

Microscopic and submicroscopic *Plasmodium* infections in indigenous and non-indigenous communities in Colombia.

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Research

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Abstract

Background The indigenous population is considered a highly susceptible group to malaria because they usually live in areas with high Anopheles exposure and poverty, and have low access to health services. There is a great diversity of indigenous communities in Colombia living in malaria-endemic areas; however, the burden of infection in these populations has not extensively studied. This study aimed to determine the prevalence of Plasmodium infections in indigenous and non-indigenous communities in two malaria-endemic areas in Colombia.

Methods A Community-based cross-sectional survey was conducted in seven villages of Turbo and El Bagre municipalities; three of these villages were indigenous communities. All inhabitants of all ages that were willing to participate were included. Sociodemographic and clinical data were recorded as well as household information. The parasitological diagnosis was performed by microscopy and nested PCR. The prevalence of microscopy and submicroscopic infection was estimated. An adjusted GEE model was used to explore risk factors associated with the infection.

Results Among 713 participants, 60.1% were from indigenous communities. Plasmodium spp. was detected in 30 subjects (4.2%, CI 95% 2.9-5.9); from those, 29 were in the indigenous population, 47% of infections were afebrile, and most of them submicroscopic (10/14). Microscopic and submicroscopic prevalence was 2.5% (CI 95% 1.6-3.9) and 1.7% (CI 95% 0.9-2.9) respectively. In El Bagre, all infections occurred in indigenous participants (3.9%, CI 95% 2.2-7.1), and 81% were submicroscopic. By contrast, in Turbo, the highest prevalence occurred in indigenous people (11.5%; CI 95%: 7.3-17.5), but 88.8% were microscopic. Living in an indigenous population increased the incidence rate of infection compared with a non-indigenous population (IRR 19.4; CI 95% 2.3-166.7).

Conclusion There is a high proportion of Plasmodium infection in indigenous communities. A substantial proportion of asymptomatic and submicroscopic carriers were detected. The identification of these infections, not only in indigenous but also in the non-indigenous population, as well as their associated factors, could help to implement specific malaria strategies for each context.

Introduction

Although significant advances have been made towards malaria elimination in several endemic countries, malaria remains a significant public health problem [1]; the World Malaria Report 2018 estimated that the global malaria burden was around 219 million reported cases and 435000 deaths worldwide [2]. Besides, the situation in the Americas comes up with further challenges for control and malaria elimination, given the high proportion of cases by *Plasmodium vivax* [3]. Particularly in Colombia, the number of malaria cases officially reported in 2018 was 63143, being Chocó, Nariño, Cordoba, and Antioquia, the departments with the highest number of malaria cases (27.0%, 20.6%, 15.6%, and 8.8% respectively [4].

In the Americas region, the indigenous population is considered one of the most vulnerable groups to suffer malaria. The highest vulnerability can be explained not only because they live in areas with a high Anopheles bite exposure, but also because they have high poverty rates and usually do not have access to diagnostic and treatment services [5]. Information about the health of these populations is not always collected, so their risk is not well understood, but in general it is known indigenous communities have poor health indicators as compared to non-indigenous populations, including morbidity and mortality due to transmissible diseases, child undernutrition, infant mortality rates, and years of potential life lost [6].

In Colombia, 3.4% of the population is indigenous, and there are around 710 indigenous communities located in 27 departments [7], many of them living in malaria-endemic regions. Between 2009 to 2014 years, 75.8% of the Colombian indigenous population was at risk of being infected by any microorganism, where *Plasmodium* spp caused 46.7% of the total infections [8]. Unfortunately, there are few studies in the indigenous population, so that the risk is not well understood. Only eight of the Twenty-one endemic countries of the Americas Region reported cases of ethnic groups and indigenous peoples in 2014 [5]. Without adequate data, it is difficult to follow up on malaria trends, recognize the risk factors in these communities, and establish malaria control strategies. In Colombia, the majority of malaria cases occurs at the pacific coast and amazon regions and affects mainly Afro-Colombian and indigenous populations [1, 9], during 2018, 62141 cases of malaria were reported, of these, 14714 (23.7%) were in the indigenous population [10]. It has also been reported autochthonous malaria transmission mainly among indigenous communities in Chocó (Pacific coast) [11].

