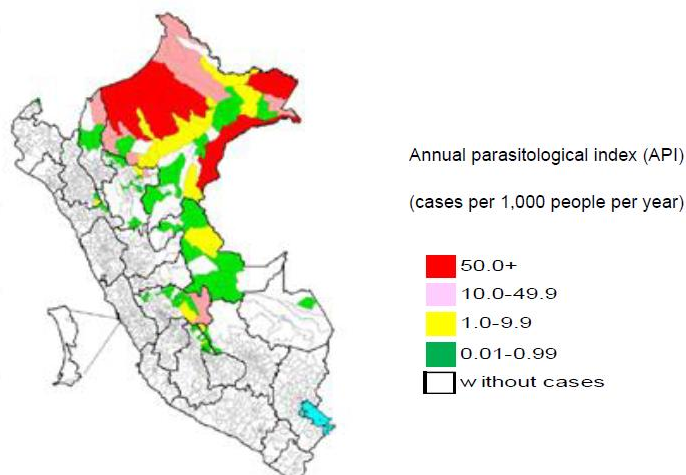
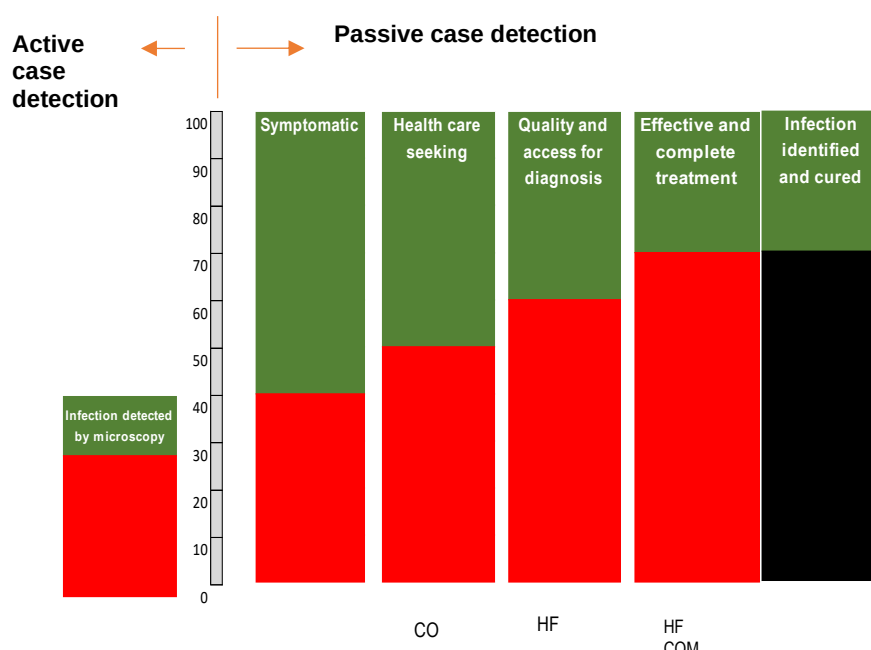


Figure 3. Malaria risk map of Peru and Loreto Region 2021 [2]

2.3. Malaria surveillance in the Peruvian Amazon

Malaria surveillance in Peru is based on passive case detection (PCD) and active case detection (ACD) using mainly light microscopy (LM) for malaria diagnosis. PCD identifies symptomatic malaria in patients who seek medical care at a health facility, while ACD is a reactive intervention based on household visits and screening of the total population in communities with a high number of cases by PCD [7].

PCD using LM has several limitations for detecting asymptomatic and symptomatic infections with limited access to health services, poor quality and limited access for diagnosis by LM, and incomplete treatment. Furthermore, asymptomatic patients do not seek health care, while in ACD, most asymptomatic infections are not detected by LM. Consequently, a highly sensitive test is necessary to identify malaria infections, such as molecular methods [7] (Figure 4).



Abbreviations: Health facility (HF); Community (COM)

Figure 4. Limiting factors on passive case detection and active case detection on malaria control and elimination [7].

Studies conducted in areas where both *P. vivax* and *P. falciparum* coexist indicate that incidence of *P. vivax* decreases less rapidly than that of *P. falciparum*. Hence, ACD for *P. vivax* control and elimination faces unique challenges [27]. Specific biological challenges that make it harder to diagnose, treat, control and eliminate *P. vivax* than *P. falciparum*, i.e. *P. vivax* gametocytes are present earlier (one or two weeks previous) in the progression of an infection compared to *P. falciparum*, as a result malaria transmission can occur before symptoms onset and thus before diagnosis or anti-malarial treatment.

In addition, *P. vivax* gametocytes are transmitted more efficiently to *Anopheles* mosquito vectors than those of *P. falciparum*. The preference of *P. vivax* for invading reticulocytes leads to low parasite densities that are more difficult to detect than *P. falciparum*, which invades blood cells of any age. Despite lower blood parasite loads, *P. vivax* immunity is acquired more rapidly than *P. falciparum* and may result in an earlier age-prevalence peak in areas of high transmission. In areas where *P. vivax* predominates, mosquitoes bite outdoors and early in the evening. As a result, conventional malaria control tools that work well against indoor-feeding and night-biting mosquitoes, such as indoor residual spraying and insecticide-treated nets, may be less effective against *P. vivax*. Within the mosquito, *P. vivax* sporozoites develop faster than *P. falciparum* at equivalent temperatures, which contributes to its wider geographic distribution [28]. Perhaps the most epidemiologically important feature of *P. vivax* biology is its ability to relapse weeks, months or even years after the primary infection via a dormant liver stage known as the hypnozoite. ACD for *P. vivax* may require several rounds to capture individuals when their infections relapse, often without causing clinical symptoms [27]. Furthermore, this feature represents a challenge for control and elimination of *P. vivax* malaria because it is undetectable using currently available diagnostic and requires a radical cure.

2.4. Asymptomatic malaria

2.4.1. Heterogeneity of the asymptomatic malaria reservoir

The main types of malaria infections are symptomatic and microscopic, asymptomatic, and microscopic, and asymptomatic and sub-microscopic. In both high and low transmission settings, asymptomatic infections far outnumber symptomatic infections (Fig 5). However, in low transmission settings, most of the asymptomatic infections are composed of submicroscopic parasitemia [29].

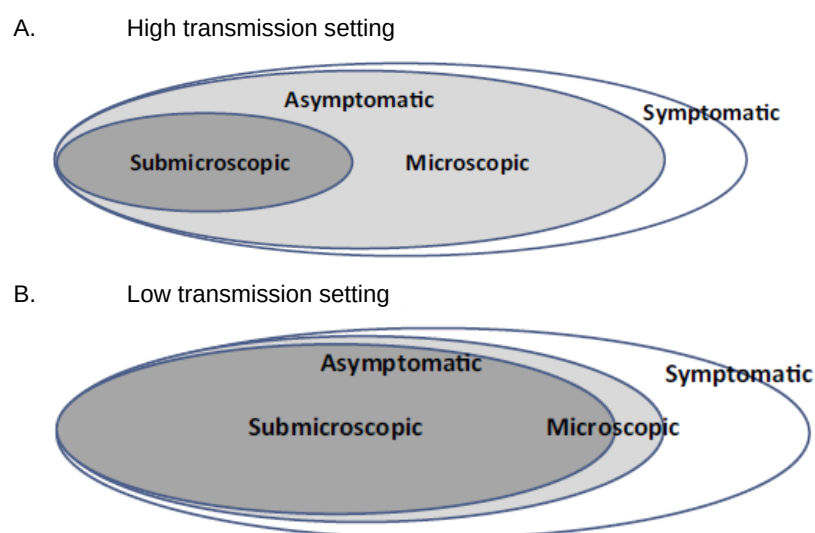


Fig 5. The asymptomatic malaria reservoir [29].

The contribution of the asymptomatic infections to malaria transmission is mediated by the duration of infection, incidence of gametocyte carriage, and is ultimately determined by mosquito infectivity. The probability of a mosquito becoming infected after feeding on an infected human is dependent on the density of gametocytes. At an individual level, this probability of infection is higher from those with higher density

symptomatic infections than those with low-density asymptomatic infections, provided there has been enough time for gametocytes to fully mature (8–12 days). At a population level, contributions to malaria transmission from each of the three types of infections may be more balanced as there will be more individuals with low-density infections than high-density infections. In the context of control and elimination, symptomatic infections often have a very short duration because symptomatic individuals are more likely to seek and receive treatment and have their infections curtailed. This is more pronounced if treatment is given early after establishment of blood stage infection, as gametocytes will be at relatively low densities and may not reach highly infectious levels.

Malaria cases can be asymptomatic for several reasons, but naturally acquired immunity is probably the main contributing factor. Naturally acquired immunity is intrinsically linked with past exposure and age. In areas of high endemicity, the frequency of symptomatic cases and the risk of mortality begin to decrease between the ages of two to five. The rate of development of naturally acquired immunity depends on the level of transmission. In areas of high endemicity, where the entomological inoculation rate is high, the exposure is greater, so immunity develops at an earlier age. Adults are less likely to be symptomatic than children, and in those adults that are symptomatic parasitemias are lower. However, in children under six months of age, parasitemias are often <100 parasites/ μ l due to protection from maternal antibodies. In low transmission areas, malaria exposure is lower, so there is less opportunity for immunity to develop. A longitudinal study from Kenya comparing low and high transmission areas showed that children with asymptomatic parasitemia were at a reduced risk of developing the symptomatic disease in a

hyperendemic area but had an increased risk of developing symptoms in an area of low transmission.

Another essential factor to consider is the multiplicity of infection and superinfection. Outbreaks, whether by *P. falciparum* or *P. vivax*, suggest a lack of immunity against new clones [30].

Regarding parasitaemia dynamics over time, an individual with a submicroscopic asymptomatic infection can have different potential infection outcomes: An individual may have been recently infected and will develop a microscopic and symptomatic infection or may become a chronic parasite carrier, remaining asymptomatic for weeks or months. Conversely, individuals with submicroscopic infections may also be at the tail end of an infection and will only remain minimally infectious for a short period (short submicroscopic). Another possible outcome is that parasitaemia fluctuates between detectable and undetectable levels by microscopy. These scenarios could be perturbed by re-infection, resulting in either continued asymptomatic infection or the development of symptoms.

2.5. Malaria case management in the Peruvian Amazon

Malaria case management, consisting of early diagnosis and prompt, effective treatment (within 24-48 h of the onset of malaria symptoms), remains a vital component of malaria control and elimination strategies [31]. However, in the Peruvian Amazon, many malaria patients with uncomplicated malaria receive diagnosis and treatment late because there is a delay in seeking care among these patients [32–34].

According to the World Health Organization (WHO), every suspected malaria case should be confirmed by light microscopy (LM) or rapid diagnostic test (RDT) before treatment, every confirmed case should be treated with a quality-assured antimalarial medicine, and every malaria case should be tracked in a surveillance system [31,35]. This service is provided free of charge by Peruvian Ministry of Health (MoH) through a network of health facilities, but in communities with a lack of health facility is provided by a voluntary community health worker (CHW), who is selected by the local community and trained by the MoH [36].

2.5.1. Diagnostics and asymptomatic malaria

In asymptomatic individuals, the low-level parasitemia limits the sensitivity of LM and RDT. Detection of parasites on Giemsa-stained blood smears by LM is the standard tool for malaria diagnosis; with detection of malaria parasites at densities as low as 4 - 20 parasites/ μ l of blood in expert hands, but according to the Peruvian guidelines, the limit of detection of malaria parasites by LM is 40 parasites/ μ l. LM allows identification of the *Plasmodium* species and quantification of parasitemia but requires continuous training of medical laboratory technicians to minimize misdiagnosis and improve case management, especially for other febrile illnesses. Because RDTs can be performed quickly with instant availability of results at the point of care, RDTs have been recommended by WHO for use in clinical care of malaria when LM is not available [31]. RDT detect *Plasmodium* antigens such as histidine-rich protein (HRP-2), lactate dehydrogenase (LDH) and aldolase. HRP-2 is only present in *P. falciparum*, while LDH and aldolase are present in all other human malaria species.

A significant limitation of the HRP-2-based RDT is the lack of sensitivity (detection limits 200 parasites/μL of blood, especially in patients with low-density infections, while LDH and aldolase-based RDTs seem to lack sensitivity below 1,000 parasites/μL of blood. Furthermore, HRP2 may cross-react with another member of the HRP gene family called histidine rich protein 3 (HRP3), and both HRP2 and HRP3 genes were missing in strains of *P. falciparum* in Peru [37]. These deletions of HRP2 and HRP3 genes are associated with false-negative RDT results, especially in asymptomatic carriers of low-density infections. Another constraint of HRP-2-based RDT is a false positive result due to HRP2 persist in the blood several days after infection clearance.

Nucleic acid amplification tests (NAATs) such as nested PCR, quantitative real-time PCR (qPCR), reverse transcriptase-qPCR (qRT-PCR), Loop-mediated isothermal amplification (LAMP), reverse transcriptase-LAMP (RT-LAMP), Nucleic Acid Sequence-based Amplification (NASBA) and real-time quantitative-NASBA (QT-NASBA) have been evaluated in the detection of asymptomatic malaria.

As PCR detected 2.2-fold higher *Plasmodium* infections than microscopy, PCR-based diagnosis has been suggested as a public health tool for ACD in Peru [24], but its large-scale use remains constrained by its high cost and complexity. LAMP could be an alternative molecular point-of care test due to similar performance as PCR but at very-low-density infection, the confirmatory *P. falciparum* test may become false-negative and interpreted as a non-falciparum result [38]. Other ultrasensitive methods such as RT-LAMP and QTNASBA have achieved a limit of

detection below 0.1 parasites/μl of blood, but their application is limited to research studies (Table 3) [39].

Table 3. Limit of detection of malaria diagnostics [39]

Type	Method	Detection target	Limit of detection parasites/μl	Quantitative
LM	Expert microscopy	Parasite morphology	4-200	yes
RDT	Standard RDT (Pf)	HRP-2, LDH, Aldolase antigen	200-500	no
	Standard RDT (non-Pf)	LDH, Aldolase antigen	500-1000	no
qPCR	qRT-PCR (RNA based)	18S rRNA 18S rRNA, mitochondrial	0.02	yes
LAMP	LAMP (DNA based)	DNA	1-100	no
NASBA	NASBA	18S rDNA	0.04	no
	QT-NASBA (RNA based)	18S rRNA	0.02	yes

LM: light microscopy, RDT: rapid diagnostic test, qPCR: quantitative polymerase chain reaction, LAMP: loop isothermal amplification, NASBA: Nucleic Acid Sequence-based Amplification

2.5.2. Treatment for uncomplicated malaria

Anti-malarial treatment is species-specific. For uncomplicated malaria vivax, chloroquine (CQ) is used against asexual stages (blood schizonticidal), a total adult dose of 1500 mg/base is given over three days (total dose of 25 mg/kg) and primaquine (PQ) for radical cure during seven days (total dose of 3.5 mg/kg). This regimen has been used since 1997 in the Peruvian Amazon. Currently, PQ is the only drug available for radical cure in Peru. The shorter seven-day regimen is used to reduce poor compliance associated with the more extended fourteen-day regimen preserving the total dose (mg/kg weight), but the compliance remains low [34]. Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency can develop hemolytic anemia and efficacy may be reduced in patients with impaired cytochrome P-450 isozyme 2D6 (CYP2D6) function due to genetic polymorphisms. Tafenoquine

administered for a single dose is an alternative drug for radical cure [40,41]. However, tafenoquine also may cause problems in G6PD-deficient participants.

The exact prevalence of G6PD deficiency in the Peruvian Amazon is not yet fully characterized, but some studies carried out in Peruvian Amazon found low prevalence rate of G6PD-deficiency [41,42]

In 2001, in response to the spread of CQ and sulfadoxine/pyrimethamine-resistant strains, Peru was among the first countries in the Americas to introduce an artemisinin-based combination therapy (ACT) as the first-line treatment for uncomplicated *P. falciparum* malaria; this ACT comprised artesunate (AS) for three days (4 mg/kg/day) plus mefloquine (MQ) for two days (12.5 mg/kg/day). A single dose of PQ (0.75 mg/kg) as a gametocytocidal was added to ACT in 2015 to prevent *P. falciparum* transmission.

Regarding *P. falciparum* - *P. vivax* co-infections, these are first treated with AS plus MQ, and then an extended period of PQ for seven days was added (0.5 mg/kg/day). Both regimens, AS plus MQ regimen for *P. falciparum* and CQ plus PQ for *P. vivax*, are efficacy in the Peruvian Amazon [40,41,43].

2.6. The economic burden of malaria

Peru has logged several consecutive years of positive economic growth in the past two decades. Peru's gross national income (GNI) per capita reached 3,990 US dollars in 2008, and since 2009 its economy has been classified as an upper-middle-income country [44]. While poverty fell nationwide from 54% in 2001 to 30.1% in 2020, a large part of the population cannot benefit from such economic growth, with poverty rates exceeding 40% in rural areas, mainly in the Andean and the Amazon Regions [45].

Malaria episodes often occur in rural areas with poor healthcare systems and limited access to health care, which can influence treatment-seeking behaviour and the demand for care. Furthermore, repeated malaria episodes can increase vulnerability to other diseases, such as malnutrition and anemia [46]. In the same way, the poorer and marginalized communities might be more likely to suffer from malaria than non-less poor communities, because their geography and environment are more hospitable to mosquitoes than areas inhabited by non-poor communities [47]. Hence, malaria control should be seen as a poverty reduction strategy.

At global level, total funding for malaria control and elimination in 2019 was estimated at US\$3.0 billion but there is a gap between the amount invested and the resources needed to fight malaria, increasing from US\$ 1.3 billion in 2017 to US\$ 2.3 billion in 2018, and to US\$ 2.6 billion in 2019 [48].

In the Peruvian Amazon, activities for malaria control from 2005 to 2010 were primarily supported by the Global Fund (GF) PAMAFRO project, with a reduction in the malaria incidence from 87,805 in 2005 cases to 23,060 in 2011.

However, after the end of PAMAFRO with only domestic funding, malaria cases increased each year, reaching 63,189 cases in 2015. Recently have been implemented Malaria Zero Plan (MZP) with about 60% reduction in malaria from 63,189 in 2015 to 15,520 in 2020 [9]. Hence, the gains in malaria depend upon sufficient and sustained investment.

As resurgence is more expensive than control, the cost of failing to sustainably control and eliminate malaria will be catastrophic. If the coverage of malaria interventions drops, then dramatic resurgence will ensue, this can lead to even higher prevalence than at baseline because, as the number of cases drops, people's acquired immunity to malaria declines, leaving them more vulnerable to clinical disease and severe illness. Resurgence also increases the risk of drug resistance, and leads to human sickness, suffering and death across all age groups. Failure costs will create a burden for economies, businesses and health systems, and could extend to countries that share borders with resurging countries. In particular, the brunt of these costs will be borne by households, with the poorest families paying the highest price. Such failure would fundamentally undermine the Sustainable Development Goals (SDG) of seeking to end extreme poverty by 2030 [49].

Background

3. INTEGRATING PARASITOLOGICAL AND ENTOMOLOGICAL OBSERVATIONS TO UNDERSTAND MALARIA TRANSMISSION IN RIVERINE VILLAGES IN THE PERUVIAN AMAZON

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3.1. Abstract

Background: Remote rural riverine villages account for most of the reported malaria cases in the Peruvian Amazon. As transmission decreases due to intensive standard control efforts, malaria strategies in these villages will need to be more focused and adapted to local epidemiology.

Methods: By integrating parasitological, entomological, and environmental observations between January 2016 and June 2017, we provided an in-depth characterization of malaria transmission dynamics in 4 riverine villages of the Mazan district, Loreto department.

Results: Despite variation across villages, malaria prevalence by polymerase chain reaction in March 2016 was high (>25% in 3 villages), caused by *Plasmodium vivax* mainly and composed of mostly submicroscopic infections. Housing without complete walls was the main malaria risk factor, while households close to forest edges were more commonly identified as spatial clusters of malaria prevalence. Villages in the basin of the Mazan River had a higher density of adult *Anopheles darlingi* mosquitoes and retained higher prevalence and incidence rates compared to villages in the basin of the Napo River despite test-and-treat interventions.

Conclusions: High heterogeneity in malaria transmission was found across and within riverine villages, resulting from interactions between the microgeographic landscape driving diverse conditions for vector development, housing structure, and human behavior.

Keywords: malaria, transmission, heterogeneity, Amazon, incidence, prevalence, human biting rate, entomological inoculation rate, Peru.

3.2. Background

The history of malaria in Peru shows that important reductions in the malaria burden can be obtained with intensive and comprehensive standard control measures, but also that this progress can quickly be lost if there is not a long-term country plan to consolidate the achievements and prevent malaria resurgence [50]. For instance, the Global Fund-sponsored PAMAFRO project supported the strengthening of microscopic diagnosis, the implementation of active test-and-treat interventions, and the distribution of long-lasting insecticidal mosquito nets (LLINs), with a community-based intercultural approach, between 2005 and 2010 in the Peruvian Amazon. During this period, malaria declined drastically in the most affected department of Loreto from 54,291 reported cases (25% due to *Plasmodium falciparum*) in 2005 to 10,504 cases (20% due to *P. falciparum*) in 2010 [50,51]. Regrettably, the marked reduction of international donors' support and the unusually heavy rains that caused floods in riverine villages starting in 2012, led to a 5-fold increase in cases between 2010 and 2015 (60,302 cases) [50,52].

The political and financial commitment from the Peruvian government was crucial not only to respond to this malaria resurgence, but also to establish a long-term initiative called Plan Malaria Cero started in 2017, with the ambitious aim of eliminating malaria in the country by 2036 [53]. Intensive malaria control interventions based on the PAMAFRO experience in high-risk malaria villages contributed to a substantial reduction in the malaria incidence in Loreto, reporting 22,037 cases in 2019 [54]. As transmission decreases, malaria strategies need to be more focalized and adapted to the local malaria epidemiology determined by the

complex interactions among *Plasmodium* parasites, human behavior, and highly variable environments driving changes in mosquito vector behavior and habitat suitability [50,55,56].

Rural riverine villages, characterized by poverty and limited access to health facilities, concentrate an important proportion of reported malaria cases in Loreto [57]; however, transmission dynamics in these villages remain understudied [5,58]. By accounting for the ecological heterogeneity of 2 distinctive river basins, we identified 4 riverine villages (2 on each river) in the endemic district of Mazan in Loreto department and calculated the prevalence of malaria infections by quantitative real-time PCR (qPCR) in March and September 2016, and March 2017. These parasitological measurements were then related to incidence rates of reported malaria episodes between January 2016 and June 2017, and to available entomological and environmental observations in the same period [59,60], to better characterize malaria transmission in these villages.

3.3. Material and methods

Study Area

The district of Mazan is home to approximately 13 900 inhabitants, with one-third of them living in Mazan town near the confluence of the Napo River and its tributary the Mazan River about 50 km from Iquitos city (capital of Loreto). Mazan was among the districts in Loreto with the highest annual parasite index in 2017 (annual parasite incidence, 96.4 cases per 1000 inhabitants; *P. vivax*, 1066 cases; *P. falciparum*, 374 cases) [54,61]. Most cases (>80%) occurred in approximately 20 villages located in

the Mazan and Napo River basins. The exophilic, exophagic, and anthropophagic *Anopheles darlingi* is the main malaria vector [60]. The primary interventions prior to this study were routine passive case detection, seasonally indoor residual spraying, and the delivery of LLINs in the first months of 2016.

Design and Selected Villages

This study integrated available sociodemographic, parasitological, entomological and environmental data from Libertad (LIB) (3.496°S, 73.234°W) and Visto Bueno (VIB) (3.449°S, 73.317°W) in the Mazan basin, and Salvador (SAL) (3.445°S, 73.154°W) and Urco Miraño (URC) (3.361°S, 73.064°W) in the Napo basin (Figure 1 and Supplementary Table 1).

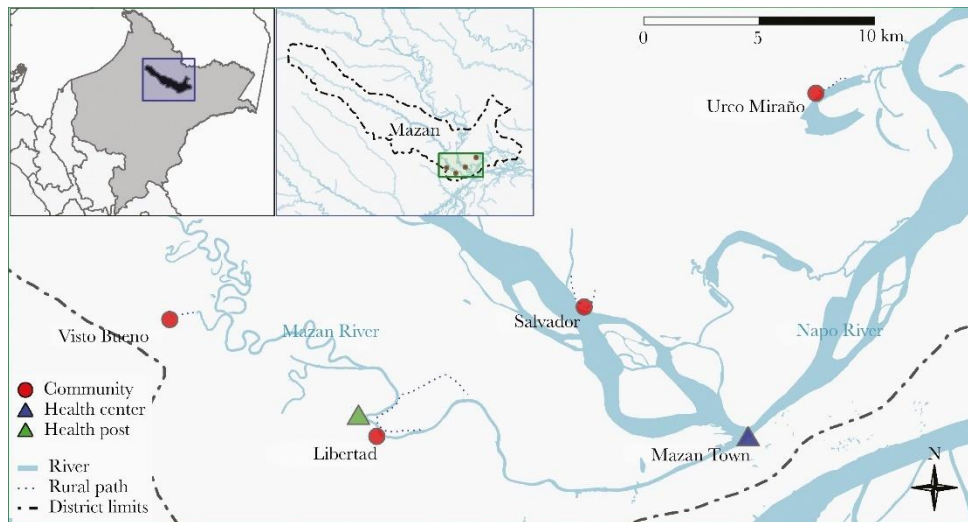


Figure 1. Study area, 4 riverine villages in Mazan district, Loreto department.

