

## EVALUATION OF SPf66 MALARIA VACCINE EFFICACY IN BRAZIL

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**Abstract.** This paper reports the efficacy results of the randomized, placebo-controlled, field trial of SPf66 malaria vaccine in Costa Marques, Rondonia, Brazil. This region is characterized by the seasonal distribution of *Plasmodium falciparum* and *P. vivax* infections, and the recent occupation by migrants from nonendemic areas. A total of 800 individuals of both sexes, ranging in age from seven to 60 years, were included in the study. Of the initial cohort, 572 participants completed the vaccination schedule. Clinical and parasitologic evaluations were obtained by active and passive searches on a periodic basis. The overall protective efficacy against *P. falciparum* infections was -1.6% (-32.9% to 22.4%), and 14.1% (-17.0% to 36.9%) for the first episode. The overall protective efficacy for *P. vivax* infections was -19.7% (-44.8% to 1.03%), and -10.8% (-41.1% to 12.8%) for the first episode. No statistical evidence of an overall significant protective effect of SPf66 malaria vaccine against *P. falciparum* and *P. vivax* malaria was obtained in this trial.

In the past 20 years, considerable attention and resources have been focused on the research and development of malaria vaccines. A synthetic vaccine composed of sequences of three peptides from *Plasmodium falciparum* merozoite and erythrocytic stages and a peptide from the circumsporozoite protein designated SPf66 has recently been tested in several populations of endemic areas in South America, Africa, and Asia.<sup>1-10</sup> Although these studies have provided evidence that the vaccine is safe and immunogenic, the results on the efficacy are controversial, with estimates varying from -9% to 60.2%.

A randomized, placebo-controlled, field trial with the SPf66 vaccine was carried out in nonimmune residents of an endemic area in Brazil from 1991 to 1993. This is the first report of the vaccine efficacy evaluation.

### SUBJECTS AND METHODS

**Study area and population.** The study was carried out in the municipality of Costa Marques, State of Rondonia in the Brazilian Amazon basin (12°26'S, 64°14'W). The annual incidence rate of malaria has been reported to be 500 cases per 1,000, one of the highest in the country.<sup>11</sup> The region is characterized by the seasonal distribution of *P. falciparum* and *P. vivax* infections, and the recent occupation by migrants from nonendemic areas. *Anopheles darlingi* is the main local vector. Its spatial and seasonal distribution are closely related to the water levels of the local rivers and the rainy seasons.<sup>12-15</sup>

During the early stage of human colonization, the number of malaria cases increased dramatically. In 1983, a total of 3,125 cases were reported in contrast to 16,029 in 1987, with *P. falciparum* infections being more frequent.<sup>16</sup> Cases were observed mainly in migrants in all age groups and parasitemia was usually accompanied by classic symptoms. Subclinical infection was an exceptional finding.<sup>17</sup> Disease control measures consisted of insecticide spraying of all houses with DDT until 1991 and with lambda-cyhalothrin since then, as well as the treatment of cases detected by active and passive search. Since 1988, a reduction in the number of cases was observed. In 1990, 7,532 cases were reported, with *P.*

*vivax* infections being more frequent. A preliminary survey that preceded the vaccine trial reported an overall prevalence of 10%, with the ratio of *P. falciparum* to *P. vivax* infections being 1:1.<sup>18</sup>

*Plasmodium falciparum* infections are treated with a combination of quinine plus tetracycline in a 10-day schedule because resistance to chloroquine (81.8%), sulfadoxine-pyrimethamine (46.6%), and quinine alone (18.2%) has been reported.<sup>19</sup> Mefloquine is available and prescribed in cases of drug resistance that is confirmed parasitologically. Although malaria is considered to be the main health problem in the municipality, mortality is low, probably due to the easy access to diagnosis and specific treatment.<sup>18</sup> Cases accompanied by clinical signs of possible complications such as dehydration, jaundice, and conscience alterations are referred to the main health center in the village.

