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Case Report: Successful Sporozoite Challenge Model in Human Volunteers with *Plasmodium vivax* Strain Derived from Human Donors

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Abstract

Successful establishment of a *Plasmodium vivax* sporozoite challenge model in humans is described. Eighteen healthy adult, malaria-naïve volunteers were randomly allocated to Groups A–C and exposed to 3 ± 1 , 6 ± 1 , and 9 ± 1 bites of *Anopheles albimanus* mosquitoes infected with *P. vivax*, respectively. Seventeen volunteers developed signs and symptoms consistent with malaria, and geometric mean prepatent periods of 11.1 days (9.3–11) for Group A; 10.8 days (9.8–11.9) for Group B; and 10.6 days (8.7–12.4) for Group C, with no statistically significant difference among groups (Kruskal-Wallis, $P = 0.70$). One volunteer exposed to eight mosquito bites did not develop a parasitemia. No differences in parasite density were observed and all individuals successfully recovered after anti-malarial treatment. None of the volunteers developed parasite relapses within an 18-month follow-up. In conclusion, malaria-naïve volunteers can be safely and reproducibly

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infected with bites of 2–10 *An. albimanus* mosquitoes carrying *P. vivax* sporozoites. This challenge method is suitable for vaccine and anti-malarial drug testing.

INTRODUCTION

An estimated 80 million cases of *Plasmodium vivax* malaria occur annually worldwide¹ and failure of classic malaria control measures has prompted the search for a malaria vaccine. Progress has been achieved in the development of *P. vivax* pre-erythrocytic subunit vaccines such as the circumsporozoite (CS) and thrombospondin-related adhesion proteins (TRAP)² and vaccines based on asexual blood stage antigens such as the merozoite surface protein-1 (MSP-1) and the Duffy-binding protein (DBP).^{3,4} Several preclinical trials using both synthetic peptides and recombinant proteins representing these and other antigens have been carried out in non-human primates.² Additionally, results from Phase 1 clinical trials conducted with different formulations of *P. vivax* CS-derived subunit vaccines indicate that these vaccines are safe and immunogenic.^{4,5}

Evaluation of the safety and efficacy of pre-erythrocytic and possibly also erythrocytic stage malaria vaccines is greatly facilitated by a safe, reliable, and reproducible method of infecting human volunteers. With the development of continuous *P. falciparum* cultures,⁶ a successful model of infection with this species through mosquito bites was developed two decades ago,⁷ which in turn led to progress in the development of RTS,S/AS02A, a vaccine based on the *P. falciparum* CS protein,^{8–10} and other vaccine candidates.¹¹ Experimental human sporozoite challenge has also contributed to systematically assessing the efficacy of *P. falciparum* irradiated sporozoite vaccines in malaria-naïve volunteers and to propose its development as a cryopreserved, non-replicating vaccine against malaria.^{12,13}

At present, without long-term *in vitro* cultures for *P. vivax*, an experimental sporozoite challenge model would require parasites derived from the blood of *P. vivax*-infected patients. The main objective of this study was to develop a reproducible *P. vivax* sporozoite challenge model that is urgently needed for assessing the protective efficacy of *P. vivax* subunit or attenuated parasite vaccines. Herein, we report findings on the development of a *P. vivax* sporozoite challenge model in malaria-naïve volunteers exposed to bites of *Anopheles* mosquitoes infected by blood from infected patients.

MATERIALS AND METHODS

Study participants

Eighteen healthy malaria-naïve subjects (19–45 years of age) participated as challenge volunteers, and 15 *P. vivax*-infected patients served as parasite donors. All participants were recruited after the protocol was approved by the Ethics Committee of the Universidad del Valle, the Fundación Clínica Valle del Lili, and Springfield Committee Research Involving Human Subjects (SCRIHS) appointed by the World Health Organization (WHO). The trial complied with the International Conference on Harmonization (ICH) E-6 Guidelines for Good Clinical Practices.

During recruitment, potential risks of participation were explained, including exposure to potential unknown pathogens harbored by *Anopheles* mosquitoes and to *P. vivax* parasites. Similarly, the potential symptoms associated with malarial infection and to anti-malarial therapy were explained. Study participants were provided ample opportunity to read the consent forms, to ask questions of the study investigators, and to consult with family and friends. Separate written informed consents were obtained from each volunteer for enrollment and for human immunodeficiency virus (HIV) screening. Participants were

allowed to withdraw voluntarily from the study at any time. Individuals were excluded from the study if they had conditions that would increase the risk of an adverse outcome or abnormal laboratory test values (Table 1).

