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MATERIALS AND METHODS

Study site

The study was performed in the village of Arso PIR I, Irian Jaya, Indonesia. Arso PIR I is one of 22 government-constructed villages making up a community of approximately 25,000 persons covering approximately 600 km². This village (140°47'E, 2°56'S, elevation < 55 meters) is located 60 km south of Jayapura, the provincial capital, and has a population of approximately 1,000 persons composed of approximately equal numbers of 1) persons native to Irian Jaya who have been exposed to significant levels of malaria transmission all their lives and 2) persons native to Java who transmigrated to Arso PIR I 2.5 years prior to the beginning of the study and who experienced very little or no exposure to malaria transmission prior to their arrival in Irian Jaya.¹⁻³ Malaria transmission in Arso PIR I has previously been characterized as holoendemic or hyperendemic depending on the categorizing standard used.¹ Point prevalence surveys for parasitemias in adults were performed at the start of each of these studies. At the beginning of the low transmission season, the prevalence of all malaria in 2-9-year-old children (n = 16) was 63% (*P. falciparum* 50%, *P. vivax* 13%), while at the start of the high transmission season, the prevalence of all malaria among 2-9-year-old children (n = 116) was 45% (*P. falciparum* 27%, *P. vivax* 18%). The prevalence values for adults were low transmission season: all malaria 32% (*P. falciparum* 23%, *P. vivax* 9%); high transmission season: all malaria 33% (*P. falciparum* 20%, *P. vivax* 13%).

Study population

The study protocol was approved by officials of the National Institutes of Health Research and Development of the Republic of Indonesia and the Protection of Human Subjects Committee at the U.S. Naval Medical Research Unit No. 2 Detachment in Jakarta. Inclusion factors for voluntary admission into the study were 1) males > 15 years of age (doxycycline should not be given to pregnant women due to the effects of the drug on developing teeth and bone), 2) full-time residency in Arso PIR I, and 3) a normal glucose-6-phosphate dehydrogenase (G-6-PD)

level normal as determined by G-6-PD deficiency screen (Sigma, St. Louis, MO) to prevent the administration of primaquine to G-6-PD-deficient volunteers. The study was described at a village meeting to Arso PIR I residents who were then invited to volunteer. Participation in the study was strictly voluntary, informed consent was obtained after each volunteer was advised of possible adverse effects and the right to leave the study at any time.

Study design

Since the incidence rate of malaria transmission was not characterized in Arso PIR I prior to these studies, there were no figures upon which to base rationally sample size calculations. We therefore recruited into the study as many volunteers as logistics permitted. The two populations were evaluated and followed prospectively as fixed cohorts, one during May through October (low transmission season) and the other during January through April (high transmission season). The number of subjects completing different phases of the study is shown in Table 1. The studies were terminated during each of the two seasons when both populations reached approximately 50% positive. This required 20 weeks during the low transmission season study and 12 weeks during the high transmission season study. The subjects were tested weekly for parasites by blood film examination.

Treatment and surveillance

Once enrolled, the subjects were radically cured of malaria with quinine sulfate (600 mg three times a day for three days), doxycycline (100 mg twice a day for seven days), and primaquine (15 mg once a day for 14 days). Consumption of medication was observed by a research team member. After the drug regimen was completed, blood films were taken weekly from each subject until the subject had a blood film positive for *P. falciparum* or until study completion. Blood films were stained with Giemsa and 200 high-magnification fields (1,000X) were viewed. Subjects positive for malaria parasites were referred to the local health facility for treatment. Subjects who became positive for blood-stage parasites during the first two weeks after the completion of the drug reg-

TABLE 3

Attack rates for *Plasmodium falciparum* during both transmission seasons presented as annual rates for ease of comparison (number of cases per person per year)

	Low transmission season	High transmission season
Transmigrants	1.89	3.04
Irianese	1.14	2.73
All	1.56	2.91

ative risk) were calculated according to Rothman⁴. Estimation of power ($1 - \beta$) and sample sizes were determined as described by Browner and others.⁷

RESULTS

Study population

The mean ages of the subjects completing the study are shown in Table 2.

Attack rates

The attack rates for *P. falciparum* during both transmission seasons are presented in Table 3 as annual rates for ease of comparison (number of cases per person per year).

Analysis of the attack rates (Table 4) revealed a statistical difference between transmigrants and Irianese during the low transmission season but not during the period of high transmission. During the low transmission season, the transmigrants were at approximately 1.65 times the risk of becoming *P. falciparum* blood-stage positive; the 95% confidence interval indicates that the true relative risk was between 1.17 and 2.34. Both ethnic groups had higher attack rates after 12 weeks in the high transmission season than during the low transmission season (Table 5).

The Irianese had significantly different attack rates during the two seasons in contrast to those seen in the transmigrants (Table 5). During the low transmission season, 50% of the transmigrants were parasite positive by week 10, 43% of the Irianese were positive by week 17. During the high transmission season, 50% of the transmigrants were positive by week 6 and 50% of the Irianese were positive by week 9 (Figure 1).