Antioquia was one of the departments with the highest malaria prevalence in Colombia for many years. However, since 2008, the number of cases has decreased markedly from 20511 cases for that year to 4971 in 2017 [12]. This was due to several malaria control strategies implemented between 2007 to 2010, as vector control activities, strengthen of diagnosis network, insecticide-treated bed nets (ITNs) distribution, and chemoprophylaxis. Despite a reduction, a significant proportion of malaria cases is related to gold-mining activities, which play an important role in the maintenance of malaria transmission and are considered as an important barrier to malaria elimination [13]. Like miners, indigenous populations are also considered as an important reservoir of malaria transmission. Unfortunately, these populations have been scarcely studied, and there is not enough information about the Plasmodium prevalence in them.

One of the main challenges for the success of malaria control programs is the early diagnosis and treatment, not only for symptomatic but also for asymptomatic infections, which represent a silent reservoir of parasites [14]. Compare to patients with acute malaria disease, who generally seek treatment in health facilities, people with low-density infections that often are asymptomatic, do not seek medical attention or anti-malarial treatment [15, 16]. These infections can contribute to local transmission in an endemic region [17]. It has been reported that in Peru, 50% of *P. falciparum* and 22% of *P. vivax* asymptomatic infections can harbor gametocytes [18]; similarly, in Colombia, 57% of the samples from asymptomatic volunteers were infective to mosquitoes [19].

In Colombia, the prevalence of low-density infections by *Plasmodium* has been previously explored, finding frequencies of the infection in general population from 2% to 15%, most of these infections were submicroscopic [20–22] and in pregnant women from 1.1% in peripheral to a 2.1% in placental blood [23]. In Urabá Region in Colombia, the prevalence of asymptomatic infections detected by PCR was 2.6% [24]. Together these studies suggest that in low endemic settings such as Colombia, molecular tests are more useful than microscopy to detect this kind of infection [25–27].

Most of the studies about low-density infections in Colombia have been conducted in the general population and pregnant women, but there are not reports in special populations as indigenous people. We aimed to determine the prevalence of microscopic and submicroscopic Plasmodium infections in indigenous and non-indigenous communities in two malaria-endemic areas in Antioquia-Colombia and to explore the associated factors to the Plasmodium infections.

Methods

Study design

A cross-sectional study was conducted in seven endemic malaria villages of Turbo and El Bagre towns between November 2016 to June 2017. Villages in every town were selected based on the historical records of malaria cases, and the accessibility for field staff. Three of these villages were indigenous communities (Los Aguacates and Los Almendros in El Bagre and Arqua in Turbo).

Study sites description

The two towns selected for this study are located in the Antioquia department. Turbo (8° 05' 42'' N, 76° 44' 123'') is located in the Uraba region of Colombia (Figure 1), has an area of 3055 m², an altitude of 2 meters above sea level and an average temperature of 28°C. The main economic activity is banana production and export [28, 29]. El Bagre (7 ° 35'25" N, 74 ° 48'27"), is located in the Bajo Cauca region of Colombia (Figure 1), has an area of 1563 m², an average temperature of 37°C and an altitude of 50 meters above sea level [29, 30]. The main economic activity is gold mining. The annual parasite index (parasite incidence per 1,000 population) reported for 2017 was 0.77 in Turbo and 21.29 in El Bagre, in both, *P. vivax* was the predominated species [12].

Study population and data and sample collection

A census was carried out in each village to know the number of houses and people; all individuals were invited to participate in the study. Individual and household data regarding sociodemographic, self-report of previous malaria episodes, and characteristics of the household were collected. Axillary temperature was measured, and 6mL of whole blood was collected by venipuncture in tubes with anticoagulant EDTA (BD Vacutainer, BD Franklin Lakes, USA). Samples were stored at 4°C until processing at the laboratory of the local hospital in each municipality. The sample was used to perform diagnosis by microscopy using thick and thin blood smears the molecular diagnosis from blood spots on Whatman filter paper #3 (Fisher, Ref 1003–917), and to measure hemoglobin levels using by a HemoCue (Hb 201+, Lake Forest, California).

Laboratory procedures

Conventional microscopy

Field-stained thick and thin blood slides were read in the field by an expert microbiologist according to national guidelines [31]. Parasitaemia was estimated against 200 leukocytes (assuming a standard value of 8,000 leukocytes/μL of blood) and was expressed as parasites/μL (p/μL). A sample was considered negative if, after the examination of 200 microscopic fields at 100x magnification, no parasites were observed. As a quality control, a second reading was performed in all PCR positives samples and 10% of negative ones. Discordant results were solved by a third reading. All participants with positive thick smear received anti-malarial treatment according to national guidelines [31].