Study design

A randomized, open-label clinical trial was designed to standardize a *P. vivax* sporozoite challenge model in malaria-naïve volunteers, specifically, the relationship between the number of infectious mosquito bites and the likelihood of developing patent parasitemia detectable by Giemsa-stained thick blood smear (TBS). The study was divided into two steps. Step A was designed to produce mature *P. vivax* sporozoites suitable for inoculation into humans; and Step B was designed to assess the safety and reproducibility of the sporozoite challenge. For Step A, patients with infective *P. vivax* gametocytes detected by TBS were recruited from the outpatient clinics at the Immunology Institute (IDIV) in Cali and Buenaventura, Colombia. Patients donated 35 mL of whole blood, which was screened for co-infections that could potentially represent a threat to the health of volunteers. The blood was then artificially fed to *Anopheles* mosquitoes.¹⁴ For Step B, malaria-naïve subjects were exposed to the bites of 3 ± 1 , 6 ± 1 , or 9 ± 1 infected mosquitoes to determine the prepatent period of the infection and its reproducibility.

Blood donation and blood quality assurance

The EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) were used to collect 35 mL of whole blood from each infected patient, which were divided into two aliquots: a 15 mL sample for mosquito feeding and a 20 mL sample that was transported to the blood bank of the Valle del Lili Clinic for routine screening for a panel of common infectious agents (viral, bacterial, parasitic), including confirmation of *Plasmodium* species (*P. vivax*, *P. falciparum*, *P. malariae*) by polymerase chain reaction (PCR) (Table 2).¹⁵ The rationale behind the blood screening and direct mosquito feeding on human volunteers was based on two premises: 1) standard blood bank screening is universally accepted as a safety procedure that ensures that no pathogens are transmitted from human-to-human during a blood transfusion. Therefore, blood samples determined to be negative for pathogens other than *P. vivax* by standard blood bank screening should be safe enough to be transferred from human-to-human through a mosquito bite. 2) *Anopheles* mosquitoes have not been reported to transmit any pathogen other than *Plasmodium*. Before the study, we carried out an extensive bibliographic search and consulted with U.S. and Colombian experts on virology, parasitology, and entomology about potential pathogens that might be transmitted by *Anopheles* and could represent a threat for the volunteers exposed to challenge. There was a consensus that as yet no other pathogen had been reported to be transmitted by *Anopheles* mosquitoes, including those comprised in the blood bank screening. However, mosquitoes were discarded using a biosafety procedure if after feeding blood was confirmed to have any co-infection.

Mosquito infection

Anopheles albimanus mosquitoes Buenaventura strain, have been reared under laboratory conditions at IDIV in Cali for about 10 years. This mosquito colony has been successfully used to regularly produce sporozoites^{14,16} and for studies on malaria transmission-blocking immunity.¹⁷ Here, mosquitoes were fed within 2–3 hours after the *P. vivax*-infected blood (gametocytemia $\geq 0.1\%$) was collected in Buenaventura. The 15 mL blood was centrifuged at $500 \times g$ for 5 minutes at room temperature, plasma was removed, and cells were washed once with RPMI 1640 medium (Gibco Cell Culture Systems, Invitrogen, Grand Island, NY). Parasitized erythrocytes were reconstituted to 50% hematocrit with pooled human AB non-immune, complement-inactivated serum obtained from the Red Cross blood bank and were used to infect lots of 4,000 female mosquitoes (3–4 days old) using a water-jacketed

membrane apparatus at 37°C, described previously.¹⁴ Infected mosquitoes were maintained under strict biosafety conditions at $27 \pm 1^\circ\text{C}$ and relative humidity of 82% and a sugar solution supplemented with 0.05% para-aminobenzoic-acid was provided.¹⁸ Samples of fed mosquitoes were dissected and microscopically examined 7–8 days after the blood meal to determine the presence of oocysts in the midgut, and on Days 14 and 15 to assess the presence of sporozoites in salivary glands.¹⁹ Mosquito infections were graded as 1+ (1–10 spz), 2+ (11–100 spz), 3+ (101–1000 spz), and 4+ (> 1001 spz).⁷

