Transactions of the Royal Society of Tropical Medicine and Hygiene 104 (2010) 343–350

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Epidemiology and control of frontier malaria in Brazil: lessons from community-based studies in rural Amazonia

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ARTICLE INFO

Article history:
Received 19 May 2009
Received in revised form
21 December 2009
Accepted 22 December 2009
Available online 27 January 2010

Keywords:
Plasmodium vivax
Plasmodium falciparum
Epidemiology
Cohort study
Spatial clustering
Amazonia

ABSTRACT

We describe the epidemiology of malaria in a frontier agricultural settlement in Brazilian Amazonia. We analysed the incidence of slide-confirmed symptomatic infections diagnosed between 2001 and 2006 in a cohort of 531 individuals (2281.53 person-years of follow-up) and parasite prevalence data derived from four cross-sectional surveys. Overall, the incidence rates of *Plasmodium vivax* and *P. falciparum* were 20.6/100 and 6.8/100 person-years at risk, respectively, with a marked decline in the incidence of both species (81.4 and 56.8%, respectively) observed between 2001 and 2006. PCR revealed 5.4-fold more infections than conventional microscopy in population-wide cross-sectional surveys carried out between 2004 and 2006 (average prevalence, 11.3 vs. 2.0%). Only 27.2% of PCR-positive (but 73.3% of slide-positive) individuals had symptoms when enrolled, indicating that asymptomatic carriage of low-grade parasitaemias is a common phenomenon in frontier settlements. A circular cluster comprising 22.3% of the households, all situated in the area of most recent occupation, comprised 69.1% of all malaria infections diagnosed during the follow-up, with malaria incidence decreasing exponentially with distance from the cluster centre. By targeting one-quarter of the households, with selective indoor spraying or other house-protection measures, malaria incidence could be reduced by more than two-thirds in this community.

1. Introduction

Despite five decades of intensive control efforts, malaria remains a major public health concern in Brazil.[1] The annual incidence of malaria experienced a 10-fold increase in this country between 1970 and 1985, associated with the massive migration of non-immune individuals involved in farming, timber extraction and open-cast gold mining in the fringes of the Amazonian rainforest.[2] In 2007, 458 041 slide-confirmed malaria cases were reported countrywide, 99.9% of which were acquired in the Amazon Basin. These figures represent 57.4% of all clinical malaria

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doi:10.1016/j.trstmh.2009.12.010

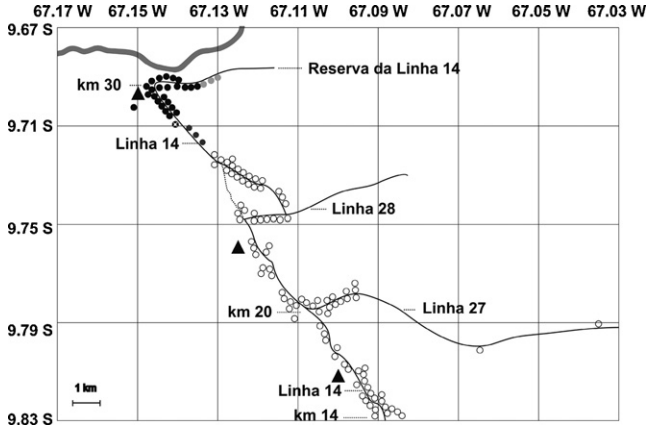

Figure 1. Location of 122 dwellings (comprising 123 households) in Granada, northwestern Brazil. Circles representing each dwelling are either filled with black ($n = 24$), dark grey ($n = 3$) or light grey ($n = 3$) shading, partially filled ($n = 1$) or open ($n = 91$), to indicate whether or not the dwellings were included in high-incidence clusters. Black or partially filled circles represent the cluster of *Plasmodium falciparum* malaria; *Plasmodium vivax* malaria episodes were clustered into dwellings represented as black, partially filled, dark grey or light grey circles. Black or light grey circles represent the cluster of malaria of any type. Black triangles indicate the three malaria diagnosis outposts in the area.

cases recorded in the Americas and the Caribbean in 2007.[3]

Frontier agricultural settlements have played a major role in maintaining malaria transmission in Brazil over the past decades, not only by inducing massive environmental changes that favour human–vector contact but also by clustering non-immune migrants close to vector habitats.[4] We have investigated the transmission dynamics of malaria, between 2004 and 2005, in the Pedro Peixoto Project, one of the largest agricultural settlements of Amazonia. We showed that the continuous arrival of new settlers to the fringes of the original settlement, opened in the 1980s, perpetuates the cycle of environmental change that enhances malaria transmission.[5] Here we analyse malaria morbidity data collected in this community between 2001 and 2006 to describe temporal and spatial patterns of malaria transmission. We discuss the prospects for malaria control in frontier settlements across the Amazon Basin.

