

OPPORTUNITIES FOR EVALUATING MALARIA VACCINES IN INDONESIA

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ABSTRAK

KEMUNGKINAN UNTUK MENGEVALUASI VAKSIN MALARIA DI INDONESIA

Di Indonesia, khususnya di Irian Jaya dan daerah malaria tinggi lainnya terdapat kesempatan yang sangat baik untuk mengevaluasi vaksin malaria. Hal ini antara lain disebabkan tersedianya data epidemiologi termasuk insidens, risiko malaria yang tinggi dan merata pada berbagai kelompok umur, jenis kelamin dan pekerjaan, adanya kelompok masyarakat yang sesuai untuk penelitian (transmigran dan angkatan bersenjata), lokasi desa yang memungkinkan randomisasi serta tersedianya fasilitas laboratorium di dekat daerah penelitian.

Pemberantasan malaria dengan vaksinasi diharapkan akan menjadi fokus dari penelitian kesehatan menjelang akhir abad ke-20. Indonesia yang terdiri dari berbagai pulau memungkinkan konsolidasi pemberantasan malaria secara bertahap. Pengalaman yang diperoleh dengan pemberantasan malaria di suatu pulau akan sangat bermanfaat untuk menghadapi masalah yang lebih berat yakni malaria di daratan luas seperti Afrika atau daratan Asia Tenggara.

INTRODUCTION

Despite decades of intensive, worldwide effort, malaria remains one of the leading causes of morbidity and mortality in tropical countries. While vector control, antimalarial drugs, bednets and other measures have had a positive impact in limiting the disease, none has achieved the long term goal of eradication, and in some areas the situation is worsening despite these control measures. Nevertheless, there is a feeling of optimism in the malaria research

community as we develop our newest weapon in the fight against this disease: vaccines. It is hoped that vaccines will significantly reduce malaria morbidity and mortality, and, in conjunction with other measures, lead to malaria eradication.

Despite economic growth and progress in development, Indonesia continues to suffer from malaria. In the eastern provinces, including some developed areas such as northern Sulawesi, malaria remains a leading cause of

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illness. Populations particularly heavily affected include indigenous populations in Irian Jaya, transmigrants moving to eastern Indonesia, and military personnel deployed to eastern Indonesia. The latter two groups may lack immunity to malaria and thus are susceptible to severe disease.

Eradicating malaria is difficult in tropical countries because the warm climate supports year round vector reproduction, resulting in high rates of transmission. A more radical interruption of the malaria cycle is needed than, for example, was needed in Europe or North America, where standard control measures easily reduced transmission below the threshold required for the disease to persist. Thus vaccines are likely to be especially helpful (particularly for groups such as transmigrants and military personnel) in improving health conditions in tropical countries, where more intense intervention is needed.

For this reason, it is important for Indonesia to participate in vaccine development. Vaccine trials conducted here in Indonesia will help us to understand their potential contribution to malaria control and eradication on Indonesian soil. This article describes the study sites and study populations in Indonesia that could participate in an evaluation of vaccine efficacy.

Malaria vaccines target different stages of the *Plasmodium falciparum* parasite according to the antigens from which they are formulated. Vaccines will thus have a differential (or stage specific) impact on the parasite according to their antigenic composition. For this reason, the outcome variables to be measured in a vaccine trial and thus the design of the trial depend upon the antigenic components of the malaria vaccine. This presentation on the opportunities for malaria vaccine trials in

Indonesia is divided into sections according to vaccine composition, as the outcome variables and corresponding study design are different in each case.

SPOROZOITE VACCINES

The first vaccine tested in blinded controlled trials, R32ToxA from the Walter Reed Army Institute of Medical Research, was based on sequences from the *P. falciparum* circumsporozoite protein¹. Its potential efficacy relied upon inducing an immune response that could affect sporozoites during the brief period of circulation (as little as five minutes) prior to binding to hepatocytes, thereby preventing hepatocyte invasion and further development. The immune response to this vaccine would have little effect on blood stage parasites, and break-through infections would be expected to show normal parasitemia levels and clinical severity. For sporozoite vaccines such as R32ToxA, the most important outcome variable to measure in a vaccine trial is the incidence of malaria infection following vaccination, as detected by newly developing parasitemia. To achieve this, cohorts of volunteers cleared of malaria (or never infected) are followed longitudinally with periodic blood smears to actively detect new parasitemias.

Indonesia has two populations where protection from malaria with an effective vaccine would be vitally important and which could support the trial design required for a blinded evaluation of vaccine efficacy: transmigrants and military personnel. The best developed site for both populations, Arso District, is located 70 km (one and a half hours by car) from Jayapura. Trials involving either transmigrants or military personnel are possible at this or similar sites in eastern Indonesia.

