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Investigation of a Sudden Malaria Outbreak in the Isolated Amazonian Village of Saül, French Guiana, January–April 2009

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Abstract. Malaria is endemic in French Guiana. *Plasmodium falciparum* and *Plasmodium vivax* are the predominant species responsible and *Anopheles darlingi* is described as the major vector. In mid-August 2008, an increase in malaria incidence was observed in Saül. A retrospective cohort survey was performed. *In vitro* susceptibility profiles to antimalarials were determined on *P. falciparum* isolates. Collections of mosquitoes were organized. The malaria attack rate reached 70.6/100. The risk of malaria increased for people between 40 and 49 years of age, living in a house not subjected to a recent indoor residual insecticide spraying or staying overnight in the surrounding forest. All isolates were susceptible. *Anopheles darlingi* females and larvae were collected in the village suggesting a local transmission. Our results strongly support a role of illegal mining activities in the emergence of new foci of malaria. Therefore, public health authorities should define policies to fight malaria at a transborder level.

INTRODUCTION

French Guiana, a French overseas territory located in the Northeast part of South America, 7,000 km away from Europe, is part of the Guyana Shield, extending from Colombia to Brazil. The Maroni and Oyapock rivers constitute natural French Guianan borders with Surinam to the West and Brazil in the South and East (Figure 1). The climate is equatorial and the Amazonian rainforest covers more than 90% of the territory. The French Guianian population of 200,000 inhabitants lives mainly in the coastal area.

Malaria has always been considered a major public health problem in French Guiana and fighting the disease remains a priority.[1,2] Over the last decade, 4,000 cases have been detected each year.[3] Currently, malaria is endemic in the inland part of the territory and along the main rivers where transmission is permanent, fluctuating with seasonal and geographical variations. In the coastal area, the disease incidence remains low with autochthonous transmission emerging regularly in local foci.

Epidemiological surveillance of malaria is performed by the "Cellule de l'Institut de veille sanitaire en Régions Antilles-Guyane" (Cire AG). Private and hospital laboratories, as well as health centers, report malaria cases to this central database.

Plasmodium falciparum and *Plasmodium vivax* are the predominant species, each responsible for approximately half of the cases.[3] Diagnosis is usually based on parasite identification either on a thin or thick smear or through rapid immunological tests. Malaria control includes particularly, treatment of patients according to recommendations validated by local experts[4] and vector control performed by the "Direction de la Démoustication et des Actions Sanitaires" (DDAS).

The standard treatment of *P. vivax* infection consists of administering Chloroquine (Nivaquine). Primaquine is authorized for temporary use but requires a G6PD blood dosage before its prescription. As patients almost never have this information, Primaquine is not prescribed as a first line treatment of *P. vivax* attack and relapses occur frequently. Treatment of *P. falciparum* classical attack includes, for people ≥ 12 years of age or ≥ 35 kgs, Riamet (20 mg of artemether + 120 mg of lumefantrine) or Malarone (250 mg of Atovaquone + 100 mg of Proguanil) or oral quinine (Quinimax) with doxycycline.

In endemic areas, indoor residual sprays of insecticides are routinely applied every 3 or 4 months. In the coastal areas, systematic interventions are implemented and vector control is conducted for 3 or 4 months and up to 2 years after the last *P. falciparum* autochthonous case was declared[5]; the main target of this control is the widely distributed malaria vector *Anopheles darlingi*. Other anopheline species may also be found at high densities in the human environment but their role in malaria transmission has yet to be confirmed in French Guiana.[6,7]

Our study took place in Saül, located in the center of French Guiana (Figure 1), in the Amazonian forest. Saül is isolated and can only be reached by an hour flight from Cayenne, the main town of French Guiana (North-East of Saül). In 2001, malaria transmission in Saül was moderate with an incidence rate of 20/1,000 inhabitants.[5] However, since the end of 2001, the incidence has drastically increased. During this period, the main change in Saül's surroundings was the development of illegal gold mining activities. Since then, illegal gold prospectors have regularly entered the village to consult the health center nurse or to buy basic grocery goods. In mid-August 2008, the Cire AG reported an increase in malaria cases observed predominately among the illegal gold miner population. At the end of 2008, the disease was observed in a large number of the permanent inhabitants of Saül. Sanitary authorities suspected local malaria transmission within the village. In 2008, *P. falciparum* was recorded