Incidence density

Incidence density data are shown in Table 6. During the low transmission season, an incidence density of 4.81 *P. falciparum* cases per 100 person-weeks was calculated. The incidence density for transmigrants alone was 6.46 cases per 100 person-weeks as compared with 3.09 cases per 100 Irianese person-weeks. The estimated relative risk during this 20-week period was 2.09 (95% confidence interval 1.28, 3.43). During the high transmission season, 8.49 cases of *P. falciparum* malaria occurred per 100 person-weeks; the rate was 8.92 for transmigrants and 7.92 for the Irianese subjects. The estimated relative risk for the transmigrants during this 12-week period was 1.13 (95% confidence interval 0.72, 1.77). There was a significant difference between the transmigrant and Irianese incidence densities during the low transmission season but not during the high transmission season (Table 7).

Entomology

Collections made at the beginning of the low and high transmission seasons indicated that *Anopheles koliensis* was the predominant vector (98.8% and 98.5% of the anopheline catch, respectively). Entomologic findings are presented in Table 8.

TABLE 4

Analysis of attack rates: comparison of transmigrants with Irianese during both transmission seasons

	Transmigrants*	Irianese*	P†	RR‡	95% confidence interval	1 - β §
LTS¶	48/66 (73)	22/50 (44)	0.003	1.65	(1.17, 2.34)	85%
HTS#	43/61 (70)	29/46 (63)	0.545	1.12	(0.83, 1.47)	8%

* Values are *Plasmodium falciparum* cases/units exposed (%).

† Chi-square with continuity correction factor.

‡ RR = Relative risk.

§ Probability of detecting a real difference between the two groups.

¶ LTS = low transmission season, 20-week follow-up.

HTS = high transmission season, 12-week follow-up.

TABLE 5

Analysis of attack rates: comparison of effect of the high and low transmission seasons on the transmission of malaria

	HIS ^a	LTS ^b	FS ^c	FIR ^d	95% confidence interval	P ^e
Transmigrants	43.61 (70)	42.66 (64)	0.528	1.11	(0.87, 1.41)	8%
Irianese	29.46 (63)	16.50 (32)	0.005	1.97	(1.24, 3.12)	82%

^a Values for HIS and LTS are *Plasmodium falciparum* cases per 1000 per year.

^b HIS = high transmission season 12 week follow-up.

^c LTS = low transmission season 12 week follow-up.

^d FS = square with continuity correction factor.

^e FS = relative risk.

FS = results of detecting a difference between the two groups.

Failure to comply

During the low transmission season, 20 transmigrant and 91 Irianese blood films were not collected. This represents failure to comply rates of 2.7% and 12.8%, respectively. The overall rate for the low transmission season was 7.6%. Equivalent figures for the high transmission season are 2.1% for the transmigrants, 9.0% for the Irianese, and 5.1% overall.

Plasmodium vivax

During the low transmission season study, 18 of 66 transmigrants (27%) and 16 of 50 Irianese subjects (32%) developed blood-stage *P. vivax* infections prior to reaching a study endpoint. In the high transmission season, seven of 61 transmigrants (11%) and four of 46 Irianese subjects (8.7%) also developed *P. vivax* infections prior to reaching a study endpoint.

DISCUSSION

To establish convincingly that a malaria vaccine is effective, field trials conducted under conditions of natural transmission are required. Because the nature and intensity of *P. falciparum* transmission varies greatly worldwide, it is important to evaluate candidate vaccines under a variety of conditions of transmission and in different populations including children and both immune and nonimmune adults. This will require the establishment of several field sites around the world. For example, it will be important to determine the performance of a candidate vaccine under intense transmission pressure such as that seen in western Kenya,² but a failure to perform under those circumstances does not mean that the vaccine in question will not be effective in areas of low or high seasonal transmission. Before a vaccine can be tested at any field site, however, a minimum characterization of the site must be made so that the vaccine can be properly designed. Arso FIR I was evaluated as a potential study site for the testing of *P. falciparum* malaria vaccines currently under development. In addition, this site has the potentially important attribute of a mixed population; half of the villagers have a history of

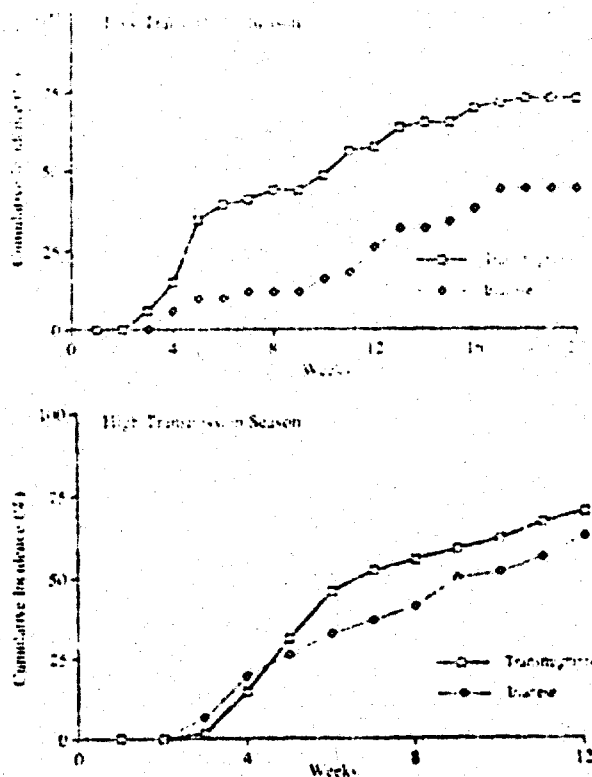


FIGURE 1. Attack rates presented as the cumulative incidence of *Plasmodium falciparum* during both the low transmission and the high transmission seasons.