2. Materials and methods

2.1. Study area

Granada, in the eastern corner of the state of Acre, northwestern Brazil (Supplementary Figure 1), became part of the Pedro Peixoto Agricultural Settlement Project in 1982. We focus on two adjacent localities in the Granada area: (1) Ramal do Granada, which includes households along the last 16 km of Linha 14, an unpaved road originating from the paved BR-364 highway, and a few dwellings along nearby secondary roads; and (2) Reserva da Linha 14, an area of more recent occupation, which includes all households along a 2.5 km secondary road that runs parallel to the Iquiri River (Figure 1). We located all dwellings using a global positioning system (GPS) receiver, with a positional accuracy within 15 m. The settlement area, mainly inhabited by migrants from Southern and South Brazil, has three government-run malaria diagnosis outposts (represented by black triangles in Figure 1) that provide free malaria diagnosis and treatment. We obtained monthly rainfall data between 2001 and 2006 from the meteorological station in Rio Branco (120 km west of the study site).

2.2. Cohort study

We initiated a population-based prospective cohort study in March 2004 to identify socioeconomic, immunological, genetic and environmental risk factors associated with malaria morbidity in Granada.[5] We analysed malaria morbidity data both prospectively (between March 2004 and October 2006, after the baseline survey) and retrospectively (between January 2001 and February 2004, before the baseline survey).

The outcome analysed in the cohort study was clinical malaria (malaria illness), defined as an episode of parasitaemia confirmed by thick-smear microscopy, irrespective of parasite density, diagnosed in a symptomatic individual through active case detection (ACD) or passive case detection (PCD). We examined all records of slide-confirmed malaria diagnosed at the three diagnosis outposts and in that located in the nearest town, Acrelândia (50 km away from Granada), between January 2001 and October 2006. This strategy is assumed to detect virtually all episodes of symptomatic malaria in cohort subjects, as: (1) there are no other public or private facilities providing laboratory diagnosis of malaria in the area; and (2) microscopic diagnosis is required to obtain antimalarial drugs in Brazil, which are distributed free of charge by the Ministry of Health and cannot be purchased in local drugstores. However, malaria infections diagnosed outside the study area and neighbourhoods may have been missed by our search strategy. A minimal interval of 28 d between two or more consecutive episodes was required to count the latter positive episode as a new, independent malaria infection. When different species were detected in samples obtained less than 28 d apart, the subject was considered to have a single episode of mixed-species infection.

The date of entry in the cohort was considered as: (1) the date of birth; (2) the date of arrival in the study area; or (3) 1 January 2001–whatever was the most recent. Census data obtained at the baseline were updated during three additional cross-sectional surveys of the whole study population (see section 2.3). Subjects who left the study area before October 2006 were considered lost to follow-up since the date they moved away. The dates of entry and exit were considered when estimating the number of person-years at risk during the follow-up.

2.3. Cross-sectional prevalence surveys

The participants in the baseline survey (survey A) aged 5 years of age or older were eligible for contributing venous blood samples for malaria diagnosis, irrespective of any clinical symptoms; 388 blood samples were collected. We define this case-detection strategy as aggressive active case detection (AACD).[6] Additional parasite prevalence surveys of the whole study population aged 5 years or older were carried out in September–October 2004 (survey B, 378 fingerprick blood samples collected), February–March 2005 (survey C, 329 venous blood samples collected) and October–November 2006 (survey D, 351 fingerprick blood samples collected). Of 438 cohort subjects aged 5 years or older, 249 (56.8%) contributed blood samples in all cross-sectional surveys. The outcome measured in cross-sectional surveys was malaria infection, defined as the presence of malaria parasites detected by conventional microscopy, PCR or both, regardless of any symptom at the time of blood collection.