The Direction of Loreto (477–2016), Universidad Peruana Cayetano Heredia (UPCH, 64746), and WHO Ethics Review Committee (0002669) approved entomological collections, while UPCH (66235) approved sociodemographic and parasitological surveys.

Baseline Data

Households were censused, georeferenced, and updated during surveys in March and September 2016 and March 2017. Mobile devices programmed with Open Data Kit allowed for the collection of sociodemographic and epidemiological data, including questions about LLIN use, and the time to go to sleep and wake up.

Prevalence Data

Malaria prevalence by both microscopy and qPCR were estimated from datasets of a pilot study that assessed the effects and costs of population-wide malaria test-and-treat interventions (in addition to passive case detection) before the high malaria transmission season in the Peruvian Amazon [61,62]. Visits to all censused households in VIB and URC were made in March and September 2016 and March 2017. Available household members in March 2016 and March 2017 had axillary temperature taken and history of fever or any other malaria-compatible symptom documented. Finger-prick blood samples were collected for immediate microscopy in the field and dried blood spots for later analysis by qPCR. Health workers from the Ministry of Health accompanied personnel conducting field research activities and treated microscopically confirmed infections regardless of symptoms according to national guidelines [63]. The same procedures were carried out in September 2016,

but microscopic diagnosis was not performed by the pilot study; individuals with any malaria-compatible symptom were referred to health facilities for diagnosis and treatment.

Additional household visits in LIB and SAL were made in April 2016 and April 2017 (30 days after visits in March). A supplementary intervention in these villages consisted of making qPCR results available within 3 days of the sample collection in March and April of 2016 and 2017, allowing for the treatment of qPCR-positive individuals who were negative in an initial microscopic test. These qPCR tests were processed at the local research laboratory in Iquitos (Supplementary Material).

Incidence Data

Passive case-detection data of microscopically confirmed malaria episodes between January 2016 and June 2017 were obtained from the Ministry of Health surveillance systems [54] and verified with data registered at health facilities. Monthly incidence rates were calculated as the number of registered episodes ($\times 100$) divided by the number of individuals censused during the baseline survey. In cases of recurrent registered episodes, intervals ≥ 60 days for *P. vivax* and ≥ 30 days for *P. falciparum* were used to identify independent episodes [64].

Entomological and River Level Data

Published data of indoor and outdoor 12-hour mosquito collections by human landing catch were used to estimate human biting rates (HBRs), the hourly biting behavior of *An. darlingi*, and entomological inoculation rates (EIRs) in March, June, September, and November 2016, and March 2017 [60]. Available vector and human

behavior data were used to provide a rough estimate of the proportion of human exposure to *An. darlingi* occurring indoors, in scenarios in which people are or are not protected by LLIN, as described by Monroe et al [65]. Larval sampling data from water bodies located by ground inspection within 1 km of each village (September and November 2016, and March 2017) identified *An. darlingi* breeding sites [59]. Daily water levels of the Napo River measured by a hydrological station near the confluence of the Napo and Mazan rivers were averaged monthly [66].

Data Analysis

Baseline characteristics between villages were compared using the χ^2 test. Trend charts related monthly incidence rates, malaria prevalence, HBRs, EIRs, and river levels. Uni- and multivariate mixed-effects logistic regression models determined risk factors for malaria infection by qPCR in March 2016. The following potential risk factors were assessed as fixed effects: age, sex, occupation, malaria antecedent, LLIN ownership, LLIN use, and housing structure. Random effects accounted for individuals nested within households, and households nested within villages. Factors with P values < .2 for the Wald test in the univariate analysis were included in the full multivariate model. Manual backward elimination guided by the minimization of the Akaike information criterion reduced the number of factors in final models. Likelihood ratio tests compared nested models.

Households were mapped and their age-standardized malaria prevalence estimated considering the population's age structure. Global Moran index (I) assessed the spatial autocorrelation of malaria prevalence in villages. Local Moran I with correction by false discovery rate assessed the variation in spatial autocorrelation

over the village area, allowing for the classification of each household into 5 categories: high-high (a high prevalent household surrounded by high prevalent households), low-low (a low prevalent household surrounded by low prevalent households), outliers high-low and low-high, and not significant. A maximum neighborhood distance criterion of 550 meters was chosen for LIB, URC, and SAL [10], whereas it was set at 200 meters for VIB given the smaller distance between households. Malaria prevalence was weighted according to the inverse of the distance to the neighboring house. All analysis and maps were done using R version 2.15 software (R Development Core Team, R Foundation for Statistical Computing).

3.4. Results

A total of 205 households and 1015 residents were censused and distributed as follows: LIB (60 households, 297 individuals), VIB (15 households, 60 individuals), URC (53 households, 284 individuals), and SAL (77 households, 374 individuals) (Supplementary Table 1). The overall ratio of female to male was 0.9, with most individuals aged younger than 20 years (median, 16.2). Secondary or higher education level was higher in URC (28.7%) than in other villages (approximately 10%) ($P < .001$). Forest-related economic activities predominated (31.2%–41.7%) with no differences between villages. Compared to LIB and SAL, residents in URC and VIB more frequently reported malaria antecedent in the past year as well as appropriate LLIN household coverage ($P < .01$). LLIN use the previous night ranged between 75.3% and 81.4%, with no differences across villages. Housing structures without complete wooden walls were common (63.2%), especially in LIB (73.7%)

($P < .001$). In contrast to VB and LIB, in URC and SAL, tin frequently replaced palm leaves as ceiling material and more participants reported having electricity at home ($P < .001$) (Supplementary Table 2 and Supplementary Table 3). This latter finding was negatively associated with the proportion of individuals sleeping at 20:00 hours in URC (75%) and SAL (70%) in comparison with those in VB (88%) and LIB (96%) ($P < .001$).

Samples for qPCR analysis were available for 823 individuals in March 2016, 843 in September 2016, and 800 in March 2017. Malaria prevalence was the highest in March 2016 for all villages (LIB, 40.9% [95% confidence interval, CI, 34.5%–47.5%]; VIB, 27.5% [95% CI, 16.3%–42.0%]; SAL, 9.2% [95% CI, 6.4%–13.0%]; and URC, 30.7% [95% CI, 24.7%–37.4%]), with *P. vivax* to *P. falciparum* ratios (LIB, 5:1; VIB, 1.3:1; SAL, 28:1; and URC, 21:1) and submicroscopic to microscopic infection ratios (LIB, 93:1; VIB, 13:1; SAL, 29:1; and URC, 16:1) varying widely across villages (Table 1, Figure 2A, Figure 3A, Figure 4A, and Figure 5A). Submicroscopic mixed infections were found in LIB (6 infections) and SAL (1 infection). The prevalence in September 2016 decreased for all villages (LIB, 11.8% [95% CI, 8.1%–16.7%]; VIB, 23.6% [95% CI, 13.7%–37.3%]; SAL, 3.8% [95% CI, 2.0%–6.7%]; and URC, 13.4% [95% CI, 9.4%–18.7%]), but the *P. vivax* to *P. falciparum* ratio continued to fluctuate across villages (LIB, 6:1; VIB, 3:1; SAL, 2:1; and URC, 28:1).

The lowest malaria prevalence, composed of mainly submicroscopic infections, were found in March 2017 (LIB, 8.1% [95% CI, 4.9%–12.8%]; VIB, 6.1% [95% CI, 1.6%–17.9%]; SAL, 4.4% [95% CI, 2.5%–7.4%]; and URC, 1.8% [95% CI, 0.6%–4.9%]) with *P. vivax* to *P. falciparum* infection ratios ranging between 1:2 and 2:1.

Table 1. Prevalence of Malaria by qPCR in March 2016, September 2016, and March 2017

Infection Detected	March 2016		September 2016 ^a		March 2017	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Libertad						
Total samples (n)	230		238		211	
Overall	94 (40.9)	34.5–47.5	28 (11.8)	8.1–16.7	17 (8.1)	4.9–12.8
<i>P. vivax</i>	73 (31.7)	25.9–38.2	24 (10.1)	6.7–14.8	11 (5.2)	2.8–9.4
Submicroscopic	72 (31.3)	25.5–37.8	...	...	7 (3.3)	1.5–7.0
Microscopic	1 (0.4)	.0–2.8	...	...	4 (1.9)	.6–5.1
<i>P. falciparum</i>	15 (6.5)	3.8–10.7	4 (1.7)	.5–4.5	6 (2.8)	1.2–6.4
Submicroscopic	15 (6.5)	3.8–10.7	...	...	6 (2.8)	1.2–6.4
Microscopic	0 (0.0)		...	...	0 (0.0)	
Mixed	6 (2.6)	1.1–5.9	0 (0.0)		0 (0.0)	
Visto Bueno						
Total samples (n)	51		55		49	
Overall	14 (27.5)	16.3–42.0	13 (23.6)	13.7–37.3	3 (6.1)	1.6–17.9
<i>P. vivax</i>	8 (15.7)	7.5–29.1	9 (16.4)	8.2–29.3	2 (4.1)	.7–15.1
Submicroscopic	8 (15.7)	7.5–29.1	...	...	2 (4.1)	.7–15.1
Microscopic	0 (0.0)				0 (0.0)	
<i>P. falciparum</i>	6 (11.8)	4.9–24.6	3 (5.5)	1.4–16.1	1 (2.0)	.1–12.2
Submicroscopic	5 (9.8)	3.7–22.2	...	...	1 (2.0)	.1–12.2
Microscopic	1 (2.0)	.1–11.8	...	...	0 (0.0)	
Mixed	0 (0.0)		1 (1.8)	.1–11.0	0 (0.0)	

Table 1. Cont.

Infection Detected	March 2016		September 2016 ^a		March 2017	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Salvador						
Total samples (n)	327		319		318	
Overall	30 (9.2)	6.4–13.0	12 (3.8)	2.0–6.7	14 (4.4)	2.5–7.4
<i>P. vivax</i>	28 (8.6)	5.9–12.3	7 (2.2)	1.0–4.7	9 (2.8)	1.4–5.5
Submicroscopic	27 (8.3)	5.6–11.9	...	...	7 (2.2)	1.0–4.7
Microscopic	1 (0.3)	.0–2.0	...	...	2 (0.6)	.1–2.5
<i>P. falciparum</i>	1 (0.3)	.0–2.0	4 (1.3)	.4–3.4	5 (1.6)	.6–3.8
Submicroscopic	1 (0.3)	.0–2.0	...	...	5 (1.6)	.6–3.8
Microscopic	0 (0.0)		...	...	0 (0.0)	
Mixed	1 (0.3)	.0–2.0	1 (0.3)	.0–2.0	0 (0.0)	
Urco Miraflores						
Total samples (n)	215		231		222	
Overall	66 (30.7)	24.7–37.4	31 (13.4)	9.4–18.7	4 (1.8)	.6–4.9
<i>P. vivax</i>	63 (29.3)	23.4–35.9	28 (12.1)	8.3–17.2	1 (0.5)	.0–2.9
Submicroscopic	59 (27.4)	21.7–34.0	...	...	1 (0.5)	.0–2.9
Microscopic	4 (1.9)	.6–5.0	...	...	0 (0.0)	
<i>P. falciparum</i>	3 (1.4)	.4–4.4	1 (0.4)	.0–2.8	2 (0.9)	.2–3.6
Submicroscopic	3 (1.4)	.4–4.4	...	...	2 (0.9)	.2–3.6
Microscopic	0 (0.0)		...	...	0 (0.0)	
Mixed	0 (0.0)		2 (0.9)	.2–3.4	1 (0.5)	.0–2.9

Abbreviations: CI, confidence interval; *P. falciparum*, *Plasmodium falciparum*; *P. vivax*, *Plasmodium vivax*; qPCR, quantitative polymerase chain reaction.

^aMicroscopic diagnosis not performed.

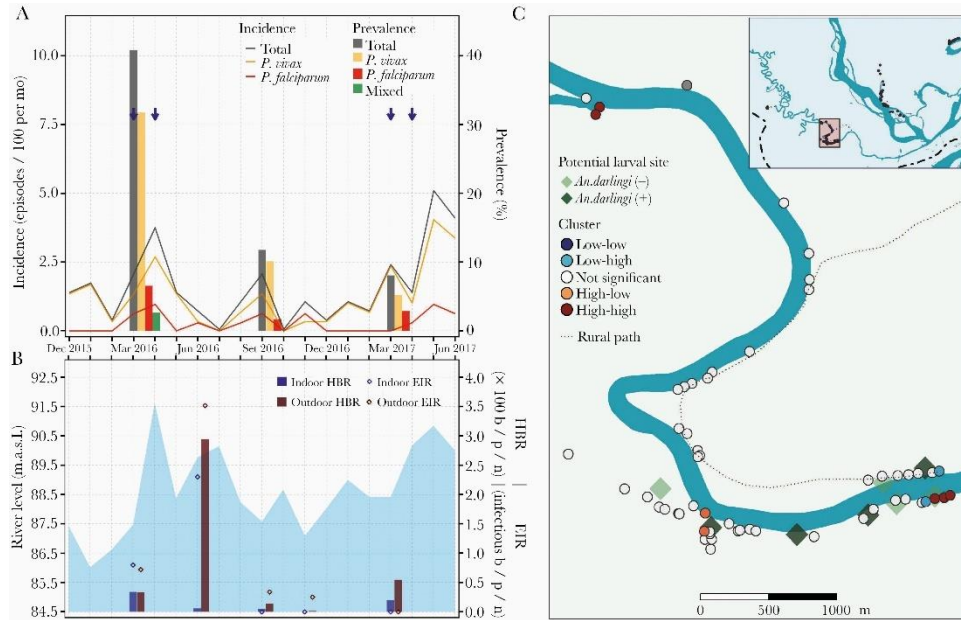


Figure 2. Parasitological, entomological, and river-level observations between January 2016 and June 2017, and spatial clusters of malaria prevalence in March 2016 in Libertad village.

A, Evolution of malaria prevalence and incidence rates: Arrows indicate months when test-and-treat interventions were conducted with polymerase chain reaction (PCR) result available for malaria treatment within 3 days of the sample collection. B, Bars represent human biting rates (HBR) and points entomological inoculation rates (EIR) estimated in March, June, September and November 2016 and in March 2017. Shaded area represents monthly water levels of the Napo River. C, Spatial distribution of households classified according to local Moran I as high-high, high-low, not significant, low-high, and low-low clusters of malaria prevalence in March 2016. Abbreviations: b/p/n, bites/person/night; MASL, meters above sea level.

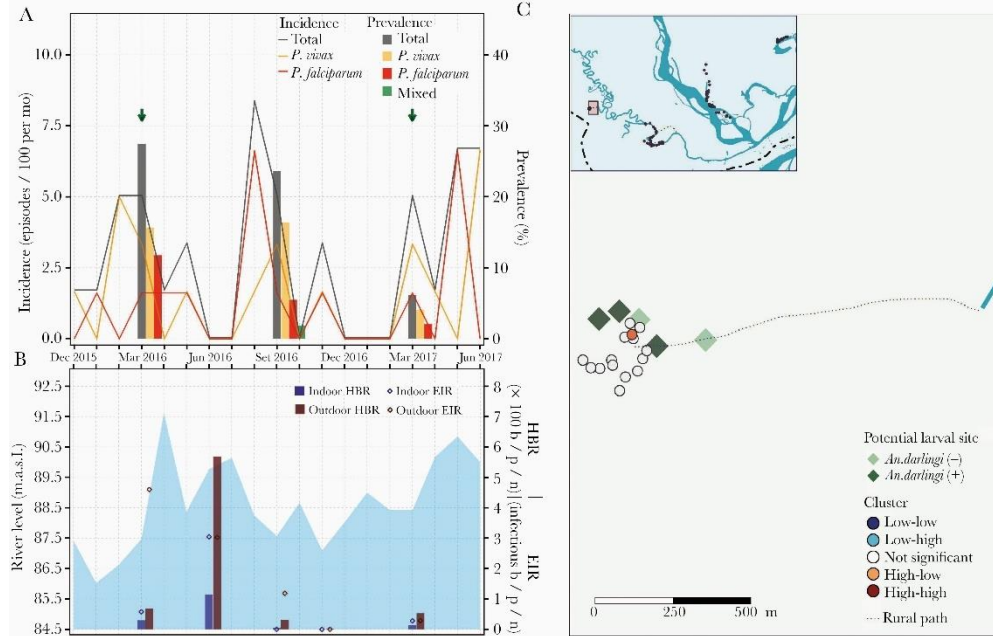


Figure 3. Parasitological, entomological, and river level observations between January 2016 and June 2017, and spatial clusters of malaria prevalence in March 2016 in Visto Bueno village.

A, Evolution of malaria prevalence and incidence rates: Arrows indicate months when test-and-treat interventions were conducted using microscopy. B, Bars represent human biting rates (HBR) and points entomological inoculation rates (EIR) estimated in March, June, September, and November 2016 and in March 2017. Shaded area represents monthly water levels of the Napo River. C, Spatial distribution of households classified according to local Moran I as high-high, high-low, not significant, low-high, and low-low clusters of malaria prevalence in March 2016. Abbreviations: b/p/n, bites/person/night; MASL, meters above sea level.

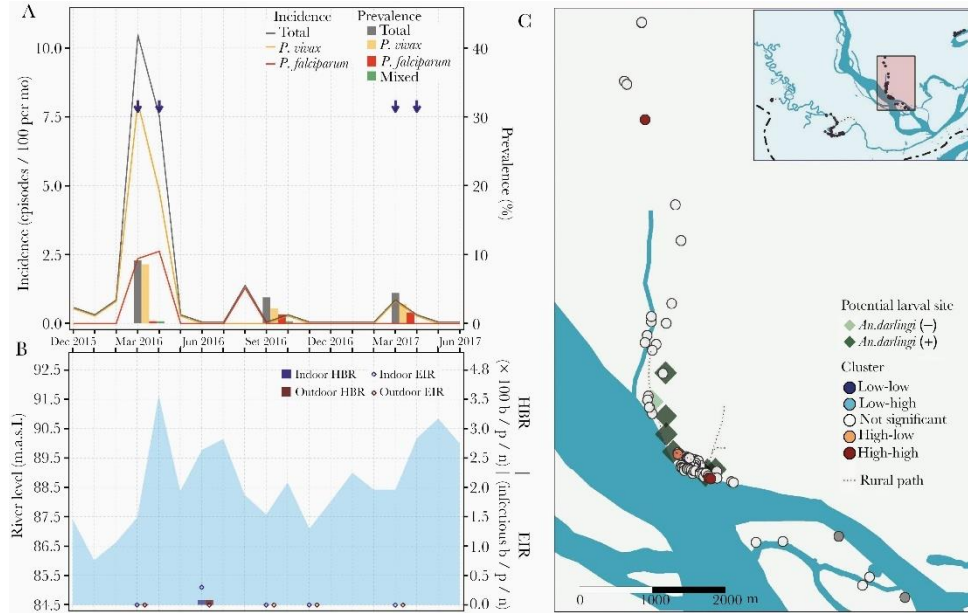


Figure 4. Parasitological, entomological, and river level observations between January 2016 and June 2017, and spatial clusters of malaria prevalence in March 2016 in Salvador village.

A, Evolution of malaria prevalence and incidence rates: Arrows indicate months when test-and-treat interventions were conducted with polymerase chain reaction (PCR) result available for malaria treatment within 3 days of the sample collection. B, Bars represent human biting rates (HBR) and points entomological inoculation rates (EIR) estimated in March, June, September, and November 2016 and in March 2017. Shaded area represents monthly water levels of the Napo River. C, Spatial distribution of households classified according to local Moran I as high-high, high-low, not significant, low-high, and low-low clusters of malaria prevalence in March 2016. Abbreviations: b/p/n, bites/person/night; MASL, meters above sea level.

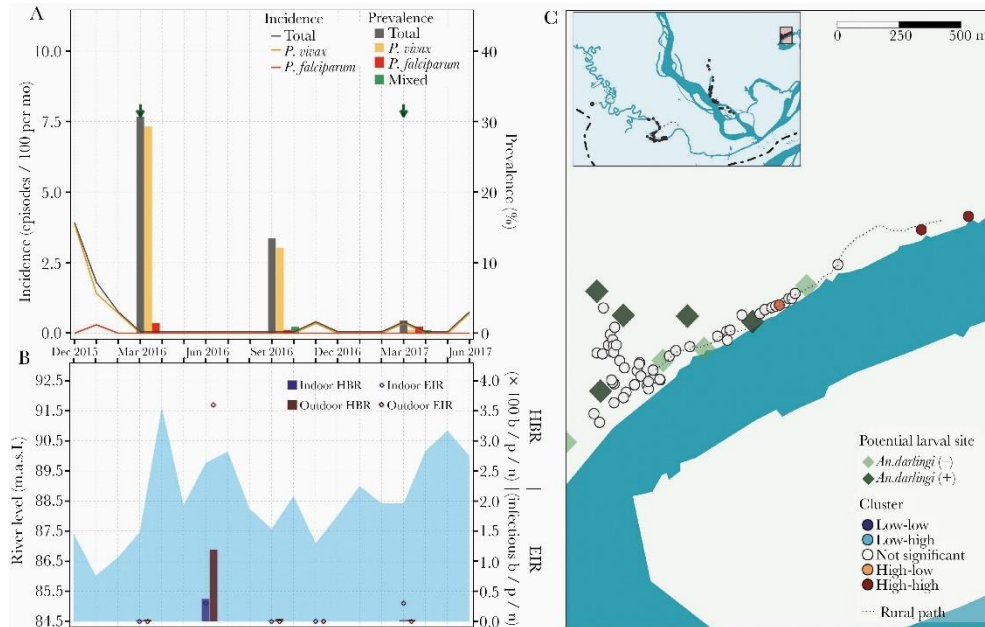


Figure 5. Parasitological, entomological, and river level observations between January 2016 and June 2017, and spatial clusters of malaria prevalence in March 2016 in Urco Miraño village.

A, Evolution of malaria prevalence and incidence rates: Arrows indicate months when test-and-treat interventions were conducted using microscopy. B, Bars represent human biting rates (HBR) and points entomological inoculation rates (EIR) estimated in March, June, September, and November 2016 and in March 2017. Shaded area represents monthly water levels of the Napo River. C, Spatial distribution of households classified according to local Moran I as high-high, high-low, not significant, low-high, and low-low clusters of malaria prevalence in March 2016. Abbreviations: b/p/n, bites/person/night; MASL, meters above sea level.

In all villages, HBRs reached the highest levels 2 months after the peak of the Napo River (June 2016) (Figure 2B, Figure 3B, Figure 4B, and Figure 5B), and were followed by an increase in incidence in August–September 2016 (second peak) of predominantly *P. vivax* episodes in LIB and *P. falciparum* episodes in VIB and SAL.

Only in VIB, the highest EIR was found in March 2016, not coinciding with the highest HBR. In comparison with villages in the Napo basin (SAL and URC), villages in the Mazan basin (LIB and VIB) had higher HBR and EIR levels, and maintained higher prevalence and incidence rates despite test-and-treat interventions. In contrast to the highest HBR levels outside houses in most surveys, differences between outdoor and indoor EIR values were less obvious. About 90% of human exposure to *An. darlingi* bites between 18:00 and 6:00 hours occurred indoors where LLINs were not available and/or used, according to the matched analysis of mosquito and human behavior data. The current LLIN usage in villages would prevent approximately 60% of human exposure (Supplementary Table 4).

The multivariate model for malaria infection by qPCR in March 2016 confirmed the influence of clustered data at household and village level, and the increased risk for individuals living in houses with incomplete walls (adjusted OR [aOR], 1.7; 95% CI, 1.1–2.6) (Table 2). Malaria prevalence was also slightly higher in individuals between 10 and 29 years old (aOR, 1.5; 95% CI, 1.0–2.3; $P = .073$) and men (aOR, 1.4; 95%CI, 1.0–2.0; $P = .067$) compared respectively with younger children and women, but not significantly.

Table 2. Uni- and Multivariate Risk Factor Analysis for Malaria Infection in March 2016

Risk Factor	n/N %	OR	OR 95%CI	aOR	aOR 95% CI
Age group, y					
<10	60/ 290 (20.7)	Ref			
10–29.9	70/ 243 (28.8)	1.5**	.9–2.2	1.5**	1.0–2.3
≥30	74/ 290 (25.5)	1.2	.8–1.8	1.2	0.8–1.8
Sex					
Female	89/ 399 (22.3)	Ref			
Male	115/ 424 (27.1)	1.3	.9–1.9	1.4**	1.0–2.0
Occupation					
Not forest related	128/ 515 (24.9)	Ref			
Forest related	76/ 308 (24.7)	1.0	.7–1.4		
Malaria in the past year					
No	90/ 395 (22.8)	Ref			
Yes	111/ 413 (26.9)	1.3	.9–1.9		
Sleep under a LLIN the previous night					
Yes	154/ 640 (24.1)	Ref			
No	48/ 176 (27.3)	1.2	.8–1.9		
1 LLIN for 2 people					
Yes	89/ 390 (22.8)	Ref			
No	115/ 433 (26.6)	1.3	.9–1.9		
Indoor residual spray in past year					
Yes	154/ 484 (31.8)	Ref			
No	49/ 336 (14.6)	0.9	.5–1.5		
Number of external walls in house					
Complete, 4	52/ 288 (18.1)	Ref			
None/incomplete, 0–3	152/ 535 (28.4)	1.6*	1.1–2.5	1.7*	1.1–2.6
Ceiling material					
Tin, calamina	63/ 283 (22.3)	Ref			
Palm leaves	141/ 540 (26.1)	1.0	.6–1.6		
Electricity available					
Yes	74/ 345 (21.4)	Ref			
No	130/ 478 (27.2)	0.8	.5–1.3		
Distance to <i>Anopheles darlingi</i> larval site					
≥ 200 meters	140/ 485 (28.9)	Ref			
< 200 meters	63/ 314 (20.1)	0.8	.5–1.2		

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; n, number of malaria infected people; N, total number of people; OR, odds ratio; Ref, reference.

