The Acre Project: the epidemiology of malaria and arthropod-borne virus infections in a rural Amazonian population

Projeto Acre: epidemiologia da malária e das arboviroses em uma população rural amazônica

Mônica da Silva-Nunes [1,2]
Rosely dos Santos Malafronte [2,3]
Bruna de Almeida Luz [1]
Estéfano Alves de Souza [1]
Lívia Carício Martins [4]
Sueli Guerreiro Rodrigues [4]
Jannifer Oliveira Chiang [4]
Pedro Fernando da Costa Vasconcelos [4]
Pascoal Torres Muniz [2,5]
Marcelo Urbano Ferreira [1,2]

[1] *Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.*
[2] *Centro de Saúde de Acrelândia, Acrelândia, Brasil.*
[3] *Laboratório de Protozoologia, Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil.*
[4] *Seção de Arbovirologia e Febres Hemorrágicas, Instituto Evandro Chagas, Belém, Brasil.*
[5] *Departamento de Ciências da Saúde, Universidade Federal do Acre, Rio Branco, Brasil.*

Correspondence
M. U. Ferreira
Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo.
Av. Prof. Lineu Prestes 1374, São Paulo, SP
05508-900, Brasil.
muferrei@usp.br

Abstract

The authors describe the baseline malaria prevalence and arbovirus seroprevalence among 467 subjects in an ongoing cohort study in rural Amazonia. Most subjects (72.2%) reported one or more previous episodes of malaria, and 15.6% had been hospitalized for malaria, but only 3.6% of individuals five years or older had malaria parasites detected by microscopy (10 with Plasmodium vivax *and 4 with* P. falciparum*). Antibodies to* Alphavirus, Orthobunyavirus, *and/or* Flavivirus *were detected by hemagglutination inhibition (HI) in 42.6% of subjects aged five years or older, with a higher seropositivity rate among males (49.2%) than females (36.2%). Since 98.9% of subjects had been immunized for yellow fever, the presence of cross-reactive antibodies to dengue and other* Flaviviruses *cannot be ruled out, but at least 12 subjects (3.3%) with IgM antibodies to dengue virus detected by ELISA had a putative recent exposure to this virus.*

Malaria; Dengue; Arboviruses; Arbovirus Infections; Amazonian Ecosystem

Introduction

Since early 2003, the county (municipality) of Acrelândia in the easternmost area of the State of Acre, Brazil, has been the site of a collaborative research project involving the Universidade Federal do Acre (Federal University of Acre – UFAC), the Instituto Evandro Chagas (Evandro Chagas Institute – IEC), and the Universidade de São Paulo (University of São Paulo – USP), aimed at investigating the population's health conditions and proposing possible targets for public health interventions. Acrelândia is located in an area with high malaria transmission, with an annual parasite index (number of malaria cases per year per thousand inhabitants) of 104.3 in 2002 [1]. It is also one of the priority counties in the Amazon Basin for dengue control activities [2]. The follow-up of a cohort of 467 individuals began in March 2004 (including all age brackets) in an agricultural settlement in rural Acrelândia. The principal objective of this prospective study is to identify risk and protective factors associated with acute febrile diseases, particularly malaria and arbovirus infections. The current article describes the baseline malaria prevalence and arbovirus seroprevalence in March-April 2004.

Methodology

Study area and population

Acrelândia, a county (municipality) founded in January 1993 through repartitioning of the counties of Plácido de Castro and Senador Guiomard, has a population of 8,697 (estimated for 2003 by the IBGE, Instituto Brasileiro de Geografia e Estatística, or National Census Bureau, 2000 Census; unpublished data). The county has a territory of 1,607.5km^2 and is located between the Abunã and Iquiri Rivers (the latter also known as the Ituxi) in the Acre River Valley (Figure 1). The estimated human development index (HDI) in 2000 for the total population was 0.680 [3], with an infant mortality rate of 70.75 per thousand live births (IBGE estimate for 1998; unpublished data). The overall illiteracy rate is 26.7%, reaching 31.9% in the rural area [3], which includes 57% of the population. Acrelândia is located 112km east of Rio Branco, capital of the State of Acre, and borders on the counties of Senador Guiomard and Plácido de Castro in Acre as well as the Brazilian States of Amazonas and Rondônia and the country Bolivia (Figure 1).