Sporozoite challenge

The proposed infective biting dose was to be 3, 6, and 9 mosquitoes, respectively. However, a range of plus or minus one bite per dose was allowed. Therefore, screen-meshed boxes ($7 \times 7 \times 7$ cm) were filled with 4, 7, and 10 mosquitoes, to have a better chance of achieving the targeted mosquito dose in a single biting round. Participants who did not complete the minimal targeted dose were subjected to a second round of biting. Eighteen malaria-naïve volunteers were randomly assigned to one of three groups ($N = 6$) and were exposed by group, from the fewest (3 bites) to the greatest (9 bites) number of bites. Sporozoite challenge was carried out under strict adherence to experimental protocol in a secure room in the entomology unit at the IDIV. Volunteers were asked not to use any topical chemicals (e.g., soap, deodorant, perfume) that could affect mosquito feeding. Mosquitoes were allowed to bite the flexor side of the forearm for a 10 to 15-minute period, previously determined to be sufficient for full *An. albimanus* engorgement. After biting, all mosquitoes were dissected to confirm the presence of blood meal and sporozoites. Mosquitoes were considered positive if any number of sporozoites (> 1) was detected microscopically. Study participants were observed for 1 hour at the entomology unit, followed up by phone 8 hours later, and examined again 24 hours thereafter to assess the response to challenge.

Malaria diagnosis and patient follow-up

From Day 7 post-challenge onward, volunteers had daily follow-up visits during which symptoms and signs of malaria were assessed and blood was collected for TBS and *Plasmodium* PCR (the latter performed retrospectively).

The TBS were performed using 50 μL of whole blood collected by finger prick that were spread over a rectangular area of approximately 1.5 sq cm and were stained with freshly prepared 10% Giemsa stain.²⁰ A total of 300 microscopic fields were examined before a slide was considered negative. When TBS were found positive, parasitemia was quantified by estimating the number of parasites counted in presence of 300 leukocytes and calculating absolute parasitemia according the leukocyte counts per μL . Smears were read by experienced microscopists and a random sample of TBS was subjected to quality control by a microscopist from the malaria control program.

Participants were treated with anti-malarial drugs as soon as parasites were detected by TBS. Treatment consisted of chloroquine (1,500 mg chloroquine base provided orally in divided doses: 600 mg initially followed by 450 mg given 24 and 48 hours later) and primaquine (two 15 mg doses given once per day for 14 days). Volunteers were provided with intravenous fluids, analgesics, and antiemetics as needed. Signs and symptoms consistent with malaria were assessed by clinical examination and were graded 1–5 according to their severity following the National Cancer Institute (NCI) criteria for adverse events (CTCAE) as follows: Grade 1 = Low, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Serious, and Grade 5 = Death. Pruritus was graded from 1 to 3 (1 = mild or localized; 2 = intense or widespread; and 3 = intense or widespread and interfering with activities of daily living). Because there is no common terminology AE agreement, for edema and erythema following mosquito bites, an adaptation from the CTCAE v 3 for “injection site/extravasation changes”

was used as follows: Grade 1 = local pain/edema and erythema; Grade 2 = pain or swelling with inflammation or phlebitis; Grade 3 = ulceration or necrosis. Pain was scored as Grade 1 = mild not interfering with function; Grade 2 = moderate pain (pain or analgesics interfering with function, but not interfering with activities of daily living); Grade 3 = severe pain (pain or analgesics severely interfering with activities of daily living).

Clinical laboratory tests

Clinical laboratory screening including an electrocardiogram confirmed the health status of volunteers 1 month before challenge and after the study was completed (Table 1). Any abnormality found in the electrocardiogram was considered as an exclusion criterion. Tests for hemoglobin, white blood cell count, platelet count, and total bilirubin were performed again on Days 11 and 53 post-challenge.

Statistical methods

Sample size ($N = 6$ per group) was based on the minimum number of individuals that would be allowed to observe the occurrence of rarer events (e.g., events that occur in approximately 5% of individuals) with reasonable probability based on a binomial assumption. The overall sample size of 18 would be sufficient to detect even rare events. A titration of mosquito bites (biting doses) was established; beginning with that arbitrarily considered the minimum number required causing an infection. Prepatent periods and duration of symptoms were expressed as geometric means. Differences among the groups were estimated by the Kruskal-Wallis test, and were considered statistically significant at P values less than 0.05.