* $P < .05$, ** $P < .01$.

Global Moran I confirmed spatial autocorrelation of age-standardized household prevalence in March 2016 in 2 villages: LIB ($I = 0.13$, $P = .04$) and URC ($I = 0.09$, $P = .04$). Local Moran I identified high-high households (high-risk clusters) in LIB, URC, and SAL, but not in VIB (Figure 2C, Figure 3C, Figure 4C, and Figure 5C). High-risk clusters in LIB and URC were, respectively, 5 and 2 of the outermost households in the villages, while in SAL this included 2 households in the most populated area and 1 outermost household near a narrow river gorge that joins the Napo River. In addition, high-low outliers (high prevalent households surrounded by low prevalent households) were identified in all villages, located mainly in the most populated areas. High-risk clusters were not closer to *An. darlingi* larval sites when compared with other households ($P > .05$).

3.5. Discussion

The combined analysis of parasitological, entomological, and environmental observations between January 2016 and June 2017 allowed for an in-depth characterization of malaria transmission dynamics in riverine villages in Mazan, an Amazonian district where poverty, limited accessibility to health care, and village-specific ecology create challenges for malaria control. Despite some degree of heterogeneity across villages, malaria prevalence in the survey in March 2016 was high ($> 25\%$ in 3 villages), comprising mostly *P. vivax* submicroscopic infections. Whereas the housing without complete walls was the main risk factor for malaria, the proximity to *An. darlingi* breeding sites was not associated with malaria. Interestingly, villages in the Mazan basin had the highest entomological indicators

and maintained the highest prevalence and incidence rates among study villages despite test-and-treat interventions.

The earlier high malaria incidence rates in LIB, VIB, and SAL in March–April 2016 in comparison to historically seasonal patterns of malaria incidence in the Mazan district (with peaks usually in June–July) are mainly explained by increased test-and-treat efforts in those months as part of a concurrent intervention pilot study. These efforts may have affected the temporal dynamics of malaria transmission, bringing a second peak of malaria incidence in these villages 2–3 months after their highest HBR levels in June 2016. The submicroscopic parasite carriers may have contributed to a silent infectious reservoir able to maintain mild-moderate transmission, despite population-wide malaria testing and treatment of confirmed infections in March–April of 2016 and 2017, and the drop in vector-human contact rates in the dry season of 2016. Indeed, several cross-sectional surveys in the Peruvian Amazon have consistently observed that a majority of *P. vivax* and *P. falciparum* infections could only be detected by sensitive molecular tests, suggesting the important role of these submicroscopic infections in sustaining malaria transmission [67–69]. For instance, a recent cohort study that screened full populations for malaria in riverine endemic villages showed that most low-density infections escaped standard surveillance using microscopy, even if residents were consecutively screened at intervals of 10 days over a month [62].

The persistently higher prevalence and incidence rates in the Mazan basin compared to those in the Napo basin (regardless of the type of test-and-treat

intervention implemented) were in agreement with higher HBRs and EIRs in Mazan villages compared with the Napo ones. Contrary to what was expected, *An. darlingi* breeding sites in the Mazan basin were not the most numerous and densely inhabited by larvae among the study villages. Indeed, our group recently showed that SAL (Napo basin) was the village with the greatest number of *An. darlingi* larvae and anopheline larvae in general during the same study period, despite having the lowest density of adult mosquitoes [59,60]. Notably, the presence and density of mosquito larvae in aquatic habitats and consequently the number of adults capable of malaria transmission are regulated by a variety of ecosystem processes operating and interacting at several organizational levels and spatial and temporal scales [70]. Understanding the patterns in the productivity of larval habitats requires knowledge about the local interactions between abiotic (eg, hydrology, temperature, shade, pH, salinity, and nutrients) and biotic (eg, predation and competition) factors [71–73]. A recent study of larval habitats in Mazan and Napo river watersheds found a correlation between *An. darlingi* larval sites and low forest coverage, low sunlight, and emergent vegetation [59]. Biotic factors, however, such as larval competition remain understudied [74]; it would be interesting to know, for instance, whether the proportion of *An. darlingi* larvae among total anopheline larvae (higher in water bodies in LIB and VIB than that in SAL) is a better determinant of the abundance of adult mosquitoes than larval densities (higher in SAL than in LIB and VIB).

The microgeographic landscape composition may also be an important determinant of the productivity of larval breeding sites, the abundance of adult mosquitoes, and ultimately malaria transmission in villages [74]. A recent study by our group, with

high-resolution imagery captured by drones, showed remarkably heterogeneous land cover composition in study villages, suggesting a high diversity of locations at a microgeographical scale where *An. darlingi* can breed [75]. Published orthomosaics from this imagery [75] together with additional field visits confirmed that all outermost households classified as spatial clusters of high malaria prevalence (high-high) in LIB, SAL, and URC were located near forest edges. Habitats in marsh and forest edges (especially in early stages of settlement) are able to promote malaria transmission by both increasing entomological risk and increasing human–vector contact as a result of human behavioral patterns associated with economical activities [76]. In an urban area in Senegal, for instance, *An. arabiensis* densities and EIR estimates showed a negative correlation with the distance to the marsh edges [77], while in rural villages in south-eastern Tanzania, high densities of malaria vectors were observed to be clustered in peripheral houses [78]. Numerous studies in the Brazilian Amazon also found a relationship between malaria and proximity to forest, finding high densities of larval and adult *An. darlingi* in forest fringes, as well as increased malaria infections in populations living or working near forest edges [79,80].

With the main malaria vector *An. darlingi* biting predominantly outdoors, it was not surprising to find a trend of the association of men and individuals aged 10–29 years with increased malaria infection. Similar relationships have been detected in many cross-sectional surveys in the Amazon, explained by the increased outdoor-, social, and forest-related activities of men, adolescents, and young adults, usually when mosquitoes are more active [67,68,81]. However, housing without complete walls as

the main risk factor for malaria (observed in other studies in the Amazon) [82,83] and indoor EIRs as high as outdoor EIRs in some villages (at different times) suggests that intradomiciliary transmission is common in some settings. Indeed, accounting for human behavior in villages [65], we estimated that approximately 90% of human exposure to *An. darlingi* at dusk and dawn would occur indoors where LLINs were not available and/or used. Several studies in Africa reported that main vectors are not predominantly endophagic, but they overwhelmingly preferred feeding at times when most humans are indoors [84–87]. This feeding pattern driven by the mosquito preference for humans in the indoor environment has also been observed in coendemic settings for *P. vivax* and *P. falciparum* in Solomon Islands [88] and Colombia [89], which are dominated by vectors that feed predominantly outdoors.

The successful application of different discipline methodologies to understand malaria transmission dynamics in endemic and remote riverine villages in Mazan together with the comprehensive integration of parasitological, vector, and environmental data prospectively collected during 18 months can be underscored as the main strengths of our study. Nevertheless, difficult access to villages together with budget constraints limited the number of collections of *An. darlingi* mosquitoes during the study period, preventing the statistical assessment of the relationship between parasitological and entomological indicators. Similarly, water level data of the Mazan River were not available to relate to those indicators in LIB and VIB; however, the fact that the highest HBR in these villages occurred 2 months after the peak level of Napo River as in SAL and URC, suggests that water levels of the Mazan River follow the same trends as those of the Napo. Moreover, there are

logistical limitations to ground exploration for the identification of all potential breeding sites, and the assessment of proximity to them as a malaria risk factor. Finally, we only performed the risk factor analysis and the spatial analysis with prevalence data of March 2016, because the prevalence of other surveys was likely affected by the deployment of test-and-treat interventions.

Our study confirmed higher prevalence and incidence rates associated with higher density of adult *An. darlingi* in the Mazan basin compared with the Napo basin. High microheterogeneity in malaria transmission was observed within the same villages, as a result of interactions between the microgeographic landscape driving diverse conditions for malaria vector development, the housing structure, and human behavior. Considering the evidence for indoor malaria transmission in the study villages, we recommend the delivery and opportune replacement of LLINs to increase coverage and prevention levels to *An. darlingi* exposure, and education interventions to insure their appropriate use and conservation. Local initiatives to improve housing conditions should be encouraged because they are not only able to reduce malaria, but also they improve health and well-being in general. New vector strategies such as spatial repellents [90] and attractive toxic sugar baits [91], capable of altering mosquito feeding behavior and reducing mosquito densities, both indoor and outdoor, can complement the use of LLINs to further reduce malaria transmission. Similarly, community environment-based management, aimed at removing and/or reducing temporary water collections near houses, and larval source management of permanent and large breeding sites, have been found to be effective vector measures in some endemic settings [92,93]. The feasibility,

operability, and effectiveness of new vector control strategies need to be assessed prior to implementation in the heterogeneous Amazon basin landscape.

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Author contributions. A. R. A., M. M., N. S., and D. G. conceived and designed the study. D. M. G., M. M., M. S., M. G., J. B., F. A., K. A., G. C. E., and K. P. supervised the fieldwork. M. S., J. C. M., and K. P. supervised the laboratory assays. A. R. A., K. A., and G. C. E. contributed to the data management and the consolidation of the fieldwork and laboratory data. A. R. A., D. M. G., and N. S. conducted the analysis. A. R. A. prepared the figures and tables and wrote the first draft of the paper. M. M., D. M. G., N. S., J. E. C., J. V., A. L. C., and D. G. contributed to the result interpretation and writing of the paper. All authors read and approved the final manuscript.

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4. ECONOMIC COSTS ANALYSIS OF UNCOMPLICATED MALARIA CASE MANAGEMENT IN THE PERUVIAN AMAZON

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4.1. Abstract

Background. Case management is one of the principal strategies for malaria control. This study aimed to estimate the economic costs of uncomplicated malaria case management and explore the influence of health-seeking behaviours on those costs.

Methods. A knowledge, attitudes and practices (KAP) survey was applied to 680 households of fifteen communities in Mazan-Loreto in March 2017, then a socio-economic survey was conducted in September 2017 among 161 individuals with confirmed uncomplicated malaria in the past 3 months. Total costs per episode were estimated from both provider (Ministry of Health, MoH) and patient perspectives. Direct costs were estimated using a standard costing estimation procedure, while the indirect costs considered the loss of incomes among patients, substitute labourers and companions due to illness in terms of the monthly minimum wage. Sensitivity analysis evaluated the uncertainty of the average cost per episode.

Results. The KAP survey showed that most individuals (79.3%) that had malaria went to a health facility for a diagnosis and treatment, 2.7% received those services from community health workers, and 8% went to a drugstore or were self-treated at home. The average total cost per episode in the Mazan district was US\$ 161. The cost from the provider's perspective was US\$ 30.85 per episode while from the patient's perspective the estimated cost was US\$ 131 per episode. The average costs per *Plasmodium falciparum* episode (US\$ 180) were higher than those per *Plasmodium vivax* episode (US\$ 156) due to longer time lost from work by patients with *P. falciparum* infections (22.2 days) than by patients with *P. vivax* infections (17.0 days). The delayed malaria diagnosis (after 48 h of the onset of symptoms) was associated with the time lost from work due to illness (adjusted mean ratio 1.8; 95% CI 1.3, 2.6). The average cost per malaria episode was most sensitive to the uncertainty around the lost productivity cost due to malaria.

Conclusions. Despite the provision of free malaria case management by MoH, there is delay in seeking care and the costs of uncomplicated malaria are mainly borne by the families. These costs are not well perceived by the society and the substantial financial impact of the disease can be frequently undervalued in public policy planning.

Keywords: Economic, Cost, Malaria, Management, Health care-seeking behaviour, Peru

4.2. Background

The good economic performance together with targeted social spending and enhanced anti-poverty programs led Peru to have significant success in improving health outcomes during the Countdown to the 2015 Millennium Development Goals (MDGs) [94]. Currently, the 2030 Sustainable Development Goals (SDGs) “*to ensure healthy lives and promote wellbeing for all at all ages*” [95] challenge the health sector, not least because Peruvian health expenditure are among the lowest in the Americas (about 5% of Gross Domestic Product-GDP) [96], substantial health inequities persist [97], and communicable diseases such as malaria resurge [57,98].

Malaria remains an important public health problem in the country despite several decades of intense control efforts [50]. Peru had the third highest increase in malaria incidence (after Venezuela and Nicaragua) since 2010 in the Americas, [99], having reported about 72% more malaria cases in 2017 (54,309 cases) compared with 2010 (31,545 cases) [26]. The Amazon Region, mainly the department of Loreto, is commonly affected by malaria due to both *Plasmodium vivax* and *Plasmodium falciparum* (*P. vivax*/*P. falciparum* ratio: 4/1), accounting for more than 95% of Peru’s malaria cases in 2017 (52,280 cases) [26]. The complex association between poverty and malaria is well known, which likely operates in both directions in the poorest districts of Loreto: poor households are more exposed to infectious mosquitoes due to occupational hazards (i.e. subsistence farming and hunting) and less able to afford prevention, and the higher burden of malaria may push these same households deeper into poverty (due to high productivity losses following

multiple episodes throughout a year) [81,100]. Indeed, a former economic evaluation conducted in 1998 from the perspective of society found that malaria costs were mainly borne by the families [100].

In the last two decades, the Peruvian Ministry of Health (MoH) has implemented strategies to improve the population's access to healthcare, especially for people not covered by the contributory social health insurance system (EsSalud), the Armed Forces (FFAA), National Police (PNP), or the private sector [101]. Thus, the Integrated Health Insurance scheme (IHIS), launched in 2009 by MoH, allowed for the expansion of former benefit packages beyond maternal and child health, and the increase of healthcare coverage to the poor and vulnerable populations of all ages [102–104]. Currently, the IHIS and EsSALUD cover about 65% and 17% of the total population in Loreto, respectively [105]; however, in poor Amazonian districts that consist mostly of rural communities, the IHIS is often the only possibility to provide health services to the population. The IHIS has reinforced the main pillar interventions for malaria control in Loreto, such as malaria surveillance and case identification through passive case detection (PCD, i.e. malaria detection at health facility or by community health workers (CHW) using standard diagnosis among symptomatic individuals seeking healthcare) or active case detection (ACD, i.e. malaria detection in the community through household visits) [62,106], and the effective, free, and timely management of confirmed malaria infections. In the past, those interventions were mainly supported by funding specifically assigned to the National Malaria Control Program (NMCP) at MoH [100].

From an economic perspective, it was described that the healthcare-seeking behaviours among individuals with malaria-like symptoms can significantly affect the direct and indirect costs associated with malaria detection and management [107]. In areas like Loreto, early and appropriate healthcare-seeking behaviours can save direct costs by halting transmission from confirmed malaria infections, and by avoiding additional costs associated with complications of such infections [100,108]. Conversely, delayed behaviours, self-medication, and seeking healthcare from traditional healers can increase the latter direct medical costs, impacting household budgets due to the lost wages and transportation costs during the care-seeking process and the illness period [108]. This study aimed to estimate the economic costs associated with the PCD and management of uncomplicated malaria episodes in riverine communities in the Peruvian Amazon from both the provider's and patient's perspectives, and explore whether healthcare-seeking behaviours significantly influence those costs.

4.3. Material and methods

Study design and study area

An epidemiological and knowledge, attitude and practice (KAP) survey, followed by a socio-economic survey were conducted in Mazan district, in the northeastern Peruvian Amazon Department of Loreto. Mazan, with a population of about 13,900 inhabitants living in 70 riverine communities, is considered one of the districts with the highest risk for malaria transmission in Peru [26]. In 2017, its annual parasite index (API) reached 96.4 cases per 1000 inhabitants. Malaria is predominantly

caused by *P. vivax* (74%), and less by *P. falciparum* (26%), while its main vector is *Anopheles (Nyssorhynchus) darlingi* in Peru [58]. The capital and largest village in Mazan, i.e. Mazan town (MT), is located at the confluence of the Mazan and Napo Rivers (3.503° S, 73.094° W), at about 55–60 km (1 hour by speedboat) from Iquitos city (capital of Loreto).

Malaria diagnosis and treatment in healthcare-seeking individuals with malaria-like symptoms are provided free of charge by the MoH in all Peruvian malaria endemic areas. In Mazan, a health centre in MT and health posts located in six riverine communities are responsible for these activities [109]. With a laboratory service available 6 days a week and two microscopists with accredited competency in species identification according to World Health Organization (WHO) standards [110], the health centre in MT is the only health facility in Mazan that provides microscopic diagnosis of malaria and immediate anti-malarial treatment to microscopically-confirmed infected individuals. Unless rapid diagnostic tests (RDTs) are available, health posts need to collect blood smear samples from symptomatic individuals, send them to the health centre, and wait for the parasitological confirmation to initiate anti-malarial treatment. In communities with lack of health facilities, these activities are performed by CHWs, who are regularly trained and supervised by the health facilities. The available RDT during the study period was the SD Bioline Malaria Ag Pf/Pf/Pv (Standard Diagnostics, South Korea), which detects three malaria antigens in human whole blood: the histidine-rich protein II (HRP-II) antigen of *P. falciparum*, the *Plasmodium* lactate dehydrogenase (pLDH) of *P. falciparum* and the pLDH of *P. vivax* [111].

According to national guidelines, *P. vivax* malaria episodes without criteria of severity and hospital admission (i.e. uncomplicated malaria) are treated immediately with chloroquine (CQ) for 3 days (10 mg/kg on days 1 and 2, and 5 mg/kg on day 3) plus primaquine (PQ) (0.5 mg/kg/day) for 7 days; while the first-line treatment for uncomplicated *P. falciparum* malaria consists of artesunate (AS) for 3 days (4 mg/kg/day), mefloquine (MQ) for 2 days (12.5 mg/kg/day) and PQ (0.75 mg/kg) in single dose. Uncomplicated *P. falciparum*–*P. vivax* coinfections receive AS and MQ at the same doses than those for uncomplicated *P. falciparum* infections, but together with an extended period of PQ (0.5 mg/kg/day) for 7 days [63]. In vivo clinical studies have found that both CQ and the combination MQ-AS remain efficacious for the treatment of respectively uncomplicated *P. vivax* and *P. falciparum* infections in the Peruvian Amazon [112,113].

Study population

The study population were all households from fifteen communities in Mazan district (Fig. 1). These communities were chosen among the total 70 communities in Mazan, because they accounted for 80% of reported cases in the district in the period 2016–2017. The Table 1 provides information of study communities about the availability of health facilities, distance in minutes to the health centre, and population. Inhabitants live mainly in open or semi-open wooden houses built on stilts. Subsistence farming is the main economic activity, followed by seasonal logging. Mestizos comprised the majority of the study population [114]; Yagua, Maijuna and Kichwa ethnic groups were present in three communities [115].

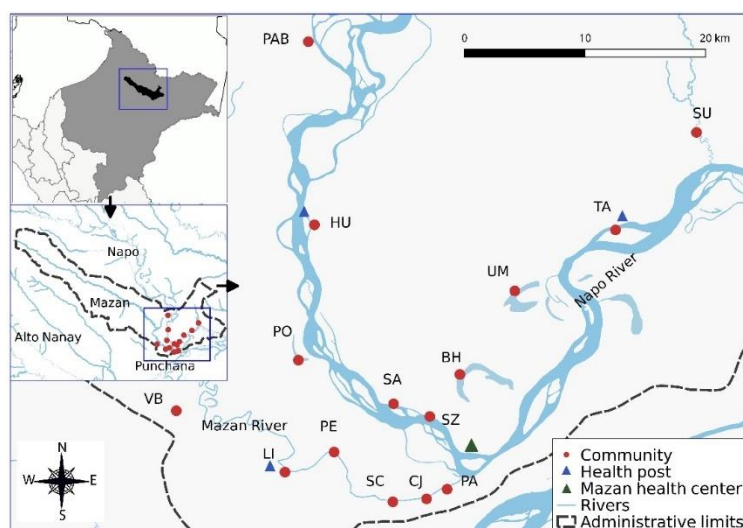


Fig. 1. Study communities in Mazan Loreto, Peru. Bello Horizonte (BH), Catorce de Julio (CJ), Huaman-Urco (HU), Libertad (LI), Puerto Abeja (PAB), Puerto Alegre (PA), Primero de Enero (PE), Puerto Obrero (PO), Salvador (SA), San Antonio de Zambrano (SZ), Santa Cruz (SC), Sucusari (SU), Tamanco (TA), Urco Miraño (UM), Visto Bueno (VB).

Table 1. Health services availability in communities, time to health centre and knowledge, attitudes and practices (KAP) survey

River basin	Community	Availability		Time to the Health Centre	KAP survey		
		Health post ^a	Community health worker	Minutes ^b	Inhabitant n	Household n	%
Mazan	Primero de Enero	No	Yes	135	133	25	3.7
	Visto Bueno	No	Yes	275	79	15	2.2
	14 de Julio	No	Yes	30	136	23	3.4
	Santa Cruz	No	Yes	45	439	73	10.7
	Libertad	Yes	Yes	155	374	64	9.4
	Puerto Alegre	No	Yes	15	353	57	8.4
	Subtotal Mazan				1514	257	37.8
Napo	Huaman Urco	Yes	Yes	195	502	85	12.5
	San Antonio de Zambrano	No	Yes	30	124	24	3.5
	Urco Miraflores ^c	No	No	225	371	54	7.9
	Bello Horizonte	No	Yes	80	285	47	6.9
	Puerto Abeja ^c	No	Yes	315	109	19	2.8
	Tamanco	Yes	No	180	228	49	7.2
	Puerto Obrero	No	No	105	109	21	3.1
	Salvador	No	Yes	45	456	91	13.4
	Sucusari ^c	No	Yes	270	179	33	4.9
	Subtotal Napo				2363	423	62.2
Total					3877	680	100

^a Health post without capacity for malaria diagnosis by microscopy

^b Time from community to the health centre in Mazan town using a 13 horsepower motor on a local boat

^c Includes ethnic groups (Urco Miraflores: Yagua; Puerto Abeja: Kichwa; Sucusari: Maijuna)

Data collection

The study field team, composed of two trained health technicians and two nurses, traveled to selected communities in motorized boats and collected data over a few days. An epidemiological and KAP survey was conducted in all available households in March 2017. Prior informed consent, household members were censused, the house location geo-referenced, and the socio-demographic characteristics were collected (e.g. household size, predominant construction materials in house, ownership of bed nets, and availability of essential services such as water for domestic use, sanitation facility and electricity). A KAP survey questionnaire was applied to the household's head or the designate to gather information on family's attitudes, understanding of malaria transmission, recognition of signs and symptoms, perceptions of cause, care-seeking patterns, preventive measures and practices. Questionnaires were programmed using the Open Data Kit (ODK) application on mobile devices without a network connection.

A second survey (socio-economic survey) was conducted in September 2017 to obtain detailed information about the household's care-seeking process and the cost of illness. The survey questionnaire was applied to a sub-population of households, of which at least one member had been diagnosed with uncomplicated malaria in the previous 3 months before the survey (June–August 2017). Households were visited up to three times in a period of three days to maximize study participation. When recurrent episodes were recorded for an individual in the past 3 months, only those recorded at intervals of more than 28 days were considered as independent episodes. Besides the registration of socio-demographic data and clinical features

of malaria episodes on mobile devices, participants or responsible relatives (in case of minors) were also asked to complete a semi-structured questionnaire on treatment seeking. The latter contained questions on all the places they visited to seek care/treatment, whether they had blood tests and the type of these tests, the time between the onset of symptoms and the confirmation of malaria infection, and the type of treatment they received. The questionnaire also allowed obtaining specific data for the estimation of direct and indirect household costs associated with malaria episodes. These included costs of transport during the care-seeking process, time lost from work due to illness for patients, their carers/companions (i.e. individuals who care informally for the patient at home, and/or accompany him/her during the healthcare seeking) and substitute labourers (i.e. economically active individuals who substitute malaria patients at work).

Data analysis

The analysis was performed using STATA 12.1 software (Statacorp, College Station, TX, USA) and R v.2.15 software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Household characteristics from KAP survey and individual socio-demographic data from the socio-economic survey were described using absolute and relative frequencies.

Univariate and multivariate generalized linear models with gamma distribution were used to test the association between the time lost due to illness and the time to malaria diagnosis (i.e. time from onset of symptoms to malaria confirmation by diagnostic test) in economically active patients (≥ 15 years) [31]. Adjusted mean ratios (Adj.MR) and the corresponding 95% confidence intervals (95% CI) measured

how much more time patients lost due to illness, considering the *Plasmodium* species that caused the malaria episode, the diagnostic test that confirmed malaria, gender, education level and the time to the health centre in MT as potential confounders. All statistical analysis considered a p value < 0.05 as statistically significant.

Cost estimates

Costs associated with uncomplicated malaria episodes in Mazan were estimated for the year 2017 from both the provider and patient perspectives. These estimates drew on the number of malaria episodes registered by the PCD's surveillance in the district, as well as base case parameters (i.e. cost parameters or unit costs). Table 2 shows that the socio-economic survey provided data of cost parameters for estimating costs from the patient perspective; while costing databases from the MoH were required to estimate costs from the provider perspective since malaria detection and case-management is provided free-of-charge at health facilities [116]. Units costs in Peruvian Nuevos Soles (PEN) were converted into United States Dollars (US\$) using the average exchange rate for 2017 (one US\$ = 3.3 PEN) [117].