Molecular diagnostic

DNA was extracted from a half blood-spot filter (approximately 30 μ L of blood) using the QIAamp DNA Mini Kit following the manufacturer's recommendations (QIAGEN, Germany). Nested PCR was performed as a two-step procedure following the protocol described by Singh et al. [32]. Amplification products were resolved in a 1.5% agarose gel stained with GelRed™ (Biotium, United States) and visualized under UV light. This protocol consists of a universal PCR that detects and amplifies a region of 18S ribosomal gene from the *Plasmodium* genus. Positive samples for *Plasmodium* spp were processed by using species-specific primers for *P. falciparum* and *P. vivax*.

Ethical considerations

This study was reviewed and approved by the Ethics Committee at Medicine school, Universidad de Antioquia in Medellín, Colombia (Record 011 dated 28 July 2016). Before to start the fieldwork, permission from community leaders was approved in each village. Signed informed consent was obtained before the interview and blood sampling for all participants. In the case of individuals <18 years old, an additional informed consent from parents or legal guardians was also obtained..

Statistical analysis

All data from questionnaires and forms were entered into a Microsoft Access database, and statistical analyses were conducted in STATA 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). A description of the population was carried out for both, individual (anemia, sex, age group, occupation, residence time in endemic region, number of malaria episodes, malaria in the previous year, municipality, use of mosquito nets) and housing factors (inhabitants per household, draining of stagnant water, access to electricity, water and sewer system). Frequencies were expressed as numbers and percentages for qualitative variables and medians with the interquartile range for the quantitative ones. The global prevalence of *Plasmodium* infection was estimated by diagnostic tests (microscopy and nPCR), locality (Turbo and El Bagre), and type of community (indigenous and non-indigenous communities). 95% confidence intervals (CI) are shown for each estimation. A generalized estimating equation (GEE) model [33] was used to handle the nested structure of sampled data (713 individuals nested within 212 households), GEE model was fitted for a Poisson family with a logarithmic link and an exchangeable correlation. Crude (IRR) and adjusted (aIRR) Incident Rate Ratios (by occupation and malaria in the previous year) were calculated for the *Plasmodium* infection (outcome) with 95% confidence intervals for each factor.

Results

Socio-demographic and household characteristics

In this study, 713 subjects from 212 houses were enrolled in seven villages of two municipalities in Antioquia department (Figure 1). El Bagre town had more indigenous people ($n = 276$; 38.7%) than Turbo ($n = 157$; 22.0%). The proportion of *Plasmodium* infection in both municipalities was higher in indigenous communities than in non-indigenous communities (6.69% vs. 0.36%).

The characteristics of participants, according to the residence in the indigenous communities, are shown in *Table 1*. There was a slightly higher proportion of anemia in non-indigenous people (15.4%) compared to indigenous (12.7%). The gender and age group distribution were similar in both groups, and the majority of people have lived in an endemic malaria region for more than four years (72.5% and 75% in non-indigenous and indigenous communities respectively). Most of the indigenous people lived in houses with more than five people (59.1%) and had animals in their homes (97%). Additionally, a high proportion of them did not drain standing water in their homes (93%), and 34% had no access to electricity.

Plasmodium infections prevalence

The overall prevalence of *Plasmodium* spp infection was 4.21% (95% CI 2.95%–5.96%), 60% of these infections were detected by microscopy, and *P. vivax* was the predominant species (3.09%; 95% CI 2.04%–4.65%). The prevalence of infection was higher in Turbo compared to El Bagre town (5.67%, 95% CI 3.64%–8.73% versus 2.91%, 95% CI 1.62%–5.19% respectively); most of the infections in Turbo were detected by microscopy (16/19, 84.2%) and 93.75% (15/16) had malaria symptoms (*Table 2*); most of the infections were asymptomatic (temperature <37.5°C) and submicroscopic (9/11). In both towns, the higher prevalence of *Plasmodium* was found in indigenous communities.

Association between Plasmodium infections and risk factors

An analysis of the association of the subjects and household characteristics with Plasmodium infections is shown in Table 3. The prevalence of Plasmodium infections was higher in males (5.7%) than females (2.9%), with an aIRR for the male sex of 2.38 (95% CI: 1.07–5.31). People who have lived in the endemic region for more than four years had a higher prevalence of infection than those living in the endemic region for less time (4.2% and 2.3%, respectively). The infection was also higher in people with previous self-reported malaria as compared to people without malaria history (4.8% and 3.2%, respectively). Furthermore, living in an indigenous community increase the incidence rate of infection as compared to the non-indigenous population (aIRR 17.86, 95% CI: 2.12–150.19). Regarding household factors, it was found that participants without electricity services in their households have a higher rate of Plasmodium infection (aIRR 4.62, 95% CI: 1.89–11.32). The IRR could not be calculated for the association with water and sewage services (the convergence was not achieved in the GEE model); because none of the infected people had access to these services.