The study area encompasses rural localities known as Linha 14 Gleba Q and Reserva da Linha 14 (9º41'-9º49'S, 67º05'-67º07'W), demarcated by the Coordinating Body for the Control of Endemics under the Acre State Secretariat of Health and Sanitation (SESACRE). Linha 14 Gleba Q includes both sides of the last 16 kilometers of Linha 14, an unpaved road that begins at the highway connecting Rio Branco to Porto Velho (BR-364); Reserva da Linha 14 includes the edges of an unpaved road that begins at the end (km 30) of Linha 14 and is perpendicular to it, running parallel to the Iquiri River. In this article, these sites will be referred to collectively as Ramal do Granada, the name used by the local population to refer to Linha 14.

Ramal do Granada is located in the largest farming settlement in the State of Acre, the Pedro Peixoto Organized Agricultural Settlement (Figure 1), implemented by the National Institute for Colonization and Agrarian Reform in the mid-1970s. The main economic activities are coffee and banana growing and beef and dairy cattle-raising. There are three malaria diagnostic outposts maintained by the SESSACRE at kilometers 16, 24, and 30 of Ramal do Granada; the largest, at km 16, also has nursing technicians trained in primary health care.

During the census conducted by our fieldwork team, all buildings (both inhabited and uninhabited) in the study area were located (with the aid of maps provided by the local teams from the Coordinating Body for the Control of Endemics under SESACRE) and visited by our team. The household census identified 473 inhabitants (226 males, 247 females), ranging in age from one day to 90 years (mean 23.5 years), distributed in 114 households. All members of these households were considered eligible for entry into our cohort and were invited to participate in the study.

Field study

During the cross-sectional survey from March 16 to April 29, 2004, all households in the study area were visited by the field team (two physicians, a biologist, and a research assistant). The project objectives were explained to each family in order to obtain written informed consent. The following procedures were also performed: (a) application of an extensive questionnaire to obtain demographic, socioeconomic, and prior disease history data and a detailed description of the households, (b) clinical examination of all household members by a field team physician, (c) collection of venous blood samples from all household members aged five years or older, and (d) determination of the household's exact location using portable navigation equipment (eTrex®, Garmin International, Olathe, USA), with an accuracy of 15 meters. Data obtained during this cross-sectional survey provide the baseline for the prospective cohort, maintained since then under clinical and epidemiological surveillance for acute febrile diseases.

Microscopic diagnosis of malaria

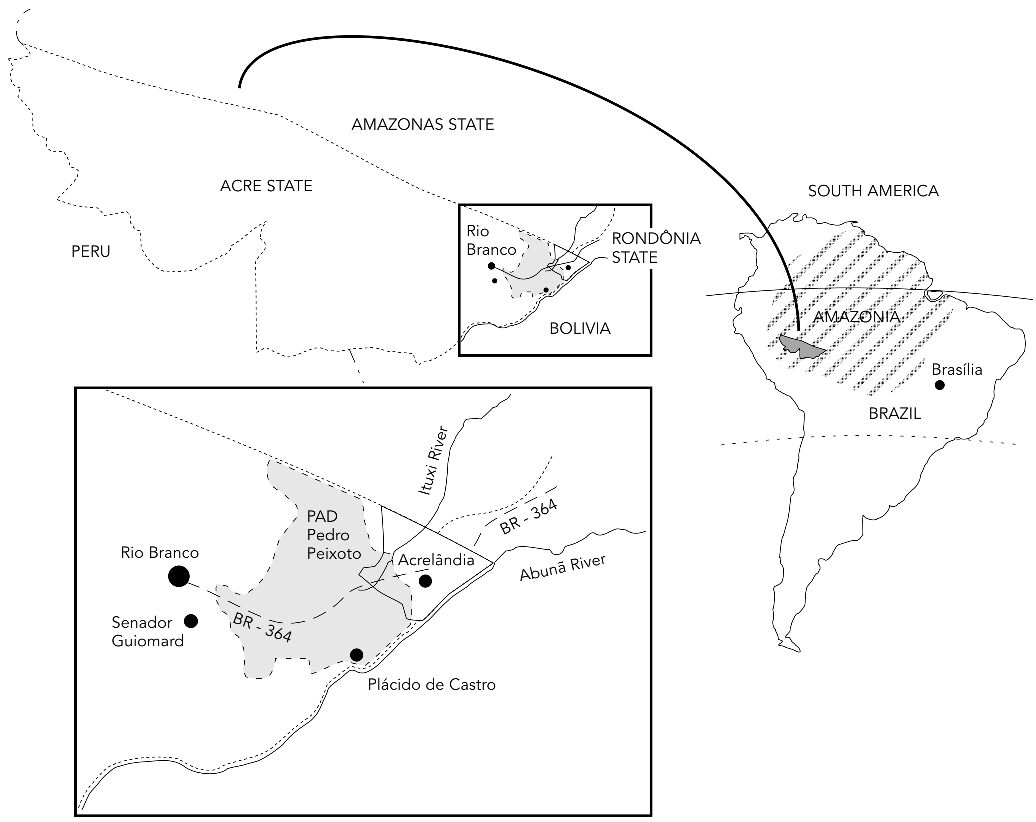
Microscopic diagnosis of malaria used thick blood smears stained with Giemsa according to the Walker technique and thin smears fixed with methanol and stained with Giemsa [4]. At least 200 high-magnification (700x) microscopic fields were examined by two experienced microscopy technicians before defining the result for each slide [5]. Individuals with a malaria diagnosis received free medication provided by the SESACRE based on Ministry of Health treatment protocols [4]. The drugs used were: (a) chloroquine and primaquine for *Plasmodium vivax* and (b) mefloquine or a quinine-doxycycline combination (in addition to primaquine in cases with patent gametocytemia) for *P. falciparum*.