The National Health Foundation (FNS) of the Brazilian Ministry of Health is responsible for conducting all control measures in the area, including treatment. According to their local records, the total population of the municipality was 23,000 inhabitants in 1991, of whom 5,975 were settlers along the main road. They constitute a very unstable group since their permanency in the area depends mainly on economic success and health conditions. Once settled, their main occupations are agriculture and lumbering. Costa Marques can be considered as an agricultural frontier, with a mesoendemic level of malaria transmission, recently colonized by nonimmune individuals.<sup>20</sup>

**Vaccine.** The synthetic vaccine SPf66 was developed at the Instituto de Immunologia, Universidad Nacional de Colombia (Bogota, Colombia) by the research group of Dr. M. E. Patarroyo.<sup>21</sup> The vaccine, as well as the placebo (tetanus toxoid for the first dose and aluminum hydroxide for the second and third doses), were bottled in clear glass vials containing 10 doses each and coded in Colombia with the letters L and S, respectively. Vaccine and placebo preparations were visually indistinguishable and were kept at 4°C in insulated boxes. Investigators were kept blind during the execution of the trial and until the completion of the statistical analysis. The code was broken in August 1995.

The preparations were applied in three doses: the first on

day 0, the second on day 30, and the third on day 180. The recommended dose of 0.5 ml was applied subcutaneously in the deltoid region.<sup>22</sup> A surveillance system for adverse reaction detection was set up during every planned vaccination. Frequency, intensity, and description of any adverse effect were systematically recorded.<sup>23</sup>

**Study design and ethical considerations.** The objective of this trial was to evaluate the efficacy, immunogenicity, and safety of the SPF66 vaccine in nonimmune residents of a Brazilian endemic region. With respect to vaccine efficacy, we intended to estimate the overall field direct effectiveness for all, as well as the first and second episodes of malaria infections separately for *P. falciparum* and *P. vivax*. The term field direct effectiveness applies to situations in which attempts are made to control for the unknown amount of exposure to infection in the analysis, and when indirect effects of the vaccine interact with estimates of the direct protective effect (vaccine efficacy).<sup>18</sup> A further objective was to detect possible differences in asexual blood-stage parasite densities.

The study consisted of a randomized, double-blinded, placebo-controlled efficacy trial. The required sample size was calculated according to the World Health Organization guidelines for phase III field studies.<sup>24</sup> We assumed a prevalence of 10% and a vaccine efficacy of 80% (as established in the first field trial<sup>1</sup>). The sample size to detect such a difference at the 5% level of significance with 80% power was 134 individuals in each group. Assuming a 30% loss to follow-up, the sample size was increased to 179 subjects. Finally, assuming a design factor of 2, a total of 358 subjects in each treatment group seemed appropriate. We included 400 individuals in each group.

Individuals who fulfilled the selection criteria (male or female, 7–60 years of age, residing in the rural settlements along the road, and freely agreed to participate in the study) were randomly assigned a number between one and 800. All participants with an odd number received the preparation labeled L and those with an even number received the preparation labeled S. The choice of this sampling method was based on its simplicity, and thus facilitated the application of the preparations without errors and allowed for the comparability between the treatment groups. All participants signed an informed consent after receiving a clear explanation of its content. The participation of children was authorized by their parents or guardians. Pregnant women and individuals with acute or severe diseases, as well as with a history of allergies, were excluded. Both the medical investigator in charge of vaccinating, as well as the recipients, were kept blind with respect to which preparation was being applied. No schizonticidal drug was administered to any of the participants before the beginning of the trial. Blood samples were collected during every scheduled vaccination and immediately examined. All participants with asexual parasitemia (either species) were adequately treated within 24 hr, according to the FNS therapeutic schemes. Participants were followed-up for 18 months after the completion of the vaccination schedule. Malaria control measures remained unchanged during the trial.

The trial was approved by the Ethical Committees of the University of Brasilia, University of Sao Paulo, and the School of Medicine of Uberaba. The study was initiated after

approval from the Ministry of Health and the local authorities. Written consent was obtained from every participant.

**Clinical and parasitologic follow-up.** Periodic visits were scheduled on days 0, 30, 45, 90, 180, 195, 240, 300, 360, 450, 540, 630, and 720. Evaluation of spleen size was performed by palpation in the supine position on every vaccination day, and on days 540 and 720. Blood samples were collected at every visit. Access to four FNS diagnostic and treatment stations along the road and to the village health center was available to all study participants throughout the study period, including the intervals between each scheduled visit. Microscopists in charge of the stations were responsible for registering all blood samples collected during the follow-up period. Subjects' names, identification numbers, dates, and parasitologic examination results were registered in specific notebooks.