Table 2. Parameters, base case estimates and uncertainty distributions for sensitivity analysis

Parameter	Distribution	Base case (Mean, proportion)	Standard error (SE)	Source
Malaria in Mazan in 2017, episodes	-	1344	-	EDB
Malaria episodes caused by Pf, proportion	-	0.23	-	EDB
Economically active (EA) Pv patient, probability	Beta	0.56	0.04	SES
Economically active (EA) Pf patient, probability	Beta	0.69	0.06	SES
Economically active (EA) companion of a Pv patient, probability	Beta	0.83	0.03	SES
Economically active (EA) companion of a Pf patient, probability	Beta	0.73	0.06	SES
Confirmed malaria by LM or RDT among symptomatic individuals, proportion	-	0.16	-	EDB
Healthcare-seeking with CHW (and not health facilities), proportion	Beta	0.17	0.03	SES
Utilization of RDT (instead of LM) at health facilities for malaria confirmation, proportion	Beta	0.21	0.03	SES
Cost of complete Pv malaria treatment, unit cost, US\$	Gamma	1.32	0.38	CDB
Cost of complete Pf malaria treatment, unit cost, US\$	Gamma	8.76	2.76	CDB
Cost of consultation, staff-related unit cost, US\$	Gamma	1.81	0.05	CDB
Cost of consultation, non staff-related unit cost, US\$	-	0.77	-	CDB
Cost of diagnosis using LM, staff-related unit cost, US\$	Gamma	0.50	0.01	CDB
Cost of diagnosis using LM, non staff-related unit cost, US\$	-	0.21	-	CDB
Cost of diagnosis using RDT, staff-related unit cost, US\$	Gamma	0.50	0.01	CDB
Cost of diagnosis using RDT, non staff-related unit cost, US\$	-	0.40	-	CDB
Cost of diagnosis using RDT, test-related unit cost, US\$	Gamma	1.07	0.33	CDB
Cost of transport Pv patient, unit cost, US\$	Gamma	2.36	0.35	SES
Cost of transport companion of Pv patient, unit cost, US\$	Gamma	1.94	0.31	SES
Cost of transport Pf patient, unit cost, US\$	Gamma	2.78	1.17	SES
Cost of transport companion of Pf patient, unit cost, US\$	Gamma	0.64	0.23	SES
Time lost due to illness in an EA Pv patient, person-days	Gamma	10.21	0.99	SES
Time lost due to illness in an EA Pf patient, person-days	Gamma	11.80	1.48	SES
Time lost in EA companions of a Pv patient, person-days	Gamma	7.73	0.50	SES
Time lost in EA companions of a Pf patient, person-days	Gamma	6.85	0.90	SES

Abbreviations: Pv (*P. vivax*), Pf (*P. falciparum*), LM (light microscopy), RDT (rapid diagnostic test), CHW (community health worker), CDB (cost database), EDB (epidemiologic database), SES (socio-economic survey)

Provider perspective, direct costs

Direct costs from the provider perspective are costs related to the malaria detection (i.e. initial consultations), diagnosis confirmation (using LM or RDTs), appropriate treatment (i.e. antipyretic drugs, anti-malarial drugs) and follow-up (consultation and LM after treatment). The unit costs for care services provided by voluntary CHW were estimated using the standard shadow price approximation based on the 2017 national minimum wage of US\$ 257.6 per month. Moreover, the unit cost's components (i.e. staff costs, drug/supply costs, or other non-staff costs) that most contributed to the unit cost's variability of care services provided by either health facilities or CHW were identified. As expected, the different salary levels between health staff, resulting in different costs of their time dedicated to a given care service, contributed most to the variability of unit costs. A malaria positivity rate of 15.5% (base case parameter in 2017) among symptomatic individuals seeking for care was assumed to estimate the number of initial consultations that allow for the identification of malaria cases. It was assumed that a symptomatic individual seeking for care had only one initial consultation. The standard treatment of malaria episodes by species [63] was costed considering that patients ≥ 15 years old had an average of 60 Kilograms (Kg); whereas, in patients < 15 years old were considered half of the total doses. Similarly, the administration of the antipyretic acetaminophen was costed considering that adults receive 10 tablets (500 mg) and that children take syrup (120 mg/5 ml) [63,118]. Follow-up costs considered that *P. vivax* and *P. falciparum* episodes require respectively one and two follow-ups as national guidelines states [63].

Patient perspective, direct costs

Direct costs from the patient perspective, comprised other medications not provided free-of-charge by the provider, such as analgesics, the transport costs for the patient and companions, and other costs (e.g. consumed food during the care-seeking process). Cost calculations were based on the volume and unit cost data collected through the socio-economic survey (Table 2).

Patient perspective, indirect costs

Indirect costs from the patient perspective included the lost wages due to malaria per patient, substitute labourers and companions. Lost wages were calculated for only individuals ≥ 15 years old only (i.e. economically active sub-population) [117,119], by multiplying the reported time lost from work in days (cost parameter expressed in person-days) by the amount of money lost in 1 day for not working (person-day unit cost). Person-day unit costs (US\$ 9.91) for patients, substitutes and companions were calculated using the monthly minimum wage of 2017 (US\$ 257.6) as reference, by dividing it by the number of working days per month [120].

Total costs and average cost per episode

The sum of costs from the provider and patient perspectives yielded the average total costs for all malaria episodes during 1 year, and the division between these average total costs by the number of episodes the average cost per episode. Both costs were calculated overall and by species.

Uncertainty and sensitivity analysis

Multi-way probabilistic sensitivity analysis (PSA) estimated the confidence in the average cost per malaria episode. This considered the uncertainty that surrounded the base case parameters (point estimates) which were used in the calculation of such average cost [121]. Since base case estimates based on few data are more uncertain (less confidence) than estimates based on large datasets, it was accounted by this uncertainty by first defining probability distributions for the relevant cost parameters (Table 2). The rationale for the assumed distribution for each parameter has been described elsewhere [121,122]. The beta distribution was chosen for the probability of being economically active either among malaria-infected individuals or companions; while the gamma distribution was used for unit costs (i.e. unit costs related to malaria detection, diagnosis and treatment, and costs associated with the transport of patients and their companions) and the time lost from work due to malaria (Table 2). Second, Monte Carlo simulations were conducted in R software to propagate uncertainty by sampling values from all the probability distributions jointly in 10,000 iterations and calculating the corresponding values for the cost per malaria episode. Third, upper and lower 95% uncertainty limits (2.5 and 97.5%) for the average cost per malaria episode were obtained from these simulations and reported using tornado diagrams.

A one-way PSA was also used to assess how sensitive the average cost per species-specific episode was to changes in individual uncertain parameters by sampling from the uncertainty distribution of a single parameter, while fixing the other uncertain parameter to their base case value. The gross domestic product (GDP) of Peru in

2017 [123] was also tested as reference in the sensitivity analysis to estimate person-day unit costs (US\$ 21.1) for patients, substitutes and companions, and to adjust the staff-costs associated with the health care services. The latter adjustment consisted in a multiplication by 2.1 (division of person-day unit cost using the GDP reference by that using the monthly minimum wage reference).

4.4. Results

Household characteristics

A total 680 households and 3877 residents were censused (Table 1). Household with more than three people per habitable room (overcrowding) (57.2%) and housing structures with incomplete walls (60.5%) were frequent in the study area. Most individuals lived in houses with wooden walls and roofs composed of palm leaf. Most households had no sanitation facility and lack of electricity. Main sources of water for domestic use were the river and the rain (Table 3).

Table 3. Baseline household characteristics of the study area

Characteristics	N=680	%
Housing structure (number of external walls)		
0	99	14.6
1-3	312	45.9
4	269	39.6
Main material in walls (n=581)*		
Wood	551	94.8
Brick	2	0.3
Others (adobe, straw, palm)	28	4.8
Main material in floor		
Wood	659	96.9
Cement or other fine finish	12	1.8
Soil	9	1.3
Main material in roof		
Palm leaf, straw	446	65.7
Tin	233	34.3
Missing	1	
Overcrowding		
No	291	42.8
Yes	389	57.2
Sanitation facility		
No facility, field	528	77.9
Pit latrine, ground dug	150	22.1
Missing	2	
Source of water for domestic use		
River, rain	601	88.4
Open well, public tap	79	11.6
Electricity available		
No	473	69.7
Yes	206	30.3
Missing	1	
Radio available		
No	302	44.4
Yes	378	55.6
Ownership of bed nets		
At least one insecticide treated net (ITN) for every two people		
No	453	66.6
Yes	227	33.4
At least one untreated net for every two people		
No	358	52.7
Yes	322	47.4
At least one ITN/untreated net for every two people		
No	109	16.0
Yes	571	84.0
Indoor residual spraying (previous 12 months)		
No	310	46.1
Yes	363	53.9
Missing	7	

*Only in household with external walls

Malaria knowledge, attitudes and practices (KAP)

Knowledge

Although most of the 680 responders knew that malaria is transmitted by infected mosquitoes; 22% of responders pointed out that malaria was acquired by drinking stagnant water and nobody knew that malaria could be transmitted through blood transfusion. Fever, headache and chills were identified as the most common symptoms (about 80% of responders) in individuals with malaria disease; myalgia/arthritis, nausea/vomiting and general discomfort were also recognized as malaria symptoms (> 15% of responders). The majority (> 90%) of responders knew that malaria can be acquired more than once and that medication can cure the disease. Health workers followed by CHW were referred as the main sources of information about malaria (Additional file 1: Table S1).

Attitudes

The majority of responders (80.6%) would go to a health facility as first action in case of malaria symptoms, despite the perception (53.5% of responders) that getting malaria was common and normal (Additional file 1: Table S2). The MoH was identified as the main responsible for malaria control (about 60% of respondents), followed by CHW. Almost all responders (> 98%) referred that a finger-prick blood smear was necessary to determine if a person had malaria. Regarding the anti-malarial treatment, about half the responders were aware that interrupting the treatment could lead to a severe illness/death, or to malaria recurrences. The majority of responders perceived that the presence of standing water increased the

risk of malaria (88.7%), that the presence of mosquitoes was a nuisance to them (94.3%), and that the use of bed net was not annoying (90.9%).

Practices

When they or their relatives had malaria, the majority of responders (79.3%, 525/662) indicated they consulted with health services or with CHW (12.7%, 84/662) (Additional file 1: Table S3). Regarding indoor malaria prevention measures, the most common practices was cleaning the house (51.4%) followed by using bed nets (23.0%), drinking boiled/chlorinated water (22.7%) and covering water containers (19.0%). A majority (58.6%) also responded that cleaning of house surroundings was the main outdoor malaria prevention measure, followed by clearing objects that can accumulate water (23.2%) and vegetation (21.4%).

Socio-economic survey

In September 2017, 161 individuals (in 112 households) were enrolled in the socio-economic survey of 251 individuals registered by health services as having had malaria between June–August 2017. Non-enrolled individuals were recent migrants or were not available at household during the survey. Of the total 161 enrolled individuals, 147 (91.3%) individuals presented only one uncomplicated malaria episode, and 14 (8.7%) individuals two malaria episodes. Among the total 175 registered episodes, 124 (70.9%) were by *P. vivax*, 47 (26.9%) by *P. falciparum*, and four (2.3%) co-infections by both parasite species (Additional file 1: Table S4).

Overall, males outnumbered females (ratio male/female = 1.15), and economically active individuals (≥ 15 years) representing about half of total patients reported subsistence farming (81.5%) as the most commonly reported occupation (Table 4). Monthly income in the latter age group ranged from US\$ 0 to US\$ 121. Moreover, the highest educational attainment of the majority of individuals ≥ 18 years was primary school level (60.3%) with no statistical differences between basins. More than 95% of enrolled individuals had health insurance (i.e. IHIS) and reported having used a bed net the previous night. Canoeing (by dugout canoe “peke-peke”) (47.2%) and walking (47.2%) were the main modes of transportation to reach the point of diagnosis and treatment.

Table 4. Socio-demographic characteristics of malaria patient

Characteristics	N=161	%
Gender		
Female	75	46.6
Male	86	53.4
Age (years)		
1 - 4	24	14.9
5 - 14	56	34.8
≥15	81	50.3
Education (≥18 years) n=68		
None	8	11.8
Primary school	41	60.3
Secondary school	19	27.9
Main occupation (≥15 years) n=81		
Logger	2	2.5
Farmer	66	81.5
Housewife	3	3.7
Student	6	7.4
Merchant	3	3.7
Distiller	1	1.2
Malaria species		
<i>P. vivax</i>	113	70.2
<i>P. falciparum</i>	44	27.3
Co-infection (<i>P. vivax</i> - <i>P. falciparum</i>)	4	2.5
Monthly Income amount (≥15 years)		
None	10	12.4
US\$ 3-121	71	87.6
Type of transportation to the point of diagnosis/treatment		
None (live in the same place)	2	1.2
Foot	76	47.2
Mototaxi/Motorcycle	2	1.2
Dugout canoe	76	47.2
Row-boat	1	0.6
Foot + Mototaxi/Motorcycle	1	0.6
Foot + dugout canoe	2	0.6
Van	1	1.2

Table 5 shows the place where individuals with malaria sought care for the first time. CHWs (71, 40.6%) were the most consulted, followed by health posts (59, 33.7%), the health centre (43, 24.6%), a hospital (1, 0.6%) and a pharmacy (1, 0.6%). When the first consultation was with CHWs, 40.8% (29/71) of the patients had immediate diagnosis with RDT and treatment, however two patients visited the health centre later (because treatment was not available). The remaining 59.2% (42/71) of the

patients had a blood smear taken which was sent to the health centre through an informal fluvial courier for a malaria microscopic diagnosis. With the exception of a single patient who went to the health centre to receive his/her diagnosis and treatment, everyone else came back two-six hours later or the following day for the malaria diagnosis and treatment. Malaria result was communicated by radio to the CHW when radio was available in the community or sent back through the informal fluvial courier. When the first consultation was at health post, 49.2% (29/59) of the patients had immediate diagnosis with RDT and treatment, and 30 (50.8%) had a blood smear sent to the health centre for malaria microscopic diagnosis. The latter patients came back two-six hours later or the following day for the malaria diagnosis and treatment. Malaria result was communicated by radio to the health posts. The initial seeking for the care at the health centre (43, 24.6%) and at hospital (1, 0.6%) always involved microscopic diagnosis by LM, except in one case diagnosed by RDT at the health centre. An additional individual sought for initial care at the pharmacy, but the malaria diagnosis was done at health centre.

Table 5. Characteristics of malaria episodes by diagnosis test

Variable	LM		RDT		Total		Time to diagnosis (days)		Time lost (days)	
	n	%	n	%	n	%	LM	RDT	LM	RDT
							median(IQR)	median(IQR)	median(IQR)	median(IQR)
Consultation (N=175)										
Health post	30	50.9	29	49.2	59	100				
Health centre	42	97.7	1	2.3	43	100				
Hospital	1	100	0	0	1	100				
CHW	41	60.3	27	39.7	68	100				
Pharmacy then health centre	1	100	0	0	1	100				
CHW then health centre	1	33.3	2	66.7	3	100				
Malaria species (N=175)										
<i>P. vivax</i>	76	61.3	48	38.7	124	100	3(2-7)	3(2-3)		
<i>P. falciparum</i>	40	78.4	11	21.6	51	100	3(2-7)	2(2-6)		
Malaria species in EA individuals (N=85)*										
<i>P. vivax</i>	38	69.1	17	30.9	55	100	4.5(3-7)	3(2-3)	7.5(5-15)	7(4-13)
<i>P. falciparum</i>	24	80	6	20	30	100	3(2.5-7)	2.5(2-7)	8.5(7-16.5)	8.5(5-14)

Abbreviations: CHW (community health worker); EA (economically active); LM (light microscopy); RDT (rapid diagnostic test); IQR (interquartile range)

**p* value < 0.05 for differences in time to diagnosis (LM vs RDT)

Only 65 (37.1%) episodes were confirmed within 48 h of the onset of symptoms. Time to diagnosis had a median (mdn) of 3 days and interquartile range (IQR) of 2-5 days, with no differences between malaria episodes by *P. vivax* and *P. falciparum* (Table 5). Considering episodes in only economically active individuals, time to malaria diagnosis was significantly shorter when diagnosis was done by RDT than by microscopy ($p = 0.01$). The analysis by species, showed that these differences were especially for *P. vivax* episodes ($p = 0.01$). However, there were not significant differences in the time lost due to malaria between individuals with episodes diagnosed by RDT and those by microscopy ($p = 0.50$ for *P. vivax*, $p = 0.62$ for *P. falciparum*). Gamma distribution models found a significant association between the reported time to malaria diagnosis and the time lost due to malaria illness in economically active patients, with near twice the time lost due to illness in patients who had the malaria diagnosis after 48 h of the onset of symptoms in comparison with those who had the diagnosis before (Adj. MR 1.8; 95% CI 1.3, 2.6) (Table 6).

Table 6. Generalized linear models of time lost due to malaria in economically active patients (>= 15 years old)

Variable	Univariate		Multivariate			
	N	PD Mean (SD)	Mean Ratio	[95%CI]	Mean Ratio	[95%CI]
Delayed diagnosis						
No (0-48 hours)	26	7.2 (7.4)	Ref		Ref	
Yes (>48 hours)	59	12.3 (8.5)	1.7	[1.2;2.4]	1.8	[1.3;2.6]
Diagnosis test						
RDT	23	9.4 (7.4)	Ref		Ref	
Microscopy	62	11.3 (8.7)	1.2	[0.8;1.7]	1.2	[0.8;1.7]
Malaria species						
<i>P. vivax</i>	55	10.2 (8.3)	Ref		Ref	
<i>P. falciparum</i>	30	11.8 (8.7)	1.2	[0.8;1.7]	1.2	[0.8;1.6]
Gender						
Female	39	11.5 (9.1)	Ref		Ref	
Male	46	10.1 (7.8)	0.9	[0.6;1.2]	0.9	[0.6;1.3]
Education level						
Primary or higher	76	10.3 (8.0)	Ref		Ref	
No education	9	14.3 (11.3)	1.4	[0.8;2.5]	1.6	[1.0;2.8]
Time to the health centre						
<120 min	46	9.5 (7.1)	Ref		Ref	
>=120 min	39	12.3 (9.7)	1.3	[0.9;1.8]	1.2	[0.8;1.7]

Abbreviations: PD (person-day) ; 95%CI (95% confidence interval); SD (standard deviation)

Costs for uncomplicated malaria episodes

The total costs, for patients and providers combined, associated with 1344 uncomplicated malaria episodes in Mazan in 2017 were US\$ 217,006: US\$ 160,285 corresponding to 1029 *P. vivax* episodes and US\$ 56,721 to 315 *P. falciparum* episodes (Table 7). Therefore, average costs amounted to US\$ 161 per average episode or US\$ 156 per *P. vivax* episode and US\$ 180 per *P. falciparum* episodes. The indirect costs (due to time lost from work) incurred by patients contribute to 78% of the total costs (Table 7).

From the provider perspective, estimated costs in Mazan were US\$ 41,466 (US\$ 30.85 per episode), including US\$ 29,451 associated with 1029 *P. vivax* episodes

(US\$ 28.62 per episode) and US\$ 12,015 with 315 *P. falciparum* episodes (US\$ 38.14 per episode). As Additional file 1: Tables S5 and S6 show, costs associated with initial consultations and malaria screenings (by LM or RDT) accounted for most of provider costs for both *P. vivax* (76.40%) and *P. falciparum* (57.35%). Moreover, costs of anti-malarial treatment and follow-up control after treatment for one *P. falciparum* episode (US\$ 13.13 and US\$ 6.41) were higher than those for one *P. vivax* episode (US\$ 1.98 and US\$ 3.20).

From the patient perspective, estimated costs for uncomplicated malaria episodes in Mazan were US\$ 175,540 (US\$ 131 per episode), including US\$ 5848 (US\$ 4.35 per episode) as direct costs and US\$ 169,693 (US\$ 126 per episode) as indirect costs. The analysis by species showed that the average costs per *P. falciparum* episode (US\$ 142) were higher than those per *P. vivax* episode (US\$ 127) (Table 7). The longer time lost by patients due to *P. falciparum* episodes (22.20 days) in comparison with *P. vivax* episodes (17.0 days) determined the higher indirect costs for *P. falciparum* episodes (US\$ 138 per episode) in comparison with those for *P. vivax* episodes (US\$ 123 per episode) (Additional file 1: Tables S7 and S8).

Uncertainty and sensitivity analysis

Multi-way PSA, accounting for the uncertainty of all relevant cost parameters at the same time and considering the monthly minimum wage as reference, found confidence intervals (95% CI) for the average cost per episode between US\$ 139 and US\$ 174 for *P. vivax*, and between US\$ 152 and US\$ 211 for *P. falciparum* (gray bars in Fig. 2a, c).

According to the one-way PSA, the uncertainty about the four parameters related to the cost of loss of productivity due to malaria, induced most of the uncertainty in the average cost per malaria episode (second, third, fourth and fifth coloured bars in Fig. 2a, c). Of these four, the uncertainty about the time lost due to malaria in an economically active patient had most impact on the average cost per episode, with estimates between US\$ 146 and US\$ 167 per *P. vivax* episode, and between US\$ 162 and US\$ 200 per *P. falciparum* episode. The uncertainty about the proportion of patients ≥ 15 years (economically active individuals) was less influential, with estimates of average cost per episode between US\$ 147 and US\$ 165 per *P. vivax* episode, and between US\$ 164 and US\$ 195 per *P. falciparum* episode. Similarly, the uncertainty about having economically active companions resulted in average costs between US\$ 150 and US\$ 160 per *P. vivax* episode, and between US\$ 172 and US\$ 188 per *P. falciparum* episode. But, again the uncertainty in the time lost in economically active companions during the healthcare-seeking, malaria diagnosis and treatment of a malaria patient caused more uncertainty in the average cost estimates: between US\$ 148 and US\$ 164 per *P. vivax* episode and between US\$ 168 and US\$ 193 per *P. falciparum* episode. Uncertainties about the cost of anti-malarial drugs, the cost of malaria detection and diagnosis, and the cost of transport resulted in the smallest uncertainty intervals of the average costs per episode.

When GDP was used as reference to estimate person-day unit costs, the base case average costs increased to US\$ 314 per *P. vivax* episode, and to US\$ 358 per *P. falciparum* episode (Table 7). Multi-way PSA found averages cost ranges between

US\$ 278 and US\$ 352 per *P. vivax* episode, and between US\$ 299 and US\$ 423 per *P. falciparum* episode (gray bars in Fig. 2b, d). The most influential parameters for the average cost per malaria episode were also those that determined the cost of loss of productivity due to malaria according to the one-way PSA.

Economic costs analysis of uncomplicated malaria case management

Table 7. Total cost from both patient and provider perspectives for uncomplicated malaria episodes in Mazan district 2017 (US\$)

Perspective	<i>P. vivax</i> (n=1,029)				<i>P. falciparum</i> (n=315)				Total (N=1344)					
	Monthly minimum wage		GDP per capita		Monthly minimum wage		GDP per capita		Monthly minimum wage			GDP per capita		
	Sub-total costs	Cost per uncomplicated episode	Sub-total costs	Cost per uncomplicated episode	Sub-total costs	Cost per uncomplicated episode	Sub-total costs	Cost per uncomplicated episode	Total cost	Cost per uncomplicated episode	%	Total cost	Cost per uncomplicated episode	%
Provider														
Direct cost	29,450.57	28.62	49,861.25	48.46	12,014.98	38.14	19,126.51	60.72	41,465.55	30.85	19.1	68,987.80	51.3	15.8
Patient														
Direct cost	4,723.11	4.59	4,723.11	4.59	1,124.55	3.57	1,124.55	3.57	5,847.66	4.35	2.7	5,847.70	4.4	1.3
Indirect cost	126,111.69	122.56	268,003.24	260.45	43,581.01	138.35	92,615.14	294.02	169,692.70	126.26	78.2	360,618.40	268.3	82.8
Direct and Indirect costs	130,834.80	127.15	272,726.35	265.04	44,705.56	141.92	93,739.69	297.59	175,540.36	130.61	80.9	366,466.00	272.7	84.2
Total cost	160,285.36	155.77	322,587.60	313.5	56,720.54	180.07	112,866.20	358.31	217,005.91	161.46	100	435,453.80	324	100

Monthly minimum wage 2017: US\$257.6; GDP (Gross domestic product) per capita 2017:US\$ 6,571.9

Economic costs analysis of uncomplicated malaria case management

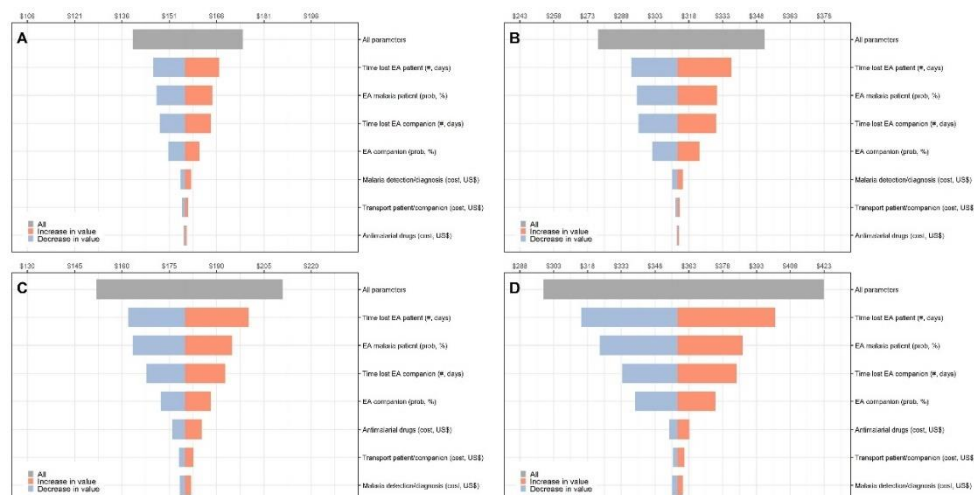


Fig. 2 Tornado diagram showing results of probabilistic sensitivity analysis (PSA) exploring the effect of key parameters on average costs per uncomplicated malaria episode in Mazan, 2017: A. *P. vivax* episode, monthly minimum wage as reference to estimate person-day costs, B. *P. vivax* episode, gross domestic product as reference to estimate person-day costs gross domestic product, C. *P. falciparum* episode, monthly minimum wage as reference to estimate person-day costs, D. *P. falciparum* episode, gross domestic product as reference to estimate person-day costs. One-way PSA (example: the lower the time lost in economically active (EA) patient, blue color, the lower the average costs per uncomplicated malaria episode); Multi-way PSA (gray color).