RESULTS

Study population

Eighteen volunteers (9 men, 9 women) that received sporozoite challenge had a mean age of 27 years and were randomly assigned to three groups of six volunteers (3 men and 3 women per group) with no differences in age between the groups (Kruskal-Wallis, $P = 0.70$) (Table 4). The 15 *P. vivax*-infected donors (11 men, 4 women) had a mean age of 30 years.

Mosquito infection

The 15 patients provided written informed consent and served as donors of infected blood. Whole blood samples were examined using standard blood bank screening for co-infections; of those a total of four presented co-infections such as *P. falciparum*, Hepatitis B, and Hepatitis C and were discarded (Table 3). Blood used for mosquito feeding had parasite densities ranging from 80–8,720 (mean 3,693) *P. vivax* asexual erythrocytic stage parasites/ μL and gametocytemia ranging from 40–3,360 (mean 934) parasites/ μL .

Four mosquito lots were discarded because of co-infection in blood used for feeding. One parasite donor had a mixed *P. vivax*/*P. falciparum* infection and three blood samples carried the hepatitis B or hepatitis C virus; patients were treated and referred for medical follow-up. Six of the remaining 11 lots of mosquitoes showed infections with $> 50\%$ of the mosquitoes having sporozoites in salivary glands, whereas the other five were considered ineligible for challenge because of low percentage ($< 50\%$) of sporozoite-infected mosquitoes. On the day before challenge, two mosquito lots examined microscopically showed sporozoite rates of 97% and 86%, respectively. The 97% lot (3 + sporozoite score in salivary glands) was selected for use.

Sporozoite challenge

Sixteen volunteers were exposed to infection during the first day and the other two (from Group C), were challenged on Day 2. The number of mosquitoes required to complete the biting dose and the number of cycles required for each dose is indicated in Table 4. Some individuals developed minor, transient discomfort that was not associated with the number of bites. Local pruritus was observed most frequently in Group B (four of six volunteers) but disappeared within 2 days. One volunteer from Group C developed mild arm pain and localized pruritus that lasted for 5 days (Table 5).

Prepatent period and clinical follow-up

Seventeen volunteers developed malaria as confirmed by TBS and PCR, and one volunteer subjected to eight mosquito bites was determined negative for malaria in a total of 20 thick smears and 15 PCR performed during the 30-day follow-up period. We believe that this volunteer who was a paramedic, surreptitiously used anti-malarials; however, as serum levels of anti-malarial drugs were not measured, we are not able to make an unequivocal confirmation. The latter (volunteer no. 213) was given anti-malarial treatment on Day 30 and was followed up as per protocol (Table 4). Patent parasitemia was confirmed between 9 and 13 days (geometric mean 11 ± 2 days) after the mosquito bites without any correlation between the challenge dose and the prepatent periods, which were 11.1 days, confidence interval (CI) 95% (9.3–11), for Group A; 10.8 days, CI 95% (9.8–11.9), for Group B; and 10.6 days, CI 95% (8.7–12.4), for Group C. No statistically significant difference was observed in the prepatent period of the different groups (Kruskal-Wallis $P = 0.70$) (Table 1). In five cases, parasites were detected earlier by PCR than by TBS, whereas in 11 cases both methods were equally sensitive. In one case, parasites were detected earlier by TBS than by PCR. Parasitemia ranged from 75 to 420 parasites/ μ L (Table 4).

All 17 infected-volunteers started to develop signs and symptoms consistent with malaria on Day 9 post-challenge, but some of the clinical manifestations appeared only 4 to 5 days later. The most frequent were chills, fever, headache, arthralgia, myalgia, and malaise followed by weakness and nausea (Table 6). Fever (axillary temperature $> 38^{\circ}\text{C}$) was documented in 10 of the volunteers and six more reported having had fever without confirmation. All individuals cleared parasitemia between 24 and 48 hours after initiating anti-malarial treatment, and all successfully recovered within 2 to 3 days without any severe or serious adverse events (Table 6). No parasites including gametocytes were observed after treatment. The most frequent symptoms associated with treatment were epigastralgia with duration of 1 to 21 days; nausea, 1–5 days; and vomiting, 1–3 days.

In addition, five patients developed blurred vision that lasted for 2 to 3 days after treatment initiation (Table 6). None of the volunteers required hospitalization, but because of nausea and vomiting, seven required fluid therapy that was administered at the MVDC outpatient clinic. The volunteer who did not become infected was treated with the anti-malarial protocol after a month of follow-up.