Malaria infections were diagnosed by the microscopic examination of thick blood films prepared as recommended by the Pan American Health Organization.<sup>25</sup> All samples were examined by the permanent staff of FNS in the field. Results were considered negative after the observation of 200 microscopic fields read with a 100× oil-immersion lens and 10 eyepieces. Specific treatment was provided within 24 hr, following the detection of a positive slide. All samples were sent to the Malaria Laboratory of the Nucleo de Medicina Tropical e Nutricao, University of Brasilia, where a blind re-examination was carried out as a measure of control for reliability of results. Asexual blood-stage parasite densities were calculated by counting the number of asexual forms per 500 white blood cells and multiplying this figure by 5,500 (the average white blood cell count/ $\mu$ l obtained for the study population), and expressed as the number of parasites/mm<sup>3</sup>. Mortality surveillance relied on information obtained from hospital registers and/or the FNS records and from members of the community.

Capillary blood samples were collected from the study subjects during each scheduled visit. Plasma samples were sent to the reference laboratory at the University of Brasilia for the detection of IgG antibodies against asexual forms of *P. falciparum*, as well as antibodies against the vaccine peptide for evaluation of immunogenicity.

**Case definition and statistical methods.** A malaria case was defined as the presence of *P. falciparum* or *P. vivax* asexual blood-stage parasitemia. A new infection caused by either species was defined as a positive parasitologic examination result in a participant observed to be free of parasitemia for 30 days since a previous positive slide and adequate treatment. All positive slides between that interval were considered either as a relapse, recrudescence, and/or treatment failure.

The main analysis concerns the estimation of the field vaccine effectiveness for all, the first, and the second episodes of malaria infections detected by active and passive methods in individuals who completed the vaccination schedule. *Plasmodium falciparum* and *P. vivax* were considered separately. The total number of episodes (excluding possible relapses, recrudescences, and/or treatment failures), as well as total person-weeks at risk, were calculated for each participant. Total person-time was calculated by accumulating the individual length of time followed during the study period, which started 30 days after the injection of the

third dose (induction period) and ended 1.5 years later, after subtracting 30 days for each malaria episode caused by any species. This approach was adopted after considering that asexual blood forms of *P. vivax* are sensitive to the anti-malarials used for treating *P. falciparum* infections, as well as a proportion of *P. falciparum* infections being sensitive to chloroquine. Participants lost to follow-up contributed to the total person-time up to the moment when they were last observed. The overall incidence density rates for the vaccinated and placebo groups (IDv and IDp, respectively) were calculated by dividing the total number of events in each group by its corresponding person-week. The crude estimate of the overall vaccine effectiveness was determined to be 100 (1 - IDv/IDp).

The same criteria were used to calculate person-time at risk for the first episode, the follow-up ending at the event of interest (first episode) or when the study participant was last observed. The follow-up period for the second episode began four weeks after the detection of the first episode. Confidence intervals (CIs) (95%) were calculated by the normal approximation to the log incidence density ratio (IDR = IDv/IDp). Significance tests for the null hypothesis (IDR = 1, no efficacy) were done using the normal approximation of the Mantel-Haenszel test.<sup>26</sup> Vaccine efficacy was calculated as VE = 100(1 - IDR) and 95% CIs were calculated as 100(1 - IDR's CI upper limit) to 100(1 - IDR's CI lower limit).<sup>27</sup> A similar analysis was done to estimate vaccine efficacy for the period between the second and third doses.

Further analysis was performed to explore the possible presence of heterogeneous effects of the vaccine in specific subgroups. The stratification intended to create epidemiologic categories that correlated with exposure and susceptibility of the participants to infection. The following covariates were considered: sex, age, time since arrival in the endemic region, baseline serologic titers against asexual forms of *P. falciparum*, and concomitant parasitemia (of either species) during the application of any dose of the vaccine and its induction periods. To explore the latter condition for each participant, time zero was defined as the date of detection of parasitemia plus 30 days. Stratum-specific incidence density ratios and their CIs were calculated. Survival curves were plotted according to the Kaplan-Meier approach.