Investigation of arbovirus antibodies

All serological assays were performed at the Division of Arbovirology and Hemorrhagic Fevers

Figure 1

Location of the County (Municipality) of Acrelândia, Acre State, Brazil, and the Pedro Peixoto Organized Settlement Project where the study area is located.



at IEC in Belém, Pará. Serum samples were obtained by centrifuging the venous blood in the field laboratory and storing at -20ºC in dry ice until shipping by airplane to Belém. The samples were initially submitted to microplate hemagglutination inhibition (HI) [6] against standardized antigens from 19 types of arbovirus, including four from genus *Alphavirus* (Eastern equine encephalomyelitis [EEE], Western equine encephalomyelitis [WEE], Mayaro, and Mucambo), nine from genus *Flavivirus* (two samples of yellow fever virus [wild and vaccinal], Ilhéus, São Luís, Rocio, dengue [DEN] 1, DEN-2, DEN-3, and DEN-4), and six from genus *Orthobunyavirus* (Caraparu, Catu, Guaroa, Maguari, Oropouche, and Tacaiuma). Positive sera according to HI (titer ≥ 1:20) against the Mayaro virus were submitted to a confirmatory enzyme-linked immunosorbent assay (ELISA) for IgM antibodies [7]. Regardless of the HI results, the serum samples were also tested by ELISA for IgM antibodies to the dengue and yellow fever viruses.

Statistical analysis

Two or more proportions were compared using the χ^2 or linear trend χ^2 tests. Odds ratios and respective 95% confidence intervals (95%CI) were calculated for the target associations using 2x2 tables. The 95% confidence intervals were obtained for proportions. These analyses used the open-access software Epidat (http://www.paho.org/spanish/sha/epidat.htm; Pan-American Health Organization).

Median exposure time until the first episode of malaria and median age at the first episode, along with their respective 95%CI, were estimated by weighted linear regression models, commonly applied to dose-response type situations in which the proportions of responses are transformed into probits [8]. In this analysis the sample population was stratified by age or time of residence in Amazonia, and an exposure "dose" was attributed to each stratum, corresponding to mean age or time of residence in Amazonia, in years. The "doses" were log-transformed to adjust the probit models. In the probit model, the proportion of individuals in each stratum who reported at least one prior episode of malaria corresponds to the proportion of "responses". The same analytical procedure was applied separately to the proportion of individuals who reported at least one prior episode of *P. falciparum* and *P. vivax* malaria. Calculations used a program written in BASIC [8]. The study adopted a 5% significance level.