2.4. Laboratory diagnosis of malaria

Giemsa-stained thick blood smears had at least 100 fields examined on site for malaria parasites under 700× magnification by two experienced microscopists. Positive slides and 10% of the negative ones were routinely revised by a reference microscopist in Rio Branco, the capital of Acre. In addition, all slides collected from PCR-positive subjects during the first three cross-sectional surveys (irrespective of the result of on-site microscopy) were sent for review by an expert microscopist at the National Reference Laboratory of the Ministry of Health of Brazil, Brasília. Malaria patients were treated according to therapy guidelines in use at that time (2001–06) in Brazil (chloroquine–primaquine for vivax malaria and mefloquine or quinine–doxycycline followed by primaquine for falciparum malaria). No attempt was made to evaluate the adherence of patients to prescribed drug treatments. During the four cross-sectional surveys, nested PCR-based amplification of a species-specific segment of the *18S rRNA* gene of *Plasmodium falciparum, P. vivax* and *P. malariae* was used, in addition to conventional microscopy, to detect malaria parasites. We used PCR assay protocols described in detail by Win and colleagues.[7]

2.5. Data analysis

A database was created with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Incidence rates were expressed as the number of cases per 100 person-years at risk and their exact Poisson 95% CIs were calculated. Rate ratios were compared with Fisher's exact test. Spatial scan statistics was used to identify significant spatial clusters of malaria episodes, with the version 7.0.3 of SaTScan.[8] This software creates circular windows that are centred on each of the households and moved systematically throughout the geographic space to identify significant clusters of infections, with the maximum window size set to include 50% of the households. SaTScan performs a likelihood ratio test to evaluate whether or not infections are more prevalent within each window compared with the outside. A Poisson model was used, with the null hypothesis that the expected number of cases in each circular window is proportional to the number of person-years of follow-up in that area. We analysed separately: (1) infections with any malaria parasite during the follow-up; (2) infections with *P. falciparum*; and (3) infections with *P. vivax*. *P*-values were determined by 100 000 Monte Carlo replications of the dataset, with the level of significance set at 5%.

3. Results

3.1. Malaria incidence

At the baseline (March–April 2004), 466 consenting dwellers of all age groups (98.5% of the permanent residents in the area) were enrolled; additional individuals (mostly newcomers to the area) were enrolled between September and October 2004 ($n = 43$) and February–March 2005 ($n = 22$). We followed 277 males and 254 females (male:female ratio, 1.09:1) aged from <1 month to 90 years for an average of 4.30 years (range, 30 d to 5.83 years), with a total of 2281.53 person-years of follow-up.

Of 599 slide-confirmed episodes of symptomatic malaria diagnosed between January 2001 and October 2006 (incidence, 26.2/100 person-years at risk), *P. vivax* accounted for 443 (74.0%), *P. falciparum* for 129 (21.5%) and *P. falciparum* and *P. vivax* for 27 (4.5%). Overall, we found *P. vivax* (alone or in mixed-species infections) in 470 episodes (incidence, 20.6/100 person-years at risk; 95% CI 18.8–22.5/100 person-years at risk) and *P. falciparum* (alone or in mixed-species infections) in 156 episodes (incidence, 6.8/100 person-years at risk; 95% CI 5.8–8.0/100 person-years at risk), with a 3.01:1 species ratio (95% CI 2.51:1–3.63:1), similar to that reported for the whole country. No severe or complicated malaria was diagnosed.

Possibly as a result of their more frequent involvement in forest-related high-risk activities,[5] males had a higher malaria incidence (30.7 [95% CI 27.6–34.0] episodes per 100 person-years at risk) than females (21.4 [95% CI 18.7–24.3] episodes per 100 person-years at risk), with a rate ratio of 1.39 (95% CI 1.17–1.64, $P < 0.001$ by Fisher's exact test). As in other areas where both species coexist,[9–11] *P. vivax* incidence peaked earlier than that of *P. falciparum* (Figure 2A), but whether these age-incidence patterns reflect species-specific differences in exposure, acquired immunity or both remains unclear. Because most adults in our cohort are migrants from malaria-free regions, their ages do not translate into cumulative exposure to malaria. We thus examined whether malaria incidence was affected by the

