

## Evidence-based public health and prospects for malaria control in Brazil

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### Abstract

Despite intensive control efforts over the past decades, Brazil still accounts for more than 50% of the malaria burden in the Americas and the Caribbean, with 458,041 slide-confirmed cases reported countrywide in 2007. The reason malaria has proved so difficult to control in this middle-income country with a reasonable health infrastructure remains unclear. Here we examine whether four strategies that were largely successful in other countries (aggressive active case detection, improved anti-relapse therapy for *P. vivax* infections, distribution of insecticide-treated bed nets, and selective house spraying with residual insecticides) are likely to work in Brazil. We review evidence from field and laboratory studies and identify gaps in our knowledge that require further investigation with well-designed large-scale trials.

**Key words:** malaria control, Brazil, Amazonia, *Plasmodium falciparum*, *Plasmodium vivax*

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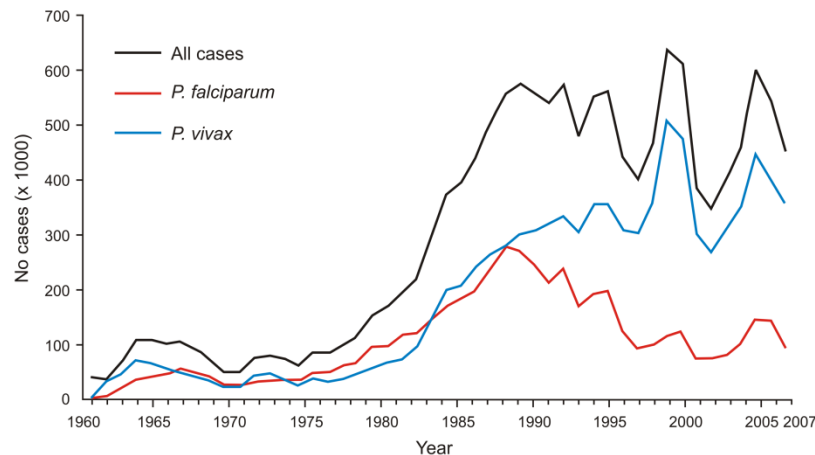
### Introduction

Despite five decades of intensive control efforts [1], malaria remains a major cause of morbidity in Brazil. The annual incidence of malaria experienced a ten-fold increase in this country between 1970 (when only 52,000 cases were recorded) and the mid-1980s (), associated with the massive migration of non-immune subjects to participate in farming, timber extraction, and open-cast gold mining in the fringes of the rainforest [2]. The most recent available data are for 2007, when 458,041 slide-confirmed malaria cases were reported countrywide, 99.9% of which acquired in Amazonia. These figures represent 57.4% of all clinical malaria cases recorded in the Americas and the Caribbean in 2007 [3].

At a time when the Bill and Melinda Gates Foundation, supported by the World Health Organization (WHO), advocates eradication as the ultimate goal of malaria control strategies worldwide [4], the reason malaria remains so difficult to control in Brazil, a middle-income country with low levels of transmission, is still unclear. Some initiatives, such as the Program for Malaria Control in the Amazon Basin (known as PCMAM after the Portuguese acronym), launched in 1999 and financed with US \$73 million from the World Bank [5], had a clear

short-term impact. Malaria morbidity decreased by 60% between 1989 and 1996 [6]. The PCMAM strategy, focused on early diagnosis and treatment of malaria cases to reduce transmission and mortality, was suggested to be more cost-effective than widespread house spraying with residual insecticides [6]. The existing network of malaria outposts was reinforced and expanded across Amazonia to provide early and free diagnosis (based on thick smear microscopy) and free treatment of slide-confirmed malaria episodes (with standardized drug regimens), while house spraying was gradually phased out [7]. However, the gains were not sustained over the next years, since strategies implemented by PCMAM were gradually modified. For instance, (a) federal control of the malaria program was first switched to state control and then to local control; (b) under new employment rules, the human task force was changed on a yearly basis, reducing task efficacy; (c) with increasing case reduction during the first years, early diagnosis was not readily available everywhere. As a result, the number of malaria cases increased by 34% between 1998 and 1999 [1]. Further intensification of malaria control through early diagnosis and treatment [8] once again resulted in a non-sustained decrease in malaria incidence between 2000 and 2002 (Figure 1).

**Figure 1.** Number of slide-confirmed symptomatic malaria episodes recorded yearly in Brazil, by the Ministry of Health, between 1960 and 2007. Ministry of Health of Brazil, unpublished data [46].