Transmigrants

Adults and children without previous malaria exposure move from the densely populated islands of Java and Bali (less than one case of malaria per 10,000 person years) to frontier areas in Irian Jaya characterized by frequent malaria transmission (three to four cases per person year). An epidemic of malaria usually occurs within six months of the establishment of a new transmigration community, with accompanying morbidity and mortality. Studies by Baird and colleagues² suggest that by eighteen months to two years, transmigrants develop a degree of immunity to parasitemia and to clinical symptoms associated with parasitemia, although this seems more pronounced in adults than children. Transmigrants thus provide a continuum of immunological experience with malaria, from naive to semi-immune. This is important because immune status may affect vaccine efficacy. It is possible to find populations in Arso with malaria exposure histories of zero, one year, two years, three years, up to more than ten years.

The Arso subdistrict contains approximately 25,000 people, 80% of which are transmigrants from Java, living in approximately twenty villages. Transmigration village population size varies from 1000 to 1500 people in 250 to 350 houses that are arranged geometrically in grids. Villages are discrete units, with agricultural land or forest extending between villages. While all four species of malaria occur in Arso, 99% of infections are due to *P. falciparum* or *P. vivax*, which are generally found in a two:one ratio during prevalence surveys. Slide positivity rates during prevalence surveys vary from 5 to 50% for *P. falciparum* and from 5 to 30% for *P. vivax*. Transmission occurs year round with a relative peak season extending from December through February. *Anopheles koliensis* (*An. punctulatus*

group) is the primary vector. Malaria attack rates average about two *P. falciparum* infections per person per year and about one *P. vivax* infection per person per year. Table 1 contains several incidence measurements made during the past five years in Arso.

An important aspect of malaria transmission by *An. punctulatus* complex in Arso is that malaria risk derives from local exposure near people's homes and appears uniformly distributed according to age, sex and occupation. This is because 1) the vector prefers open, sunlit breeding places such as occur on the roads and the disturbed land around people's homes, 2) the vector feeds indoors and outdoors with equal frequency exposing both those who are sleeping and those who are outdoors at night and 3) the vector feeds throughout the night with only slight increases at around midnight relative to the rest of the feeding period. Because these factors even out risk among individuals, there are no particular groups at greater risk of infection who are likely to introduce a bias and thus confound interpretation of vaccine trial results.

Both *P. falciparum* and *P. vivax* are highly resistant to chloroquine in Arso. Resistant rates range from 80 to 90% for *P. falciparum* and from 70 to 80% for *P. vivax* as measured in vivo. Despite this, chloroquine remains the standard first line drug for treating malaria infections in Arso because a majority of individuals experience a satisfactory clinical response to chloroquine therapy, even if their infection is not eradicated. Fansidar is the second line drug, and quinine is used for clinically resistant or severe cases. There is no evidence for quinine resistance in Arso. Chloroquine, Fansidar and quinine are readily available in local pharmacies, while artemisinin derivatives, mefloquine and halofantrine are not licensed for use in Indonesia and are not available.

Table 1. Summary of *P. falciparum* Attack Rates Measured in Arso, Irian Jaya.

Village	Season	Population	Years TMG	Follow-up	Attack Rate	Yearly Adjusted (infections/person/yr)
Arso PIR I**	LTS	transmigrants	2.5	20 weeks	73%	1.9
	HTS	transmigrants	2.5	12 weeks	70%	3.0
	LTS	Irianese	NA	20 weeks	44%	1.1
	HTS	Irianese	NA	12 weeks	63%	2.7
Arso PIR IV	LTS	transmigrants	0	10 weeks	37%*	1.9
Arso X	HTS	transmigrants	0	16 weeks	33%**	1.1
Arso XI	Both	transmigrants	0.5	52 weeks	36%***	0.4
Military posts	LTS	soldiers	NA	14 weeks	43%	1.6

+ other sites unpublished data (PIR IV and X, JKB; XI, DJF; military, C. Ohrt).

* Attack rate while on chloroquine prophylaxis (unsupervised).

** Attack rate while on chloroquine prophylaxis (supervised).

*** Attack rate expressed as cumulative incidence to adjust for drop-outs due to *P. vivax* infections.

Other attack rates are crude (# new falciparum infections X 100 / size of cohort at start, for the specified interval under "follow-up).

Years TMG = years since transmigration

LTS = low transmission season

HTS = high transmission season

NA = not applicable.