## RESULTS

**Study population and follow-up.** The present study was carried out from January 1991 to February 1993. A total of 800 volunteers (13.4% of the total population living in the rural settlement) received the first dose of either the vaccine or placebo (400 in each group). Most of them were migrants (99.6%), natives of nonendemic areas, and residents along the road. Both groups were comparable in relation to baseline characteristics at enrollment.<sup>18</sup>

Of the initial cohort, 714 received the second dose and 572 received the third dose (28.5% lost to follow-up). The proportion and the average time of survival of the participants lost to follow-up up to the third dose were comparable for both treatment groups (113 vaccinees versus 115 in the placebo group;  $P = 0.9$ , by chi-square test and 12.5 versus 11.5 weeks of survival, respectively;  $P = 0.5$ , by Student's *t*-test). Comparability between lost participants and survivors

TABLE 1

Baseline characteristics of participants completing the vaccination schedule

Characteristic	SPf66 no. (%)	Placebo no. (%)	<i>P</i> *
Age (years)			
< 11	51 (18)	63 (22)	0.45
11-20	93 (32)	96 (34)	
21-50	118 (41)	101 (35)	
> 50	25 (9)	25 (9)	
Sex			
Male	177 (62)	171 (60)	0.68
Female	110 (38)	114 (40)	
Time since arrival (years)			
< 1	111 (39)	111 (39)	0.95
1-2	54 (19)	56 (20)	
>2-3	46 (16)	48 (17)	
>3	76 (26)	70 (24)	
Previous malaria episodes			
None	24 (8)	35 (12)	0.30
1-3	72 (25)	67 (24)	
Several	191 (67)	183 (64)	
IgG antibodies to <i>Plasmodium falciparum</i>			
Positive	89 (31)	81 (28)	0.49
Negative	198 (69)	204 (72)	
Spleen			
Palpable	71 (26)	70 (26)	0.99
Unpalpable	207 (74)	204 (74)	
Prevalence of infection at enrollment			
<i>P. falciparum</i>	9 (2)	9 (2)	0.63
<i>P. vivax</i>	16 (4)	11 (3)	
Negative	367 (94)	370 (95)	

\* *P* value for chi-square tests for differences between groups.

was maintained with respect to all characteristics, and losses seemed to be independent of the disease and of secondary reactions to the vaccine or placebo. The main causes of losses were absence of the participants (117) and/or the presence of any acute disease during the scheduled vaccination (38), and emigration from the study area (36). Deaths (2) were not related to malaria or the vaccine/placebo preparations. Losses were evenly distributed between both groups and the randomization effect was not compromised.<sup>18</sup>

Both groups were considered comparable with respect to the baseline characteristics at the moment of application of the third dose (Table 1). Although comparability was achieved, the study population was heterogeneous within each group in relation to age, sex, and previous contact with malaria infection, indicating a heterogeneous exposure to the source of infection and susceptibility to it.

**Adverse effects.** No severe adverse reactions were detected with either dose of application or in any group. Local reactions such as mild inflammation, nodules, and pain or erythema, frequently accompanied by pruritus, were the most common reactions detected in both groups (3.8%, 29.1%, and 8.5% for the first, second, and third doses, respectively, in the SPf66 group, and 4.0%, 7.6%, and 2.5% in the placebo group), and were significantly higher in vaccinees after the application of the second and third doses ( $P < 0.0001$  and  $P < 0.0035$ , respectively, by chi-square test). Local side effects after the second dose were more frequent in females in the vaccinated group ( $P < 0.0001$ , by chi-square test). Systemic side effects consisted mainly of unspecific general symptoms reported by the participants, and

TABLE 2  
Stratum-specific vaccine efficacy estimates for the first *Plasmodium falciparum* malaria episode after the third dose