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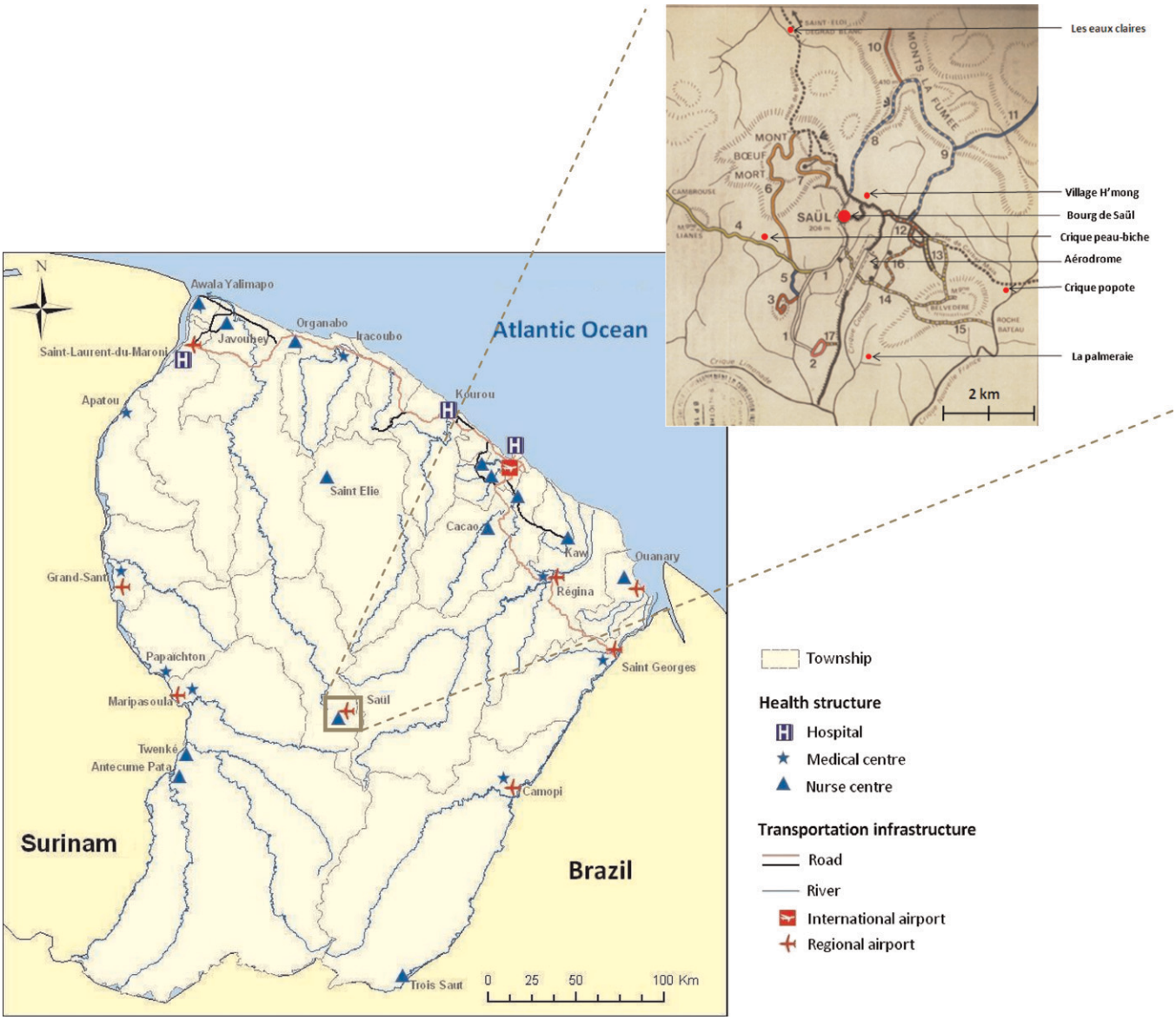