Clinical laboratory follow-up

Hematologic tests indicated that except for one individual that had a moderately decreased hemoglobin (Hb) level on Day 11 (Hb = 10.3 g/dL), all volunteers maintained normal Hb levels throughout the study. Eight volunteers developed mild leucopenia (2,100–4,000 white blood cell [WBC]/ μ L) on Day 12 post-challenge, and three had platelet counts below 150,000/ μ L, with the lowest being 96,000/ μ L. Only one of the individuals (no. 226) had a decrease in all three hematologic parameters. All abnormal hematologic values rapidly returned to normal after treatment. Four individuals had abnormal alanine aminotransferase (ALT) on Day 12 and two of them maintained mildly increased levels on Day 53. Except for

two individuals that had slightly high levels of blood urea nitrogen (BUN) on Day 53, BUN values remained normal in all volunteers throughout the study. BUN values in these two participants returned to normal levels at follow-up visit 1 month later.

DISCUSSION

We showed that malaria-naïve volunteers can be safely and reproducibly infected by bites of *An. albimanus* mosquitoes carrying *P. vivax* sporozoites. Although prepatent periods in the 17 infected volunteers varied between 9 and 13 days, all developed malaria symptoms at Day 9 post-challenge. Fever was not as frequent as expected and it was not an early symptom. Over half of the individuals presented with their first febrile episode after Day 10. Malaise, arthralgia, myalgias, and headache occurred more frequently and appeared earlier than fever.

Prepatent periods (geometric mean, range 9.3–11) were similar to those reported for *P. vivax* in a previous study.²¹ We were, however, surprised that there was no association between the number of mosquito bites and the length of the prepatent period. In previous studies conducted with *P. falciparum*, an inverse correlation between the number of infectious bites and the prepatent period was reported.^{22,23} Nevertheless, in a recent study by Verhage and others,²⁴ only a weak inverse relationship between the number of *P. falciparum*-infected mosquito bites (1–2 bites versus 4–7 bites) and the prepatent period was found. Of note, all the volunteers were exposed only to one mosquito lot infected from a single donor. It is feasible that the prepatent period, and other pathogenic features, might rely more on the infectious strain than on the amount of inoculated parasites. Challenges including mosquito lots infected from several donors might result in more variable prepatent periods.

As expected, because of prompt diagnosis, there was no great difference in parasite density (75–420 parasites/ μ L) between the groups, and no relationship between parasitemia and malaria symptoms was observed. Interestingly, parasitemia was cleared in most cases (13/17) within the first 24 hours of treatment and the remainder cleared within 2 days. The fact that parasitemia, including gametocytemia, was not observed after anti-malarial treatment, provides confidence on the efficacy of the treatment particularly to prevent any further malaria spreading through the infected volunteers. Furthermore, the study site (Cali) is not endemic for malaria transmission because of the absence of local malaria vectors. Even though all volunteers presented with symptoms beginning at Day 9 post-challenge, prepatent periods were variable. The narrow prepatent window observed in this study would be suitable for vaccine testing as it would allow the use of smaller experimental groups to determine differences between controls and immunized volunteers. This model would allow assessment of whether vaccinated individuals become completely immune (no parasitemia) or develop partial protection (prolonged prepatent periods).

In conclusion, the challenge system used in our study was found to be safe and to result in only mild to moderate clinical signs and symptoms, which resolved quickly without complications. Careful volunteer selection and follow-up is advisable to avoid spreading of the infection after travel to areas with capable vectors, and to provide timely therapy in case of relapses. Most laboratory abnormalities were mild and resolved after treatment.

Because there is as yet no system to cryopreserve *P. vivax* gametocytes for further mosquito infection, new trials may be required to conclusively determine the reproducibility of this method with different wild *Plasmodium* isolates. For this purpose, we plan to conduct a new challenge trial using different *P. vivax* isolates at the lowest dose (2–4) that proved to induce reproducible infections. To ascertain the reproducibility of infection in all exposed volunteers, it would be advisable to consider the determination of anti-malarial consumption

in challenged volunteers. Furthermore, to address the variability that is likely to result from the fact that every challenge will have a different donor strain, we plan to establish a bank of parasite isolates for further genotyping. The availability of this *P. vivax* challenge model, in conjunction with new methodologies such as transcriptome analysis of infected individual volunteers, should facilitate a better understanding of the natural history of *P. vivax* infection.^{25,26} Most importantly, the development of this model is an essential step in the critical path to effective vaccines and anti-malarial drugs against *P. vivax* infections.