Stratum	SPf66			Placebo			VE (%) (95% CI) <sup>†</sup>
	No. of cases	Person-weeks	IDv*	No. of cases	Person-weeks	IDp*	
Crude	76	12,178	0.0062	85	11,698	0.0073	14.11 (-17.0-36.9)
Sex							
Males	48	7,273	0.0066	44	7,196	0.0061	-7.94 (-62.5-28.3)
Females	28	4,905	0.0057	41	4,503	0.0091	37.30 (-1.4-61.2)
Age (years)							
< 11	12	2,404	0.0049	19	2,861	0.0066	24.8 (-54.9-63.5)
11-20	27	3,628	0.0074	21	4,034	0.0052	-43.0 (-152.9-19.16)
21-50	34	4,985	0.0068	38	3,599	0.0106	35.39 (-2.6-59.32)
> 50	3	1,162	0.0026	7	1,203	0.0058	55.63 (-71.6-88.53)
Time since arrival (years)							
<1	36	4,408	0.0089	40	3,913	0.0102	13.0 (-36.5-44.5)
1-2	17	2,227	0.0076	14	2,271	0.0062	-23.9 (-151.3-38.9)
>2-3	9	2,239	0.0040	16	2,233	0.0072	43.91 (-26.9-75.2)
>3	14	3,664	0.0038	15	3,280	0.0046	16.44 (-73.1-59.7)
IgG antibodies to <i>Plasmodium falciparum</i>							
Negative	55	8,302	0.0066	58	8,453	0.0069	3.45 (-39.6-33.2)
Positive	19	3,632	0.0052	24	3,229	0.0074	29.62 (-28.5-61.45)
Intercurrent malaria in vaccine applications							
<i>P. falciparum</i>	13	1,653	0.0079	17	1,594	0.0107	26.24 (-51.9-64.2)
<i>P. vivax</i>	28	2,743	0.0102	20	2,678	0.0075	-36.7 (-142.6-23.0)
None	35	7,783	0.0045	48	7,426	0.0065	30.42 (-7.6-55.0)

\* IDv and IDp = incidence density rates in the vaccinated and placebo groups.

† VE (%) = vaccine efficacy estimates; CI = confidence interval.

were most frequent after the first dose in both groups (4.3% in the SPf66 group and 3.0% in the placebo group).<sup>23</sup>

**Passive and active case detection.** After the third dose, 5,684 blood samples were collected either by the active (3,427) or passive (2,257) surveillance systems, 2,891 in the vaccinees and 2,793 in the placebo group. A total of 967 positive slides were detected, 520 in the vaccine group (179 corresponding to *P. falciparum* infections and 341 to *P. vivax*), and 447 in the placebo group (175 *P. falciparum* and 272 *P. vivax*). Excluding relapses and/or treatment failures, 212 *P. falciparum* infections were confirmed, 107 in 76 participants in the vaccine group, 24 through the active periodical searches, and 83 through the FNS surveillance system, and 105 episodes were detected in 85 participants in the placebo group, 23 through the active periodical searches, and 82 through the FNS surveillance system. A total of 427 *P. vivax* infections were detected, 233 in 138 participants in the vaccine group and 194 in 127 participants in the placebo group.

**Protective effectiveness for *P. falciparum* malaria episodes.** The crude estimate of the overall direct vaccine effectiveness was -1.6% (-32.4% to 22.4%) and 14.1%

(-17.0% to 36.9%) for the first episode. For the second episode, the estimate was -50.9% (-172.7% to 16.4%). Although not significant, marked differences in point estimates of vaccine efficacy for the first episode can be observed in all subgroups, especially those related to sex, age, and the presence of concomitant parasitemia with either species during application of any dose of the vaccine during its induction periods (Table 2). In the subgroup of participants free of intercurrent malaria during vaccine applications, the overall protective effect was 17.2% (-19.9% to 42.8%), and 30.4% (-7.6% to 55.0%) for the first episode. The stratified analysis for the first episode showed closer estimates of vaccine efficacy between females and males, but differences in age subgroups were marked. Adults more than 20 years of age showed a significant level of protection (Table 3). Vaccine efficacy for the second episode was -27.3% (-171.9% to 40.4%). The frequency distribution of all *P. falciparum* malaria episodes and total person-weeks at risk for participants completing the vaccination schedule and Kaplan-Meier survival curves for the first episode under both analytical approaches are presented in Table 4 and Figure 1, respec-