FIGURE 1. Localization of the different sites situated in and around the village of Saül.

as the main parasite species circulating in Saül[8]; an entomo-epidemiological investigation, coordinated by Cire AG and the "Institut Pasteur de la Guyane" (IPG) was carried out. The objectives of this investigation were to confirm and characterize the malaria outbreak and to identify environmental and behavioral risk factors.

MATERIALS AND METHODS

Study site. The entomo-epidemiological study was carried out in the area of Saül, which includes 100 inhabitants. Eighty-five percent of the population lives in the center of Saül, the other 15% live in five isolated sites situated within a 5 km range, such as « La Palmeraie » or « Crique popote » (Figure 1).

Epidemiological investigation. An epidemiological investigation was carried out from January 14–16, 2009. This retrospective cohort study was based on the inhabitants of Saül and surrounding areas present during the 3-day survey. The illegal gold miners, subjects visiting the village during this period (tourists, visitors, etc.), the inhabitants of Saül who were out of the area between August 1 and December 31, and those living < 4 days per week in the study area were excluded from the investigation.

A malaria case was defined as the onset of fever (temperature ≥ 38.5°C) between August 1 and December 31, 2008, biologically confirmed by a rapid diagnostic test (OptiMal test, Bio-Rad, Diamed AG, 1785 Cressier FR, Switzerland) performed at the health center. The OptiMal test discriminates *P. falciparum* infection from all *Plasmodium* species. Malaria cases caused by *Plasmodium malariae* have a very low incidence in French Guiana (2.6% of cases) and their distribution is restricted to the Maroni river.[3] Therefore, in our study, *P. falciparum* and *P. vivax* were considered as the only implicated *Plasmodium* species. Malaria attacks occurring within 2 weeks of a first attack and considered to be from the same species were defined as recrudescence and were not counted as new cases.

A standardized questionnaire was completed during a face-to-face interview with each person included in the study. Data were collected on date of birth, gender, clinical data (number and date of malaria attacks, *Plasmodium* species, gravity of attacks, treatment, etc.), places visited between July and December 2008, mosquito bite prevention measures, house description (« open » or « closed ») and information on insecticide spraying carried out inside and/or outside the house. A house was considered as closed if all the openings were closed by windows or mosquito nets. If the kitchen, living room, or bedroom was not closed by a window or mosquito net, the house was considered « open ». Insecticidal formulation used for indoor residual spraying (IRS) was Cislin 25 V-O (Bayer, Leverkusen, Germany) at a dose of 25 mg of deltamethrin/m^2.

Student *t* test or Mann-Whitney test were used to analyze the quantitative variables; χ^2 or Fisher's test was used to analyze the qualitative variables. A logistic regression with random effect was carried out. Each record was composed of the data for one person over 1 month. If data were available for the 5 months of the study, this person represented five person-months (five records).

Variables significantly associated with malaria infection ($P \leq 0.2$) were used for multivariate analysis. Statistical analyses were carried out using the software Stata 9.0 (Stata, College Station, TX).

***Plasmodium falciparum in vitro* drug susceptibility assay.** The "Centre National de Référence de la Chimiorésistance du Paludisme" (CNRCP) at IPG regularly collects *P. falciparum* isolates and determines their *in vitro* susceptibility profiles for a panel of antimalarials using the isotopic method.[9]

Entomological investigation. Two entomological surveys were organized in the village of Saül and its surroundings from January 12–14, 2009 and from April 6–10, 2009. In January 2009, larvae were collected in various types of breeding places in the village, its close surroundings, and in the two localities of "Village H'mong" and "Crique Popote" (Figure 1). Human landing collections were organized in the evening from 6:30–7:30 PM and in the morning from 6:00–7:00 AM in the village, inside and outside of the houses. In April 2009, larvae were collected only in the village and its close surroundings. Indoor and outdoor human landing collections were carried out in the evening, from 6:30–8:30 PM and in the morning from 5:30–7:30 AM in the village and in the distant locality of "La Palmeraie." Night collections were also performed using five Centers for Disease Control and Prevention (CDC) light traps installed in the close surroundings of the village and in the distant locality of "La Palmeraie." Finally, females at rest were collected early in the morning inside the houses of the village.