Due to living in an indigenous community as well as some characteristics of the houses were associated with a higher incidence rate of Plasmodium infections, a household description by indigenous and non-indigenous communities is detailed in Table 4. A total of 125 from 212 houses were from indigenous communities (59%), 30.4% of these did not have electricity service, and 91.2% did not drain the standing water (91.2%); these proportions were substantially higher in indigenous compared to non-indigenous communities.

Discussion

This study evaluated the prevalence of microscopic and submicroscopic Plasmodium infections in indigenous and non-indigenous communities from Antioquia, Colombia, and its associated factors, to describe the distribution of disease prevalence among heterogeneous populations; this knowledge is necessary to implement proper control strategies for each context [34]. We found that the prevalence of Plasmodium infections was higher in indigenous communities than in non-indigenous communities in both municipalities. Even more, all infections in El Bagre were detected in indigenous communities (11/11), and most of them were asymptomatic and submicroscopic (9/11). On the contrary, most of the infections in indigenous communities in Turbo were symptomatic and microscopic (84.2%).

It is known that malaria in Colombia is characterized to have different transmission intensity in each endemic region [34]; in this way, our findings could be explained for differences in malaria profile in each municipality. Although the general prevalence of malaria in Antioquia has decreased during last years, the number of cases in El Bagre have been higher than in Turbo (from 190.45 cases/1000 people in 2007 to 21.29 cases/1000 people in 2017 in El Bagre and from 61.53 cases/1000 in 2007 to 0.77cases/1000 people in 2017 in Turbo).

As previously reported, malaria immunity is determined by previous Plasmodium exposure, where an anti-disease immunity is first achieved, and as a result, there is a reduction in severe malaria and mortality. Then, an anti-parasitic immunity is slowly acquired and confers protection against high parasitic densities, which in turn protect against the severe form of the disease [35]; this could explain the highest prevalence of submicroscopic infections in El Bagre, where 50.3% of individuals had more than one malaria episode over life compared to 35.8% in Turbo (*Supplementary table 1*). Nevertheless, we could not find an association of this variable with the Plasmodium infections using a GEE analysis. On the contrary, a previous study in Nariño Colombia showed that have suffered more than one malaria episode was associated with an increased risk of having asymptomatic infections (aOR 2.4, 95% CI 1.1–5.4) [22]. These differences could be explained because our model included not only Asymptomatic but also symptomatic infections.

Household factors are also associated with malaria risk [36]. We found that had no access to electricity was associated with an increase in the malaria rate. These findings are in agreement with previous studies that reported that the poorest households had a 29% greater risk of microscopic parasitemia compared to the less poor houses (aRR 1,29; 95% CI 1,07–1,55) [37]. Additionally, lack of household electricity increased the childhood mortality in Rwanda, including malaria mortality (aOR: 1.4, 95% CI: 1.0–1.8) [38]. The above is important because housing quality can affect malaria risk through its effect on house entry of the malaria vector [37].

Taken together, the individual and housing characteristics could help to understand why the indigenous population has a higher prevalence than its counterpart non-indigenous does. Ethnicity is an important determinant of the health conditions of people, influencing morbidity and mortality rates in different ethnic groups and interfering with access to health services for certain population strata 2018 [39]. In Colombia, the exclusion of indigenous people is reflected in poverty rates, lack of land and employment, school desertion, unsatisfied basic needs, the access difficulty to health services, and an epidemiological profile where transmissible diseases exhibit a higher prevalence as than the general Colombian population. [40]. Furthermore, and due to the indigenous population frequently live close to rainforest or wetlands where they have more vector exposure, they have an increased risk of getting sick by vector-borne diseases such as malaria [8].

It is possible to suggest that the diversity of epidemic characteristics of malarial infection among the Colombia subpopulations account for an ideal environment for parasite evolution. In this environment, the parasite can interact at the same time with asymptomatic individuals and susceptible populations from different ethnics and under different public health interventions [34]. We suggest prevention efforts should be population-specific and vary according to the individual, housing, and environmental characteristics. Given the heterogeneity in the prevalence of malaria in Colombia, it is become necessary to target malaria control activities according to each population and context.