Chloroquine prophylaxis is administered to new transmigrants for a period of three months following arrival in Arso. A recent trial showed that as a prophylactic agent, chloroquine is no different from placebo in preventing malaria infection in Arso. As mentioned, however, its efficacy in preventing disease (as opposed to infection) probably provides a level of protection for new transmigrants.

In order to conduct a vaccine trial, it would be ideal if transmigrant populations could be identified in Java or Bali before departure so that a six month series of three immunizations could be completed prior to arrival in Irian Jaya. However, this has not proved feasible. The most realistic vaccine trials in naive transmigrants would provide the first immunization on arrival. The cohort could be maintained on malaria prophylaxis during the

period of immunization. Following the third immunization with malaria vaccine, prophylaxis would be stopped and the cohort followed prospectively to measure malaria incidence (attack rate) in those who had received vaccine and those who had received placebo. With the use of a placebo in such trials (required by WHO direction), it is important that study participants not be subjected to malaria risks any greater than would normally occur if they weren't participating. Usually it is possible to *reduce* the risk of malaria illness or death relative to the normal situation, despite the use of a placebo, by providing excellent on-site diagnostic and treatment capability during the trial.

With regard to the best drug for prophylaxis during vaccination, Fryauff and colleagues have shown that daily primaquine is an effective prophylactic agent against chloroquine resistant *P. falciparum* and chloroquine resistant *P. vivax* in Arso (DJF, unpublished data). Unlike chloroquine, primaquine does not appear to suppress the immune response to vaccine components (DJF, unpublished data). In a recent study, tetanus toxoid was administered to transmigrants who had been on primaquine prophylaxis for one year and to a similar number of transmigrants who had been on placebo for one year. There were no significant differences in either serological or cellular immune responses to the tetanus antigen between the primaquine and placebo groups, indicating that prophylaxis with primaquine during vaccine administration should not interfere with the development of an immune response to vaccine antigens.

An alternative design would be to provide vaccine to transmigrants already in residence in Arso and thus already exposed to malaria. Prior to immunization the cohort would receive radical cure. This is the study design of the

recent trial of SPf66 malaria vaccine in Tanzania³. The Ministry of Health in collaboration with NAMRU-2 has shown that the combination of quinine sulfate for four days, doxycycline hyclate for ten days, and primaquine phosphate for 14 days has 100% efficacy in achieving the radical cure of both the blood and liver stages of malaria in Arso. Currently, we are evaluating the combination of halofantrine and primaquine, which is easier to administer and better tolerated. Following radical cure, the cohort would be immunized and then either maintained on prophylaxis for six months until the third injection was completed or given radical cure a second time, either to the whole cohort or to only those individuals becoming parasitemic during the six month interval. Following the third immunization, the surveillance period would begin.

Primaquine administration is an important part of radical cure. Without clearing the liver of hypnozoites, a large proportion of study subjects will develop *P. vivax* relapses during the surveillance period. If this occurs, these study subjects can not be counted when determining vaccine efficacy.

Military

Military posts are present throughout the Arso district. Malaria naive (or remotely exposed) soldiers are deployed to Arso for periods of one year. Deployment dates are scheduled far in advance and a battalion could be fully immunized prior to arrival in Arso. Attack rates appear to be higher in soldiers than in transmigrants, making the military an ideal study population for benefitting from a malaria vaccine. In a recent field trial (C. Ohrt, personal communication), attack rates indicate five to six infections per soldier per year

(roughly four *P. falciparum* infections and two *P. vivax* infections). It is reported that in other locations besides Irian Jaya, such as Timor Timur, soldiers experience high attack rates of malaria. Thus battalions deployed to other sites besides Arso could significantly benefit from participation in vaccine trials.

ASEXUAL BLOOD STAGE VACCINES

The most widely tested *P. falciparum* vaccine, SPf66 (developed in Colombia by Dr. Manuel Patarroyo and colleagues) includes sequences from three blood stage proteins (also included is a short peptide sequence derived from circumsporozoite protein). For blood stage vaccines, an important outcome variable is the incidence of new parasitemia, just as with sporozoite vaccines. This is because the blood stage vaccine could prevent parasitemia from reaching patent levels after the organism leaves the liver, even if the liver stages themselves are not affected. Additional outcome variables important to blood stage vaccines are the level of the parasitemias that occur (the vaccine could reduce parasitemia levels even if parasitemia is not prevented), and the degree of clinical severity associated with parasitemia (the immune response to the vaccine could blunt the pathological expression of blood stage infections by neutralizing malaria proteins). To measure these variables, cohorts are followed longitudinally as with sporozoite vaccines, but level of parasitemia and degree of clinical illness become important outcome variables in addition to incidence of parasitemia.