Adult mosquitoes and larvae were sorted by genera and anopheline specimens were identified morphologically according to identification keys in use in the Amazonian region.[10,11]

RESULTS

The number of inhabitants living at least 4 days per week within the study area was estimated at 74 from which 68 were questioned (91.9%).

Attack rate. From August 1 to December 31, 2008, 48 subjects presented a total of 90 malaria attacks, representing an attack rate of 70.6/100 (48 of 68) and an incidence density rate of 6.0/100 person-weeks. Among these 48 subjects, 22 presented one malaria attack and one presented five attacks. *Plasmodium falciparum* was responsible for 57.8% of the malaria attacks (52 of 90). The epidemic curve showed a peak in November 2008 with 43.1% (22 of 51) and 44.7% (17 of 38) of *P. falciparum* and *P. vivax* cases, respectively (Figure 2). During this month, a total of 39 malaria attacks occurred including 24 (61.5%) first malaria attacks. Two malaria attacks occurring within 14 days were identified in two distinct people; these attacks were removed from the study. Median duration between two *P. vivax* attacks was 41 days ($N = 8$ subjects with 20 *P. vivax* attacks; range: 18–111). The median duration between two *P. falciparum* attacks was 28.5 days (range: 18–111).

Subject characteristics and prevention measures. The male/female sex ratio was 1.3. The median age was 39 years (range: 1–83). Of the 68 subjects questioned, 57 (83.8%) were living in the village of Saül or its close surroundings (< 5 km from the center) and 57.4% (35 of 61) lived in a closed house. Indoor spraying by DDAS had occurred in 23.5% (16 of 68) of the houses, between April and November. Among respondents, 79.0% (49 of 62) declared using a bednet; among those, 51% (24 of 47) used an insecticide-treated bednet; 77.4% (48 of 62) declared using domestic insecticides and 63.9% (39 of 61) using topical mosquito repellents.

Risk factors. Malaria risk was three times higher for people from 40 to 49 years of age ($P = 0.01$) than the others (Table 1). Furthermore, the risk of getting malaria was multiplied by nearly three for people living in a house, which did not receive IRS during the last 6 months before the malaria attack. Finally, hunting and going to « Crique Popote » and « La Palmeraie » represented a risk activity (Table 1), especially when staying overnight in those areas (between 6:00 PM and 6:00 AM, odds ratio [OR] = 3.3, 95% confidence interval [CI] = [1.1–9.5]). No individual prevention practices used in the home had a significant impact on malaria risk (Table 1). Measures of prevention practices used during night activities outside of the usual residence were not reliable enough to be analyzed.

Treatment of malaria attacks and drug susceptibility assay. Three malaria attacks involving three subjects 58, 59, and 70 years of age, required hospitalization. Artemether/lumefantrine (Riamet) was prescribed in 90.2% (46 of 51) of the *P. falciparum* cases. Chloroquine (Nivaquine) was prescribed in 91.7% (33 of 36) of *P. vivax* infections. Two *P. falciparum* attacks were self-treated with halofantrine (Halfan). In mainland France, quinine (Quinimax) and atovaquone/proguanil (Malarone) were prescribed for three *P. falciparum* attacks; two *P. vivax* attacks were treated with artemether/lumefantrine and one with quinine.

For 79 malaria attacks, people reported correct observance of the treatment, except one during his fourth *P. falciparum* infection treated by artemether/lumefantrine.

Vomiting was the most frequent undesirable side effect, reported in 33.9% (21 of 62) of malaria treatments.