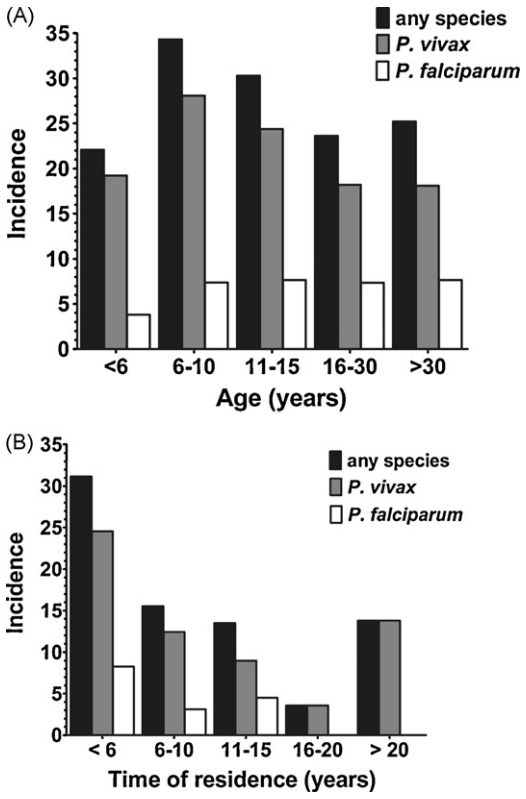

Figure 2. Malaria incidence according to parasite species (number of episodes/100 person-years at risk) in relation to subjects' age (in years) (**A**) or time (in years) of residence in the study area, a proxy of cumulative exposure to malaria (**B**). Numbers of person-years at risk in each age group are: <6 years, 421.5; 6–10 years, 338.1; 11–15 years, 287.2; 16–30 years, 516.6; >30 years, 718.2. Numbers of person-years at risk in the cumulative exposure strata are: <6 years, 1646.2; 6–10 years, 322.1; 11–15 years, 177.91; 16–20 years, 55.9; >20 years, 29.0 (time of exposure missing for 50.4 person-years of follow-up).

time of residence in the study site, a proxy of cumulative exposure to malaria.[5] The incidence of malaria peaked among settlers with less than 6 years of residence in Granada and declined thereafter (Figure 2B), with no *P. falciparum* infection diagnosed among those with more than 15 years of residence in Granada. We have previously shown that the decline in malaria morbidity in Granada, between 2004 and 2005, with increasing time fo residence in the area remained statistically significant after controlling for potential confounding covariates such as the place of residence (newcomers tend to settle in forest fringes, where transmission is more intense) and occupation (newcomers are often involved in high-risk activities, such as land clearing, before they can earn a living from their crops and cattle ranching).[5]

Recurrent episodes of infection with *P. vivax* were more frequent than those with *P. falciparum* (Table 1). Because malaria is transmitted in Granada year-round, recrudescences, relapses and new infections may all originate *P. vivax* recurrences. To address this issue, we used multilocus microsatellite typing to compare haplotypes in 28 pairs of consecutive *P. vivax* infections diagnosed in Granada between 2004 and 2006.[12] Only two pairs of isolates were genetically identical, consistent with recrudescences or relapses originating from the reactivation of homologous hypnozoites; most recurrent infections were either new infections or relapses due to the reactivation of heterologous hypnozoites.

We found no clear-cut temporal association between malaria incidence and monthly rainfall (Figure 3), cumulative rainfall, rainfall anomalies or changes in greenness levels in the study site (Joseph Ssentongo, Maria Gabriela M. Gomes and NSdS, unpublished observations). *Plasmodium vivax* incidence ranged between 7.7 (2005) and 48.3 (2001) episodes/100 person-years at risk, with *P. falciparum* incidence ranging between 3.2 (2003) and 13.3 (2004) episodes/100 person-years at risk. Between 2001 and 2006, *P. vivax* and *P. falciparum* incidence rates decreased by 81.4 and 56.8%, respectively.

3.2. Malaria parasite prevalence in cross-sectional surveys

We examined 1496 thick smears and 1427 DNA samples collected during four cross-sectional surveys. Nested PCR revealed 5.4-fold more infections than conventional microscopy (Table 2), with all samples positive by microscopy also being DNA-positive. Most microscopy-positive samples (17 of 30; 56.7%) had low parasitaemias (<200 parasites/μl of blood). Mixed-species infections were detected by PCR in 22.8% DNA-positive samples; no mixed-species or *P. malariae* infections were diagnosed by microscopy.