Ethical aspects

The research protocol was analyzed and approved by the Research Ethics Committee for Experimentation in Human Beings at the Institute of Biomedical Sciences, USP (reviews 318/ CEP, July 19, 2002, and 538/ CEP, January 7, 2004).

Results

Malaria: prior exposure and prevalence of current infection

The study participants included 98.7% of the 473 eligible individuals identified during the household census; four males and two females refused to participate or were not found in the residences after at least three attempts. The sample population consisted of 467 individuals ranging in age from 1 day to 90 years (mean, 23.3 years) distributed in 113 households, with 42.4% under 15 years of age. The slight predominance of males in the sample population (1.08 males for every female) is similar to that of the overall population in Acrelândia (1.15:1), according to estimates by the IBGE for 2003 (unpublished data). Table 1 shows the sample population distribution by gender and age bracket.

The impact of malaria as a cause of prior morbidity in the sample population can be inferred from the data, obtained through individual interviews and shown in Tables 2 and 3. The vast majority (72.2%) reported at least one previous episode of malaria diagnosed by microscopy and treated (mean of 9 episodes, among the 337 who reported prior malaria), and 73 (15.6%) of these reported at least one hospitalization due to malaria. As shown in Tables 2 and 3, the proportions of individuals with a history at least one episode of malaria correlates positively with age (linear trend χ^2 = 72.70, 1 degree of freedom, $p < 0.00001$) and time of residence in Amazonia (linear trend χ^2 = 88.64, 1 degree of freedom, $p < 0.00001$).

Based on probit models adjusted to the data presented in Table 3, median residence time in Amazonia since the first episode of malaria was estimated at 3.3 years (95%CI: 1.9-4.7 years). In other words, after living 39 months in Amazonia, half of the sample population had already experienced at least one symptomatic malaria infection, confirmed by microscopy and treated. Probit models were also adjusted to the proportions of those reporting at least one episode of malaria attributed to *P. falciparum* and *P. vivax,* resulting in median residence times in Amazonia of 11.9 years (95%CI: 8.3-17.5 years) and 4.8 years (95%CI: 1.8-7.9 years), respectively, for the first documented episode of malaria attributed to each *Plasmodium* species.

The probit model adjusted to data in Table 2 estimated a median age of 4.8 years (95%CI: 3.2-6.4 years) for individuals presenting their first episode of malaria. The probit models were also applied separately to data on history of *P. falciparum* and *P. vivax* infection, resulting in medians of 20.2 years of age (95%CI: 16.2-25.4 years) and 6.4 years (95%CI: 3.7-9.1 years), respectively, at the time of primary infection by these *Plasmodium* species.

Of the 399 individuals five years of age or older, eligible for drawing venous blood samples, 387 (97.0%; mean age 27.4 years) had at least one thick and thin blood smears analyzed by microscopy for malaria diagnosis. Additional capillary blood samples were drawn by finger prick, at the request of the parents or guardians, in 23 children under five years of age, totaling 410 samples submitted to microscopic examination. Of these, 16 were positive for *P. vivax* and four for *P. falciparum* (4.9% positivity rate among the samples analyzed), with no case of co-infection by the two species. No individual with infection diagnosed during the cross-sectional survey, through active search, was classified as asymptomatic. However, three presented no signs or symptoms of malaria at the moment of blood sampling: (a) one adult who presented symptoms during the two weeks following the home visit and appeared at the SESACRE malaria diagnostic outpost for laboratory confirmation; (b) one child who had gone for medical care during the two weeks prior to the home visit due to an ill-defined fever; and (c) a pregnant woman on chloroquine chemoprophylaxis at the time of blood

Table 1

Sample population distribution (n = 467) by gender and age bracket. Ramal do Granada, Acre State, Brazil, 2004.

Age bracket (years)	Female		Male		Total	
	n	%	n	%	n	%
< 1	5	2.2	6	2.5	11	2.4
1-4	27	12.1	30	12.3	57	12.2
5-14	67	29.9	63	25.9	130	27.8
15-30	63	28.1	58	23.9	121	25.9
31-60	53	53.7	71	29.2	124	26.6
> 60	9	4.0	15	6.2	24	5.1
Total	224	100.0	243	100.0	467	100.0

The percentages in each column refer to the proportions of individuals from each gender in each age bracket.