In vitro antimalarial chemosusceptibility of *P. falciparum* isolates coming from five subjects included in the study were tested. All five isolates were susceptible to the tested antimalarials, i.e., chloroquine, quinine, artemether, lumefantrine, atovaquone, and doxycycline.

Entomological results. In January 2009, three anopheline mosquitoes identified as *An. darlingi* were collected on

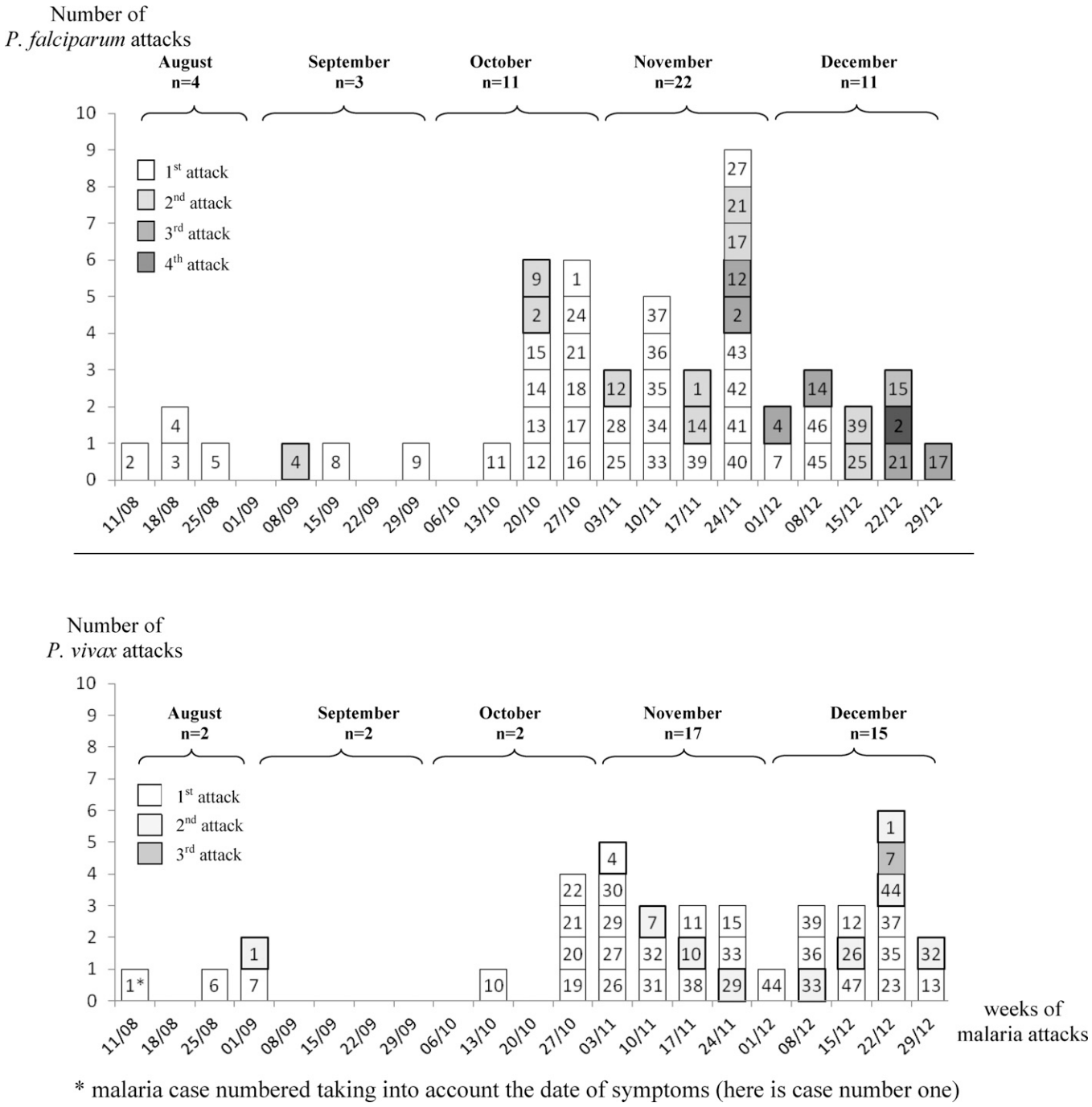

FIGURE 2. Epidemic curves of malaria infections by *Plasmodium* species between August 1 and December 31 2008 (Saül, January 2009).