3.3. Spatial clustering of malaria

We examined spatial clustering patterns of morbidity data collected yearly and found that both the location and the size of the significant clusters remained remarkably constant over time (data not shown). Figure 1 shows clustering patterns for the whole dataset (2001 to 2006), with a single significant high-incidence cluster ($P < 0.001$)

Table 1
Number of repeated episodes of infection with the same malaria parasite species diagnosed between 2001 and 2006 in Granada, Acre, Brazil

	Number of consecutive infections per subject															
Species	None	1	2	3	4	5	6	7	8	9	11	12	13	14	15	17
P. vivax	353 (66.5)	87	33	18	6	9	9	7	4	2	–	–	2	–	1	–
P. falciparum	432 (81.3)	63	21	11	3	–	1	–	–	–	–	–	–	–	–	–
Total	329 (62.0)	78	46	34	6	7	6	7	7	5	1	1	1	1	1	1

Values in parentheses are percentages.

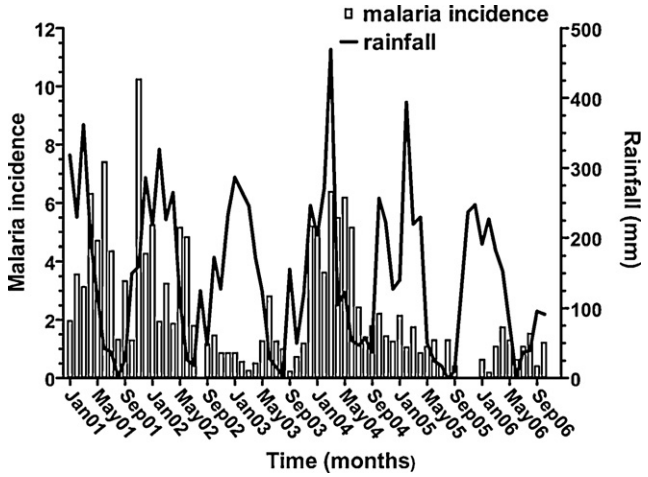

Figure 3. Monthly incidence (number of episodes per 100 person-months at risk) of slide-confirmed malaria in Granada between January 2001 and October 2006 in relation to monthly rainfall (mm) measured in the nearest meteorological station.

with 414 (69.1%) cases of malaria (both species considered) found in 27 dwellings (22.3% of the total) within a 2.2 km radius. *Plasmodium falciparum* and *P. vivax* malaria, when considered separately, were also significantly clustered ($P < 0.001$); 108 (69.2%) *P. falciparum* infections occurred in 25 households (20.7% of the total) within a 2.1 km radius, whereas 348 (74.0%) *P. vivax* infections occurred in 31 (25.6%) households within a 2.3 km radius.

We next analysed household-specific incidence of clinical malaria in relation to the distance between each dwelling and the centre of the circular cluster identified by spatial scan statistics. Malaria incidence between 2001 and 2006 decreased exponentially with distance from the cluster centre, reaching a plateau after 8 km distance (Figure 4). This pattern is consistent with the known dispersal range of the main local vector, *Anopheles darlingi* (5 km or even more),[13] but contrasts with the typical gradient of incidence decline seen in Africa, which extends over no longer than 2–3 km away from the area of highest transmission.[14]

Table 2
Number of malaria infections detected by conventional microscopy and nested PCR during four consecutive cross-sectional surveys of the population aged 5 years or older in Granada, Acre, Brazil

		Cross-sectional survey (date)							
		A (Mar–Apr 2004)		B (Sep–Oct 2004)		C (Feb–Mar 2005)		D (Oct–Nov 2006)	
Symptoms?	Species	Giemsa	PCR	Giemsa	PCR	Giemsa	PCR	Giemsa	PCR
Yes	*P. falciparum*	3	12[a]	5	9[d]	0	2[h]	0	1
	P. vivax	7	14[a]	3	8[d]	4	11[h]	0	0
	No. tested	19	19	17	17	28	28	7	7
No	*P. falciparum*	3	23[b]	1	26[e]	0	20[i]	1	7
	P. vivax	0	16[b]	0	25[e,f]	0	17[i]	3	8
	P. malariae	0	0	0	2[f]	0	0	0	0
	No. tested	369	367	361	362	301	300	344	327
Total	*P. falciparum*	6	35[c]	6	33[g]	0	22[j]	1	8
	P. vivax	7	30[c]	3	35[f,g]	4	28[j]	3	8
	P. malariae	0	0	0	2[f]	0	0	0	0
	No. tested	388	386	378	379	329	328	351	334
Overall parasite prevalence (%)[k]									
	P. falciparum	1.5	9.1	1.6	8.7	0	6.7	0.3	2.4
	P. vivax	1.8	7.8	1.0	9.2	1.2	8.5	0.8	2.4
	P. malariae	0	0	0	0.5	0	0	0	0