4.3. Discussion

This research provides total costs and average costs per episode associated with the detection and management of uncomplicated malaria in Mazan, an exemplar district in the Peruvian Amazon where poverty, limited accessibility from communities to health facilities, and healthcare-seeking behaviour challenge malaria control efforts. Estimated total costs for the 1344 uncomplicated malaria episodes registered in Mazan in 2017 were US\$ 217,006 with most of these costs (78%)

corresponding to indirect costs incurred by patients. Average costs per episode were higher for *P. falciparum* (US\$ 180) than for *P. vivax* (US\$ 156), mainly due to differences in costs of the anti-malarial treatment and in time losses due to illness. Indeed, the length of time lost due to illness in a patient, found to be associated with delayed confirmatory malaria diagnosis, was the parameter that caused most uncertainty in the average costs per malaria episode for both species.

As in Colombia [124], Brazil [125] and other endemic areas outside the Americas [107,126,127], uncomplicated malaria episodes in Mazan imposes significant costs on households, pushing poor families further into poverty. In several Amazonian communities with high risk of malaria transmission, the situation can become catastrophic due to the occurrence of repeated episodes in the same individuals and/or the same households every year [100,128–131]. In contrast to the low costs of transport and medications paid out of pocket by families, indirect costs of malaria episodes were the key determinant of the disease's overall costs. This is because adults have to interrupt their normal activities to care for ill family members, or the disease directly afflicts the economically active population [100,126,132].

The indirect costs of a *P. vivax* episode is markedly higher in Mazan in 2017 (US\$ 260.45 with GDP; US\$ 122.56 with minimum wage) than in Afghanistan, Ethiopia, Indonesia and Vietnam in 2016 [133] and Papua New Guinea in 2013 [133]. This is partially due to Peru having a higher GDP per capita, which was used to estimate the lost wages of patients and their carers/companions. Specifically, in Papua, Indonesia, malaria associated costs may have been underestimated since they were

calculated based on data collected in localities with high access to health facilities by road [126]. In the study area, the limited access to health facilities would also have increased the time lost due to illness with corresponding uncertainty for economically active patients and carers/companions during the period in which healthcare is sought for diagnosis, treatment and follow-up. As a result, the costs of case management of one *P. vivax* episode in Mazan (US\$ 313.50 based on GDP per capita, US\$ 155.77 based on minimum wages) were higher than the estimates in Papua, Indonesia (US\$ 44.50 based on mean wage) [126].

The association between longer time losses in a malaria patient and delayed malaria diagnosis could reflect a more compromised clinical case (without the need for hospitalization) that requires more recovery time. Indeed, several studies have shown an increase in malaria severity with a delay in malaria case management [108,134,135], thus the WHO's recommendation of insuring access to early diagnosis, and prompt, effective treatment within 24-48 h of the onset of malaria symptoms [118]. The reduced group of participants (37.1%) with malaria diagnosis within 48 h of onset of symptoms suggests that the delay in the malaria diagnosis remains a pitfall of the malaria case management in the rural and widely dispersed population of the Peruvian Amazon, as previously found by a longitudinal study (2001–2003) in riverine and road-associated rural populations of peri-Iquitos districts [136,137].

It was not surprising to find that the availability of RDTs in the first place to which enrolled participants sought for healthcare (i.e. CHW or health posts) reduced the

time from the onset of symptoms to malaria diagnosis. The impact of incorporating the use of RDTs to get timely diagnosis and appropriate treatment has already been demonstrated in rural Amazonian communities [136,137]. Where RDTs were not available in the study communities, unreliable or irregular fluvial transport and limited access to the health centre in MT may have delayed any patient reference and/or sending of blood smear samples for malaria confirmation [33]. However, the findings show that even with an easy access to malaria diagnosis, an early malaria case management of participants was not guaranteed [136]. The time to diagnosis of three or more days in several participants who went directly to health facility or CHW with RDTs available, suggest a delay in seeking care. The community's knowledge, misperceptions (e.g. perceiving malaria as a common, uneventful, and a mild and self-limiting disease), the lack of household involvement in community control of malaria, and initial self-treatment (e.g. use of antipyretics and refreshing herbs at home) reported in the KAP survey may be associated with such delay to seek care [34,138,139].

Several studies have highlighted the need for economic research on the household costs of illness, household responses, and their implications for poverty [132]. The lack of knowledge about the economic burden of illness for households and/or the poor understanding about the effects on the quality of life and well-being in the population may lead health stakeholders to plan and deliver health services with the main goals of increasing coverage and reducing disease burden. This decision-making process may be more obvious in the case of services that are provided free-of-charge like the detection, diagnosis and treatment of malaria provided by the

Peruvian MoH in the entire country [140,141]. Increased access to prevention measures (e.g. delivery of long lasting insecticide treated bed nets) and malaria behaviour change communication (BCC) strategies would remain as the best options to avoid indirect and direct household costs due to malaria.

Similar to other studies conducted in Peru [100,137], the provider in Mazan had to use more resources for delivering diagnosis and treatment services to an individual with a *P. falciparum* episode than one with a *P. vivax* episode. This was also observed in Papua, Indonesia, where the cost differences between both species were associated with additional consultations, transportation to the health facility and lost wages per episode [126]. The difference in costs of first-line treatment regimens between species (US\$ 8.76 for *P. falciparum* and US\$ 1.32 for *P. vivax*) explains well the higher provider costs of a *P. falciparum* episode. Delays in adjusting the daily dosage of one of the drugs (MQ) that make up the anti-malarial combined regimen for *P. falciparum* (MQ + AS + PQ) according to national policies have made difficult and expensive the procurement of separate presentations of these drugs because low volumes are less interesting for pharmaceutical companies, *ceteris paribus*. These delays have also prevented the purchase of cheaper drug presentations (AS-MQ fixed dose combinations instead of separate drug blisters) [142], which are used in other South-American countries such as Bolivia, Brazil and Venezuela [143,144]. The uncertainty in the unit cost of the anti-malarial treatment for *P. falciparum* likely depends on these procurement issues.

This study has a number of limitations. First, budget constraints did not allow to include all the 70 communities of Mazan and to visit households more than three times to maximize participation in the socio-economic survey; however, the chosen communities (accounting for 80% of malaria incidence) and the cost parameters obtained from the survey likely reflect the case-management's situation in the district. Second, verbal report of time losses due to illness and costs may affect the accuracy of the cost estimates from the patient perspective. This possibility was reduced with a short recall. Third, productivity changes may occur because of absence from work (absenteeism) or because of reduced productivity while at work (presenteeism). This information may be derived from existing data sources, such as registrations from occupational health service companies. Nonetheless, such registrations are typically available only for salaried workers and not for self-employed workers, such as subsistence farmers [145]. Fourth, from a patient perspective, indirect costs for children were considered zero as they should not be economically active. However, children represent a risk group and as 41% of the population were children, the methods for valuing the productivity costs could lead to biased estimates; this could be due to educational losses. Fifth, malaria infection can cause macroeconomic costs, which cannot be assessed in this study at the household level. For example, malaria can affect foreign direct investments, international trade and tourism. Sixth, mental stress and social costs of families with sick members were not included, which are in general very difficult to evaluate over a short period (and are typically not monetised in health economic evaluations). Finally, as this study was focused on uncomplicated malaria, costs may have been underestimated due to the exclusion of the cost of primaquine-induced haemolysis

and the cost of adverse effects. Primaquine-induced haemolysis was not included in any of the estimates due to uncertainty related to its frequency and severity. In countries that are prescribing primaquine without testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency, as in Peru, it is possible that haemolysis already occurs, however in previous studies, Peru has reported low prevalence rate (< 2%) of G6PD-deficiency [42].

4.4. Conclusions

Access to prompt diagnosis and treatment is one of the principal strategies for malaria control and elimination. This includes two components, one dependent upon the patient to seek care when sick and the other being the availability of accessible diagnosis and treatment facilities. Although malaria case management is provided free-of-charge by the MoH in Peru, this study confirmed that malaria poses a significant economic burden on rural households and individuals in terms of indirect costs due to the loss of working days. These costs are not well perceived by society and the substantial financial impact of the disease may be undervalued in public policy planning. Malaria control policies need to be integrated into development and poverty reduction programmes as socioeconomic development may be an effective and sustainable intervention against malaria in the long term.

5. EFFECTIVENESS OF A MALARIA SURVEILLANCE STRATEGY BASED ON ACTIVE CASE DETECTION DURING HIGH TRANSMISSION SEASON IN THE PERUVIAN AMAZON

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5.2. Abstract

Background: Faced with the resurgence of malaria, malaria surveillance in the Peruvian Amazon incorporated consecutive active case detection (ACD) interventions using light microscopy (LM) as reactive measure in communities with an unusual high number of cases during high transmission season (HTS). We assessed the effectiveness in malaria detection of this local ACD-based strategy.

Methods: A cohort study was conducted in June–July 2015 in Mazan, Loreto. Four consecutive ACD interventions at intervals of 10 days were conducted in four riverine communities (Gamitanacocha, Primero de Enero, Libertad and Urco Miraflores). In each intervention, all inhabitants were visited at home, and finger-prick blood samples collected for immediate diagnosis by LM and on filter paper for later analysis by quantitative real-time polymerase chain reaction (qPCR). Effectiveness was calculated by dividing the number of malaria infections detected using LM by the number of malaria infections detected by delayed qPCR.

Results: Most community inhabitants (88.1%, 822/933) were present in at least one of the four ACD interventions. A total of 451 infections were detected by qPCR in 446 participants (54.3% of total participants); five individuals had two infections. *Plasmodium vivax* was the predominant species (79.8%), followed by *P. falciparum* (15.3%) and *P. vivax-P. falciparum* co-infections (4.9%). Most qPCR-positive infections were asymptomatic (255/448, 56.9%). The ACD-strategy using LM had an effectiveness of 22.8% (detection of 103 of the total qPCR-positive infections). Children aged 5–14 years, and farming as main economic activity were associated with *P. vivax* infections.

Conclusions: Although the ACD-strategy using LM increased the opportunity of detecting and treating malaria infections during HTS, the number of detected infections was considerably lower than the real burden of infections (those detected by qPCR).

Keywords: malaria; diagnosis; asymptomatic; active case detection; Peru

5.2. Background

Malaria remains an important problem in global public health with about 212 million cases and 429,000 deaths estimated worldwide in 2015 [146]. In the Americas, intensive control efforts over the past 15 years have resulted in a significant reduction in the malaria burden in the region from about 2.3 million confirmed cases and 838 malaria-related deaths in 2000 to near 800,000 cases and 490 deaths in 2015 [146]. By 2015, seven countries (Paraguay, Belize, El Salvador, Costa Rica, Mexico, Ecuador, Suriname) were attempting to eliminate malaria and many others considering doing so in the near future [146,147].

Intensified control efforts in the Amazonian Region mainly supported by the Global Fund (GF) PAMAFRO project contributed to a significant decline in malaria reported case incidence from 87,805 cases in 2005 to 23,060 cases in 2011 (about 70% reduction). Regrettably, this achievement rapidly vanished in the following years after the end of the GF project, reaching 63,153 reported cases in 2015 [26,148,149]. The vast majority of these malaria cases (about 95%) in the Peruvian territory were concentrated in Loreto Department [26]; where transmission is seasonal and highly heterogeneous, caused by *Plasmodium vivax* and *P. falciparum* (ratio of *P. vivax*/*P. falciparum*: 5/1) and mainly transmitted by the mosquito *Anopheles darlingi* [3,150]. Faced with the malaria resurgence in Loreto, the Peruvian government and the Regional Health Direction of Loreto (RHDL) pledged to improve the malaria case detection and case management to ensure prompt and accurate diagnosis, and

opportune treatment with effective antimalarial drugs, as recommended by World Health Organization (WHO) [35].

The improved malaria action plan considered the limitations of the routine malaria surveillance relying on passive case detection (PCD) [9,106] using light microscopy (LM) for detecting both asymptomatic infected individuals (i.e., asymptomatic parasite carriers) and symptomatic individuals that do not attend the health services (i.e., symptomatic individuals with limited access to healthservices, and/or poor treatment-seeking behaviors) [63,151]. Indeed, several prevalence studies using LM and polymerase chain reaction (PCR) in endemic rural villages near Iquitos city (capital of Loreto) have repeatedly found that the majority (more than 60%) of the people carrying the parasite were asymptomatic. This indicates there is a large “hidden” human infectious reservoir able to maintain transmission [67,152,153].

Malaria surveillance in Loreto was enhanced by the addition of active case detection (ACD) through household visits as a reactive measure in selected communities with unusually high numbers of cases previously reported by PCD, especially during high transmission season [154]. The local strategy consists of 2–4 consecutive screenings for malaria using LM over a period of one month in all individuals living in the selected communities and the treatment of confirmed infections irrespective of symptoms. In theory, consecutive ACD interventions would increase malaria detection by microscopically identifying parasite carriers who were in a latent state at the time of the previous ACD (i.e., development of blood asexual parasites after sporozoite inoculation), and by increasing the coverage of the intervention through

including individuals who were absent during the previous ACD [27]. Nevertheless, this local ACD-based strategy requires evidence on its effectiveness in different epidemiological settings before it can be implemented on a wider-scale, particularly in endemic areas where most infected individuals have low parasitemia levels below the threshold for detection by LM [10–13]. In this paper, we assessed the short-term effectiveness of this household-based malaria surveillance in four riverine communities of Mazan district in the Peruvian Amazon, in terms of the proportion of malaria infections detected by field LM in consecutive ACD interventions among the total malaria infections detected by delayed quantitative real-time polymerase chain reaction (qPCR).

5.3. Material and methods

Study Design and Study Area

A population-based cohort study was conducted in Mazan, one of the districts in Loreto with the highest annual parasite index (API): 121 cases per 1000 inhabitants in 2015 [155]. According to census projections in Mazan for 2015, 13,779 inhabitants lived in about 70 riverine communities located on the banks of Mazan or Napo River (including three communities inhabited by Orejón, Quichua and Yagua ethnic groups) [156]. People in riverine communities live mainly in open or semi-open wooden houses built on stilts. Subsistence farming is the main economic activity, followed by seasonal logging. The latter activity requires individuals to stay far out in the jungle for several weeks during the rainy season, where they usually sleep unprotected against mosquito bites.

The capital and largest community of Mazan, i.e., Mazan Town (MT), is located on the confluence between Mazan and Napo Rivers (3.503°S, 73.094°W), at about 55–60 km (one hour by speedboat) from Iquitos city (capital of Loreto). Due to its geographic situation, MT mediates the commerce between all communities located in margin of the Napo River and Iquitos city. With predominance of *P. vivax* (76.2%) over *P. falciparum* cases (23.8%), malaria in Mazan is seasonal, epidemic [157], and mainly transmitted by the highly anthropophilic *An. darlingi* [58]. All age groups are at risk of infection, though adults more so than children. The peak of cases is usually after the rainy season [3], between May and September. The main Health Centre in Mazan is also located in MT and has a laboratory service responsible for providing malaria diagnosis using LM and complete treatment in confirmed malaria infections six days a week. Malaria diagnosis and treatment in the area follow MoH standard procedures and treatment guidelines. Chloroquine (CQ) for 3 days (10 mg/kg on days 1 and 2, and 5 mg/kg on day 3 plus primaquine (PQ) (0.5 mg/kg/day) for 7 days is given in individuals with confirmed *P. vivax* malaria infections, while mefloquine (MQ) for 2 days (12.5 mg/kg/day) plus artesunate (AS) for 3 days (4 mg/kg/day) in confirmed *P. falciparum* infections [63]. A number of drug trials have confirmed the efficacy of CQ for *P. vivax* and of the combination MQ-AS for *P. falciparum* in the Peruvian Amazon [112,113].

Selection of Communities

Ten communities that accounted for about 80% of the total malaria cases in the district in the past two years (2013–2014) were censused and their eight-week slide positivity rates (8w-SPR) weekly analysed since the first week of March 2015. The 8w-SPR was weekly calculated by dividing the number of microscopically confirmed slides/cases in the past 8 weeks according to PCD surveillance by the total individuals screened for malaria by thick/thin blood slides in the past 8 weeks. The evolution of 8w-SPR values in malaria endemic communities is considered in the decision of RHDL for implementing additional control interventions [9,106,158]; thus, 8w-SPR values above the threshold of 0.05 in two consecutive weeks indicating unusual increase of malaria cases (potential outbreak) commonly trigger the onset of ACD interventions.

By May 2015, four communities exceeded the triggering threshold and were selected for the cohort study: Gamitanacocha (GAM), Primero de Enero (PRI), Libertad (LIB) and Urco Miraño (URC). GAM (3.426°S, 73.318°W), PRI (3.494°S, 73.221°W) and LIB (3.496°S, 73.234°W) are located along Mazan River, while URC (3.361°S, 73.064°W) along Napo River. They are only reachable from MT (about 3–5 h upstream) using a 9–13 hp motor on a dugout canoe, locally known as a “peke-peke” (Figure 1). Only LIB has a health post, where health technicians can take a finger-prick of blood from individuals with malaria-like symptoms and subsequently send the blood smears to the main health centre in MT for malaria diagnosis (no laboratory service in LIB). The health post in LIB can also provide antimalarial treatment to individuals with confirmed infections referred from the main health centre in MT.

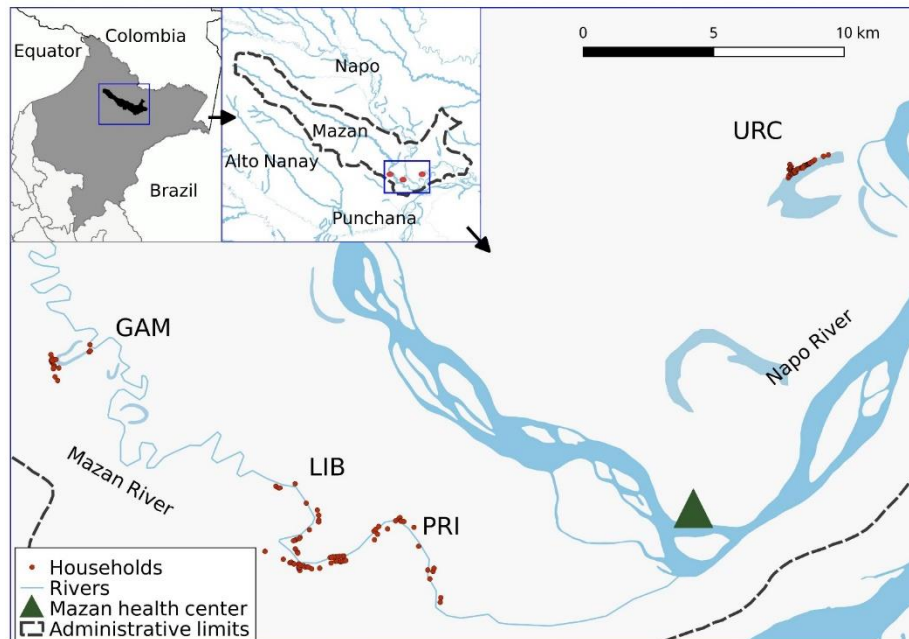


Figure 1. Study communities in Mazan, Loreto, Peru. Gamitanacocha (GAM), Primero de Enero (PRI), Libertad (LIB) and Urco Miraflores (URC).

ACD Interventions and Data Collection

During the full population census in March 2015, each household in the selected communities was encoded (6-digit numeric code) and geo-referenced using a Global Positioning System (GPS) hand device (Garmin's GPSMAP 60CSx, Garmin International Inc., Olathe, KS, USA).

In June–July 2015, four consecutive ACD interventions were conducted at intervals of 10 days in selected communities. In each ACD intervention, all community inhabitants were visited at home, and after informed consent they had axillary temperature taken, history of fever or any other malaria-compatible symptoms documented through use of a semi-structured questionnaire, and finger-prick blood

samples collected for immediate microscopy (thick and thin blood smears) and on filter paper (Whatman grade 3, Whatman, Springfield Mill, Philadelphia, PA, USA) for later analysis by qPCR (two–three months after the field-work). Household visits also allowed the collection of household and individual data (e.g., household characteristics, age, education, gender, socioeconomics, recreation and occupational activities, malaria history, previous antimalarial treatment, bed net use, travel) through questionnaires programmed onto Open Data Kit (ODK) application on mobile devices without network connection. Data were synchronized with the project's server in Lima, and linked to laboratory results using 9-digit unique numerical codes.

ACD interventions were designed and coordinated with health authorities and workers from RHDL and the health centre in MT. Field teams included interviewers and nurses in charge of the data entry and sample collection, microscopists and laboratory technicians dealing with the diagnostic tasks, and trained health technicians from the health centre in MT who were in charge of the malaria case management including the treatment of all microscopically confirmed infections according to national guidelines. Between ACD interventions, community health volunteers visited individuals with microscopically confirmed infections to improve the adherence to the antimalarial treatment. Field teams travelled to the communities in motorized boats and settled there for a few days (staying overnight in semi-closed huts and/or tents). During the screenings, the field staff moved around with the boats to reach the scattered households (most of them surrounded by water) and, by the time samples were sequentially delivered at the community “base-camp”, where

microscopists proceeded with the blood-slide diagnostic by using mirror-illuminated microscopes (no electricity available). Households were visited up to three times in a period of 3 days to maximize subject participation in each ACD intervention.

Laboratory Procedures

Light Microscopy (LM)

Microscopy examination was performed the same day of sample collection in each ACD survey. Thick and thin smears were stained for 10 min with a 10% Giemsa solution. A slide was judged to be negative if no malaria parasite was seen after examining 100 high-powered fields [159]. Both the presence of asexual and sexual forms of *Plasmodium* species were determined during slide readings. Quality control was done blindly on all positive slides and 10% of randomly chosen negative slides by a senior technician at Reference Laboratory of RHDH. Parasite density in the field was reported using semi quantitation system ("plus system" scale) according to MoH standard procedures and national guidelines [159]: <+/2 (<40 parasites per 100 thick films fields); +/2 (40 to 60 parasites per 100 thick films fields); + (1 parasite per one thick film field); ++ (2 to 20 parasites per one thick film field); +++ (21 to 200 parasites per one thick film field); ++++ (>200 parasites per one thick film field).

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Filter paper areas containing blood were cut into ~6 mm² sections for the parasite genomic DNA extraction using E.Z.N.A.® Blood DNA Kit (Omega Bio-Tek®, Norcross, GA, USA), following manufacturer instructions with slight modifications—

addition of TEN (20 mM Tris-HCl, pH 8.0; 2 mM EDTA, pH 8.0; 0.2 M NaCl) buffer, supplemented with SDS 10% w/v—and stored at 4 °C for immediate use and at –20 °C for further analysis.

qPCR testing was done following a modified protocol from Mangold et al. [160] using PerfeCta SYBR Green Fast Mix (Quanta Biosciences, Gaithersburg, MD, USA). Briefly, this method amplifies the 18SSU rRNA gene sequence of the *Plasmodium* species-specific region. After the amplification, analysis of the differences in melting curves provided an accurate differentiation between *Plasmodium* species.

Multiplex Real Time PCR (mPCR)

For improving the detection of *P. vivax*/*P. falciparum* co-infections, all samples of individuals who presented consecutive positive qPCR results in which the identification of *Plasmodium* species changed across ACD visits—in absence of previous antimalarial treatment—were further analysed using the mPCR assay described by Cnops et al. [161]. Briefly, DNA was extracted from dry blood spots using Qiagen DNA mini kit (Qiagen Benelux, Venlo, The Netherlands), according to the manufacturer's guidelines except the addition of 10 µL of an internal control (DiaControlDNA, Diagenode, Liège, Belgium). A couple of primers were used to detect *P. vivax* (Pviv) and *P. falciparum* (Pfal) in combination with the 2 species-specific probes and reverse plasmo2 primer (Eurogentec, Seraing, Belgium). Primers and probes of DiacontrolDNA were used to test the extraction and amplification process. The mix consists of 12.5 µL of 2x Taqman Universal PCR master mix (Applied Biosystems, Foster City, CA, USA), 200 nM of Pfal and *P. falciparum* specific probe, 100 nM of Pviv and *P. vivax* specific probe, 2.5 µL of

DiaControlDNA primers and probes and 5 µL of DNA extract. Water was added to obtain a final volume of 25 µL. PCR conditions were as follows: initial step of 50 °C for 2 min, 95 °C for 10 min and 45 cycles of 95 °C for 15 s followed by 1 min. at 60 °C. PCR was run on an ABI 7500 thermocycler (Foster city, CA, USA).