TABLE 6
Incidence density data

	LTS*		HTS*	
	Transmigrants	Irianese	Transmigrants	Irianese
Person-weeks	743	713	482	366
Pf cases†	48	22	43	29
ID‡	6.46	3.09	8.92	7.92

* LTS = low transmission season.

† HTS = high transmission season.

‡ Pf = *Plasmodium falciparum*.

§ ID = incidence density, cases/100 person-weeks.

life-long exposure to malaria, the other half are effectively naive.¹⁻³

The most important determination required for planning a vaccine trial is the rate at which the infection-specific outcome variable occurs in exposed persons. In this study, development of patent parasitemia was the malaria-specific outcome variable. The occurrence of this outcome is usually presented as an incidence or attack rate, the number of new cases of infection occurring in the population during a specified time. In this study, both the attack rate and incidence density figures showed significant levels of difference between the two populations during the low transmission season. During the high transmission season, the power calculations indicate that the sample sizes did not provide sufficient power to resolve easily a difference between the two groups. When transmission pressure was evaluated by incidence density, a difference between the transmigrant and Irianese rates was found in the low transmission season study. A determination of relative risk based on the attack rates indicates that the transmigrants had at least a 17% greater risk than the Irianese to develop *P. falciparum* parasitemia during the low transmission season (point estimate of risk = 65%). When incidence density data were used in the same analysis, the transmigrants had at least a 28%

increase in risk of becoming parasitemic (point estimate of risk = 109%). Long-term exposure to malaria induces a level of protective immunity. This analysis condenses to a measure of relative risk the amount of risk experienced by the nonimmune transmigrants compared with the semi-immune Irianese.

Another way to interpret these incidence density data is to calculate the mean time until onset of *P. falciparum* parasitemia (the inverse of the rate).⁴ During the low transmission season, for example, the mean time until onset is 15 weeks for the transmigrants and 32 weeks for the Irianese. This difference, as with the relative risk, is reflective of the effect of long-term exposure to *P. falciparum* transmission.

Exclusion criteria exercised at the time of data calculation eliminated those subjects who had the greatest degree of failure to comply. The remaining subject groups had failure percentages of 2.1% to 12.8%. Evaluation of the data based on person-weeks rather than attack rates accounts for these lost slides (lost observations) because the rate is not based on number of cases per population per time period but rather number of cases per person-week. A person-week is represented by a slide, positive or negative, and in theory, the likelihood of any given slide being positive within any group is the same. While this method eliminates the effect of lost slides, it

TABLE 7
Analysis of incidence densities: comparison of transmigrants with Irianese during both transmission seasons

	Transmigrants*	Irianese*	P†	RR‡ (95% confidence interval)
LTS§	48/743	22/713	0.004	2.09 (1.28, 3.43)
HTS¶	43/482	29/366	0.695	1.13 (0.72, 1.77)

* Values are *Plasmodium falciparum* cases/person-weeks.

† Chi-square with continuity correction factor.

‡ RR = relative risk.

§ LTS = low transmission season.

¶ HTS = high transmission season.

one, and we are forced to accept the null hypothesis (there is no difference between the placebo and the vaccinated groups). As the third entry in Table 9 shows, however, a small increase in the n value to 65 per group with the same 40% efficacy would result in a significant difference in the risk ($P = 0.032$), a 95% confidence interval of 6% and 61%, and rejection of the null hypothesis. The last entry in Table 9 shows what is required if one wishes to narrow the width of the 95% confidence interval for vaccine efficacy to approximately $\pm 10\%$ (40%, 58%); the sample size required would increase to 500 subjects per group. These analyses highlight the need to consider not just statistical significance as demonstrated by a P value. Of equal importance is the need to consider the magnitude of the difference in estimated relative risk between the vaccinated and placebo groups." When selecting samples sizes, investigators need to decide what level of uncertainty (confidence interval) they are willing to accept considering the logistic constraints they face.

Once the sample size has been selected, additional factors must be considered. In this study, an average of 11% of high transmission season subjects became positive for *P. vivax* prior to reaching a study endpoint and an attrition rate of 18% occurred between recruitment and study completion. Therefore, larger numbers of subjects would be required at the start of a placebo-controlled vaccine study. Once adjustments for these losses are made, 690 rather than 500 subjects would be needed in both the placebo and experimental groups to complete a study as described above. This study highlights the need to calculate the sample size based not only on predicted vaccine efficacy and estimated subject attrition but also on a desired width of confidence interval for the observed vaccine efficacy.

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