humans from a capture effort of six man-hours. In April 2009, in the village of Saül, three anopheline mosquitoes belonging to the species *An. darlingi*, *Anopheles nuneztovari*, and *Anopheles neivai*, were identified from human landing collections, after a capture effort of 32 man-hours. In the site of « La Palmeraie », one female *An. nuneztovari* was collected on a human, after a capture effort of 16 man hours. The five light traps yielded three anopheline mosquitoes belonging to the species *Anopheles squamifemur*. No anopheline mosquitoes were found by the collections performed inside the dwellings of the village.

In January and April 2009, 13 and 11 larval breeding places, respectively, were found positive for anopheline mosquitoes. *Anopheles darlingi* larvae were found within the village in a rainwater puddle in January, but none found in April. During the two field trips, larvae of *Chagasia bonneae* were found in creeks inside the village and its surroundings. Larvae of *Anopheles mediopunctatus* were collected in the neighborhoods of the village in various breeding places. In January, larvae of *Anopheles ininii* were found in « Village H'mong » and « Crique Popote » in various breeding places. In April

TABLE 1
Results of the multivariate analysis* (N = 243 persons-month, Saül 2009)

				Initial model			Final model		
	OR	95% CI	P	aOR†	95% CI	P	aOR†	95% CI	P
Age (years)			0.08			0.04			0.01
0–20	1.0			1.0			1.0		
21–39	1.9	0.8–4.4		0.7	0.2–2.2		0.7	0.2–2.0	
40–49	3.3	1.2–8.5		3.0	1.0–8.5		3.2	1.2–9.0	
≥ 50	1.4	0.6–3.3		0.9	0.4–2.5		0.9	0.3–2.4	
Type of house									
Closed	1.0			1.0					
Open	1.6	0.8–2.9	0.16	1.0	0.4–2.3	0.96			
Residence inside the village									
Yes	1.0			1.0					
No	3.5	1.8–6.9	< 0.001	1.0	0.4–3.5	0.94			
Indoor residual spraying									
≤ 6 months	1.0			1.0			1.0		
> 6 months	2.7	1.3–5.6	0.008	2.4	0.9–6.5	0.10	2.8	1.1–7.2	0.04
Domestic insecticides									
Yes	1.0			1.0					
No	3.8	2.0–7.3	< 0.001	1.5	0.6–3.8	0.35			
Frequented areas‡									
« Crique Popote »									
No	1.0			1.0			1.0		
Yes	2.3	1.2–4.4	0.01	2.0	0.8–4.8	0.11	2.4	1.1–5.4	0.03
« La Palmeraie »									
No	1.0			1.0			1.0		
Yes	2.9	1.3–6.5	0.008	2.1	0.8–5.9	0.16	2.7	1.2–6.2	0.02
Hunting activity									
No	1.0			1.0			1.0		
Yes	2.5	0.8–8.1	0.13	2.9	0.8–10.0	0.10	3.3	1.0–10.5	0.04

* Only variables with a P value ≤ 0.2 are presented.
† Adjusted odds ratio (OR).
‡ The home place was considered as a frequented area (e.g., « La Palmeraie » was considered as a frequented area for people living on the spot).

larvae of *An. ininii* and *Anopheles eiseni* were found in « Village H'mong ».

DISCUSSION

The number of inhabitants of Saül living at least 4 days per week at the study site was estimated at 74. During the survey, 68 persons (92%) were interviewed. The median age was 39 years. This figure may appear to be high but it can be explained because children > 11 years of age go to school in Cayenne. Therefore, they did not meet the criteria to be included in this study.