Table 2

Proportions of sample population (n = 467) by age bracket with a history of one or more episodes of malaria. Ramal do Granada, Acre State, Brazil, 2004.

Age bracket (years)	*Plasmodium vivax*		*Plasmodium falciparum*		Either species	
	n	%	n	%	n	%
< 1	1	9.1	0	0.0	1	9.1
1-4	20	35.1	6	10.5	21	36.8
5-14	79	60.8	45	34.6	84	64.6
15-30	82	67.8	67	55.4	102	84.3
31-60	93	75.0	79	63.7	107	86.3
> 60	19	79.2	17	70.8	22	91.7
Total	294	63.0	214	45.8	337	72.2

The percentages in each line refer to the proportion of each age bracket reporting at least one prior episode of malaria diagnosed by microscopy and treated. The numbers of individuals in each age bracket are shown in Table 1.

Table 3

Proportions of sample population (n = 467) according to time of residence in Amazonia, with a history of at least one prior episode of malaria. Ramal do Granada, Acre State, Brazil, 2004.

Time of residence in Amazonia (years)	*Plasmodium vivax*		*Plasmodium falciparum*		Either species	
	n	%	n	%	n	%
< 5 [n = 89]	30	33.7	12	13.5	32	36.0
5-9 [n = 106]	64	60.4	39	36.8	70	66.0
10-19 [n = 175]	126	72.0	93	53.1	143	81.7
20 + [n = 97]	74	76.3	70	72.2	92	94.8
Total [n = 467]	294	63.0	214	45.8	337	72.2

The percentages on each line refer to the proportion of individuals in each stratum reporting at least one prior episode of malaria diagnosed by microscopy and treated.

sampling and who turned symptomatic as soon as prophylaxis was suspended.

Among the 387 individuals five years of age or older who had blood samples taken and examined, 14 (3.6%; 95%CI: 1.6-5.6%) were positive on microscopic examination; ten had *P. vivax* infection and four had *P. falciparum*. Since this age group was sampled systematically (regardless of symptoms), this positivity rate is considered a more adequate estimate of malaria prevalence in the sample population. No statistically significant difference was observed ($p = 0.55$, χ^2 with Yates correction) in overall malaria prevalence between males (4.4%; 95%CI: 1.3-7.5%) and females (2.8%; 95%CI: 0.9-5.4%).

Prevalence of arbovirus antibodies

Of the 399 individuals aged five years of age and older and eligible for venous blood sampling, 357 (89.5%; mean age, 27.5 years) were tested for antibodies to the various types of arbovirus using HI, and 358 (89.7%; mean age 27.5 years) were tested for IgM antibodies to dengue and yellow fever using ELISA. Of these, 15 individuals with antibodies to the Mayaro virus, detected by HI, were tested by ELISA for IgM antibodies to this virus.

A total of 152 samples (42.6%; 95%CI: 37.4-47.9%) were positive according to HI. Of these, 20 (5.6%) presented monotypical reactions (i.e., antibodies to a single type of virus from this genus) to *Alphavirus*, 15 (4.2%) had antibodies to *Orthobunyavirus* (of which 11 were monotypical and four heterotypical), and 65 (18.2%), all of which heterotypical, to *Flavivirus* (including the four dengue serotypes and the wild yellow fever virus). A statistically significant difference was observed ($p = 0.02$, χ^2 test with Yates correction) in the overall prevalence of antibodies to arboviruses comparing males (49.2%; 95%CI: 41.4-56.4%) and females (36.2%; 95%CI: 29.2-43.7%), with an odds ratio of 1.69 for males (95%CI: 1.08-2.64). Table 4 shows the gender and age distribution of individuals with antibodies to one or more of the 19 arboviruses tested; the overall arbovirus seroprevalence, estimated by HI, showed a positive correlation to age (linear trend $\chi^2 = 24.62$, 1 degree of freedom, $p < 0.00001$).