A closer look at the recent epidemiological trends of malaria in Brazil reveals clear differences between parasite species. While the annual incidence of *Plasmodium falciparum* (the predominant malaria parasite species between 1985 and 1990) decreased steadily during the 1990s, that of *P. vivax* maintained an upward trend (Figure 1; see also [9,10]). *P. vivax* accounted for 79.6% of the malaria reported in this country in 2007, while *P. falciparum* contributed to nearly 20% of the disease burden. *P. malariae* is currently reported in less than one percent of slide-confirmed infections cases in Brazil, although molecular techniques have recently revealed the presence of this species in 9.4% to 11.9% of patients in selected settings [11,12]. Therefore, as in other parts of the world [13], the failure to reduce malaria incidence in Brazil over the past decade is largely a failure to control *P. vivax* transmission.

Here we discuss whether control measures that have proven successful elsewhere (see [14,15] for recent reviews) are likely to reduce the malaria burden in Brazil. We focus on four strategies (aggressive active case detection, improved anti-relapse therapy for *P. vivax* infections, distribution of insecticide-treated bed nets, and selective house spraying with residual insecticides) (Table 1). We briefly review evidence from field and laboratory studies that either lend support to or do not support the use of these strategies. We also identify gaps in our knowledge that require further investigation with well-designed large-scale trials.

### Early diagnosis and treatment of clinical malaria

Most malaria-exposed people in the Amazon Basin of Brazil are non-immune migrants from malaria-free areas [2]. These migrants, who are usually located in rural settlements created by the Brazilian government over the past three decades, typically fail to achieve the status of clinical immunity normally seen in rural African adults; all age groups are assumed to be similarly vulnerable to infection and disease and most infections are symptomatic [16-18]. Symptomless malaria infections, however, are widespread in riverine communities of native Amazonians in both Brazil [19,20] and Peru [21,22], which may be naturally isolated from contact with non-natives geographically (by rivers and large areas of forest) and legally (*i.e.*, the indigenous population), or may be in close contact with migrants, representing in this case a significant source of gametocytes for local vectors [23]. More recent data suggest that asymptomatic parasite carriage is not limited to native Amazonians. Migrants can also harbor subclinical, chronic malaria infections with very low parasitemias, most of them detected only by polymerase chain reaction (PCR), in typical frontier malaria settings such as gold-mining camps [24,25] and agricultural settlements [26].

Little is known about the acquisition of clinical immunity to malaria under conditions of low endemicity that prevail in rural Amazonia. The epidemiology of malaria in riverine settlements of native Amazonians has some features that are typical of holoendemic Africa, such as the widespread occurrence of subclinical infections and subpatent

**Table 1.** Summary of advantages and disadvantages of different control strategies. Pros and cons of each strategy revised in the paper are presented and briefly discussed.

<b>Control strategy</b>	<b>Advantages</b>	<b>Disadvantages</b>
<i>Early diagnosis and treatment of clinical malaria</i> <i>Passive case detection</i>	Can stop transmission at an early stage Cheaper and easier to implement	Does not assess asymptomatic infection properly May delay case detection May not assess asymptomatic infection properly
<i>Aggressive active case detection</i>	Can identify cases at an early stage and interrupt transmission more easily	Expensive More complex strategy May not assess asymptomatic infection properly
<i>Surveillance of antimalarial drug resistance</i>	Can detect and confirm resistance at an early stage May give evidence-based elements to treatment choices	Expensive Has to be combined with properly designed clinical trials in order to have valid results
<i>Insecticide-treated bed nets</i>	Drastic reduction in transmission	Relatively expensive for developing countries Nets have to be replaced on a frequent basis
<i>Insecticide spraying of houses and the environment</i>	May be very efficient to reduce transmission, especially by targeting high-incidence malaria clusters	May be toxic to the individual and the environment May not be suitable to all types of housing

parasitemias, and the finding of most malaria morbidity among young children. In addition, parasite rates decrease sharply with age, suggesting that these populations, after a few years of continuous exposure to infection, acquire not only anti-disease immunity but also some degree of anti-parasite immunity [25]. A recent community-based cohort study also showed a marked decline in the risk of clinical malaria after five years of residence in a typical agricultural settlement in eastern Acre, Brazil, which remained significant after controlling for putative confounding variables but was not affected by the subject's age [26]. The decreased malaria morbidity with prolonged residence suggests that the gradual acquisition of clinical immunity creates a significant reservoir of malaria (asymptomatic parasite carriers) not only in isolated and sparsely populated traditional communities [20,23] but also in more densely populated mining [24,25] or agricultural settlements [26], with clear implications for malaria control (see [27] for a review).