[a] Seven *P. falciparum–P. vivax* mixed-species infections included.
[b] Eight *P. falciparum–P. vivax* mixed-species infections included.
[c] Fifteen *P. falciparum–P. vivax* mixed-species infections included.
[d] Three *P. falciparum–P. vivax* mixed-species infections included.
[e] Ten *P. falciparum–P. vivax* mixed-species infections included.
[f] Two *P. falciparum–P. malariae* mixed-species infections included.
[g] Thirteen *P. falciparum–P. vivax* mixed-species infections included.
[h] One *P. falciparum–P. vivax* mixed-species infections included.
[i] Eight *P. falciparum–P. vivax* mixed-species infections included.
[j] Nine *P. falciparum–P. vivax* mixed-species infections included.
[k] Including both single-species and mixed-species infections.

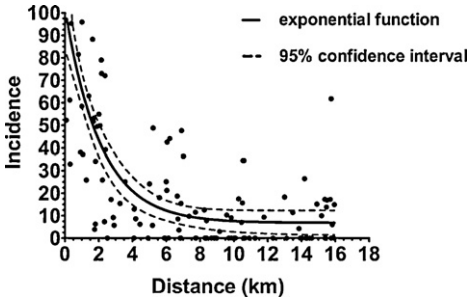

Figure 4. Malaria incidence in households (per 100 person-years at risk) between 2001 and 2006 in relation to the distance (in km) of each dwelling to the centre of the high-incidence cluster of malaria detected by spatial scan statistics. Circles represent individual data points. An exponential function was fitted to the data (coefficient of determination $r^2 = 0.576$).

4. Discussion

Despite the emerging consensus that 'public health needs to be evidence-based if it is to be done correctly',[15] translating scientific evidence into public health interventions remains challenging. Here we focus on two features of frontier malaria with clear implications for designing effective control strategies: (1) the high prevalence of asymptomatic sub-patent infections; and (2) the spatial clustering of malaria.

Non-immune migrants from malaria-free areas settled across the Amazon Basin of Brazil typically fail to achieve the status of clinical immunity usually seen in rural African adults.[16,17] Asymptomatic malaria infections, however, are widespread among native Amazonians[18,19] and may represent a significant source of gametocytes for local vectors.[20] More recent studies also revealed high prevalences of subclinical, chronic malaria infections with very low parasitaemias, most of them detected only by PCR, in typical frontier malaria settings inhabited mostly by migrants, such as gold-mining areas[21] and agricultural settlements (Table 2).[5] Asymptomatic parasite carriers may represent a significant reservoir of malaria, not only in isolated riverine communities of native Amazonians but also in densely populated mining or agricultural settlements, with clear implications for malaria control.[22]

Nearly all malaria infections in Brazil are identified through either ACD or PCD. Cases are found through PCD when febrile subjects attending malaria diagnosis outposts across the country have a blood sample tested positive for malaria parasites. ACD implies periodic visits to households, with collection of thick blood smears from every person having had fever since the last visit.[23] AACD has been limited to a few settings of very high endemicity, such as the Yanomami Indian Reservation.[6] A major limitation of ACD and PCD is that asymptomatic infections and even symptomatic but afebrile infections go undetected and untreated. The clinical spectrum of symptomatic malaria in Granada ranges from a very mild illness to a full-blown disease with periodic fever paroxysms.[24] No fever was reported in 19.1% of 230 laboratory-confirmed episodes of symptomatic malaria detected by ACD or PCD in Granada, although other symptoms (mostly headache or myalgia) were present and individuals felt ill enough to seek malaria diagnosis.[24] Therefore, ACD- and PCD-based strategies of malaria control in Brazil deal with a heterogeneous disease in which cyclical paroxysms with fever, chills and profuse sweating, the hallmark of textbook malaria, are not necessarily prominent features.