Table 5 shows the number and proportion of individuals with antibodies detected by HI to each type of *Alphavirus* and *Orthobunyavirus* tested. Of these viruses, based on seroprevalance, Mayaro is the only one that appears to circulate widely in the study area. Half of the 18 seropositive individuals from 11 to 49 years of age were natives of the State of Acre and two others had only resided previously in other States belonging to the Legal Amazonia (Rondônia and Mato Grosso). The others (ranging from 31 to 52 years of age) were originally from the South and Southeast of Brazil (States of Espírito Santo, Paraná, and Santa Catarina), but had lived between 13 and 28 years in Amazonia. Six of the seropositive individuals work frequently in timber cutting, an activity that involves frequent contact with the forest environment. However, of the 15 individuals with antibodies to the Mayaro virus and submitted to confirmation for detection of IgM antibodies, only one was positive, suggesting recent infection by this virus (i.e., in the three previous years).

Due to probable exposure of the local population to some *Flavivirus* species (dengue and wild and vaccinal yellow fever), the heterotypical reactions observed in the HI assay with the nine *Flavivirus* tested may be due largely to antibody cross-reactions to one or more of these viruses. Although 98.9% of individuals five years or older reported previous yellow fever immunization, only 66 (18.5%) of the 357 assessed with serology had antibodies (detected by HI) to the 17D vaccinal virus. Only four individuals showed IgM antibodies to the yellow fever virus detected by ELISA; in all cases, the history of vaccination may explain the occurrence of these antibodies.

Fourteen individuals (3.9%; 95%CI: 2.2-6.6%), seven males and seven females (from 17 to 79 years), had IgM dengue virus antibodies detected by ELISA. One had a history of yellow fever vaccination 30 days previously; another had IgM antibodies to the yellow fever virus, a situation in which a cross-reaction cannot be ruled out. In the remaining 12 (3.3% of the tested sample), the presence of specific IgM antibodies was interpreted as indicative of recent dengue infection. Of these 12, nine are relatives, and their households are located within a 2km radius in the study area. Two individuals with dengue IgM antibodies reported previous febrile episodes with clinical diagnosis of dengue (but without serological confirmation), 11 months and two years prior to the study, respectively.

Of the 467 interviewees, 11 (2.3%; six males and five females from 10 to 70 years of age) reported some lifetime diagnosis of dengue. In all of these cases the diagnosis was clinical, without laboratory confirmation. Of these individuals, 10 had serum samples tested by HI. All the results were *Flavivirus* antibody-negative, although two individuals (mentioned above) had dengue IgM antibodies detected by ELISA.

Discussion

Beginning in the 1970s, large-scale projects implemented by the National Institute for Colonization and Agrarian Reform (INCRA) attracted migrants from the South and Southeast of Brazil to Amazonia. The projects were of two types, the so-called Organized Settlement Projects (PAD), aimed at farmers with better technical training and financial resources, and the Integrated Colonization Projects (PIC), targeting low-income groups, specifically landless rural workers [9]. The impact of tropical diseases, especially malaria, on recently arrived migrants has been accurately described in Machadinho D'Oeste, Rondônia [10], but there are only limited data on the living and health conditions in settlements that have been consolidated over the course of several decades, and which now include the majority of the population in farming areas in Amazonia. The current article presents data on a sample population from a large-scale agricultural settlement, collected more than two decades after it was originally occupied.

Malaria

Malaria is still one of the main parasitic endemics in Brazil. From 1970 to the mid-1990s, the annual malaria incidence in Brazil increased ten-fold, then leveled off at around 500 thousand cases a year, of which 99% were acquired in Amazonia. However, in 1999 there was a new peak in incidence, reaching a record of 630 thousand reported cases [11]. In 2000, this situation prompted the Plan for Intensification of Malaria Control Activities in the Amazon Region, aimed at a major reduction in the number of cases in Amazonia in the following two years. This plan led to important decreases in malaria incidence in most of the States, with the important exception of Rondônia. The most recent available data are for 2003, with 350 thousand cases [12]. Despite the large drop in malaria incidence since 2001, Acrelândia is still among the counties at greatest risk in the State of Acre [1]. With less than 2% of the State's population, Acrelândia has nearly 20% of its reported malaria cases.

Malaria prevalence (3.6%) in Ramal do Granada in the late rainy season in 2004 is similar to that described in peri-urban (1.6%) [13] and rural populations (0.5-4.2%) [14] in the neighboring State of Rondônia, consisting predominantly of migrants who settled there at least a decade previously. The prevalence is still far lower than in mining areas (17.3-35.0%) [15,16] and isolated riverine communities (16.9%) [17] in Amazonia. However, the use of more sensitive diagnostic methods than conventional microscopy could show a higher proportion of malaria-infected individuals, with or without symptoms [17,18].