TABLE 3

Stratum-specific vaccine efficacy estimates for the first *Plasmodium falciparum* malaria episode after the third dose in participants free of intercurrent parasitemia during vaccine applications and/or induction periods

Stratum	SPf66			Placebo			VE (%) (95% CI)†
	No. of cases	Person-weeks	IDv*	No. of cases	Person-weeks	IDp*	
Crude	35	7,783	0.0045	48	7,426	0.0065	30.42 (-7.6-55.0)
Sex							
Males	21	4,669	0.0045	28	4,634	0.0060	25.57 (-31.1-57.7)
Females	14	3,113	0.0045	20	2,792	0.0072	37.22 (-24.3-68.3)
Age (years)							
<11	9	1,508	0.0059	11	1,995	0.0055	-8.2 (-161.1-55.2)
11-20	13	2,088	0.0062	10	2,336	0.0043	-45.4 (-231.6-36.2)
21-50	13	3,348	0.0039	21	2,444	0.0086	54.8 (9.7-77.4)
>50	0	837	0	6	651	0.0092	Undefined
Time since arrival (years)							
<1	18	2,136	0.0084	23	2,563	0.0089	-6.1 (-73.9-49.3)
1-2	5	1,302	0.0038	6	1,214	0.0049	22.3 (-154.5-76.3)
>2-3	6	1,805	0.0033	10	1,481	0.0068	50.78 (-35.4-82.1)
>3	6	2,439	0.0024	9	2,169	0.0041	43.05 (-60.0-79.7)
IgG antibodies to <i>Plasmodium falciparum</i>							
Negative	28	5,622	0.0049	35	5,508	0.0063	21.62 (-20.8-52.32)
Positive	7	1,941	0.0036	12	1,911	0.0063	42.56 (-4.59-77.4)

\* IDv and IDp = incidence density rates in the vaccinated and placebo groups.

† VE (%) = vaccine efficacy estimates; CI = confidence interval.

tively. No differences in the mean parasite densities between the two groups were found.

The crude protective effect for the first episode after the second dose was -20.4% (-86.1% to 22.0%). In the participants free of concomitant parasitemia during the first and/or second doses and their induction periods, the field vaccine effectiveness was -0.6% (-82.9% to 44.6%).

#### Protective effectiveness for *P. vivax* malaria episodes.

The frequency distribution of all *P. vivax* malaria episodes and total person-weeks at risk are presented in Table 5. The overall vaccine effectiveness was -19.7% (-44.8% to 1.03%) and -10.8% (-41.1% to 12.8%) for the first episode. In the subgroup of participants free of concomitant parasitemia during the injection of either vaccine dose and

TABLE 4

Frequency distribution and vaccine efficacy estimates for *Plasmodium falciparum* malaria episodes in participants who completed the vaccination schedule\*

	Analytical approach			
	All participants		In participants free of intercurrent parasitemia during vaccine applications	
	SPf66 (n = 269)	Placebo (n = 271)	SPf66 (n = 159)	Placebo (n = 165)
Number of episodes				
0	193	186	124	117
1	53	65	23	34
2	16	20	8	14
3	6	0	4	0
4	1	0	0	0
Total no. of events	107	105	51	62
Person-weeks	14,844	14,799	9,152	9,216
Incidence rate	0.0072	0.0071	0.0055	0.0067
Vaccine efficacy				
Overall (CI)	-1.6% (-32.9% to 22.4%)		17.2% (-19.9% to 42.8%)	
First episode (CI)	14.11% (-17.0% to 36.97%)		30.42% (-7.6% to 55.0%)	
Second episode (CI)	-50.9% (-172.7% to 16.48%)		-27.3% (-171.9% to 40.43%)	

\* CI = confidence interval.