Data analysis

The analysis was performed using STATA 12.1 software (Statacorp, College Station, TX, USA) and R v.2.15 software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics between communities were compared using the Chi-squared test.

All four ACD interventions/visits were taken into account to characterize malaria infections in study participants. A positive qPCR result (regardless of the presence of symptoms) defined a malaria infection in a participant, and the number of ACD visit (1st, 2nd, 3rd or 4th) in which the qPCR was positive considered as the time of detection. A consecutive positive qPCR result in the same participant at the following ACD visit (after 10 days) was considered as still being part of the infection detected at the previous ACD visit, even when the *Plasmodium* species detected on consecutive visits were different (*P. vivax* or *P. falciparum*). In this latter situation, in absence of antecedent of antimalarial treatment, the infection was considered as produced by co-infection *P. vivax*-*P. falciparum*. Two new independent infections in a participant were only considered when the participant had an earliest ACD visit with both positive qPCR and LM results (with proven administration of antimalarial treatment), and a later non-consecutive ACD visit (after 20 or 30 days) with positive qPCR result regardless of the parasite species. An infection was classified as

symptomatic if the individual had axillary temperature higher than 37.5 °C at the time of its detection by qPCR, reported history of fever in the previous seven days, or had headache, chills or general discomfort in the previous 24 h [63,162].

The effectiveness in malaria detection of the local ACD-based strategy was calculated by dividing the number of malaria infections detected through consecutive ACD interventions using LM by the total number of malaria infections detected by qPCR throughout the study period. When the local ACD-based strategy was able to detect individuals who had malaria infections, the time of this detection by LM (1st, 2nd, 3rd or 4th visit) was registered and later compared with the time of detection using qPCR. Uni- and multivariate mixed-effects logistic regression models were used to determine risk factors for species-specific malaria infection. The following potential risk factors were assessed as fixed-effects in the models: age, gender, occupation, travel in the past month to another endemic area, the history of malaria in the past year, housing structure (number of wall), electricity availability and indoor residual insecticide spraying in the past year. Random effects were included in the models to account for individuals nested within households, and households nested within communities. Factors with p values < 0.1 for the Wald test in the univariate analysis were considered for inclusion in the full multivariate model. Interactions were systematically checked for up to order two. Manual backward elimination guided by the minimization of the Akaike information criterion (AIC) allowed for the reduction of the number of factors in final models. Likelihood ratio tests (LRTs) were used to assess statistical differences between nested models.

Ethical Issues

Ethics clearance for the study was obtained from the Ethics Review Board of the Universidad Peruana Cayetano Heredia, Lima, Peru (SIDISI code # 64371). Permissions were received from health and local authorities after explaining the purpose and procedures of the study. Signed informed consent was obtained prior the study enrollment to participation and blood sampling by all adults and the parents of all participating children <18 years. In addition to parental/guardian consent, children older than 7 years provided a signed informed assent. All the methods were carried out in accordance with approved guidelines.

5.4. Results

Participants

Among the total 933 censused people—living in 158 households—in March 2015 in the study area, 822 (88.1%) people—living in 154 households—were surveyed and sampled during at least one of the four consecutive ACD visits conducted in June–July 2015, with the following distribution by community: GAM (92 individuals, 91.1% of censused people), LIB (299, 86.2%), PRI (100, 82.6%) and URC (331, 90.9%) (Table 1). While intervention coverages exceeded 70% in individual ACD visits (range between 73.4% and 78.5%), censused people that completed the four ACD visits only reached 58.3% with the lowest figures in GAM (43.6%) and PRI (44.6%).

Table 1. Number of visits and participants in census by community.

Table 1. Number of visits and participants in census by community.						
	Community					
		GAM	LIB	PRI	URC	Total
Households						
In census	N	21	58	20	59	158
With at least one ACD visit	n (%)	19 (90.5)	56 (96.6)	20 (100)	59 (100)	154 (97.5)
Individuals						
In census	N	101	347	121	364	933
With at least one ACD visit	n (%)	92 (91.1)	299 (86.2)	100 (82.6)	331 (90.9)	822 (88.1)
With one visit	n (%)	11 (10.9)	25 (7.2)	4 (3.3)	4 (1.1)	44 (4.7)
With two visits	n (%)	25 (24.8)	24 (6.9)	10 (8.3)	15 (4.1)	74 (7.9)
With three visits	n (%)	12 (11.9)	63 (18.2)	32 (26.4)	53 (14.6)	160 (17.1)
With four visits	n (%)	44 (43.6)	187 (53.9)	54 (44.6)	259 (71.2)	544 (58.3)
In the 1st visit	n (%)	64 (63.4)	242 (69.7)	67 (55.4)	312 (85.7)	685 (73.4)
In the 2nd visit	n (%)	63 (62.4)	255 (73.5)	95 (78.5)	309 (84.9)	722 (77.4)
In the 3rd visit	n (%)	73 (72.3)	258 (74.4)	87 (71.9)	314 (86.3)	732 (78.5)
In the 4th visit	n (%)	73 (72.3)	255 (73.5)	87 (71.9)	294 (80.8)	709 (76.0)

GAM: Gamitanacocha; PRI: Primero de Enero; LIB: Libertad; URC: Urco Miraflores.

Baseline socio-demographic characteristics and history of past malaria episodes of participants are presented in Table 2.

Effectiveness of a malaria surveillance strategy based on active case detection

Table 2. Baseline socio-demographic characteristics of study participants by community.

Characteristics		Gamitanacocha (GAM)		Libertad (LIB)		Primero de Enero (PRI)		Urco Miraflores (URC)		Total	
		N = 92		N = 299		N = 100		N = 331		N = 822	
		n	%	n	%	n	%	n	%	n	%
Gender											
	Male	43	46.7	159	53.2	55	55.0	174	52.6	431	52.4
Age (years)											
	<5	18	19.6	56	18.7	16	16.0	56	16.9	146	17.8
	5–14	23	25.0	72	24.1	34	34.0	113	34.1	242	29.4
	15–39	32	34.8	101	33.8	32	32.0	82	24.8	247	30.1
	≥40	19	20.7	70	23.4	18	18.0	80	24.2	187	22.8
Education (≥18 years) * n = 391											
	None	3	6.7	24	15.8	5	10.6	20	14.7	52	13.7
	Primary school	33	73.3	102	67.1	33	70.2	70	51.5	238	62.6
	Secondary school or higher	9	20.0	26	17.1	9	19.2	46	33.8	90	23.7
	Missing			8				3		11	
Main occupation (≥15 years) *											
	None or others	10	19.6	49	28.7	11	22.0	60	37.0	130	30.0
	Farmer	41	80.4	122	71.4	39	78.0	102	63.0	304	70.1
Malaria episodes (previous 12 months)											
	0	65	71.4	229	76.9	80	80.0	259	78.3	633	77.2
	≥1	26	28.6	69	23.2	20	20.0	72	21.8	187	22.8
	Missing	1		1						2	
Travel (previous month) *											
	No	80	87.0	269	90.0	81	81.0	306	92.5	736	89.5
	Yes	12	13.0	30	10.0	19	19.0	25	7.6	86	10.5
Housing structure (number of walls) *											
	0	10	11.0	42	14.2	16	16.0	44	13.3	112	13.7
	1–3	53	58.2	207	70.2	79	79.0	188	56.8	527	64.5
	4	28	30.8	46	15.6	5	5.0	99	29.9	178	21.8
	Missing	1		4						5	
Indoor residual spraying (previous 12 months) *											
	No	14	15.4	28	9.49	10	10.0	1	0.31	53	6.5
	Yes	77	84.6	267	90.5	90	90.0	325	99.7	759	93.5
	Missing	1		4				5		10	
Electricity available *											
	No	67	73.6	215	72.9	69	69.0	62	18.7	413	50.6
	Yes	24	26.4	80	27.1	31	31.0	269	81.3	404	49.5
	Missing	1		4						5	

* $p < 0.05$

Males slightly outnumbered females (ratio female/male: 0.91) without differences between sites. Individuals < 15 years represented 47.2% of the total participants with slight differences in the proportion of this age group across communities ($p = 0.117$). Among adults 18 years and older, the proportion of individuals who had completed secondary/higher education level was higher in URC (33.8%) than in other communities ($p = 0.014$). The most commonly reported occupation among individuals 15 years and older was farming (70.1%), with a greater proportion of this economic activity in GAM and PRIM ($p = 0.044$). At the time of the first ACD visit, participants reporting having experienced malaria in the past 12 months were more common in GAM (28.6%), LIB (23.2%) and URC (21.8%) than in PRIM (20.0%) ($p = 0.495$). Bed net use the previous night was almost universal in all sites, and about 10% of people reported that they had travelled outside the community in the previous 4 weeks. The proportion of individuals having electricity (81.3%) and reporting indoor residual spraying in the past 12 months at home (99.7%) were higher in URC than in other communities ($p < 0.001$), while housing structure with 4 walls was less frequent in PRI (5.0%) than in other communities ($p < 0.001$).

Malaria Infections in the study period

Overall, 451 malaria infections were detected by qPCR in 446 participants (54.3% of total participants) through the four ACD visits (Table 3): 40.4% (182/451) infections were detected at the first visit, 22.4% (101/451) at the second visit, 20.6% (93/451) at the third visit, and 16.6% (75/451) at the fourth visit. About 80% (360/451) of these infections were due to *P. vivax*, 15.3% (69/451) due to *P. falciparum*, and 4.9% (22/451) considered as coinfections *P. vivax*-*P. falciparum*. The latter coinfections

were detected in 22 individuals who had change in the species detection by qPCR in consecutive ACD visits. Additional analysis of these individuals' samples using mPCR detected DNA of both species in two individuals at a same visit (mixed infections). According to the case definitions, five participants were considered as presenting two malaria infections during the study period: all had recurrent *P. vivax* infections between 20 and 30 days after presenting a primary *P. vivax* infection treated by health workers with standard antimalarial regimen (CQ plus PQ).

Table 3 relates the time of detection of malaria infections by qPCR with the time of field detection by LM during the four-ACD local strategy and Figure 2 presents the effectiveness in malaria detection of the ACD local strategy. Only 103 (22.8%) of the total 451 qPCR-positive malaria infections were accurately detected by LM despite the intensive case detection efforts (i.e., four consecutive ACD visits using LM): 67 (65.0%) at the same ACD visit and 36 (35.0%) at the following ACD visits.

Effectiveness of a malaria surveillance strategy based on active case detection

Table 3. Time of detection of malaria infections by quantitative real-time polymerase chain reaction (qPCR) and light microscopy (LM).

Time of Detection of Malaria Infections by qPCR		Time of Detection of Malaria Infections by the Local Strategy Using LM					
		Same Visit	Next Visit (10-days Later)	After 2 Visits (20-days Later)	After 3 Visits (30-days Later)	Non Detected	Total
		n (%)	n (%)	n (%)	n (%)	n (%)	N
Overall	1st visit	22 (12.1)	9 (4.9)	5 (2.7)	8 (4.4)	138 (75.8)	182
	2nd visit	10 (9.9)	6 (5.9)	4 (4.0)	ND	81 (80.2)	101
	3rd visit	22 (23.7)	4 (4.3)	ND	ND	67 (72.0)	93
	4th visit	13 (17.3)	ND	ND	ND	62 (82.7)	75
	Total	67 (14.9)	19 (4.2)	9 (2.0)	8 (1.8)	348 (77.2)	451
<i>P. vivax</i>	1st visit	20 (14.5)	9 (6.5)	4 (2.9)	7 (5.1)	98 (71.0)	138
	2nd visit	10 (12.8)	6 (7.7)	4 (5.1)	ND	58 (74.4)	78
	3rd visit	22 (26.5)	4 (4.8)	ND	ND	57 (68.7)	83
	4th visit	13 (21.3)	ND	ND	ND	48 (78.7)	61
	Total	65 (18.1)	19 (5.3)	8 (2.2)	7 (1.9)	261 (72.5)	360
<i>P. falciparum</i>	1st visit	2 (6.7)	0 (0)	1 (3.3)	0 (0)	27 (90.0)	30
	2nd visit	0 (0)	0 (0)	0 (0)	ND	17 (100.0)	17
	3rd visit	0 (0)	0 (0)	ND	ND	8 (100.0)	8
	4th visit	0 (0)	ND	ND	ND	14 (100.0)	14
	Total	2 (2.9)	0 (0)	1 (1.4)	0 (0)	66 (95.7)	69
Co-infection *	1st visit	0 (0)	0 (0)	0 (0)	1 (7.1)	13 (92.9)	14
	2nd visit	0 (0)	0 (0)	0 (0)	ND	6 (100.0)	6
	3rd visit	0 (0)	0 (0)	ND	ND	2 (100.0)	2
	4th visit	0 (0)	ND	ND	ND	0 (0)	0
	Total	0 (0)	0 (0)	0 (0)	1 (4.5)	21 (95.5)	22

* *P. vivax*-*P. falciparum*. ND: no data.

Effectiveness of a malaria surveillance strategy based on active case detection

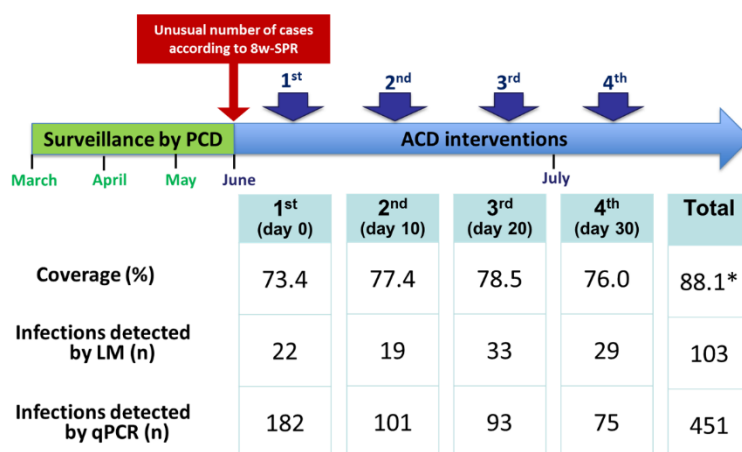


Figure 2. Effectiveness in malaria detection of the ACD local strategy. * Proportion of individuals with at least one ACD visit; eight-week slide positivity rate (8w-SPR); passive case detection (PCD); active case detection (ACD); light microscopy (LM); quantitative real-time polymerase chain reaction (qPCR).

Among these 103 infections detected by LM, 99 were *P. vivax* infections (27.5% of the total *P. vivax* infections detected by qPCR), three were *P. falciparum* infections (4.3% of the total *P. falciparum* infections detected by qPCR), and one *P. vivax*-*P. falciparum* coinfection (4.5% of the total co-infections detected by qPCR). The majority of individuals with microscopically-confirmed infections (>90%) reported had completed the treatment and had been followed by community health workers. Interestingly, the number of infections detected by qPCR in the first ACD visit (182 infections) was considerably higher than that detected by the four-ACD local strategy using LM (103 infections). The specific tracking of LM results from the 182 individuals who presented qPCR-positive infections at the first ACD visit found that only 22 (12.1%) individuals were accurately detected by LM at the same first ACD visit: 20 had *P. vivax* infections and 2 *P. falciparum* infections. Another 22 (12.1%) individuals with qPCR-positive infection at the first ACD visit were also detected by the local

ACD-based strategy but in the following visits: 9 individuals had confirmed malaria infection by LM at the second visit (10 days later), 5 individuals at the third visit (20 days later), and 8 individuals at the fourth visit (30 days later) (Table 3).

The analysis of available parasite density data of detected infections by LM showed that most microscopically confirmed *P. vivax* infections (59.8%, 58/97) had low parasite density levels (<1+ in the “plus system” scale) and that all three microscopically confirmed *P. falciparum* infections had parasitaemia $\geq 1+$ (i.e., ≥ 1 parasite per one thick film field). The unique coinfection accurately detected by LM had very low parasite densities (<1/2+). Four additional individuals that had *P. vivax*-*P. falciparum* co-infections were inaccurately detected by LM as *P. vivax* mono-infections (Table 4).

Table 5 presents the evolution of clinical status of malaria infections detected by qPCR, overall and by species. Only 128 (28.6%) of the total 448 PCR-positive malaria infections were symptomatic at the time of their detection by qPCR: 108 were *P. vivax* infections (30.2% of the total 358 *P. vivax* infections with clinical data), 16 *P. falciparum* infections (23.5% of the total 68 *P. falciparum* infections with clinical data), and four *P. vivax*-*P. falciparum* coinfections (18.2% of the total 22 co-infections). Incomplete data about symptoms in three malaria infections (two *P. vivax* infections and one *P. falciparum* infection) did not allow for the definition of their clinical status.

Table 4. Parasite density level at the time of detection malaria infection.

Parasite Density Level (Plus System Scale)	Time of Detection by the Local Strategy Using Light Microscopy (LM) in Comparison with the Time of Detection by Quantitative Real-Time Polymerase Chain Reaction (qPCR)		
	Same Visit	Following Visits (10–30 days Later)	Total
	n (%)	n (%)	N (%)
<i>P. vivax</i>			
<1/2+	24 (36.9)	16 (50.0)	40 (41.2)
1/2+	13 (20.0)	5 (15.6)	18 (18.6)
≥1+	28 (43.1)	11 (34.4)	39 (40.2)
Total	65 (100)	32 (100)	97 (100)
<i>P. falciparum</i>			
<1/2+	0 (0)	0 (0)	0 (0)
1/2+	0 (0)	0 (0)	0 (0)
≥1+	2 (100)	1 (100)	3 (100)
Total	2 (100)	1 (100)	3 (100)
Co-infection *			
<1/2+	0 (0)	1 (100)	1 (100)
1/2+	0 (0)	0 (0)	0 (0)
≥1+	0 (0)	0 (0)	0 (0)
Total	0 (0)	1 (100)	1 (100)

* *P. vivax*-*P. falciparum*.

Of the total 179 individuals with positive qPCR results at the first visit, 76 (42.5%) had symptoms at the same visit and another 30 (16.8%) individuals developed symptoms at later visits: 18 (10.1%) at the second, 10 (5.6%) at the third and 2 (1.1%) at the fourth visit. Noteworthy, an important proportion of individuals with qPCR-positive infections (40.8%, 73 individuals) at the first visit remained asymptomatic along the four ACD visits (Table 5). This latter proportion varied slightly in individuals with infections detected by the local ACD-based strategy (35.7%) and in those non-detected by the local ACD-based strategy (42.3%) ($p = 0.47$) (Supplementary Materials: Table S1).

Risk Factors by Malaria Species

Uni- and multivariate mixed effects logistic models for species-specific malaria incidence are presented in Table 6. In the final models, the estimates of the random-effects coefficients confirmed the influence of clustered sampled data at the community level for *P. vivax* infection, and at the household level for *P. falciparum*. Age and agricultural economic activities remained independently associated with *P. vivax* malaria infection in the multivariate model. While individuals between 5 and 14 years (AOR 1.7, 95% CI [1.1–2.6]) had higher odds of *P. vivax* infection than those <5 years, farmers were 1.6 times more likely to have a *P. vivax* infection than participants involved in other economic activities (AOR 1.6, 95% CI [1.0–2.4]). Considering *P. falciparum*, a non-significant trend was found for males and older adults to have higher risk of infection than women and young children, likely due to the low number of *P. falciparum* infections.

Table 5. Evolution of clinical status of malaria infections detected by quantitative real-time polymerase chain reaction(qPCR).

Time of Detection of Malaria Infections by qPCR		All qPCR-Positive Malaria Infections				
		Symptomatic				Asymptomatic
		In the Same Visit	In Following Visit (10- days Later)	After 2 Visits (20- days Later)	After 3 Visits (30- days Later)	Total
		n (%)	n (%)	n (%)	n (%)	N
Overall						
	1st visit	76 (42.5)	18 (10.1)	10 (5.6)	2 (1.1)	73 (40.8)
	2nd visit	20 (19.8)	12 (11.9)	12 (11.9)	ND	57 (56.4)
	3rd visit	22 (23.7)	11 (11.8)	ND	ND	60 (64.5)
	4th visit	10 (13.3)	ND	ND	ND	65 (86.7)
	Total	128 (28.6)	41 (9.2)	22 (4.9)	2 (0.4)	255 (56.9)
<i>P. vivax</i>						
	1st visit	60 (44.1)	13 (9.6)	8 (5.9)	1 (0.7)	54 (39.7)
	2nd visit	18 (23.1)	10 (12.8)	8 (10.3)	ND	42 (53.8)
	3rd visit	22 (26.5)	10 (12.0)	ND	ND	51 (61.4)
	4th visit	8 (13.1)	ND	ND	ND	53 (86.9)
	Total	108 (30.2)	33 (9.2)	16 (4.5)	1 (0.3)	200 (55.9)
<i>P. falciparum</i>						
	1st visit	12 (41.4)	1 (3.4)	0 (0)	1 (3.4)	15 (51.7)
	2nd visit	2 (11.8)	0 (0)	4 (23.5)	ND	11 (64.7)
	3rd visit	0 (0)	1 (12.5)	ND	ND	7 (87.5)
	4th visit	2 (14.3)	ND	ND	ND	12 (85.7)
	Total	16 (23.5)	2 (2.9)	4 (5.9)	1 (1.5)	45 (66.2)
Co-infection *						
	1st visit	4 (28.6)	4 (28.6)	2 (14.3)	0 (0)	4 (28.6)
	2nd visit	0 (0)	2 (33.3)	0 (0)	ND	4 (66.7)
	3rd visit	0 (0)	0 (0)	ND	ND	2 (100)
	4th visit	0 (0)	ND	ND	ND	0 (0)
	Total	4 (18.2)	6 (27.3)	2 (9.1)	0 (0)	10 (45.5)

* *P. vivax*-*P. falciparum*. ND: no data.

Table 6. Univariate and multivariate risk factor analysis by malaria species.

Characteristics	%	<i>P. vivax</i>						<i>P. falciparum</i>					
		n/N	Univariate		Multivariate		%	n/N	Univariate		Multivariate		
			OR	[95% CI]	AOR	[95% CI]			OR	[95% CI]	AOR	[95% CI]	
Gender													
Female	46.8	183/391	Ref				9.21	36/391	Ref				
Male	45.0	194/431	0.9	[0.7; 1.2]			12.8	55/431	1.5	[0.9; 2.4]	1.5	[0.9; 2.3] °	
Age (years)													
<5	37.0	54/146	Ref		Ref		7.53	11/146	Ref				
5–14	50.0	121/242	1.7	[1.1; 2.6] *	1.7	[1.1; 2.6] *	11.6	28/242	1.8	[0.8; 3.9]	1.7	[0.8; 3.7]	
15–39	47.8	118/247	1.6	[1.0; 2.4] *	1.2	[0.7; 2.0]	10.9	27/247	1.6	[0.7; 3.5]	1.7	[0.7; 3.9]	
≥40	44.9	84/187	1.4	[0.9; 2.1]	0.9	[0.5; 1.7]	13.4	25/187	2.1	[1.0; 4.7] °	2.2	[0.9; 5.4] °	
Main occupation													
None or others	43.86	225/513	Ref		Ref		10.5	54/513	Ref				
Farmer	49.2	152/309	1.3	[1.0; 1.7] °	1.6	[1.0; 2.4] *	12.0	37/309	1.2	[0.8; 1.9]	0.8	[0.4; 1.6]	
Malaria episodes (previous 12 months)													
0	45.7	289/633	Ref				11.1	70/633	Ref				
≥1	46.5	87/187	1.1	[0.8; 1.5]			11.23	21/187	1.0	[0.6; 1.7]			
Travel (previos month)													
No	46.3	341/736	Ref				11.1	82/736	Ref				
Yes	41.9	36/86	0.9	[0.6; 1.4]			10.5	9/86	0.9	[0.4; 2.1]			
Housing structure (number of walls)													
0	47.3	53/112	Ref				10.7	12/112	Ref				
1–3	46.3	244/527	1.0	[0.6; 1.5]			10.4	55/527	1.0	[0.4; 2.3]			
4	44.4	79/178	0.9	[0.5; 1.4]			12.9	23/178	1.4	[0.5; 3.5]			
Indoor residual spraying (previous 12 months)													
No	35.9	19/53	Ref				13.21	7/53	Ref				
Yes	46.5	353/759	1.4	[0.7; 2.5]			10.9	83/759	0.8	[0.3; 2.3]			
Electricity available													
No	44.3	183/413	Ref				11.1	46/413	Ref				
Yes	47.8	193/404	1.0	[0.7; 1.4]			10.9	44/404	0.9	[0.5; 1.6]			

* $p < 0.05$; ° $p < 0.1$; AOR: adjusted odds ratios; OR: crude odds ratios.

5.5. Discussion

This short-term population-based cohort study showed that the local ACD-based strategy in riverine Amazonian communities was able to detect about five times more microscopically-confirmed malaria infections during a high transmission season (HTS) than an intervention based on a single ACD visit. Nevertheless, detected microscopic malaria infections by this strategy only represented 22.8% of the total malaria infections detected by a delayed qPCR analysis of collected blood samples during ACD visits. Indeed, the latter highly sensitive test confirmed that more than half of the participants acquired mono-infections due to endemic *Plasmodium* parasites (*P. vivax*/*P. falciparum* ratio: 5.2/1) at any of the ACD visits, but also evidenced that co-infections by both species would be more common than previously reported in the Peruvian Amazon. Moreover, the clinical follow-up found that most qPCR-positive infections were asymptomatic and sub-microscopic at the time of their detection, and that only a small proportion of them became symptomatic and detectable by LM in following ACD visits.