#### *Aggressive active case detection for malaria control in Brazil?*

Nearly all malaria infections in Brazil are identified through active or passive case detection (ACD and PCD, respectively), which relies on the

presence of fever to diagnose malarial infections. Cases are found through PCD when febrile subjects attending one of more than 3,000 malaria diagnosis outposts across the Amazon Basin have a blood sample tested positive for malaria parasites. These outposts are located in urban, periurban, rural and riverine areas, and may be stationary or mobile. Malaria diagnosis is facilitated since access to these outposts is free to any subject, but due to the large Amazonian area it may not be readily accessed by everyone; sometimes an individual may walk or travel by boat for two or three days to be tested. ACD involves periodic visits to households in endemic areas, with collection of thick blood smears from every person having had fever since the last visit [28]. Aggressive active case detection (AACD) through mass surveys of the whole population of a given endemic area, irrespective of any clinical symptoms, has been limited to a few settings of very high endemicity, such as the Yanomami Indian Reservation [29].

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 semi-immune Amazonians ranges from a very mild, oligosymptomatic illness to a full-blown disease with

periodic fever paroxysms [30]. Therefore, ACD- and PCD-based strategies of malaria control, such as those currently used in Brazil, must deal with a heterogeneous disease in which high fever and cyclical paroxysms with chills and profuse sweating, the hallmark of textbook malaria, are not necessarily prominent features. For example, fever perceived by the patients as “intense” was found in only 52.6% of 230 consecutive episodes of laboratory-confirmed symptomatic malaria diagnosed during our prospective cohort study in rural Amazonia. No fever at all was reported in 19.1% of such episodes, although other symptoms (mostly headache or myalgia) were present and most subjects felt ill enough to seek malaria diagnosis [30].

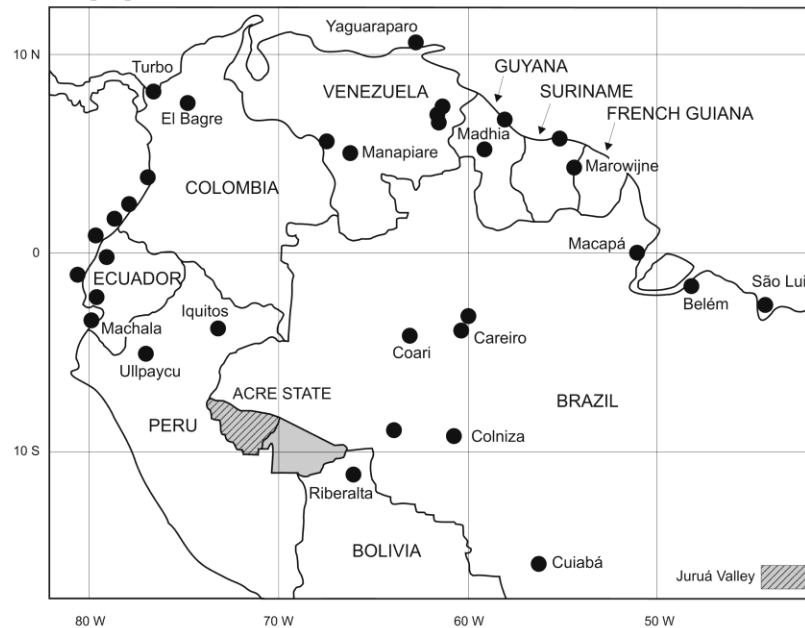
Whether long-lasting asymptomatic infections represent a major reservoir that must be addressed by AACD-based strategies in Brazil remains a matter of debate [19,25,26, 27,29,31]. Since AACD is expensive, a careful cost-effectiveness analysis is required before its large-scale use may be advocated. First of all, the relative role of asymptomatic infections in maintaining malaria transmission in Amazonia must be quantified. Although African control strategies do not rely on single strategies, mathematical models have recently incorporated asymptomatic infections to show that they may represent a crucial target for malaria eradication efforts in Africa [32]. No similar analysis has been conducted with data from other endemic settings. A pilot study showed that asymptomatic carriers of low-grade parasitemias can infect laboratory-reared *Anopheles darlingi*, the primary malaria vector in Brazil, although less efficiently than symptomatic subjects with greater parasite loads [23]. Further experiments using larger numbers of subjects and mosquitoes are needed to quantify the reservoir competence of asymptomatic parasite carriers. Second, currently available laboratory methods for large-scale use, such as microscopy and rapid diagnostic tests, are poorly sensitive to detect low parasitemias and miss a considerable proportion of subclinical infections with subpatent parasitemias, which are commonly detected only by PCR [19,21,25,26]. In addition to the low sensitivity of the test, isolates may lack the target proteins identified by the tests, such as pfhrp2/pfhrp3 [33]. PCR-based diagnosis has been suggested as a public health tool for AACD in Peru [21], but its use remains severely constrained by its high cost and complexity.