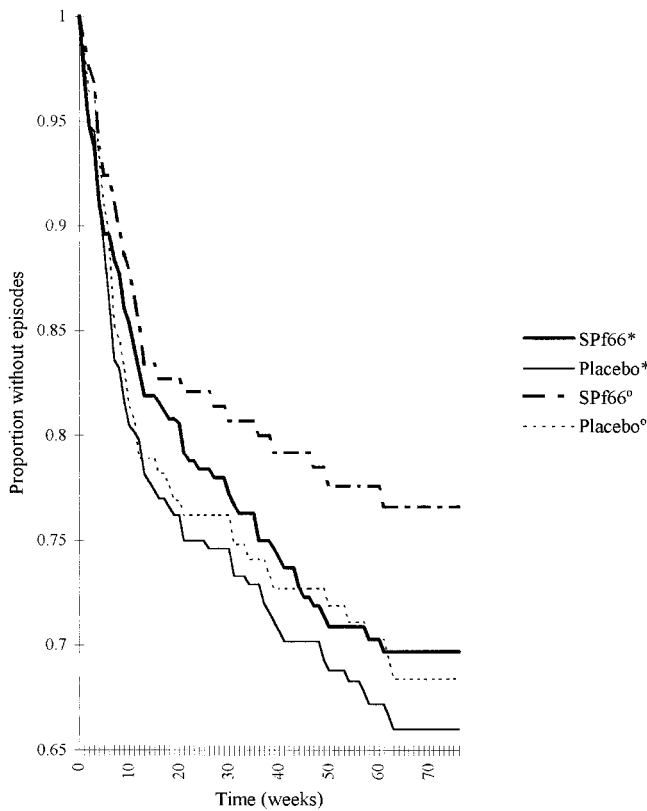


FIGURE 1. Kaplan-Meier survival curves for the first *P. falciparum* malaria episode after the third dose of SPf66 vaccine. \*All participants; °Participants without intercurrent parasitemia during vaccine applications.

its induction periods, the overall estimate was -13.4% (-47.9% to 13.0%) and -12.3% (-55.0% to 18.6%) for the first episode.

DISCUSSION

No evidence of an overall significant protective effect of SPf66 malaria vaccine against *P. falciparum* and *P. vivax* malaria episodes were obtained in this trial. Caution is advised regarding the interpretation of the results given the small sample size, which was calculated to detect an over-estimated 80% vaccine efficacy, and a significant amount of losses to follow-up, both resulting in lack of precision of the estimates as reflected in wide confidence intervals.

Three special conditions characterized this trial: 1) the participants were mainly migrants with low, if any, acquired immunity; 2) in the study area, *P. vivax* and *P. falciparum* infections coexist, and the population has easy access to diagnosis and specific treatment; and 3) no radical treatment was administered to the participants before the scheduled vaccinations. These facts raise important issues for discussion.

Migrants were natives of different regions of Brazil, representing a variety of ethnic groups, probably with distinct genetic features. There is increasing evidence that immune response to SPf66 might be genetically restricted.<sup>28-31</sup> Immunogenicity is being evaluated and we intend to clarify this point in the future.

Coexistence of both *P. falciparum* and *P. vivax* infections in the study area raises important points. First, several criteria can be used to define distinct infections and person-time at risk, with a resulting modification of vaccine efficacy estimates. Second, the use of antimalarials is widespread in the area, and it is not clear how the rapid clearance of parasitemia might affect the expected natural boosting of the immune response in vaccinated individuals. However, no ev-

TABLE 5

Frequency distribution and vaccine efficacy estimates for *Plasmodium vivax* malaria episodes in participants who completed the vaccination schedule\*

	Analytical approach			
	All participants		In participants free of intercurrent parasitemia during vaccine applications	
	SPf66 (n = 269)	Placebo (n = 271)	SPf66 (n = 159)	Placebo (n = 165)
Number of episodes				
0	131	144	82	95
1	75	80	45	45
2	42	34	26	20
3	14	8	5	3
4	6	3	1	1
5	0	2	0	1
6	0	0	0	0
7	0	0	0	0
8	1	0	0	0
Total no. of events	233	194	116	103
Person-weeks	14,844	14,799	9,152	9,216
Incidence rate	0.0157	0.013	0.0126	0.0117
Vaccine efficacy				
Overall (CI)	-19.74% (-44.86% to 1.03%)		-13.4% (-47.9% to 13.02%)	
First episode (CI)	-10.8% (-41.12% to 12.86%)		-12.3% (-55.0% to 18.66%)	
Second episode (CI)	-34.0% (-91.87% to 8.13%)		-23.0% (-107.5% to 27.11%)	

\* CI = confidence intervals.

idence of an increased protection was observed for the second episode. On the contrary, estimated vaccine effectiveness was negative, suggesting that previous infections do not boost protection.