The impact of malaria on the study population in terms of prior morbidity can be assessed by the proportion of individuals reporting at least one episode of diagnosed and treated malaria (72.2% of the sample) and at least one hospitalization due to malaria (15.6%). The historical data confirm the trend in recent years,

Table 4

Positive hemagglutination inhibition for one or more arbovirus types, by age bracket. Ramal do Granada, Acre State, Brazil, 2004.

Age bracket (years)	Individuals tested (n)	Positive individuals (n)	Positive individuals (%)
5-14	111	26	23.4
15-30	103	48	46.6
31-60	116	64	55.2
> 60	27	14	51.9
Total	357	152	42.6

The percentages on each line refer to the proportion of individuals in each age bracket with antibodies to one or more types of arbovirus; only individuals five years or older were tested.

Table 5

Number and proportion of individuals with antibodies detected by hemagglutination inhibition to each type of *Alphavirus* and *Orthobunyavirus*. Ramal do Granada, Acre State, Brazil, 2004.

Virus	Positive	
	n	%
Alphavirus		
Eastern equine encephalomyelitis	1	0.3
Western equine encephalomyelitis	0	0.0
Mayaro	18	5.0
Mucambo	1	0.3
Orthobunyavirus		
Caraparu	4	1.1
Catu	3	0.8
Guaroa	5	1.4
Maguari	0	0.0
Oropouche	6	1.7
Tacaiuma	1	0.3

Only individuals five years or older (n = 357) were tested.
The same individual can have antibodies to more than one virus.

in which *P. vivax* predominated over *P. falciparum* in most of the Brazilian Amazonia [11]; more individuals in the current sample reported previous exposure to *P. vivax* as compared to *P. falciparum* (Tables 2 and 3). The mean time between initial exposure to malaria and primary infection in Ramal do Granada is comparable to the estimate for native Amazonian populations; based on different modeling strategies, and the median age at primary infection among natives in two different areas in the Brazilian Amazon during the last decade was estimated at 4 to 6 years [8,19].

Dengue and other arbovirus infections

Epidemiological data on dengue transmission in Brazil come mostly from large cities in the Southeast, Northeast, and more recently the North of the country [20,21,22,23]. The disease has received little epidemiological, clinical, and laboratory research in small cities [24] or rural areas, and no data are available on dengue transmission dynamics and its main determinants in typical Amazonian counties, even though they represent nearly 10% of what are considered priority Brazilian counties under the Ministry of Health's National Dengue Control Program [2]. However, sero-epidemiological studies in Loreto, in the Peruvian Amazon, show equivalent prevalence rates for dengue IgG antibodies in the urban population in Iquitos (66%) and in adjacent forest-dwelling populations (32-67%) [25], indicating intense circulation of the dengue virus in rural areas. In Southeast Asian countries, recent years have witnessed a predominance of hemorrhagic dengue (a disease until recently limited to large cities) in rural populations [26].

Although the re-introduction of the dengue virus into Brazil in 1981 started from Roraima [27], it was only in 1999 that the dengue epidemic spread throughout Amazonia [2,28]. Acre was the last Amazonian State to report autochthonous dengue cases (DEN-1 and DEN-2), with a major increase (40%) in incidence from 2002 and 2003 (FUNASA, unpublished data). The only available data on prevalence of antibodies to the dengue virus and other arboviruses in populations in Acre refer to a sample in the city of Rio Branco [21].

Joint analysis of serological data and individual history suggest that at least 12 individuals in our sample (3.3% of the tested population) had recent exposure to the dengue virus, although it is impossible to determine the precise site of infection (urban versus rural, in Acrelândia versus other counties). The hypothesis of local transmission is suggested by the fact that nine of the 12 individuals with serology compatible with recent dengue virus infection reside in a geographically limited area close to km 16 of Ramal do Granada. *Aedes aegypti* and *Ae. albopictus* have never been found in entomological surveys in the rural area of Acrelândia (SESACRE, unpublished data); however, these surveys were done somewhat haphazardly and were focused primarily on the winged and aquatic forms of malaria vectors, without prioritizing probable breeding sites for *Aedes*. Theoretically, the increasing population density in Ramal do Granada could allow domiciliary infestation with *Ae. aegypti*, a hypothesis whose proof depends on systematic entomological surveys, currently in the planning phase.