### *Surveillance of antimalarial drug resistance in Plasmodium falciparum*

Chloroquine resistance of *P. falciparum* in South America was first documented in the early 1960s, and became widespread across the Amazon Basin by the mid-1980s [34]. Between 2001 and 2007, the regimens for uncomplicated falciparum malaria recommended by the Ministry of Health of Brazil were (a) quinine plus doxycycline (formerly tetracycline) for seven days, or (b) a single dose of mefloquine. Both regimens are followed by a single dose of primaquine (45 mg, adult dose), on day 6 of treatment, for gametocyte clearance [35]. Surprisingly, a recent systematic review concluded that there was limited locally-generated pharmacological and clinical evidence to support the choice of these regimens at the time when they were implemented [36]. Separate clinical trials in northern Mato Grosso, central Brazil, subsequently showed high cure rates for mefloquine (98.9% [37]) and quinine plus tetracycline (77.3% and 100% [38,39]), despite *in vitro* evidence for quinine resistance in local isolates [40]. Few drug sensitivity data are available for other endemic regions in the country.

More recent data on *P. falciparum* drug resistance in Brazil are expected to be made available soon by the large surveillance network across the Amazonian countries of South America known by its Spanish acronym, RAVREDA [41]. Established in 2001, RAVREDA has received substantial financial support from the US Agency for International Development (USAID) (US \$48.4 million between 2001 and 2006) and PAHO. The selection of sentinel sites for monitoring *in vivo* drug resistance considered factors such as malaria incidence, local health infrastructure, and logistics [42]. Figure 2 shows the locations of the RAVREDA sentinel sites. Given the strong geographical structure of Amazonian populations of *P. falciparum* [43], these sentinel sites are unlikely to provide an accurate view of countrywide patterns of drug resistance. For example, there is no sentinel site in the westernmost state of Brazil, Acre (Figure 2). Although antimalarial drug resistance has not been investigated in Acre since the late 1980s [44,45], this was the first site in the country to use the fixed-dose artesunate-mefloquine combination therapy for falciparum malaria. There are two compelling reasons that data collected elsewhere may not be representative of parasites in Acre. First, Acre borders with Peru and Bolivia, where until recently, first-line regimens for falciparum malaria differed from each other and from

**Figure 2.** Location of sentinel sites for monitoring *in vivo* drug resistance of *P. falciparum* in South America [41].



These sites participate in the surveillance network across the Amazonian countries of South America known by its Spanish acronym, RAVREDA, established in 2001 and funded by the US Agency for International Development (USAID) and Pan American Health Organization (PAHO). The shaded area in Brazil shows the location of Acre, the westernmost state in this country, bordering with Bolivia and Peru. The hatched area shows Juruá Valley, in Acre State, where the fixed-dose artesunate-mefloquine combination is currently in use.

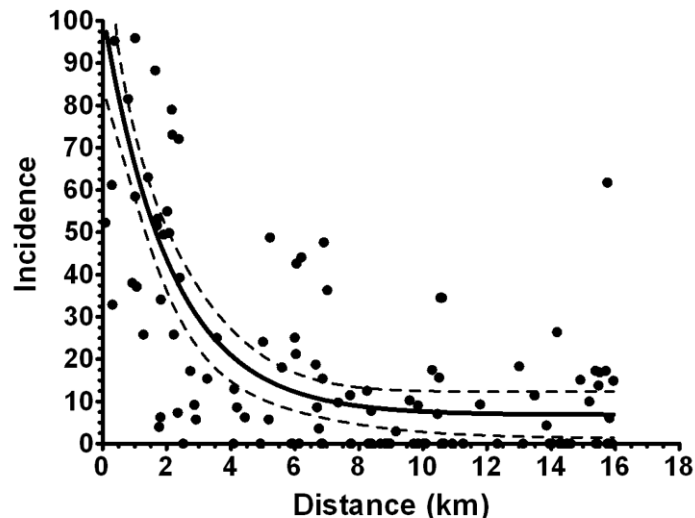
those in use in Brazil. A continuous influx of patients and parasites from these countries may affect dramatically the local patterns of drug-resistance. Second, extensive genotyping data revealed that *P. falciparum* isolates from Acre are highly divergent from those of all other populations of Brazil [43]. Different drug pressures may also affect the little-studied parasite populations from other international borders of Brazil, such as the borders with Venezuela, Suriname, or French Guyana.