This study has some limitations. First, due to cross-sectional design, the association with malaria status should be interpreted with caution, as they do not imply causality. Second, it was not possible to analyze the risk factors for asymptomatic infections exclusively due to the low number of this kind of infection. This last could, in turn, affect the accuracy of confidence intervals for some of the factors analyzed due to the sample size. Third, as we mentioned before, the villages in this study were selected based on the historical records of malaria cases, the distance to the urban area, and the accessibility for field staff, and therefore we did not make a random selection of the villages included in the study. The results of this study cannot be extrapolated to the general population; nevertheless, they are useful to exhibit the problems around the asymptomatic infections in the indigenous and non-indigenous people. Fourth, considering nowadays, there are ultra-sensitive molecular tests for the detection of low-density infections, the prevalence in this study could be underestimated due to the limit of detection of the nPCR used. By last, it is possible that other variables that we did not consider in the GEE model could explain the associated factors to Plasmodium infections. Future studies are required to improve the knowledge that we have about the risk factors of the plasmodium infections in indigenous communities. Despite these limitations, these results are useful to understand malaria transmission in studied places and to suggest prevention efforts according to the individual, housing, and environmental characteristics.

Conclusion

This study reveals that in both municipalities, most of the Plasmodium infections were in indigenous communities. Nevertheless, the infection profile was different for each town. A substantial proportion of asymptomatic and submicroscopic carriers were detected in El Bagre, while most of the symptomatic and microscopic infections were identified in Turbo. Our findings provide an understanding of the key characteristics of asymptomatic, submicroscopic, and microscopic infections in the study population: to live in an indigenous community, have previous malaria episodes and having no access to electricity, sewage system, and water services. The current malaria control efforts could benefit through the application of targeted interventions to indigenous villages using molecular tests to identify submicroscopic reservoirs that could be contributing to malaria transmission. Additionally, the identification of these infections not only in indigenous but also in the non-indigenous population, as well as demographic, social and household factors related to them, could help to implement specific malaria strategies for each context.

List Of Abbreviations

aIRR: adjusted incident rate ratio; aOR: adjusted odds ratio; CI: confidence interval; DNA: deoxyribonucleic acid; GEE: generalized estimating equation; IRR: incident rate ratio; IQR: inter quartile range; ITNs: insecticide-treated bed nets; p/μL: parasite/μL *P. falciparum*; *Plasmodium falciparum*; *Plasmodium spp.*: Plasmodium species; *P. vivax*: *Plasmodium vivax*; PCR: polymerase chain reaction; nPCR: nested polymerase chain reaction; *spp*: species; UV: ultraviolet.

Declarations

Ethical approval and consent to participate

The study was reviewed and approved by the Facultad de Medicina Ethics Committee at the Universidad de Antioquia, Medellín, Colombia (Record 011 dated 28 July 2016). Before starting any study procedure, written informed consent or an informed assent in the case of participants under 18 years old were obtained from each participant. For participants <18 years old additional informed consent from her parents or legal guardian was also obtained.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JM, LZ, AT, CS, AV conceived the design of this study. JM and LZ supervised data collection and work field. JM, LZ and DA analysed the data. JM drafted the manuscript. All authors contributed to the manuscript edit, review and revising, and approved the final version of the manuscript.

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Tables

Table 1. Clinical and demographic characteristics and malaria history in the study population

Characteristic	Non-Indigenous community n= 280		Indigenous community n= 433		Total n=713	
	n	%	n	%	n	%
	Individual characteristics					
Hemoglobin <11mg/dL	43	15.4	55	12.7	98	13.7
Sex						
Female	145	51.8	234	54.0	379	53.2
Age						
<5	27	9.6	43	9.9	70	9.8
5 -15	87	31.1	133	30.7	220	30.9
> 15	166	59.3	255	58.9	421	59.0
Occupation						
Outdoor	152	54.3	207	47.8	359	50.4
Residence time in endemic region						
≥5 years	210	75.0	314	72.5	524	73.5
Self-report of number of symptomatic malaria episodes						
0	106	37.9	173	40.0	279	39.1
1	58	20.7	66	15.2	124	17.4
>1	116	41.4	194	44.8	310	43.5
Self-report of malaria last year						
	12	4.3	51	11.8	63	8.8
Municipality						
Turbo	102	36.4	157	36.3	259	36.3
El Bagre	178	63.6	276	63.7	454	63.7
Use of bed net						
	262	93.6	405	93.5	667	93.5
Household characteristics						
Number of inhabitants per household						
1 - 4	127	45.4	162	37.4	289	40.5
>5	146	52.1	256	59.1	402	56.4
Participants who have animals in their households						
	259	92.5	420	97.0	679	95.2
Participants who draining standing water in their households						
	78	27.9	20	4.6	98	13.7
Participants who have no access to electricity in their households						
	23	8.2	149	34.4	172	24.1
Participants who have no access to water in their households						
	266	95.0	382	88.2	648	90.9
Participants who have no access to sewage system in their households						
	271	96.8	415	95.8	686	96.2

Table 2. Overall Plasmodium prevalence in the study population by diagnostic test.