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Table 1

Study participant requirements

Exclusion criteria *	Clinical laboratory test
Hepatitis B or Hepatitis C virus infections	Hematologic
HIV/AIDS infection	CBC, reticulocyte count, G-6-PD determination, Duffy phenotype, ABO and Rh group typing, hemoglobin electrophoresis and erythrocyte sedimentation rates and APTT [†]
Cardiovascular disease	Cardiovascular function
Hepatic or renal abnormalities	Electrocardiogram
Immunodeficiency	Renal function
Autoimmune diseases	Urinalysis, Creatinine, Urea and BUN [‡]
Allergies	Hepatic function
Duffy negative phenotype	ALT, AST, Bilirubin, LDH, Alkaline phosphatase, Total protein and PT [§]
Previous malaria exposure (IFAT [§] > 1:100)	Blood chemistry
Residence in a malaria-endemic area	Glucose, C reactive protein and ANA
Previous participation in experimental malaria vaccination	Infectious diseases
Pregnancy, breast feeding or plans to become pregnant during the study	HIV, HBC, HCV, HTLV-1 and -2, RPR for syphilis and Chagas disease
Plans to have surgery	Pregnancy testing
	β-HCG in serum

* Subjects must not meet any of the following exclusion criteria to be eligible for participation in this study.

[†] APTT = activated partial thromboplastin time.

[‡] PT = prothrombin time.

[§] IFAT = indirect fluorescent antibody test (Pv/Pv asexual stages).

Table 2

Microbial agents included in the blood bank screening for parasite donors*

Disease	Pathogen	Technique	Reference values	Commercial brand
Malaria	<i>P. falciparum/P. vivax</i>	PCR	Positive or Negative	Experimental
Hepatitis B	HB virus	MEIA for HBsAg Abs	OD \geq 2.0	ABBOTT
Hepatitis C	HCV-3 Antibodies	MEIA for HCV-3 Abs	OD \geq 1.0	ABBOTT
Syphilis	<i>Treponema pallidum</i>	Flocculation of reagins and cardiolysins coated coal particles	Dark aggregates	Biomerieux
PET	HTLV I/HTLV II	MicroELISA for gp46-I, p21-I, gp46-II	> Cutoff	ABBOTT
CHAGAS	<i>Trypanosoma cruzi</i>	MicroELISA for <i>T. cruzi</i> rec proteins	> Cutoff	IMMCO

* All tests have external periodical QC by the College of American Pathologist, The Medical Laboratory Evaluation, and the Colombian regulatory agency INVIMA.

Table 3

Outcomes of blood bank screening of *P. vivax* parasite donors

Individuals	PCR		HB	HC	Syphilis	HTLV I/II	Chagas	HIV	Infectivity	Eligibility
	<i>P. vivax</i>	<i>P. falciparum</i>								
1	+	+	0.86	0.42	NR [†]	0.155	0.166	0.27	68.42	E [‡]
2	+	–	0.72	0.52	NR	0.067	0.153	0.27	86.48	E
3	+	–	0.69	0.31	NR	0.07	0.144	0.24	97.34	E
4	+	–	0.51	0.35	NR	0.075	0.084	0.25	47.37	I [§]
5	+	–	0.57	0.61	NR	0.07	0.072	0.24	31.58	I
6	+	–	0.32	0.75	NR	0.071	0.048	0.35	36.84	I
7	+	–	0.62	0.54	NR	0.063	0.042	0.42	47.37	I
8	+	–	0.54	0.54	NR	0.051	0.181	0.25	Unknown	Discarded
9	+	–	0.68	0.38	NR	0.054	0.12	0.26	44.73	I
10	+	–	0.7	2.16	NR	0.055	0.16	0.32	15.79	I
11	+	–	0.62	0.73	NR	0.053	0.078	0.26	21.05	I
12	+	–	0.81	0.51	NR	0.077	0.097	0.33	Unknown	Discarded
13	+	–	0.74	0.55	NR	0.05	0.091	0.32	Unknown	Discarded
14	+	–	0.62	0.27	NR	0.05	0.219	0.24	Unknown	Discarded

* All blood samples whose outcomes were above normal values were rejected.

[†]NR = non reactive.[‡]E = eligible.[§]I = ineligible.