Since 2006, Brazil has been gradually implementing artemisinin combination therapy (ACT) as the first-line regimen for uncomplicated *falciparum* malaria. Treatment with artesunate-mefloquine or arthemeter-lumefantrine combinations had been implemented in 90.3% of the municipalities with malaria transmission in Brazil by mid-2008 [46]. The first local trial of a fixed-dose artesunate-mefloquine combination was conducted in Juruá Valley, Acre, in 2006-07 [47]. This intervention trial, financed by RAVREDA and the Ministry of Health of Brazil, involved 17,000 patients but had no comparison group. Baseline data for mefloquine, artesunate, or quinine-doxycycline resistance were not collected at the time when the intervention was implemented. This fixed-dose artesunate-mefloquine combination remains the first option for *falciparum*

malaria in Acre, while in all other states in Brazil arthemeter-lumefantrine is the first-line therapy. A small, open-label clinical trial supported by Novartis, with 27 patients from two sites (Manaus in Western Amazonia and Santarém in Eastern Amazonia) in the arthemeter-lumefantrine arm, who were followed for seven days, described a faster clearance of *P. falciparum* parasitemia with a six-dose regimen of arthemeter-lumefantrine compared to quinine-doxycycline [48,49]. No other published studies have investigated the *in vivo* efficacy of arthemeter-lumefantrine, artesunate-mefloquine or other ACT-based regimens in Brazil, although several unpublished clinical trials have been performed in RAVREDA sentinel sites [41].

Whether or not *P. falciparum* resistance to standard quinine-doxycycline and mefloquine regimens, which were used until recently, can explain some of the difficulties in malaria control in Brazil remains uncertain. A sustained decrease in the incidence of *P. falciparum* infection and hospitalizations for *falciparum* malaria was observed in Thailand with the widespread deployment of early diagnosis and mefloquine-artesunate treatment [50,51]. In Juruá Valley of Acre, Brazil, *P. vivax* now predominates in urban populations (who are more likely to have access to prompt diagnosis and

**Figure 3.** Household-specific malaria incidence in relation to the distance (in km) of each dwelling to the center of the high-incidence cluster of malaria. Data collected between 2001 and 2006 in the agricultural settlement of Granada, Acre, Brazil.



The circular high-incidence spatial cluster was detected by spatial scan statistics; incidence rates were calculated per 100 person-years at risk. Circles represent individual data points. An exponential function (continuous line) was fitted to the data (coefficient of determination  $r^2 = 0.576$ ); the 95% confidence interval is

treatment of falciparum malaria with mefloquine-artesunate), but *P. falciparum* still accounted for 50-60% of the malaria burden in isolated riverine communities in 2009 (Ministry of Health of Brazil, unpublished data).

#### Chloroquine and primaquine resistance in *Plasmodium vivax*

How effective are early diagnosis and treatment, with little vector control, to control malaria in areas where *P. vivax* predominates? Three concerns must be considered. First, the recent emergence of chloroquine resistance in *P. vivax* [52] may require the use of more expensive (and potentially more toxic) drugs. Second, because mature gametocytes are commonly found in the blood before symptoms occur and drug treatment is started, significant transmission of *P. vivax* may persist in areas where early diagnosis and treatment can be achieved [13]. Third, the distinct ability of *P. vivax* to stay dormant in the host's liver cells and cause relapses weeks or months after the primary infection, despite the use of effective blood schizonticidal drugs, further complicates treatment.

The definition of chloroquine resistance in *P. vivax* relies mainly on *in vivo* studies. Because use of a poor quality drug, poor compliance, and emesis may prevent normal drug levels from being achieved, or whole blood levels of chloroquine must be measured at the time when parasites reappear to

define true resistance. Parasites are defined as chloroquine-resistant if they persist in the blood despite concurrent blood levels of chloroquine and its main metabolite, desethylchloroquine, above 100 ng/ml (equivalent plasma levels are 10 ng/ml) [53]. Chloroquine resistance, defined according to these criteria, remains mostly confined to Indonesia, East Timor, and Papua New Guinea [52]. Whether chloroquine-resistant *P. vivax* currently represents a major public health concern in Brazil remains to be determined; parasite recrudescences up to 28 days after treatment with chloroquine alone, despite adequate plasma levels of chloroquine, were recently described within the RAVREDA framework in 11 of 109 *vivax* malaria patients followed in Manaus, Amazonas [54].

The reasons chloroquine resistance tends to spread much slower in *P. vivax* than in *P. falciparum* remain open to speculation. The recent report of synergism between primaquine and chloroquine against blood-stage parasites suggests an explanation for the late emergence of chloroquine resistance in *P. vivax*. Namely, the continuous use of an effective combination therapy might have inhibited the emergence and spread of resistant parasites. Over half a century, primaquine and chloroquine have been routinely associated with the radical cure of *P. vivax* infections (*i.e.*, eradication of blood stages and hepatic hypnozoites) in most countries except those with a relatively high prevalence of glucose-6-