Municipality	Overall prevalence			Prevalence detected by microscopy and PCR			Prevalence detected only by PCR		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
TOTAL n=713	30	4.21	2.95 - 5.96	18	2.52	1.59 - 3.98	12	1.68	0.96 - 2.94
<i>P. falciparum</i>	6	0.84	0.38 - 1.86	3*	0.42	0.14 - 1.30	3	0.42	0.14 - 1.30
<i>P vivax</i>	22	3.09	2.04 - 4.65	15**	2.10	1.27 - 3.46	7	0.98	0.47 - 2.05
<i>Mixed infection</i>	2	0.28	0.07 - 1.12	0	0.00	0.00 - 0.00	2	0.28	0.07 - 1.12
El Bagre n=378	11	2.91	1.62 - 5.19	2	0.53	0.13 - 2.10	9	2.38	1.24 - 4.52
Indigenous community n=276	11	3.99	2.22 - 7.07	2	0.72	0.18 - 2.86	9	3.26	1.70 - 6.16
Non indigenous community n=102	0	0.00	0.00 - 0.00	0	0.00	0.00 - 0.00	0	0.00	0.00 - 0.00
Turbo n=335	19	5.67	3.64 - 8.73	16	4.78	2.94 - 7.66	3	0.90	0.29 - 2.75
Indigenous community n=157	18	11.46	7.33 - 17.50	16	10.19	6.32 - 16.02	2	1.27	0.32 - 4.98
Non indigenous community n=178	1	0.56	0.08 - 3.91	0	0.00	0.00 - 0.00	1	0.56	0.08 - 3.91

*Median parasitemia (IQR: inter quartile range) [parasites/ μ l]) 360 (40-28186), ** Median parasitemia (IQR) [parasites/ μ l]) 3147 (2805-15615)

Table 3. Association between individual and household characteristics with Plasmodium infections

Characteristic	Infected by Plasmodium				Crude IRR*		Adjusted IRR**	
	No		Yes		IRR	IC 95%	IRR	IC 95%
	n	%	n	%				
Individual factors								
Sex								
Female	368	97.1	11	2.9	1		1	
Male	315	94.3	19	5.7	1.95	0.95 - 4.02	2.38	1.07 - 5.31
Age								
median (IQR)	20	(10 - 37)	17	(11 - 29)	0.99	0.97 - 1.01	0.99	0.97 - 1.01
Age								
<5	67	95.7	3	4.3	1		1	
5 -15	209	95.0	11	5.0	1.29	0.36 - 4.61	0.93	0.20 - 4.48
> 15	405	96.2	16	3.8	0.94	0.27 - 3.22	0.68	0.15 - 3.07
Residence time in endemic region								
median (IQR)	10	(4 - 20)	12	(6 -20)	1.00	0.97 - 1.03	1	0.97 - 1.03
Residence time in endemic region								
<5 years	172	97.7	4	2.3	1		1	
≥5 years	502	95.8	22	4.2	1.64	0.57 - 4.70	1.83	0.55 - 6.08
Episodes number of symptomatic malaria								
median (IQR)	1	(0 -3)	2	(0 -4)	1.02	0.99 - 1.06	1.01	0.98 - 1.05
Episodes number of symptomatic malaria								
0	270	96.8	9	3.2	1		1	
1	120	96.8	4	3.2	0.98	0.30 - 3.21	0.75	0.19 - 2.92
>1	293	94.5	17	5.5	1.77	0.78 - 4.03	1.55	0.59 - 4.10
Self-report of malaria history								
0	270	96.8	9	3.2	1		1	
≥1	413	95.2	21	4.8	1.53	0.70 - 3.36	1.26	0.50 - 3.19
Participants living in the indigenous community								
No	279	99.6	1	0.4	1		1	
Yes	404	93.3	29	6.7	20.77	2.14 - 201.12	17.86	2.12 - 150.19
Use of bed net								
Yes	638	95.7	29	4.3	1		1	
No	42	97.7	1	2.3	0.44	0.05 - 4.10	0.51	0.06 - 4.14
Household factors								
Number of inhabitants per household								
median (IQR)	5	(4 - 6)	4	(4 - 7)	1.08	0.90 - 1.31	1.05	0.88 - 1.27