Table 4

Prepatent period and malaria diagnosis

Group	Volunteer's code	Age	Gender [*]	No. mosquito bites	No. biting rounds	Prepatent period (days)		PD [§]
						TBS [†]	PCR [‡]	
A	206	41	F	4	1	11	11	152
	207	22	F	4	2	9	9	298
	208	32	M	4	1	9	9	144
	221	23	F	4	1	12	12	93
	222	46	F	2	1	13	10	280
	226	20	F	4	2	13	13	56
B	201	25	M	6	1	10	10	179
	210	22	F	6	1	11	10	256
	217	20	F	7	1	12	11	125
	220	23	M	6	2	10	9	176
	223	24	M	7	1	12	13	229
	234	25	M	7	1	10	10	115
C	215	27	M	8	1	9	9	303
	213	36	M	8	1	NI [¶]	NI	NI
	204	23	M	8	1	12	12	187
	211	23	M	10	1	12	10	109
	219	27	F	8	1	9	9	420
	224	26	F	9	2	11	10	75

^{*} F = female; M = male.[†] TBS = thick blood smear.[‡] PCR = polymerase chain reaction.[§] PD = parasite density (parasite/μL blood).[¶] NI = not infected.

Table 5

Adverse events associated with mosquito bites

Adverse events	A				B				C			
	Frequency	Duration (days)*	Severity grade [†]		Frequency	Duration (days)*	Severity grade [†]		Frequency	Duration (days)*	Severity grade [†]	
			1	2			1	2			1	2
Local Pruritus	1/6	1	X	-	4/6	1.25	X	-	1/6	5	X	-
Edema	1/6	1	X	-	1/6	1.25	X	-	0/6	-	-	-
Erythema	1/6	1	X	-	2/6	1.50	X	X	0/6	-	-	-
Arm pain	1/6	1	X	-	0/6	-	-	-	1/6	5	X	-

* Geometric mean.

[†] Grade 1 = low; Grade 2 = moderate.

Table 6

Symptoms and signs related with *P. vivax* infection

Adverse events	A			B			C		
	Frequency %	Initial day*	Duration (hour) [†]	Frequency %	Initial Day*	Duration (hour) [†]	Frequency %	Initial day*	Duration (hour) [†]
Associated to infection [‡]									
Symptoms									
Myalgias	6/6	9	74.4	6/6	10	43.2	5/5	9	57.6
Chills	4/8	9	64.8	6/6	10	50.4	5/5	9	48.0
Headache	6/6	9	108.0	6/6	9	60.0	5/5	9	67.2
Malaise	5/6	9	76.8	6/6	10	62.4	5/5	9	52.8
Weakness	5/6	10	62.4	5/6	10	81.6	4/5	10	52.8
Signs									
Temperature > 38°C	2/6	9	48.0	4/6	10	28.8	3/5	9	55.2
Jaundice	0/6	-	-	3/6	12	48.0	0/6	-	-
Tachycardia	1/6	9	24.0	0/6	-	-	1/5	9	24.0
Splenomegaly	0/6	-	-	0/6	-	-	1/5	10	24.0
Laboratories									
Lymphopenia	5/6	10	NA [§]	1/6	14	NA	2/5	10	NA
Thrombocytopenia	1/6	13	NA	NA	-	-	NA	-	-
GPT increase	1/6	11	NA	2/6	14	NA	2/5	12	NA
Hyperbilirubinemia	-	-	-	-	-	-	1/5	10	NA
BUN [¶]	2/6	53	NA	-	-	-	-	-	-
Associated with treatment [‡]									
Abdominal pain	2/6	17	96.0	0/6	-	-	2/5	1	24.0
Dizziness	3/6	15	134.4	2/6	15	72.0	3/5	16	72.0
Nausea	2/6	16	67.2	4/6	15	96.0	1/6	15	24.0
Epigastric pain	2/6	22	192.0	4/6	16	102.0	3/5	15	247.2
Vomiting	2/6	18	24.0	2/6	16	48.0	0/6	-	-
Blurred vision	4/6	19	60.0	1/6	15	48.0	0/5	-	-

* Days after parasite challenge, expressed as geometric mean.

[†] Geometric mean.

[‡] Days after treatment initiation expressed as geometric mean.

[§] NA = not applied.

^{||} GPT = glutamic pyruvic transaminase.

[¶] BUN = blood urea nitrogen.