phosphate dehydrogenase (G6PD) deficiency, because of the risk of severe primaquine-induced hemolysis. Where primaquine is used, regimens differ across countries; chloroquine and primaquine are administered either simultaneously (as in most of South America) or sequentially. The finding that primaquine reverses chloroquine resistance in *P. falciparum* [55] suggests that a similar effect might occur in *P. vivax* isolates simultaneously exposed to both drugs. Some observations support this hypothesis: (a) chloroquine-resistant *P. vivax* emerged in regions where primaquine is not routinely associated to chloroquine, because of the relatively high prevalence of G6PD deficiency; (b) the 22 published reports of chloroquine-resistant *P. vivax* infections acquired in South America (where chloroquine and primaquine are routinely co-administered) refer to patients to whom primaquine was either given after chloroquine treatment or not administered at all [52,54]; and (c) the co-administration of primaquine improved the efficacy of chloroquine against chloroquine-resistant *P. vivax* in clinical trials in Indonesia, Thailand and India (see [56] for a review). Chloroquine resistance in *P. vivax* may not be a major reason for concern in Brazil when chloroquine and primaquine are co-administered.

The early production of gametocytes in vivax malaria [13] may have also contributed to the delayed spread of chloroquine resistance to this species, compared to *P. falciparum*. In falciparum malaria, gametocytes are often produced after chemotherapy is started, and parasites that resist treatment are those producing most gametocytes. As a consequence, gametocyte populations tend to be biased towards drug-resistant strains, which are preferentially transmitted to mosquitoes. This phenomenon is absent in *P. vivax* infection.

Primaquine is the only current available alternative to eliminate dormant liver stages (hypnozoites) of *P. vivax* [57]. Across the Amazon Basin of Brazil, where the prevalence of severe G6PD deficiency is around 3-4% [58, 59], primaquine-related severe hemolysis is rarely described. Simple laboratory methods for G6PD screening prior to primaquine administration have been used in Southeast Asia [60,61] and are recommended when high-dose primaquine regimens are used in Brazil [62].

Over the last two decades, there have been numerous reports of primaquine failure to prevent *P. vivax* relapses in the Western Pacific, Southeast Asia, India, and Central and South America [57,63,64].

Data from Brazil are scarce, although primaquine resistance is a well-characterized phenotype of a local monkey-adapted *P. vivax* strain [65]. A relapse rate of 24.5% was described among 1374 *P. vivax*-infected patients given standard regimens of chloroquine (total dose in adults, 1.5 g) plus primaquine (total dose in adults, 210 mg over 14 days) and followed in non-endemic southeast Brazil [66]. A subsequent analysis of 50 patients treated with the same chloroquine-primaquine regimen in central Brazil revealed seven relapses (14.0%), most of them associated with subtherapeutic primaquine dosages [67]. More recently, however, a *P. vivax* infection acquired in Brazil was found to relapse despite the administration of 900 mg of primaquine over 30 days [68].

Assessing primaquine efficacy for relapse prevention remains challenging in malaria-endemic areas. Until recently, relapses were thought to be caused by hypnozoites that are genetically identical to the blood-stage parasites found in primary infections [69,70], suggesting that molecular methods could easily discriminate relapses (due to the same genotype found in the primary infection) and new infections with different genotypes. This view has been challenged by the recent finding of different parasite genotypes in primary infections and relapses in most *P. vivax*-infected patients from Thailand, India, and Myanmar who provided paired blood samples for multilocus analysis [71]. Further studies are needed to confirm this finding, since genotyping errors may be relatively common, especially when the primary infection comprises multiple genotypes. Errors are made when a minor subpopulation is missed in the primary infection, and a relapse of these parasites is incorrectly interpreted as a new infection.

Given the current uncertainties regarding the detection of parasite relapses in endemic areas, their relative contribution to the incidence of *P. vivax* in Brazil remains uncertain. We recently found high rates of *P. vivax* recurrence (26-40% after 180 days post-treatment) in two cohorts of rural Amazonians exposed to low levels of malaria transmission after a vivax malaria episode treated with chloroquine-primaquine. However, microsatellite analysis of 28 paired acute-infection and recurrence parasites revealed only two pairs of identical haplotypes (consistent with recrudescences or reactivation of homologous hypnozoites) and four pairs of related haplotypes (sharing alleles at 11-13 of 14 microsatellites analyzed) [72].