Number of inhabitants per household										
1 - 4	280	96.9	9	3.1	1				1	
≥5	381	94.8	21	5.2	1.47	0.62	-	3.44	1.38	0.59 - 3.20
Participants who have animals in their households										
No	24	96.0	1	4.0	1				1	
Yes	650	95.7	29	4.3	0.88	0.12		6.51	0.79	0.10 - 5.97
Participants who draining standing water in their households										
Yes	97	99.0	1	1.0	1				1	
No	577	95.2	29	4.8	3.99	0.48	-	32.88	3.90	0.49 - 30.78
Participants who have access to electricity in their households										
Yes	518	97.7	12	2.3	1.00				1	
No	154	89.5	18	10.5	4.94	2.17		11.28	4.62	1.89 - 11.32
Participants who have access to water in their households										
Yes	56	100.0	0	0.0	1				1	
No	618	95.4	30	4.6	CNA***				CNA	
Participants who have access to sewage system in their households										
Yes	18	100.0	0	0.0	1				1	
No	656	95.6	30	4.4	CNA				CNA	

*Incidence Rate Ratio, **Adjusted by occupation and malaria last year, ***Convergence not achieved

Table 4. Household characteristics by indigenous and non-indigenous communities

Variable	Non Indigenous community		Indigenous community		Total	
	n= 87		n= 125		n=212	
	n	%	n	%	n	%
Number of inhabitants						
1 - 4	55	63.2	70	56.0	125	59.0
>5	31	35.6	51	40.8	82	38.7
Possession						
Own	79	90.8	113	90.4	192	90.6
Leased	8	9.2	9	7.2	17	8.0
Electric service						
Yes	77	88.5	84	67.2	161	75.9
No	9	10.3	38	30.4	47	22.2
Water service						
Yes	5	5.7	12	9.6	17	8.0
No	82	94.3	110	88.0	192	90.6
Sewage system						
Yes	3	3.4	4	3.2	7	3.3
No	84	96.6	118	94.4	202	95.3
Kind of water bodies						
Lake	3	3.4	2	1.6	5	2.4
Stagnant rain water	6	6.9	3	2.4	9	4.2
River	77	88.5	114	91.2	191	90.1
Fumigation (vector control program)						
Yes	78	89.7	115	92.0	193	91.0
No	9	10.3	7	5.6	16	7.5
Use of insecticides						
Yes	5	5.7	10	8.0	15	7.1
No	82	94.3	112	89.6	194	91.5
Draining standing water						
Yes	23	26.4	8	6.4	31	14.6
No	64	73.6	114	91.2	178	84.0