Whether asymptomatic infections in Brazil represent a major reservoir that must be addressed by AACD remains a matter of debate.[6,18,22] As AACD is expensive and labour-intensive, a careful cost-effectiveness analysis is required before its large-scale use may be advocated. First, the relative role of asymptomatic infections in maintaining malaria transmission in Amazonia must be quantified. Mathematical models have recently shown that asymptomatic infections represent a crucial target for malaria eradication efforts in Africa,[25] but no similar analyses are available for other endemic areas. Second, laboratory methods appropriate for large-scale use, such as microscopy and rapid diagnostic tests, are poorly sensitive to detect low-grade infections. Previous studies in Brazil show that nested PCR is between 2.7-fold and 8.6-fold more sensitive than conventional microscopy in detecting malaria parasites in apparently healthy individuals.[18,19,21,26] PCR-based diagnosis has been suggested as a public health tool for AACD in Peru,[27] but its use remains severely constrained by its high cost and complexity.

The environmental impact and cost of insecticide-based vector control may be drastically reduced by a careful spatial targeting of house spraying. Because malaria risk clusters in certain households, control measures may target preferentially high-risk areas and individuals.[4,14] Detecting spatial clusters became a feasible goal for countrywide malaria control programmes, with the current availability of cheap GPS receivers and appropriate statistical methods and software for spatial analysis.

Data from our cohort study in rural Amazonia illustrate the spatial clustering of malaria. Spatial scan statistics revealed that 22.3% of the households contribute 69.1% of all malaria cases diagnosed during follow-up (Figure 1). Therefore, by targeting one-quarter of the dwellings we can theoretically reduce malaria incidence by more than two-thirds. Malaria in Granada clusters in the area of most recent occupation with ongoing land-clearing, favouring an increase in the abundance of *A. darlingi*[28] and putting the entire community at greater risk. Similar patterns of spatial clustering have been reported in agricultural settlements in Rondônia,[4] suggesting that this may be a common feature of frontier malaria in Brazil. Further analyses are clearly needed to support spatially targeted malaria control interventions, such as selective spraying of premises, in high-risk rural areas of Brazil. The choice of an insecticide remains a matter of debate; both dichlorodiphenyltrichloroethane (DDT)-based[29] and non-DDT-based[30] strategies have recently been reported to be successful in other endemic settings.

This study has three major limitations. First, malaria episodes may have been missed by our search strategy because patients could have potentially been seen at other health facilities outside Granada and neighbouring areas. Second, malaria incidence estimates were derived from routine microscopic diagnosis, which may be relatively imprecise. The performance of local microscopists

was not directly evaluated by the research team. The rather different proportions of positive results obtained by microscopy and PCR during cross-sectional surveys suggest that microscopy may have a relatively poor performance in areas with relatively little malaria transmission, especially when applied to asymptomatic carriers of low-grade parasitaemias.[18,19,21,26] Third, we were unable to distinguish new infections from late recrudescences (beyond day 28 of treatment) or relapses in our cohort. Despite these limitations, we were able to reveal major features of malaria in a typical agricultural settlement of rural Amazonia, which provide potential targets for control efforts.

Authors' contributions: NSdS, MdSN and MUF designed the study; NSdS, MdSN, RSM, CEC and MUF carried out fieldwork; MdSN, MJM, RRD'A, NTK, KKGS and EMB carried out laboratory work; NSdS managed the dataset and analysed the data with JAC and MUF; MUF drafted the manuscript. All authors contributed to and read and approved the final manuscript. NSdS and MUF are guarantors of the paper.

Acknowledgements: We thank the malaria control team in Granada for logistic support, Adamílson L. de Souza, Valdecir E. da Silva, Camila Juncansen and Vítor D. Muniz for help with fieldwork, Francisco das Chagas O. Luz (Ministry of Health, Brasília), for reviewing malaria slides, and Cassiano P. Nunes, for artwork.

Funding: This work received financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 470067/2004-7) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 03/09719-6 and 05/51988-0). Scholarships from FAPESP (MdSN and RRD'A), CNPq (NSdS, NTK, EMB and MUF) and CAPES (KKGS) are also acknowledged.

Conflicts of interest: None declared.

Ethical approval: The study protocol was approved by the Ethical Review Board of the Institute of Biomedical Sciences of the University of São Paulo, Brazil (318/2002 and 538/2004). Written informed consent was obtained from each adult participant and from the parent or legal guardian of every minor.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2009.12.010.

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