In Ramal do Granada, as well as in Rio Branco [21], the prevalence of antibodies to the vaccinal yellow fever virus (17D) was surprisingly low given the frequency of reported previous immunization. For example, in Rio Branco only 30% of individuals reporting prior yellow fever vaccination showed antibodies to the 17D virus [21]. However, we feel that the proportion of the sample population effectively vaccinated against yellow fever is higher than indicated by the serological data. For example, the State Vaccination Campaign in 1999 vaccinated 416,267 individuals, more than 80% of the population of Acre (SESACRE, unpublished data); since then, various vaccination drives have been held in Ramal do Granada, covering individuals who had still not been born or had not arrived in the area or were not eligible for vaccination in 1999. It is important to highlight that hemagglutination inhibition is not the test of choice for evaluating the immunity conferred by the yellow fever vaccine. The best technique for this evaluation is the plaque reduction neutralization test (PRNT), which was not used in this study. In the future it would be important to use PRNT to investigate the immunity induced by the 17D vaccine, whether in Acrelândia or in other counties in the State of Acre.

Current land occupation patterns in the Amazon Basin favor the emergence of various typically rural arbovirus diseases, including those produced by the Oropouche virus, associated with an epidemic in Xapuri, Acre (in 1996) [29] and the Mayaro virus [30]. However, in Rio Branco [21] and Ramal do Granada (Table 5), the prevalence of antibodies to the types of *Alphavirus* and *Orthobunyavirus* that were investigated is far inferior to that usually described in rural populations of Eastern Amazonia [31]. Although Mayaro has never been isolated in individuals from Acre, it appears to be the only *Alphavirus* circulating with a certain frequency in this State, judging by the available seroprevalence data (Table 5) [21]. This hypothesis is supported by the finding that half of the individuals in our sample with antibodies to the Mayaro virus are native Acreans.

Resumo

*Descrevem-se aqui dados de prevalência de malária e soroprevalência de arboviroses na linha de base de um estudo de coorte, envolvendo 467 indivíduos, atualmente em curso na Amazônia rural. A maioria (72,2%) dos indivíduos relatou um ou mais episódios maláricos prévios, tendo 15,6% deles tido pelo menos uma internação hospitalar motivada pela malária, mas somente 3,6% dos indivíduos com idade igual ou superior a cinco anos tinham plasmódios (*Plasmodium vivax *em dez e* P. falciparum *em quatro) diagnosticados por microscopia. Detectaram-se anticorpos contra* Alphavirus, Orthobunyavirus *e* Flavivirus, *pelo teste de inibição de hemaglutinação, em 42,6% dos indivíduos com idade igual ou superior a cinco anos, com uma taxa de soropositividade maior entre indivíduos do sexo masculino (49,2%) do que os do feminino (36,2%). Como 98,9% dos indivíduos entrevistados tinham sido previamente vacinados contra a febre amarela, a presença de reações cruzadas com o vírus do dengue e outros* Flavivirus *não pode ser descartada, mas a exposição recente ao vírus do dengue foi evidenciada pela detecção de anticorpos IgM por ELISA em 12 indivíduos (3,3%).*

Malária; Dengue; Arbovírus; Infecções por Arbovirus; Ecossistema Amazônico

Contributors

M. da Silva-Nunes, P. F. C. Vasconcelos, and M. U. Ferreira designed the field survey, which was conducted by M. da Silva-Nunes, R. S. Malaffronte, P. T. Muniz, and M. U. Ferreira. L. C. Martins, S. G. Rodrigues, J. O. Chiang, and P. F. C. Vasconcelos performed arbovirus serology, and M. da Silva-Nunes and R. S. Malafronte supervised the malaria microscopy and reviewed all the slides. B. A. Luz and E. A. Souza analyzed the data, under the supervision of M. U. Ferreira. M. da Silva-Nunes, P. F. C. Vasconcelos, and M. U. Ferreira wrote the paper, which was read and approved by all the other authors.

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