Figures

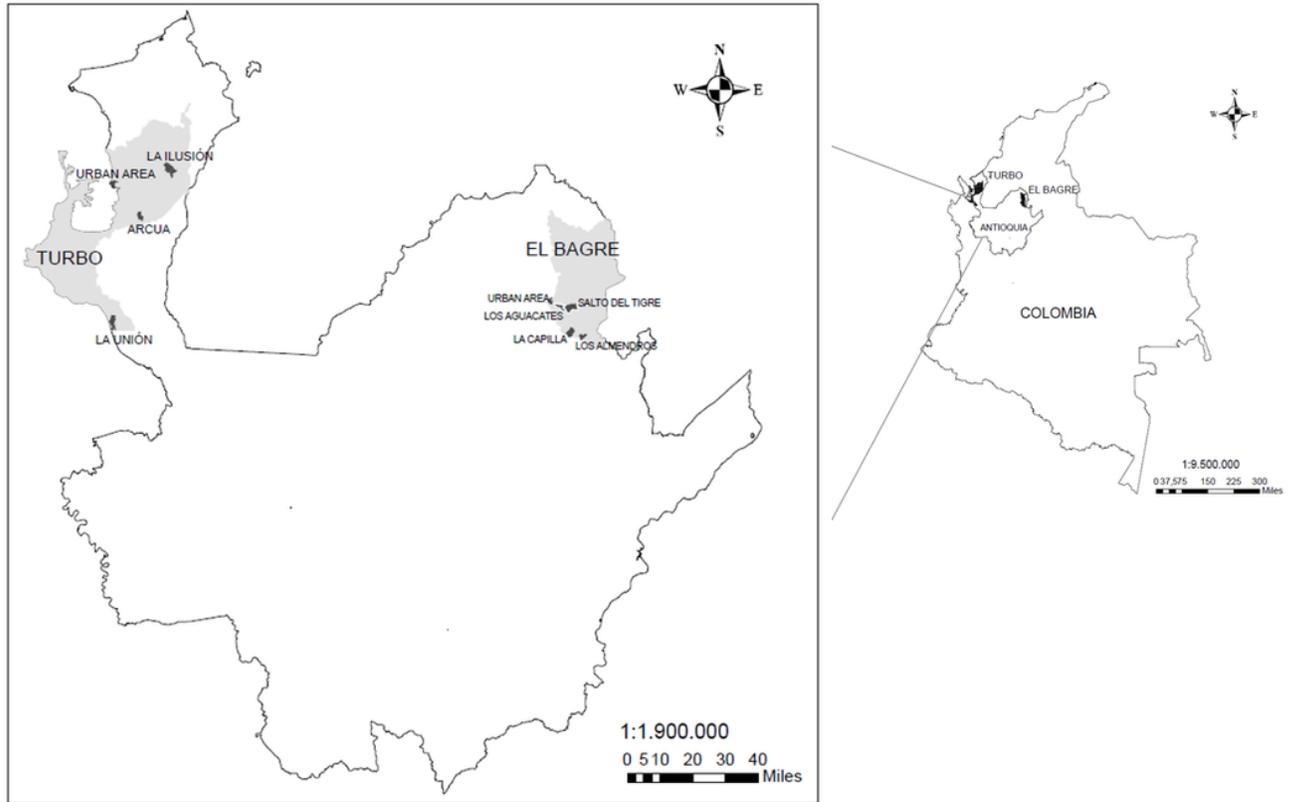


Figure 1
Study sites in Antioquia department, Colombia

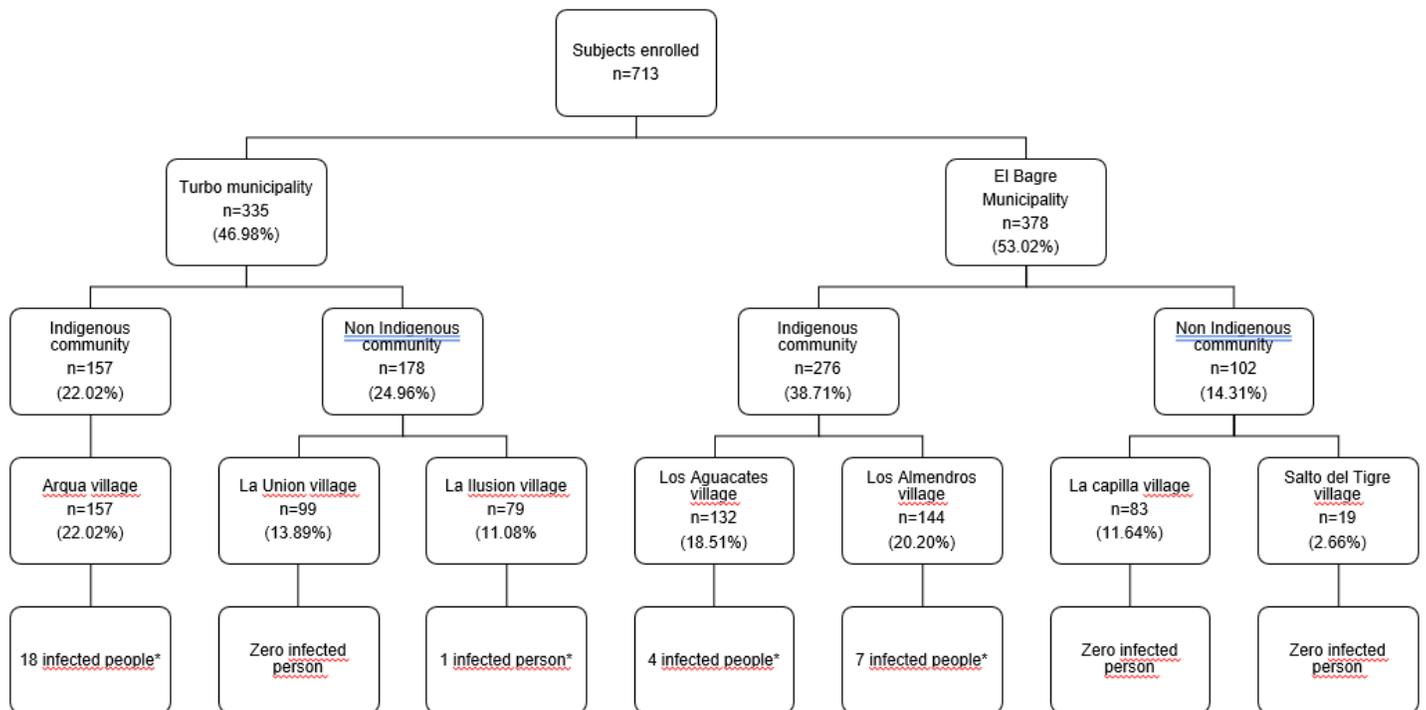


Figure 2
Flowchart of enrolled participants and proportion of infected people by villages. * Plasmodium infections detected by either microscopy or PCR

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