From August 1 to December 31, 48 people had a total of 90 malaria attacks. The malaria attack rate reached 71/100 (incidence density rate of 6.0/100 person-weeks) with a peak in November 2008. These findings were in accordance with local epidemiologic surveillance data[8]; the malaria attack rate was most likely over-estimated because of possible relapses caused by *P. vivax*. The median duration between two *P. vivax* attacks was 41 days. Hanf and others[12] studied the *P. vivax* relapse pattern in French Guiana on a cohort of children born in Camopi, a village on the Oyapock River (Figure 1). Results suggested that a *P. vivax* attack is a relapse if < 90 days separates it from the previous one. Taking into account this result, nine malaria attacks could be excluded from our survey, resulting in a significant decrease of 10% of the incidence density rate (incidence density rate of 5.4/100 person-weeks). *Plasmodium falciparum* was responsible for 58% of the malaria attacks. Malaria treatment recommendations, adapted to the chemosusceptibility profiles of parasites circulating in the area, were well followed (91%).[4] *In vitro* susceptibility data from parasites isolated in gold miners circulating in Saül support the same conclusion (data not shown). Treatment observance was presumed to be correct and vomiting was the most frequently undesirable effect reported. Multivariate analysis showed an increase in the risk of malaria for people: 1) who visit « Crique Popote » and « La Palmeraie » (2-fold increase); 2) aged between 40 and 49 years (3-fold increase); 3) living in a house not subjected to IRS in the 6 months preceding the disease (3-fold increase); and 4) practicing hunting activities (3-fold increase).

In January 2009, we asked people about the sites they visited between August and December 2008. Obviously, inaccuracies of time or place caused by respondent's recall of these activities could cause errors. Nevertheless, we analyzed the association between malaria attacks and traveling during the 2 months preceding each malaria attack on a month-scale. We thought that the use of this time scale, even if it might cause a loss of accuracy, may help people remember their whereabouts. Indeed, in case-control studies, the "cases" generally remember expositions better than "controls". Inaccuracies of time and place related to faulty memory of respondents

because of the time elapsed between potential exposure and the survey could be responsible for information bias. The impact of such bias on the results is difficult to estimate.

The villagers of Saül participate in agricultural, cattle breeding, and forest activities such as hunting, fuel, or food gathering for village consumption. Villagers 40–49 years of age could be more exposed because of their use of forest paths and hunting/fishing spots used by illegal gold miners. At the same time period of the epidemic in the village, 62 gold miners had 72 malaria attacks diagnosed at the health post, including 77.5% *P. falciparum* (data not shown). The peak of diagnosis was observed in October, 1 month before the epidemic peak observed among Saül inhabitants. In the absence of selection bias during this 5-month period, the results strongly support the hypothesis that the malaria recrudescence in the village is directly linked to the presence of illegal gold mining activities in the area. Indeed, several factors could have enhanced malaria transmission, including massive environmental changes caused by gold mining activity, altering vector population dynamics,[13] or the high turnover of the workers who live in precarious conditions with poor access to disease prevention and care. In addition, prospectors regularly go to Saül to get fresh supplies (food, material, etc.) or for health care, presenting opportunities for transmission to the permanent residents.

Because 10 villagers presented malaria infection with no report of traveling outside the village and *An. darlingi* specimens were identified in the village for the first time, a local malaria transmission in the village was highly suspected. The introduction of new foci of malaria transmission in the forest and surrounding villages following gametocyte introduction by gold miners is not unusual. Indeed, in Brazil, malaria outbreaks generated by the arrival of gold prospectors coming from endemic areas were described most recently in Monte Alegre de Minas (south east region in the state of Minas Gerais),[14] and in the early 1990s in Mato Grosso.[15]