Four consecutive ACD visits at intervals of 10 days increased the total screened population by LM from 73.4% (on the first ACD visit) to 88.1% (on any of the four ACD visits). This meant that most individuals had two or more screenings (83.3% of censused people, 94.6% of people with at least one ACD visit) during the intervention period. With this 30-day extended coverage, the local ACD-based strategy detected significantly more individuals with microscopic malaria infections than an intervention

based on a single ACD visit (first visit), included: absent individuals on the first visit (mainly due to travel and economic activities) whose malaria infection was detected by LM at later visits, individuals with latent infections at the first visit who developed microscopically detectable asexual parasites in blood at later visits, individuals with submicroscopic infections at the first visit that had higher parasite density levels detectable by LM on later visits, and individuals that acquired microscopic infections after the first visit [10,58]. The latter case implies that latent periods for both *P. falciparum* and *P. vivax* (i.e., between sporozoite inoculation and development of asexual blood stage) are shorter than the time gap between the first and the fourth ACD visit [163].

The predominance of low-density infections among the total microscopically-confirmed malaria infections anticipated some limitation in the performance of the local ACD-based strategy. The availability of qPCR results at each ACD visit not only confirmed that the local strategy missed 77.2% of the total qPCR-positive infections detected throughout the study period, but also that a single ACD intervention using qPCR would be able to detect about 40% more infections in study participants than four consecutive ACD interventions using LM. Previous studies in the Peruvian Amazon had consistently found that the large majority of prevalent malaria infections by both *P. vivax* and *P. falciparum* could only be detected by highly sensitive diagnostic tests, but their cross-sectional designs did not allow the estimation of the proportion of infections that later exceed the parasite threshold for LM detection [152,153,164]. In our study, the follow-up through consecutive ACD visits found that only a small proportion of sub-patent infections—with positive qPCR and negative

LM results—at the first ACD visit (13.8%, 22/160) became patent and detectable by LM in subsequent visits, suggesting a persistence of sub-microscopic parasite carriers.

This persistence has recently been pointed out as an important contributor for onward transmission in the Amazon region, since it likely harbors a “hidden” human infectious reservoir that remains largely undetected by conventional malaria surveillance, and therefore untreated [30,165]. Indeed, several reports using mosquito feeding-assays have indicated that mosquitoes can be infected with low-density *P. falciparum* and *P. vivax* infections, although less efficiently than with high-density infections [166]. Although conclusive data on the natural history of low-density malaria infections in different endemic settings remain elusive [167], mathematical modelling could be used to understand how the burden and persistence over time of sub-microscopic infections can compensate the lower efficiency of these infections in infecting mosquitoes [168]. Given the unknown measurable parasite density threshold below which transmission cannot occur, WHO recommends in low-transmission and elimination settings that all malaria infections (including those infections with low-density parasitaemia) should be considered as potentially infectious [167].

Data regarding the clinical evolution of malaria infections also showed that some individuals with qPCR-positive infections who were asymptomatic on the first ACD visit became symptomatic over the subsequent 30 days of follow-up, reducing the proportion of asymptomatic infections from about 60% (when only clinical data of the

first visit is considered) to about 40% (when clinical data of all ACD visits is considered). Few longitudinal studies in Amazonia have reported data of this clinical evolution. For instance, a 6-months cohort study in Zungarococha village in the Peruvian Amazon found a high proportion of malaria infections which were asymptomatic at the time of detection but gave rise to malaria symptoms after one week of follow-up, resulting in about 40% of asymptomatic infections among all confirmed cases in study communities [153]. Another study conducted in Northwestern Brazil in 2004–2005 [169], combining PCD and ACD methods over a 14-month period, found that among 93 asymptomatic qPCR-positive infections during surveys, only 10 (10.7%) developed symptoms over the subsequent 2 months of follow-up. More evidence about the clinical evolution of sub-patent infections will be provided soon by population-based longitudinal studies implemented in the Amazonia with the aim of assessing to what extent persistent asymptomatic and sub-patent infections contribute to malaria transmission [68,165].

As in the routine malaria surveillance, the *P. vivax*/*P. falciparum* ratio (5.2/1) in our study confirmed *P. vivax* as the predominant species in Mazan district. In co-endemic areas for both species like Vietnam [170], Brazil [171] and Peru [152], *P. vivax* infections tend to have lower parasitaemia and less symptoms than *P. falciparum* infections as result of different species-specific patterns in the acquisition of host immunity (i.e., earlier and faster development of malaria immunity for *P. vivax* than for *P. falciparum*) [172]. It is not clear how hypnozoite-triggered relapses representing most *P. vivax* infections/recurrences according to modelling estimations [173,174] can influence the development of malaria immunity, but

treatment-time-to-infection studies have reported that most *P. vivax* recurrences were submicroscopic and asymptomatic [172,175,176].

Moreover, lower parasite densities in *P. vivax* infections can also be explained by the inability of *P. vivax* to infect all age classes of erythrocytes in comparison with *P. falciparum* [177]. The lower proportion of asymptomatic and submicroscopic infections among the total qPCR-positive *P. vivax* infections (59.8% and 72.5%) in comparison with that among qPCR-positive *P. falciparum* infections (69.2% and 95.7%, respectively) found in our study reflects the differences in transmission intensity by species in the selected communities during the study period. Indeed, the main criterion for the selection of study communities was the confirmation of a recent and unusual increase of reported malaria cases in communities (which was mainly due to *P. vivax*) with the historically highest malaria risk in Mazan, and this confirmation immediately triggered the onset of ACD interventions. Therefore, it is probable that the study period captured the onset of malaria outbreaks in the communities, caused predominantly by *P. vivax*. Indeed, findings from the risk factor analysis showing the highest risk for *P. vivax* infection in individuals with increased exposure to infective vector bites following outdoor- and mobility-related occupational activities [5] (i.e., adult farmers and children who usually start their participation in these activities as early as at 5–7 years [58,67]), suggested that an important proportion of the total detected *P. vivax* infections in our study might be newly acquired infections rather than hypnozoite-triggered relapses. A different study design such as a treatment-time-to-infection trial incorporating molecular genotyping methods may help to estimate the contribution of relapses/new infections to the total *P. vivax* infections [178,179], however this was not the case in our study.

Occupational mobility patterns could also have facilitated the introduction and spread of new parasite strains across communities [131], leading to an increase of symptomatic and microscopically detectable malaria infections in non-immune individuals when conditions for transmission were favourable [27].

Given the limitations of molecular tests in the detection of very low parasitaemia using blood spot filter papers [180], all qPCR results in ACD visits were taken into account to characterize malaria infections in the participants. In this regard, the detection of mixed/co-infections posed a major challenge since molecular tests like qPCR frequently identify only one species (the species with the most abundant parasite DNA) [181]. Therefore, any change in the species detection by qPCR in consecutive ACD visits in study participants without history of antimalarial treatment (22 participants representing 4.9% of the total qPCR-positive infections) was considered as *P. falciparum*/*P. vivax* co-infection. This operative definition could overestimate the proportion of *P. falciparum*/*P. vivax* co-infections. However, DNA detection of both species in samples of two participants in a same visit (mixed infections) by a highly specific mPCR [161] suggests that *P. falciparum*/*P. vivax* co-infections in some communities of the Peruvian Amazonian are more common than previously reported in the routine malaria surveillance and observational studies [67,152]. Indeed, cross-sectional surveys in endemic communities around Iquitos have found that less than 1% of total infections detected by PCR were mixed infections missed by LM [152], and more recently a study that used PCR to analyse packed red blood cell (PRBC) samples of 450 individuals during a malaria outbreak in two Amazonian communities did not identify any mixed infection [69].

Conclusions

In conclusion, although the local ACD strategy based on repeated and consecutive population screenings using LM increased the opportunity of detecting and treating microscopic and symptomatic malaria infections in selected Amazonian communities during HTS, our study showed that the number of detected infections was considerably lower than the number of detected infections by qPCR consisting mainly of sub-microscopic and asymptomatic infections.

According to our study, a single one ACD using qPCR would be more effective in detecting malaria infections than the four-ACD strategy using LM. This finding should encourage Peruvian malaria stakeholders to assess the feasibility and cost-effectiveness of the incorporation of molecular tests in the malaria case detection activities for reducing malaria transmission and burden (considering different operational criteria for the implementation and malaria transmission scenarios).

These assessments may include non-conventional molecular tests based on loop-mediated isothermal amplification (LAMP) which has recently proven to provide accurate detection of asymptomatic malaria parasite carriers in isolated Amazonian communities that lack of laboratory infrastructure. Alternative interventions like mass drug administration (MDA) using effective treatment combinations for both species could also be evaluated, especially in scenarios where more than half the population acquired the infection over a short time period (outbreaks, peaks of the high transmission season). Mathematical modelling can be used to assess these tools and strategies in terms of geographic scope and long-term feasibility in different malaria endemic settings.

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6. GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES

6.1. General discussion

In order to understand malaria transmission in the Peruvian Amazon, we combined parasitological, entomological, and environmental observations (**Chapter 3**). Despite the heterogeneity across communities, the prevalence of submicroscopic malaria infections was high and mainly due to *Plasmodium vivax*. In addition, housing with incomplete walls was the main risk factor for malaria, and communities in Mazan basin had higher entomological indicators, i.e., human biting rate (HBR) and entomological inoculation rate (EIR), prevalence and incidence rates compared to communities in the Napo basin. Then, considering that routine malaria surveillance is based on passive case detection (PCD) using light microscopy (LM) for malaria diagnosis, we conducted two cross-sectional studies, i.e., a socio-economic survey and a knowledge, attitudes, and practices (KAP) survey, to estimate the economic costs associated with the PCD and management of uncomplicated malaria episodes from both the provider's and patient's perspectives and explore whether healthcare-seeking behaviours significantly influence those costs (**Chapter 4**). Although malaria case management was free, there was a delay in seeking care, and the costs of uncomplicated malaria incurred by families were mainly indirect costs due to loss of working days.

Since the Peruvian Ministry of Health (MoH) improved routine malaria surveillance by the addition to the existing system of an optimized strategy recommended by the World Health Organization (WHO) known as active case detection (ACD) to overcome the limitations of the routine malaria surveillance based on PCD using LM

for detecting both asymptomatic infected individuals and symptomatic individuals that do not attend the health services, a population-based cohort study was conducted to assess the effectiveness of this optimized strategy based on ACD using light microscopy (LM) compared with quantitative real-time polymerase chain reaction (qPCR) (**Chapter 5**). With a single ACD intervention in the communities using qPCR (the first one), it was possible to detect more cases than with four ACD interventions using LM during a whole month of work. Furthermore, qPCR results confirmed that most malaria infections were asymptomatic, submicroscopic, and mainly due to *P. vivax*.

As previously described in other studies conducted in the Peruvian Amazon [5,68,182], our study showed high heterogeneity in malaria transmission (**Chapter 3**), with a significant proportion of asymptomatic and submicroscopic malaria infections mainly caused by *P. vivax* (*P. vivax*/*P. falciparum* ratio: 5/1) (**Chapter 3 and 5**). Hence, ACD-strategy using light microscopy (LM) had low effectiveness (22.8%) compared with qPCR for the detection and treatment of these malaria infections (**Chapter 5**).

Similarly, a previous cross-sectional study conducted in another area in Peru, including ACD and PCD, found that among 144 malaria infections detected by PCR, only 38 (26.3%) were detected by LM [152]. In addition, another cross-sectional study conducted in Brazil found that malaria infections detected by LM were 42.6% (43/101) of the total malaria infections detected by PCR [171]. These studies also reported that among the PCR-positive malaria infections, about 70% were asymptomatic, and 60% were submicroscopic [152,171]. Hence, PCR-based

diagnosis, which is almost three-fold more sensitive than conventional LM in detecting asymptomatic and submicroscopic malaria infections, is an effective test for ACD; however, this test is expensive, complex, and does not provide immediate results.

Another molecular test called loop-mediated isothermal amplification (LAMP) able to detect low-density infections, would be an alternative for malaria diagnosis in remote riverine communities. This test has a relatively low marginal cost, involves less lab equipment, and provide quick results to enable prompt treatment; however, more evidence on the feasibility of its implementation is needed for the incorporation as routine diagnostic tool in malaria control programs [38,183].

Some studies have reported on the role of asymptomatic and submicroscopic parasitemia in malaria transmission. A study conducted in Peru and Brazil using membrane feeding assay showed that asymptomatic and microscopic malaria infections by *P. vivax* were more infectious (28.2% to 79.2%) to *Anopheles darlingi* than submicroscopic malaria infections (12.7% to 24.9%) [184]. Another longitudinal study carried out in Brazil [185] using the same method showed that blood from symptomatic patients infected 43.4% (199/458) of the *An. darlingi* and blood from asymptomatic infected only 2.5% (43/1,719), but several asymptomatic individuals maintained parasitemia for several weeks indicating their potential role as an infectious reservoir. Furthermore, this study showed that blood from asymptomatic patients with submicroscopic parasitemia effectively infected the vector *An. darlingi* with *P. vivax*. For *P. falciparum*, a longitudinal study conducted in Uganda showed that asymptomatic and microscopic infections infected 87.2% (389/446) of the *An. gambiae*, while asymptomatic submicroscopic infections only infected 11.9%

(53/446) [186]. Hence, asymptomatic and submicroscopic infections contribute to the transmission of *P. vivax* and *P. falciparum*.

These types of malaria infections could be targeted by elimination strategies such as mass drug administration (MDA) using a single standard ACT-based treatment to treat the asexual stages of all *Plasmodium* species and a hypnozoitocidal drug (either single-dose tafenoquine or short-course primaquine) for treating uncomplicated malaria by both *P. falciparum* and *P. vivax* [187].

Furthermore, radical cure is recommended in co-endemic areas such as the Peruvian Amazon, since there is an increased risk of *P. vivax* after the treatment of *P. falciparum* [188].

Additionally, the average costs per episode were higher for *P. falciparum* (US\$ 180) than for *P. vivax* (US\$ 156) due mainly to differences in costs of the anti-malarial treatment and in time losses due to illness (**Chapter 4**). A single standard treatment for uncomplicated malaria by *P. vivax* and *P. falciparum* could substantially reduce the economic costs of antimalarial treatment due to its short duration and the potential to improve adherence.

However, this challenge requires to use glucose-6-phosphate dehydrogenase (G6PD) quantitative test to enable the safe malaria treatment with tafenoquine or primaquine [41]. Both primaquine (PQ) and tafenoquine (TFQ) can cause haemolysis in patients with G6PD deficiency, which is an X-linked enzymopathy. Hence, G6PD testing is recommended before treatment with these drugs. In countries that are prescribing primaquine without testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency, as in Peru, it is possible that haemolysis already

occurs, however in previous studies, Peru has reported low prevalence rate (< 2%) of G6PD-deficiency [41,42].

The main difference between PQ and TFQ is the duration of treatment. According to the national guidelines, the dose of PQ is 0.5 mg/kg/day for 7 days [63] resulting in poor adherence [34] while TFQ was approved for radical cure of *P. vivax* malaria in adults 16 years of age and older a single dose of 300 mg [189].

A modelling study showed that adopting a policy of G6PD deficiency screening and provision of supervised high-dose of primaquine (total dose of 7mg/kg) could avoid 6.1 million cases and reduce the global cost of *P.vivax* by US\$93.6 million (from US\$ 359 million to US\$266 million) [190]. However, these costs may be overestimated since the cost of primaquine was calculated based on high-dose primaquine regimen. Low-dose primaquine regimen (total dose of 3.5 mg/kg) used in Peru remain efficacious for the treatment of uncomplicated *P. vivax* infections [191].

On the other hand, MDA should be implemented in combination with good access to case management, effective vector control, and surveillance. There appears to be limited potential for reintroduction because MDA rapidly reduces the prevalence and incidence of malaria in the short term. However, if malaria transmission is not interrupted or importation of malaria is not prevented, transmission eventually returns to its original level once MDA is terminated, unless the vectorial capacity is reduced and maintained at a very low level during the post MDA period [192].

Furthermore, community engagement is a key factor in determining the success of MDA in order to improve participation rates and adherence to the full treatment course of the medicine [193].

Clusters of households with high malaria prevalence (high-high), as those identified in Libertad (LIB), Salvador (SAL) and Urco Miraflores (URC) (**Chapter 3**), could be targeted by focal drug administration [8], especially if those clusters remain stable (similar locations) every year. Considering that between 60-80% of *P. vivax* infections are relapsing infections [194,195], *P. vivax* clusters would indicate the areas with increasing probability to have hypnozoite carriers.

Recently, Peruvian Ministry of Health (MoH) has approved in January 2022, the National Plan towards malaria elimination in Peru 2022-2030, which proposes the use of optimized strategies such as ACD using PCR or LAMP and MDA in delimited geographical areas [196]. The collaborations with different national and international research and control institutions will allow to define the operational criteria for the implementation of these strategies.

High heterogeneity in malaria transmission was influenced by interactions among ecological, human, and entomological factors (**Chapter 3**). Higher prevalence and incidence rates were associated with a higher density of adult *An. darlingi* in the Mazan basin compared with the Napo basin. Similarly, a study conducted in riverine communities of Mazan district found some differences between both Mazan and Napo basins, such as communities located on the bank of Mazan River considered a blackwater river had higher mosquito density and EIR than those on Napo River considered as a whitewater river suggesting that there are physicochemical differences in anopheline larval habitats located in those basins [21].

Houses without complete walls were the main malaria risk factor. Hence, there was not a physical barrier, preventing mosquito entry into homes and poor people were unprotected against *An. darlingi* bites, especially in households without use of long-lasting insecticidal mosquito nets (LLINs). Local initiatives to improve housing conditions should be encouraged because they are improved health and well-being in general. In addition, households close to forest edges were more commonly identified as spatial clusters of malaria prevalence.

Some studies conducted in Thailand [197] and Cambodia [198] suggested that targeted drug administration (TDA) in forest goers could not be feasible as control strategy as forest-goers access the forest using many different entries and exit points.

Another significant aspect of the study area was the limited access to health facilities, which would have increased the time lost due to illness and the uncertainty around this time in the search for treatment by patients and their carers/companions. However, there was a delay in seeking care due to misconceptions, attitudes and practices that interfered with appropriate health-seeking (**Chapter 4**) as the time to diagnosis was more than 48 hours from the onset of symptoms [31] in various individuals who went directly to a health facility or community health worker (CHW) with rapid diagnostic test (RDT) available. Poor healthcare-seeking behaviours can significantly affect the direct and indirect costs associated with malaria case management impacting household budgets due to the lost wages and transportation costs during the care-seeking process and the illness period [199]. By contrast, early and appropriate healthcare-seeking behaviours can save direct costs by stopping

transmission from confirmed malaria infections, and by avoiding additional costs associated with complications of such infections [199,200].

In the malaria transmission scenario described above, the costs of uncomplicated malaria case management from the patient's perspective were higher (US \$131 per episode) than the costs from the provider's perspective (US \$ 30.85 per episode). Although malaria case management was provided free of charge, these costs represented a significant economic burden on rural households and individuals in terms of indirect costs due to the loss of working days. These findings are similar to those from other malaria studies conducted in Colombia [124] and Brazil [125], where the costs can become catastrophic due to the occurrence of several episodes in the same individuals and/or the same households every year. Similarly, a prospective cohort study conducted in Peru showed that even when tuberculosis treatment is free, catastrophic costs were incurred by about 40% of households [201].

6.2. Limitations and strengths

In our research, budget constraints did not allow to include all the 70 riverine communities of Mazan and to visit households more than three times to maximize participation in the socio-economic survey; however, the selected riverine communities (accounting for 80% of malaria incidence) and the cost parameters obtained from the survey likely reflect the case-management's situation in the district. Verbal report of time losses due to illness and costs may affect the accuracy of the cost estimates from the patient perspective. This possibility was reduced with a short recall.

Productivity changes may occur because of absence from work (absenteeism) or because of reduced productivity while at work (presenteeism). This information may be derived from existing data sources, such as registrations from occupational health service companies. Nonetheless, such registrations are typically available only for salaried workers and not for self-employed workers, such as subsistence farmers. From a patient perspective, indirect costs for children were considered zero as they should not be economically active. However, children represent a risk group and as 41% of the population were children, the methods for valuing the productivity costs could lead to biased estimates; this could be due to educational losses.

Mental stress and social costs of families with sick members were not included, which are in general very difficult to evaluate over a short period of time (and are typically not monetised in health economic evaluations).

Since our research was focused on uncomplicated malaria, costs may have been underestimated due to the exclusion of the cost of primaquine-induced haemolysis and the cost of adverse effects. Primaquine-induced haemolysis was not included in any of the estimates due to uncertainty related to its frequency and severity. However, Peru has reported low prevalence rate (< 2%) of G6PD deficiency [42] and generally, uncomplicated malaria treatment is well tolerated.

On the other hand, given the limitations of molecular tests in the detection of very low parasitaemia using blood spot filter papers, all qPCR results in ACD visits were considered to characterize malaria infections in the participants. In this regard, the detection of mixed/co-infections posed a major challenge since molecular tests like qPCR frequently identify only one species (the species with the most abundant parasite DNA). Therefore, any change in the species detection by qPCR in consecutive ACD visits in study participants without history of antimalarial treatment (22 participants representing 4.9% of the total qPCR-positive infections) was considered as *P. falciparum*/*P. vivax* co-infection. This operative definition could overestimate the proportion of *P. falciparum*/*P. vivax* co-infections. However, DNA detection of both species in samples of two participants in a same visit (mixed infections) by a highly specific mPCR suggests that *P. falciparum*/*P. vivax* co-infections are common.

This thesis is the first research work that assessed an optimized strategy based on active case detection (ACD) recommended by the World Health Organization for the detection and treatment of malaria infections conducted in riverine communities in the Peruvian Amazon.

6.3. Conclusions and perspectives

Conclusions

Our study showed high heterogeneity in malaria transmission in riverine communities of Mazan district in the Peruvian Amazon and confirmed that asymptomatic and submicroscopic malaria infections mainly due to *Plasmodium vivax* are common, undetected, and untreated using a standard diagnostic test such as light microscopy due to low effectiveness. In addition, housing with incomplete walls was the main risk factor for malaria, and communities in Mazan basin had higher entomological indicators, prevalence and incidence rates compared to communities in the Napo basin. On the other hand, the economic malaria burden is substantial at the household level with the most significant component arising from lost productivity. Peruvian stakeholders should consider implementing strategies to eliminate both symptomatic and asymptomatic malaria infections.

Perspectives

In this section we list briefly to key stakeholders a series of recommendations, derived from work developed in the thesis.

To the National Malaria Control Programme

- To implement optimized strategies based on ACD using molecular tests added to PCD for reducing malaria burden in the Peruvian Amazon.
- To implement MDA in well-delimited areas i.e., in clusters of high malaria prevalence, with a single treatment using ACT plus primaquine or tafenoquine for uncomplicated malaria by both *P. falciparum* and *P. vivax* infections.
- To introduce routine screening for G6PD deficiency to support safe malaria treatment.
- To increase access to prevention measures such as the delivery of long-lasting insecticide-treated bed nets.
- To promote behaviour change communication (BCC) strategies for improving prevention measures and healthcare-seeking behaviours through developing strong linkages with other ministries and government institutions, including the Ministry of Education.
- To reduce malaria economic burden in poor households by increasing access to case management at the point of care (POC).
- To implement artesunate-mefloquine fixed-dose combination for the treatment of uncomplicated malaria by *Plasmodium falciparum*.

To researchers

- To assess feasibility and cost-effectiveness of the incorporation of molecular tests in optimized active case detection interventions to reduce malaria transmission and burden.
- To assess the feasibility, acceptability and cost-effectiveness of mass drug administration in the Peruvian Amazon to ensure optimal allocation of resources for malaria elimination.
- To assess the cost-effectiveness of G6PD screening.
- To estimate economic costs of severe malaria case management in the Peruvian Amazon.

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8. ANNEXES

Chapter 3. Integrating parasitological and entomological observations to understand malaria transmission in riverine villages in the Peruvian Amazon

Supplementary text 1. Laboratory procedures

Supplementary table 1. Study villages in Mazan

Supplementary table 2. Sociodemographic data and malaria preventive measures in study villages

Supplementary table 3. Household characteristics reported by study participants

Supplementary table 4. Indicators of human exposure to *An. darlingi* estimated from matched analysis of mosquito and human behaviour data in study villages

Chapter 4. Economic Costs Analysis of uncomplicated malaria case management in the Peruvian Amazon

Table S1. Knowledge of household heads on malaria

Table S2. Attitudes of household heads on malaria

Table S3. Malaria prevention practices among household heads

Table S4. Malaria episodes and household by basin

Table S5. Direct cost from the provider perspective for uncomplicated malaria by *P.vivax* in Mazan district 2017 (US\$)

Table S6. Direct cost from the provider perspective for uncomplicated malaria by *P.falciparum* in Mazan district 2017 (US\$)

Table S7. Direct and indirect costs from the patient perspective for uncomplicated malaria by *P.vivax* in Mazan district 2017 (US\$)

Table S8. Direct and indirect costs from the patient perspective for uncomplicated malaria by *P.falciparum* in Mazan district 2017 (US\$)

Chapter 5. Effectiveness of a malaria surveillance strategy based on active case detection during high transmission season in the Peruvian Amazon

Table S1. Evolution of clinical status of malaria infections detected and undetected by ACD-based strategy using light microscopy (LM)

Annexes chapter 3. Integrating parasitological and entomological observations to understand malaria transmission in riverine villages in the Peruvian Amazon

Supplementary text 1. Laboratory procedures

Microscopic diagnosis: Thick and thin blood smears for microscopic diagnosis were stained on two slides with a 10% Giemsa solution. One slide was immediately read in the field site and the second was read the following day at the reference laboratory in Iquitos for diagnosis confirmation. Negative slides were defined as those having no malaria parasites after examining 100 fields, and parasite density in positive slides was estimated assuming a concentration of 8,000 white blood cells (WBC)/ μ l (Ministerio de Salud del Perú, 2010). Expert quality control was done blindly on all positive slides and 10% of randomly chosen negative slides.