The adult total dose of 210 mg of primaquine currently used in Brazil often fails to prevent relapses in *P. vivax* infections acquired in several endemic regions [57,68]. (Note that 210 mg of primaquine are currently administered in Brazil over seven days [35], while a 14-day regimen [same total dose] is currently recommended by the WHO [56]). The finding that high-dose primaquine regimens are safe and effective in preventing relapses among G6PD-normal subjects in areas where standard primaquine treatment often fails (see, for example, [61]) led the US Centers for Disease Control and Prevention (CDC) to recommend 420 mg over 14 days as the standard anti-relapse regimen of primaquine for adults [73]. The WHO currently recommends a dose of 0.5 mg/kg of body weight over 14 days for preventing relapses of infections acquired in Southeast Asia and Oceania [56]. No comparison between usual and high-dose primaquine regimens has been performed in Brazil and, as a consequence, the potential impact on *P. vivax* incidence of improved anti-relapse therapy remains unknown. In addition, the comparability of the 7-day (currently used) and 14-day (traditional) regimens (same total dose, 210 mg) for relapse suppression has not been conclusively demonstrated in Brazil; the only data available derives from a small randomized trial in Rondônia, a setting where reinfections may be difficult to distinguish from relapses [74].

### Vector control

The relative impact of different control measures may be anticipated by considering our current understanding of transmission dynamics of malaria. Macdonald [75] has already shown, in the mid-1950s, that measures that reduce mosquito survival rates, such as indoor residual spraying and, more recently, the use of insecticide-treated bed nets (ITBNs), tend to reduce transmission drastically, while measures that decrease the duration of parasite carriage among humans, such as chemotherapy, have a relatively minor impact [reviewed in 14]. The recent neglect of vector control measures in Brazil [1] may therefore explain some of our difficulties in controlling malaria in Amazonia [76]. In the Amazon, epidemiological settings may dictate living conditions, which in turn, may be a factor in favor or against vector control. In the most urbanized areas, houses are generally made of brick or wood, with complete walls and a metal roof. In the rural areas, there may be a mixture of this pattern with non-walled houses or houses made of

palm leaves, that may impose a greater difficulty to vector control.

### *A role for insecticide-treated bed nets in Brazil?*

Insecticide-treated bed nets (ITBNs) emerged in the 1990s as one of the great hopes for controlling malaria worldwide. Their efficacy has been clearly demonstrated in different endemic areas, especially in Africa [77]. When ITBNs are provided free of charge and a high population coverage is achieved, their impact on malaria transmission in Africa is comparable to that in the best house spraying projects [78]. Nevertheless, Zimmerman and Voorham [79] concluded their 1997 review by stating that “it would be premature to use insecticide-impregnated mosquito nets or other materials as a major component of an integrated malaria control program in the Americas at this time” and call for well-designed large-scale trials in this region (see also [80]). The quite variable biting behavior of malaria vectors in the Americas, and particularly that of the principal vector across the Amazon Basin, *Anopheles darlingi*, is the main reason that ITBN-based malaria control programs may fail in this region. *An. darlingi* can present a marked early biting behavior and high outdoor-to-indoor biting ratio [81] in some areas, although a late biting behavior and indoor preference have also been described [82]. Very little is currently known about the biting behavior of other malaria vectors in Amazonia [81,83]. The marked regional variation in vector behavior, which cannot be anticipated without careful baseline entomological studies, has been suggested to cause large differences in the estimates of ITBN efficacy obtained in three randomized community trials in Peru and Nicaragua [84].

Surprisingly, no large community-based randomized trials of ITBNs have been conducted in Brazil since the 1997 review of Zimmerman and Voorham. However, 7,000 ITBNs were acquired and distributed by the Ministry of Health across the Juruá Valley of Acre in late 2006 [85]. These bed nets, impregnated with permethrin, were acquired in Thailand at an approximate cost of US \$18 each. The intervention was not randomized and included no comparison groups; no baseline information on biting behavior and pyrethroid resistance among local malaria vectors was available. Over the next 12 months, the incidence of malaria in the region fell by 32% [85]. Since several other control measures were simultaneously implemented in the Juruá Valley, including the large-scale use of the fixed-dose



artesunate-mefloquine combination for uncomplicated falciparum malaria mentioned earlier [47], the relative contribution of ITBNs to the observed decline in malaria incidence is difficult to determine. One decade after the publication of Zimmerman and Voorham's review, we are still waiting for "well-conceived, large-scale trials at the community or regional level" that are "based on a thorough understanding of the dynamics of malaria transmission in the areas involved" [79] to decide whether or not ITBNs are an appropriate public health tool for malaria control in Brazil. The recent initiative of the Ministry of Health of Brazil to launch a massive program of ITBN distribution in Acre (70,500 ITBNs are expected to be provided, free of charge, to malaria-exposed populations in the State of Acre through 2010) may dramatically change this scenario if an appropriate study design is used for impact assessment.