An increased incidence of *P. vivax* episodes was observed among vaccinated individuals. This may have led to an overestimation of the vaccine efficacy against *P. falciparum* infections since vaccinees would have received a greater number of doses of chloroquine as compared with the placebo group and approximately 18% of *P. falciparum* infections are considered to be sensitive to chloroquine. Although *P. vivax* infections are not associated with mortality, they are an important cause of morbidity and economic losses. A potential increase in the number of cases caused by this species following vaccination with SPf66 would also increase the burden on traditional control measures.

Since no radical treatment was administered to the participants before the scheduled vaccinations, information on the effect of intercurrent parasitemia during vaccine applications was explored. Point estimates suggest that concomitant parasitemia during either dose of vaccination and/or during the induction periods can possibly affect the protective effect of the vaccine, probably due to the immunosuppression effect associated with acute malaria. Vaccine efficacy estimates among individuals free of concomitant parasitemia during vaccine injections seem closer to the concept of biological efficacy of the vaccine against *P. falciparum* malaria episodes. No evidence of protection against the first episode of *P. falciparum* malaria was observed except among individuals more than 20 years of age. Age-related differences in vaccine efficacy estimates may reflect different levels of exposure to infection and susceptibility patterns.

Phase III field trials of SPf66 have been carried out in very heterogeneous conditions throughout the world. Studies in South America were conducted in Colombia, Venezuela, and Ecuador in areas of low endemicity and in all age groups except infants. Vaccine efficacy estimates were 33.6% (CI = 18.8–45.7%), 55% (CI = 21–75%), and 60.2% (CI = –26% to 88%), respectively.<sup>3–5</sup> A vaccine efficacy estimate of –9% (CI = –33% to 14%) in children 2–15 years of age was obtained in Thailand in area of low-to-medium endemicity.<sup>9</sup> *Plasmodium falciparum* and *P. vivax* malaria infections coexist in all of these areas. In spite of the similarities in these endemic areas, different case definitions and methods for handling *P. vivax* concomitant episodes were used. In addition, radical treatment before each programmed vaccination was provided only in Thailand. The comparison of our observations to these trials, particularly those conducted in South America, should consider additional information not available to us on how intercurrent malaria parasitemia during the vaccine applications and/or induction periods were handled in the analysis, as well as how person-time at risk was defined in case of *P. vivax* infections.

The trials in Africa were conducted in Tanzania and The Gambia, both areas of high endemicity, yet one with intense perennial malaria transmission and the other with seasonal transmission.<sup>6,8</sup> Treatment with sulfadoxine-pyrimethamine was provided before vaccination to all participants in both trials and *P. falciparum* malaria infections predominated. The vaccine efficacy estimates obtained in these trials differed markedly. An estimated protective effect of 31% (CI

= 0–52%) was obtained in Tanzanian children 2–5 years of age in contrast with a vaccine efficacy estimate in Gambian infants 6–11 months of age of 8% (CI = –18% to 29%).

When one considers that the vaccine is to be a public health tool, we cannot base our conclusions on its efficacy against the first episode, but on its overall efficacy. Overall protective efficacies of SPf66 malaria vaccine against *P. falciparum* infections were estimated in the Colombian trial (38.8%) but the CI was not reported.<sup>3</sup> More recently, a protective efficacy of 25% (CI = 1–44%) was reported for the Tanzanian trial.<sup>10</sup> Estimated overall protective efficacies for other field trials range from 3% to 66.8% (Gambia and Ecuador, respectively), but none are statistically significant.<sup>4,8</sup>

Results obtained in the several field trials to date can be formally pooled together with meta-analytic techniques. In addition to an increase in power, one could also benefit from the heterogeneous conditions in which these trials were conducted to understand the role of previous infection with malaria in modulating the immune response to the vaccine. Such procedures must take into account possible differences in immune response related to age, as well as the role of intercurrent malaria infections, on the protective effect of the vaccine.

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