As for individual vector prevention, bednets were used by 79% of the subjects among which 51% were insecticide-treated nets (ITN). In Saül, a large part (42.6%) of the population lives in « open » houses. Because this kind of house does not prevent mosquito bites, the use of bednets is strongly recommended. Contrary to generally reported results, our study found that use of bednets was not significatively associated with malaria risk. Several reasons could explain this result 1) during the interview, people might have, voluntarily or not, over-estimated their bednet use; 2) their bednets might be in poor condition (tears, holes, etc.); 3) a misuse of the bednets could allow mosquitoes to bite (a body in contact with the bednet or partially outside); and 4) anopheline mosquitoes circulating in Saül may bite during daytime or early at night when people are still active and not yet under the bednet. *Anopheles darlingi* bites predominantly outdoors during the early evening.[13,16] The efficiency of ITNs in preventing malaria in forest areas has been demonstrated in the Amazon region and in South East Asia.[17,18] Since 2006, French sanitary authorities have promoted the use of long-lasting insecticidal nets (LLIN) in French Guiana.[19] The LLIN are free for women giving birth in French Guiana and undergoing medical surveillance by the "Service de Protection Maternelle et Infantile". Furthermore, bednets are distributed when there is increased risk of vector-borne disease outbreak (flooding, malaria outbreak, etc.).[19] In other cases, no financial support is allocated for bednets, which sell for around 20 euros (€). During this investigation in Saül, the health authorities decided to distribute LLIN to all Saül inhabitants.

Only 64% of subjects reported using topical mosquito repellents at home. This use significantly decreased with age, with 60% of people 40–49 years of age declaring using topical repellents at home (data not shown). Furthermore, some of the people interviewed thought that topical repellents were ineffective or even toxic, which may contribute to a poor use of this malaria prevention measure. Even if the cost of topical repellents can discourage their widespread use, Moore and others[20] defends their role in malaria prevention in the particular situation of the Americas because of the multiplicity of malaria vector species and because of their various behaviors. Entomological results of this study in Saül showed vector multiplicity, as seven anopheline species were identified. This included *An. darlingi* and other anophelinae species, such as *An. neivai* or *An. nuneztovari*, which are putative malaria vectors in the Amazonian region.[21,22] Therefore, promotion of efficient low cost topical repellents should be encouraged in the future in the context of Saül and, on a larger scale, in all areas where populations live next to gold mining activities.

For collective vector control, IRS seemed to be effective because the absence of IRS in the previous 6 months increased the risk of malaria by three. The IRS effectiveness depends on endophilic/endophagic behavior of vector mosquito species and on the presence of walls and ceilings in the house. According to the World Health Organization (WHO),[23] IRS may be relatively ineffective in some Amazonian regions because of 1) the behavior of selvatic anopheline mosquitoes (exophilic vectors), 2) the behavior of the inhabitants (mobility of settlements), and 3) the house configurations (temporary shelters without walls to be sprayed). Our results suggest that IRS is adapted to the context of the village of Saül where houses are closed constructions and inhabitants are sedentary. Moreover, in the most stable agricultural communities of the Amazon region, well-organized spraying operations with sufficient logistical support are able to reduce malaria transmission.[23] The IRS requires a high level of coverage, in space and time, of the surfaces where the vector is likely to rest. The duration of the biocide effect of deltamethrin formulation (used by DDAS) may vary between 1 and 9 months depending on the dosage and the nature of the surfaces treated. In Saül, insecticide spraying is scheduled every 3 or 4 months by the DDAS,[24] allowing for permanent insecticide coverage. However, a high level of coverage is generally difficult to achieve because IRS is not always well accepted by villagers because of the need to cover all furniture and food, and to stay out of the house for a few hours after the spraying. It is therefore fundamental to increase the population awareness of the importance of IRS in malaria control to improve its acceptance.

To conclude, this study confirmed the hypothesis of a malaria transmission in the village of Saül even though breeding places were difficult to find and aggressive female densities observed on humans were very weak. It is clear that further entomological investigations are necessary to unravel malaria transmission in the village of Saül and its close surroundings. The role of illegal mining in the emergence of new foci of malaria transmission is very likely and this risk is shared by all countries of the Guyana Shield. Therefore, it is essential that public health authorities, in collaboration with bordering countries, develop an ambitious policy against

malaria, taking into account the presence of illegal gold miners who can introduce public health risks for local populations.

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