#### *Spatial clustering of malaria and selective house spraying*

Spraying the inside surfaces of houses with a residual insecticide, principally dichlorophenyltrichloroethane (DDT), was one of the mainstays of the Global Malaria Eradication Campaign supported by the World Health Organization (WHO) between 1955 and 1969 [86]. The attack phase included periodic spraying of all houses in the endemic areas with DDT for 3- to 5 years, to interrupt malaria transmission. Concerns about the safety of DDT for human health [78,87]) led several countries to reduce or phase out house spraying with DDT over the 1970s and 1980s. Alternative insecticides, such as carbamates, organophosphates, and pyrethroids, have replaced DDT in house-spraying programs, but they tend to have shorter residual effect and are more expensive than DDT [87,88]. There has been a marked decline, in recent years, in house spraying activities across the endemic areas [86], which may have caused malaria to re-emerge in some of them (Roberts [76]). The WHO currently promotes DDT house spraying (standard dosage, 1-2 grams per m<sup>2</sup> every six months) for malaria control in most epidemiologic settings, including areas with low and unstable transmission [86], but relatively few countries have implemented large-scale spraying programs over the past few years [89].

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 tends to have an uneven distribution, being clustered in certain households, control measures may target preferentially high-risk areas and subjects [90,91]. Identifying spatial clusters became a feasible goal for countrywide malaria control programs, given the current availability of cheap, hand-held global positioning system (GPS) receivers and appropriate statistical methods and open-source software for spatial analysis. The observed clustering of malaria risk may represent the basis for targeted house spraying, which tends to be much more cost-effective than widespread spraying strategies [90].

We use data from our recent cohort study in rural Amazonia to illustrate the spatial clustering of malaria in endemic areas in Brazil [26]. Spatial scan statistics [92] revealed a highly significant circular cluster comprising 22% of the households that contributed 69% of all malaria infections diagnosed between January 2001 and October 2006. Therefore, by targeting less than one-fourth of the households we can theoretically decrease malaria transmission by two thirds. The circular cluster had a radius of only 2.2 km. 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 decreased exponentially with distance from the cluster center, reaching a plateau after 8 km of distance (Figure 3). This pattern is consistent with the known dispersal range of the main local vector, *Anopheles darlingi* (5 km or even more) [93], but contrasts with the typical gradient of incidence decline seen in Africa, which extends over no longer than 2- to 3 km away from the area of highest transmission [90].

Spatial clustering of frontier malaria is clearly associated with land-use patterns. The high-risk cluster in our study site is located in the area of most recent colonization, close to the forest fringes. Settlers in this area are typically newcomers involved in land-clearing activities, which puts them at an increased risk of infection. Because land clearing leads to changes in relative vector species abundance that may favor malaria transmission [94], this activity plays a crucial role in maintaining malaria transmission in the entire community. Therefore, the continuous influx of settlers to forest-fringe areas perpetuates the cycle of environmental change and colonization that favors malaria transmission [26].

Similar patterns of spatial clustering have been reported in the agricultural settlements of

Machadinho, Rondônia [91], suggesting that this may be a common feature of frontier malaria in Brazil. Targeted vector control is also a feasible strategy for malaria control in densely populated urban areas in Brazil where the disease is re-emerging, such as Belém (population, 1,370,000) [9]. Further spatial analyses are clearly needed to support targeted malaria control interventions, such as selective spraying of premises in high-risk areas [90], in both urban and rural areas of Brazil. The choice of an appropriate insecticide remains a matter of debate: in the recent literature, both DDT-based [95] and non-DDT-based [96] strategies have been reported to be successful in other endemic settings.

### Conclusion

There is an emerging consensus that “public health needs to be evidence-based if it is to be done correctly” [97], but translating scientific evidence into public health interventions may be particularly challenging.

First, the external validity of well-designed controlled trials may be surprisingly limited when interventions deal with diseases with relatively complex causal pathways [98]. For example, the biting behavior of malaria vectors is a clear source of effect modification that affects the generalizability of ITBN efficacy trials [84]). Patterns of antimalarial drug resistance are also clearly regional, and a given therapy that has proven to be highly effective in some endemic settings may fail in others. Consequently, evidence to support public health interventions must be generated locally.

Second, trials that investigate the efficacy of new tools must ensure high compliance rates, which usually cannot be maintained by public health interventions under routine circumstances. Therefore, efficacy (shown in well-controlled trials with close supervision) does not necessarily translate into long-term effectiveness within large-scale public health programs. A well-known example comes from ITBN promotion programs in Africa [99].

Here we show that little locally generated evidence supports many of the malaria control interventions currently in use in Brazil. In addition, little research has addressed some alternative tools or interventions that could improve current strategies. This situation is particularly surprising for a middle-income country with relatively well-developed research capacity and commitment of funding agencies to support knowledge translation [100] and calls for an enhanced partnership between researchers

and decision-makers to face the country’s challenges in malaria control.

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