Quantitative real-time PCR (qPCR): DNA was extracted from ~6 mm²-pieces of dried blood spots (DBS) using a commercial DNA extraction kit (QIAamp DNA Blood Minikit, Qiagen, Hilden, Germany). A qPCR method targeting the 18S rRNA gene region was applied following the protocol reported by Mangold et al. (Mangold et al., 2005), to obtain melting curves that accurately differentiate *P. vivax* from *P. falciparum*. Diagnosis by qPCR provided by the local research laboratory in Iquitos was based on the result of one qPCR analysis per sample, while the confirmation of an infection provided by the National Research Laboratory in Lima was based on the results of two qPCR analyses per sample. An infection was confirmed with only one positive qPCR which identified either *P. vivax*, *P. falciparum* or mixed infection. In the case of two positive qPCRs with different species identification, the infection was considered to be a mixed infection.

Supplementary table 1. Study villages in Mazan

Village	River	Availability		Time to health centre	Houses	Population	First survey	Second survey	Third survey
		Health post	Community health worker	Minutes	n	N	n	n	n
Libertad	Mazan	Yes	Yes	180	60	297	230	238	211
Visto Bueno	Mazan	No	Yes	360	15	60	51	55	49
Salvador	Napo	No	Yes	45	77	374	327	319	318
Urco Miraflo	Napo	No	No	120	53	284	215	231	222

Supplementary table 2. Sociodemographic data and malaria preventive measures in study villages

	Libertad (N=297)	Visto Bueno (N=60)	Salvador (N=374)	UrcoMiraño (N=284)
Age group (years)*				
- <10	92 (31.0%)	23 (38.3%)	147 (39.3%)	77 (27.1%)
- 10-29.9	95 (32.0%)	20 (33.3%)	96 (25.7%)	103 (36.3%)
- >=30	110 (37.0%)	17 (28.3%)	131 (35.0%)	104 (36.6%)
Gender				
- Female	140 (47.1%)	24 (40.0%)	184 (49.2%)	131 (46.1%)
- Male	157 (52.9%)	36 (60.0%)	190 (50.8%)	153 (53.9%)
Education*				
- None	11 (3.8%)	1 (1.7%)	10 (2.8%)	8 (2.9%)
- Primary school	243 (85.0%)	53 (88.3%)	321 (88.4%)	191 (68.5%)
- Secondary or higher	32 (11.2%)	6 (10.0%)	32 (8.8%)	80 (28.7%)
Occupation*				
- Housewife/student/none	90 (30.5%)	23 (39.0%)	102 (27.6%)	121 (43.4%)
Other	82 (27.8%)	15 (25.4%)	123 (33.2%)	71 (25.4%)
- Forest related (farming, logging)	123 (41.7%)	21 (35.6%)	145 (39.2%)	87 (31.2%)
Malaria in the past year*				
- No	194 (66.2%)	30 (50.0%)	205 (55.7%)	105 (37.9%)
- Yes	99 (33.8%)	30 (50.0%)	163 (44.3%)	172 (62.1%)
At least one LLIN for two people*				
- No	167 (56.2%)	19 (31.7%)	217 (58.0%)	150 (52.8%)
- Yes	130 (43.8%)	41 (68.3%)	157 (42.0%)	134 (47.2%)
Sleep under LLIN the previous night				
- No	72 (24.7%)	11 (18.6%)	91 (24.7%)	54 (19.5%)
- Yes	220 (75.3%)	48 (81.4%)	157 (75.3%)	223 (80.5%)
Indoor residual spraying (past year)*				
- No	48 (16.4%)	21 (35.0%)	297 (79.4%)	38 (13.4%)
- Yes	244 (83.6%)	39 (65.0%)	77 (20.6%)	246 (86.6%)

*p<0.05

Supplementary table 3. Household characteristics reported by study participants

	UrcoMiraño (N=284)	Visto Bueno (N=60)	Libertad (N=297)	Salvador (N=374)
Number of external walls*				
- None	51 (18.0%)	9 (15.0%)	38 (12.8%)	50 (13.4%)
- One-three	110 (38.7%)	25 (41.7%)	181 (60.9%)	177 (47.3%)
- Four	123 (43.3%)	26 (43.3%)	78 (26.3%)	147 (39.3%)
Wall material*				
- No walls	51 (18.0%)	9 (15.0%)	38 (12.8%)	50 (13.4%)
- Wood	216 (76.1%)	41 (68.3%)	247 (83.2%)	285 (76.2%)
- Pona(palm) or other	17 (6.0%)	10 (16.7%)	12 (4.0%)	39 (10.4%)
Floor material*				
- Wood	249 (87.7%)	44 (73.3%)	280 (94.3%)	317 (84.8%)
- Pona or other	35 (12.3%)	16 (26.7%)	17 (5.7%)	57 (15.2%)
Ceiling material*				
- Palm leaves	82 (28.9%)	60 (100.0%)	279 (93.9%)	235 (62.8%)
- Tin (calamina)	202 (71.1%)	0 (0.0%)	18 (6.1%)	139 (37.2%)
Water supply				
- Open well	16 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
- River, rain	268 (94.4%)	60 (100.0%)	297 (100.0%)	374 (100.0%)
Sanitation facility*				
- Inside	9 (3.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
- Outside (latrine, dug)	95 (33.5%)	0 (0.0%)	27 (9.1%)	68 (18.2%)
- No facility, field	180 (63.4%)	60 (100.0%)	270 (90.9%)	306 (81.8%)
Electricity available*				
- No	111 (39.1%)	60 (100.0%)	268 (90.2%)	162 (43.3%)
- Yes	173 (60.9%)	0 (0.0%)	29 (9.8%)	212 (56.7%)

* $p < 0.05$

Supplementary table 4. Indicators of human exposure to *An. darlingi* estimated from matched analysis of mosquito and human behaviour data in study villages*

	UrcoMiraño	Visto Bueno	Libertad	Salvador
Proportion biting indoors	0.242	0.184	0.237	0.493
Behaviour-adjusted exposure - Unprotected individual				
Proportion of vector bites occurring indoors for an unprotected individual	0.881	0.877	0.887	0.923
Proportion of vector bites occurring while asleep for an unprotected individual	0.835	0.819	0.858	0.857
Behaviour- adjusted exposure – LLIN user				
Proportion of remaining exposure occurring indoors for a protected user	0.426	0.447	0.389	0.585
Proportion of human exposure occurring while asleep for a protected user of a LLIN	0.202	0.184	0.232	0.230
Proportion of human exposure occurring indoors but not asleep	0.224	0.263	0.157	0.355
Proportion of all vector bites prevented by using a LLIN	0.793	0.778	0.815	0.814
Behaviour-adjusted exposure - population mean				
Proportion of exposure prevented by current levels of LLIN use in the population	0.581	0.605	0.581	0.600

*Indicators were estimated using the methodology and spreadsheet proposed by Monroe et al. [1] and published data of hourly mosquito and human behaviour between 18:00-06:00 h in study villages [2]. Human behaviour data included LLIN use the night before, and the time to go sleep and wake up indoors. We assumed similar values for both the proportion of people outside houses and the proportion of people inside them but not sleeping. This assumption could overestimate the number of people outdoors in some hours, especially if electricity is not available. We also assumed a conservative personal protection of 90% when using a LLIN based on a report of the effect of permethrin impregnated bednet on blood-feeding behaviour of *An. darlingi* mosquitoes in the Peruvian Amazon [3].

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Annexes chapter 4. Economic costs analysis of uncomplicated malaria case management in the Peruvian Amazon

Table S1. Knowledge of household heads on malaria

Variable (Responded affirmatively)	N=680	%
Knowledge		
Mode of transmission†		
Mosquito bite	501	73.9
Contact with malaria patient	15	2.2
Drinking stagnant water	149	22.0
Contact with animals	1	0.2
Blood transfusion, syringes contaminated with blood	0	100
Symptoms†		
Fever	588	86.7
Chills	518	76.4
Headache	572	84.4
Profuse sweating	20	3.0
Myalgia/arthralgia	381	56.2
General discomfort	121	17.9
Nausea and vomiting	190	28.0
Diarrhea	24	3.5
Abdominal pain	36	5.3
Malaria can be cured with medicaments		
No	38	5.9
Yes	611	94.1
Missing	31	
A patient can have malaria more than once		
No	17	2.5
Yes	655	97.5
Missing	8	
Source of information†		
Friends, neighbours	50	7.4
School	8	1.2
Health workers	430	63.4
Radio or television	61	9.0
Community health worker	153	22.6
My experience/ I suffered from malaria	78	11.5
Non-governmental organization	12	1.8
Family	24	3.5
Poster	8	1.2

†More than one response was possible for these questions

Table S2. Attitudes of household heads on malaria

Variable (Responded affirmatively)	N=680	%
Attitude		
First action when you have symptoms of malaria‡		
Go to a health centre	547	80.6
Go to a community health worker	97	14.3
Go to pharmacy	6	0.9
Go to shop	5	0.7
Self-treat at home drinking herbs	37	5.5
Go to traditional healers	0	100
Getting malaria is common and normal		
No	309	46.5
Yes	355	53.5
Missing	16	
Responsible for malaria control‡		
Health centre/Ministry of health	419	61.6
Community health worker	264	38.8
Myself	51	7.5
Community	6	0.9
Blood smear is necessary to diagnosis		
No	9	1.3
Yes	664	98.7
Missing	7	
Without complete antimalarial treatment‡		
Malaria can lead to severe illness and death	344	50.7
Malaria can return	336	49.6
Transmit to your relatives	10	1.5
Malaria cannot be cured	96	14.2
Symptoms remain	24	3.5
I become weak	11	1.6
Nothing happens	3	0.4
Standing water increase risk of malaria		
No	74	11.3
Yes	583	88.7
Missing	23	
The use of bed net is annoying		
No	617	90.9
Yes	62	9.1
Missing	1	
The presence of mosquitoes bother me		
No	39	5.7
Yes	641	94.3

‡More than one response was possible for these questions

Table S3. Malaria prevention practices among household heads

Variable (Responded affirmatively)	N=680	%
Practices		
First action when you or your relatives had malaria		
Go to a health centre	525	79.3
Go to a community health worker	84	12.7
Self-treat at home drinking herbs	36	5.4
Go to drugstore	3	0.5
Go to shop	4	0.6
Other	10	1.5
Missing	18	
Indoor malaria prevention measures‡		
Use bed net	156	23.0
Use net for windows and doors	2	0.3
Fumigation	26	3.8
Use mosquito repellent	8	1.2
Cleans the house	349	51.4
Do nothing	28	4.1
Drink boiled/chlorinated water	154	22.7
Cover water containers	129	19.0
Hand washing	15	2.2
Wear long-sleeved clothing and long trousers	17	2.5
Outdoor malaria prevention measures‡		
Clean surroundings	397	58.6
Wear long-sleeved clothing and long trousers	51	7.5
Clear the vegetation	145	21.4
Drain stagnant water	74	10.9
Fumigation	4	0.6
Use mosquito repellent	7	1.0
Throw any objects that can accumulate water	157	23.2
Take a shower early	30	4.4
Do nothing	35	5.2

‡More than one response was possible for these questions

Table S4. Malaria episodes and household by basin

Communities by basin	Malaria patients		Malaria episodes								Household	
			<i>P. vivax</i>		<i>P.falciparum</i>		Co-infection*		Total			
	N	%	n	%	n	%	n	%	N	%	N	%
Mazan												
Primero de Enero	14	8.7	15	8.6	2	1.1	0	0.0	17	9.7	8	7.1
Visto Bueno	15	9.3	9	5.1	6	3.4	0	0.0	15	8.6	9	8.0
14 de Julio	6	3.7	4	2.3	2	1.1	0	0.0	6	3.4	4	3.6
Santa Cruz	37	23.0	33	18.9	5	2.9	1	0.6	39	22.3	25	22.3
Libertad	30	18.6	20	11.4	13	7.4	1	0.6	34	19.4	22	19.6
Puerto Alegre	20	12.4	15	8.6	8	4.6	1	0.6	24	13.7	14	12.5
Subtotal	122	75.8	96	54.9	36	20.6	3	1.7	135	77.1	82	73.2
Napo												
Huaman Urco	15	9.3	8	4.6	7	4.0	1	0.6	16	9.1	11	9.8
San Antonio de Zambrano	4	2.5	3	1.7	1	0.6	0	0.0	4	2.3	2	1.8
Urco Miraflores	2	1.2	1	0.6	1	0.6	0	0.0	2	1.1	2	1.8
Bello Horizonte	3	1.9	3	1.7	0	0.0	0	0.0	3	1.7	2	1.8
Puerto Abeja	1	0.6	1	0.6	0	0.0	0	0.0	1	0.6	1	0.9
Tamanco	1	0.6	0	0.0	1	0.6	0	0.0	1	0.6	1	0.9
Puerto Obrero	4	2.5	4	2.3	0	0.0	0	0.0	4	2.3	4	3.6
Salvador	5	3.1	4	2.3	1	0.6	0	0.0	5	2.9	4	3.6
Sucusari	4	2.5	4	2.3	0	0.0	0	0.0	4	2.3	3	2.7
Subtotal	39	24.2	28	16.0	11	6.3	1	0.6	40	22.9	30	26.8
Total	161	100.0	124	70.9	47	26.9	4	2.3	175	100.0	112	100.0

**P. falciparum*-*P. vivax*

Table S5. Direct cost from the provider perspective for uncomplicated malaria by *P.vivax* in Mazan district 2017 (US\$)

Cost category (Units)	Quantity	Unit costs (US\$)	Total costs (US\$)
<i>P.vivax</i> (n=1029)			
Initial consultations			
Screened individuals			
By HF	5,538	2.58	14,288.04
By CHW	1,100	0.62	684.95
Sub-total	6,638		14,972.99
Malaria diagnosis			
Screened individuals by HF			
With LM	4,400	0.71	3,124.00
With RDT	1,138	1.97	2,238.45
Screened individuals by CHW			
With LM	0	0.71	0.00
With RDT	1,100	1.97	2,163.70
Sub-total	6,638		7,526.15
Treatment consultations			
By HF	858	2.58	2,213.64
By CHW	171	0.62	106.48
Sub-total	1,029		2,320.12
Anti-malarial treatment			
Complete treatment (CQ+PQ)			
Adults	580	1.32	766.76
Children	449	0.66	296.79
Sub-total	1,029		1,063.55
Antipyretic drug			
Acetaminophen (doses for 2 days)			
Adults	580	0.09	52.20
Children	449	0.49	220.01
Sub-total	1,029		272.21
Follow-up control			
Control	1,029	3.20	3,295.56
Sub-total	1,029	3.20	3,295.56
Total (US\$)			29,450.57

Abbreviations: HF (Health facility), CHW (Community Health Worker), LM (Light Microscopy), RDT (Rapid Diagnostic Test), CQ (Chloroquine), PQ (Primaquine).

Table S6. Direct cost from the provider perspective for uncomplicated malaria by *P. falciparum* in Mazan district 2017 (US\$)

Cost category (Units)	Quantity	Unit costs (US\$)	Total costs (US\$)
<i>P. falciparum</i> (n=315)			
Initial consultations			
Screened individuals			
By HF	1,696	2.58	4,375.68
By CHW	337	0.62	209.84
Sub-total	2,033		4,585.52
Malaria diagnosis			
Screened individuals by HF			
With LM	1,348	0.71	957.08
With RDT	348	1.97	684.52
Screened individuals by CHW			
With LM	0	0.71	0.00
With RDT	337	1.97	662.88
Sub-total	2,033		2,304.48
Treatment consultations			
By HF	263	2.58	678.54
By CHW	52	0.62	32.38
Sub-total	315		710.92
Anti-malarial treatment			
Complete treatment (AS+MQ+PQ)			
Adults	217	8.76	1,899.84
Children	98	4.38	429.00
Sub-total	315		2,328.83
Antipyretic drug			
Acetaminophen (doses for 2 days)			
Adults	217	0.09	19.53
Children	98	0.49	48.02
Sub-total	315		67.55
Follow-up control			
Control	315	6.41	2,017.69
Sub-total	315	6.41	2,017.69
Total (US\$)			12,014.98

Abbreviations: HF (Health facility), CHW (Community Health Worker), LM (Light Microscopy), RDT (Rapid Diagnostic Test), AS (Artesunate), MQ (Mefloquine), PQ (Primaquine).

Table S7. Direct and indirect costs from the patient perspective for uncomplicated malaria by *P. vivax* in Mazan district 2017 (US\$)

Cost category	Unit	Number of episodes	Average person-days lost	Quantity	Unit costs	Sub-total costs (US\$)
<i>P. vivax</i> (n=1029)						
Direct costs						
Medicaments	Per episode	1029		1029	0.00	0.00
Transport cost for patient	Round trip	1029		1029	2.36	2,428.44
Transport cost for companions	Round trip	1029		1029	1.94	1,996.26
Other cost	Per episode	1029		1029	0.29	298.41
Total direct costs						4,723.11
Indirect costs						
Lost wages for patient						
1 - 4 years old	Person-day	181	2.7	492	0.00	0.00
5 – 14 years old	Person-day	268	4.1	1096	0.00	0.00
>=15 years old	Person-day	580	10.2	5922	9.91	58,685.04
Lost wages for substitute labourers						
No substitute	Person-day	980	0.0	0	0.00	0.00
1 – 4 years old	Person-day	0	0.0	0	0.00	0.00
5 – 14 years old	Person-day	8	3.0	24	0.00	0.00
>=15 years old	Person-day	41	4.8	195	9.91	1,929.97
Lost wages for companions						
No companion	Person-day	166	0.0	0	0.00	0.00
1 – 4 years old	Person-day	0	0.0	0	0.00	0.00
5 – 14 years old	Person-day	8	1.8	14	0.00	0.00
>=15 years old	Person-day	855	7.7	6609	9.91	65,496.68
Total indirect costs						126,111.69
Total costs by <i>P. vivax</i>						130,834.80

Table S8. Direct and indirect costs from the patient perspective for uncomplicated malaria by *P.falciparum* in Mazan district 2017 (US\$)

Cost category	Unit	Number of episodes	Average person-days lost	Quantity	Unit costs	Sub-total costs (US\$)
<i>P. falciparum</i> (n=315)						
Direct cost						
Medicaments	Per episode	315		315	0.00	0.00
Transport cost for patient	Round trip	315		315	2.78	875.70
Transport cost for companions	Round trip	315		315	0.64	201.60
Other cost	Per episode	315		315	0.15	47.25
Total direct costs						1,124.55
Indirect costs						
Lost wages for patient						
1 – 4 years old	Person-day	29	5.0	145	0.00	0.00
5 – 14 years old	Person-day	69	5.4	371	0.00	0.00
>=15 years old	Person-day	217	11.8	2561	9.91	25,375.55
Lost wages for substitute labourers						
No substitute	Person-day	272	0.0	0	0.00	0.00
1 – 4 years old	Person-day	0	0.0	0	0.00	0.00
5 – 14 years old	Person-day	6	7.5	45	0.00	0.00
>=15 years old	Person-day	37	7.4	275	9.91	2,728.02
Lost wages for companions						
No companion	Person-day	81	0.0	0	0.00	0.00
1 – 4 years old	Person-day	0	0.0	0	0.00	0.00
5 – 14 years old	Person-day	6	10.9	66	0.00	0.00
>=15 years old	Person-day	228	6.9	1562	9.91	15,477.44
Total indirect costs						43,581.01
Total costs by <i>P. falciparum</i>						44,705.56

Annex chapter 5. Effectiveness of a malaria surveillance strategy based on active case detection during high transmission season in the Peruvian Amazon

Table S1. Evolution of clinical status of malaria infections detected and undetected by ACD-based strategy using light microscopy (LM)

Time of detection of malaria infections by qPCR	qPCR-positive malaria infections detected by ACD-based strategy using light microscopy (LM)						qPCR-positive malaria infections undetected by ACD-based strategy using light microscopy (LM)					
	Symptomatic				Asymptomatic	Total	Symptomatic				Asymptomatic	Total
	In the same visit n (%)	In following visit (10-d later) n (%)	After 2 visits (20-d later) n (%)	After 3 visits (30-d later) n (%)	n (%)	N	In the same visit n (%)	In following visit (10-d later) n (%)	After 2 visits (20-d later) n (%)	After 3 visits (30-d later) n (%)	n (%)	N
Overall												
1st visit	20 (47.6)	5 (11.9)	2 (4.8)	0 (0)	15 (35.7)	42	56 (40.9)	13 (9.5)	8 (5.8)	2 (1.5)	58 (42.3)	137
2nd visit	5 (25.0)	4 (20.0)	2 (10.0)	ND	9 (45.0)	20	15 (18.5)	8 (9.9)	10 (12.3)	ND	48 (59.3)	81
3rd visit	14 (53.8)	3 (11.5)	ND	ND	9 (34.6)	26	8 (11.9)	8 (11.9)	ND	ND	51 (76.1)	67
4th visit	2 (15.4)	ND	ND	ND	11 (84.6)	13	8 (12.9)	ND	ND	ND	54 (87.1)	62
Total	41 (40.6)	12 (11.9)	4 (4.0)	0 (0)	44 (43.6)	101	87 (25.1)	29 (8.4)	18 (5.2)	2 (0.6)	211 (60.8)	347
<i>P. vivax</i>												
1st visit	17 (44.7)	4 (10.5)	2 (5.3)	0 (0)	15 (39.5)	38	43 (43.9)	9 (9.2)	6 (6.1)	1 (1.0)	39 (39.8)	98
2nd visit	5 (15.6)	4 (12.5)	2 (6.3)	ND	21 (65.6)	32	13 (28.3)	6 (13.0)	6 (13.0)	ND	21 (45.7)	46
3rd visit	14 (42.4)	3 (9.1)	ND	ND	16 (48.5)	33	8 (16.0)	7 (14.0)	ND	ND	35 (70.0)	50
4th visit	2 (15.4)	ND	ND	ND	11 (84.6)	13	6 (12.5)	ND	ND	ND	42 (87.5)	48
Total	38 (39.2)	11 (11.3)	4 (4.1)	0 (0)	44 (45.4)	97	70 (26.8)	22 (8.4)	12 (4.6)	1 (0.4)	156 (59.8)	261
<i>P. falciparum</i>												
1st visit	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3	9 (34.6)	1 (3.8)	0 (0)	1 (3.8)	15 (57.7)	26
2nd visit	0 (0)	0 (0)	0 (0)	ND	0 (0)	0	2 (11.8)	0 (0)	4 (23.5)	ND	11 (64.7)	17
3rd visit	0 (0)	0 (0)	ND	ND	0 (0)	0	0 (0)	1 (12.5)	ND	ND	7 (87.5)	8
4th visit	0 (0)	ND	ND	ND	0 (0)	0	2 (14.3)	ND	ND	ND	12 (85.7)	14
Total	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3	13 (20.0)	2 (3.1)	4 (6.2)	1 (1.5)	45 (69.2)	65
Co-infection*												
1st visit	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1	4 (30.8)	3 (23.1)	2 (15.4)	0 (0)	4 (30.8)	13
2nd visit	0 (0)	0 (0)	0 (0)	ND	0 (0)	0	0 (0)	2 (33.3)	0 (0)	ND	4 (66.7)	6
3rd visit	0 (0)	0 (0)	ND	ND	0 (0)	0	0 (0)	0 (0)	ND	ND	2 (100)	2
4th visit	0 (0)	ND	ND	ND	0 (0)	0	0 (0)	ND	ND	ND	0 (0)	0
Total	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1	4 (19.0)	5 (23.8)	2 (9.5)	0 (0)	10 (47.6)	21

**P. vivax*-*P. falciparum*. ND: no data

SCIENTIFIC CURRICULUM VITAE

Diamantina Moreno-Gutierrez is a medical doctor with background in infectious and tropical diseases, case management and epidemiology. Her research focuses on evaluation of strategies for the detection and treatment of asymptomatic malaria infections in the Peruvian Amazon



Education

2016 – Present	PhD student in Public Health Sciences – Medical Sciences. Université catholique de Louvain – University of Antwerp. Belgium
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2007 – 2008	Master's degree in Control of Infectious and Tropical Diseases. Universidad Peruana Cayetano Heredia. Peru
2004	Medical Surgeon. Universidad Nacional de la Amazonía Peruana. Peru
2004	Bachelor's degree in Human Medicine. Universidad Nacional de la Amazonía Peruana. Peru

Employment

2020 – 2021	Sub Investigator. Asociación Civil Selva Amazónica. Iquitos, Peru
2014 – 2017	Resident Medical in Infectious and Tropical Diseases. Regional Hospital of Loreto. Ministry of Health. Peru
2014	Epidemiologist. National Institute of Health. Peru
2011 – 2014	Head of Epidemiological Surveillance. Ministry of Health, Lima. Peru
2011	Consultant “Systematization of entomological surveillance and vector control in selected regions by the PAHO/ ECHO Project”. Pan American Health Organization. Peru
2008 – 2010	Technical Consultant. Global Fund Multi-Country Malaria Project “Malaria control on the cross border areas of the Andean Region: A community based approach”- PAMAFRO. Peru.
2006 – 2007	Medical Manager. Association of Local Community Health Administration Tamanco Viejo. Ministry of Health. Peru
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Publications, abstracts and books

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