If the goal of the vaccine is to prevent disease and not infection, active case detection using blood smears is not required. Rather, participants may be visited several times a week to ask about symptoms, and blood smears made only in symptomatic individuals. In other

words, a purely clinical outcome variable is an acceptable measurement for blood stage vaccines designed to counter disease rather than infection. This is an appropriate design for studies involving children, where it is desirable to minimize blood drawing. Relatively infrequent blood smears can be obtained to compare the incidence of parasitemia underlying the incidence of clinical illness.

Because naturally acquired immunity can limit disease expression, the participation of malaria-naive vs malaria-experienced study subjects is an important choice when designing blood stage vaccine trials. As malarial illness is more severe in naive individuals, anti-disease effects of blood stage vaccines might be picked up with greater sensitivity in study populations that have not previously been exposed to malaria. On the other hand, it is easier to justify the use of placebo in a malaria experienced study group. The use of placebo is generally a requirement of WHO sponsored vaccine trials.

Opportunities for studying blood stage vaccines in Indonesia are excellent. Both transmigrants and soldiers could be considered for prospective, longitudinal studies in which vaccine or placebo are blindly and randomly provided to the study cohort. As mentioned, a range of malaria experience is available in the migrant population. Active case detection with blood smears, symptom questionnaires or both can be used to detect outcome variables. In the case of the military, vaccination can be completed prior to exposure to malaria.

Vaccine trials should be considered for people with lifetime exposure to malaria, as is often the case in villages of indigenous peoples in Irian Jaya. It is critical that vaccines be developed that will benefit those living permanently in malaria areas. Because of their

potential efficacy in reducing clinical disease, blood stage vaccines should be targeted toward these populations.

Clinical measures are particularly important for blood stage vaccine trials. Partly to address this need, the Ministry of Health and NAMRU-2 maintain clinical and research laboratories in Jayapura that by the end of 1995 will be certifiable as Good Laboratory Practices (GLP) facilities. In addition, the presence of laminar flow hoods, CO₂ incubators, high speed centrifuges, liquid nitrogen and other laboratory equipment in Jayapura allows sophisticated immunological and other assays to be performed on site in conjunction with vaccine trials. There is 24 hour field telephone, field radio, and regular telephone and fax communication between Jayapura and Jakarta. The daily flights between the two cities facilitates transport of specimens, supplies and equipment.

GAMETOCYTE STAGE VACCINES

Transmission blocking vaccines based on gametocyte, gamete or ookinete proteins should not affect parasitemia or clinical illness in vaccine recipients, because gametocytes themselves do not cause pathology in the human host. Useful outcome variables include oocyst or sporozoite counts in mosquitoes fed directly or through membranes on the blood of parasitemic vaccinees. Alternatively, if entire villages (or defined areas of transmission) are randomized to vaccine or placebo, attack rates in those villages could be used to measure reductions in transmission associated with a gametocyte vaccine.

Appropriate study subjects for evaluating transmission blocking vaccines are semi-immune individuals living in malaria endemic areas. These individuals especially adults, have naturally acquired immunity to

clinical disease when parasitemic. Thus the administration of a vaccine that was not directly beneficial in preventing malarial illness in study participants would be acceptable.

The unique organization of Arso, with twenty transmigrant villages placed in semi-isolated pockets of cleared land cut from the jungle, offers the possibility of a large study in which placebo and gametocyte (or transmission blocking) vaccine are randomized between villages. Simple outcome variables such as point prevalence positivity, or more complex measures such as malaria incidence or entomologic inoculation rate, could be used to monitor the effect of vaccination on a particular village. It could be predicted that an effective transmission blocking vaccine would eliminate malaria from vaccinated villages. Thus by using this study design, ultimately very large benefits can be derived for vaccine recipients.

An alternative design is to use the modest prevalence of *P. vivax* as an internal control. In this way, the ratio of *P. falciparum* to *P. vivax* would be expected to decrease in villages receiving a *P. falciparum* transmission blocking vaccine. Either of these designs could be implemented in Arso, in transmigrant and indigenous populations.

MULTIPLE STAGE VACCINES

Newer vaccines under development, such as NYVAC7, include antigens from sporozoite, liver, asexual and sexual blood stages. It is difficult to measure the overall efficacy of such vaccines; if efficacy is demonstrated, it is likely to be unclear which antigens are responsible. Probably the best study design is to use a cleared cohort or naive cohort design as with sporozoite or blood stage vaccines. If parasitemia is prevented, however, the potential contribution of the transmission blocking components cannot be evaluated.

SAMPLE SIZE CALCULATIONS

Table 2 presents sample size calculations for a vaccine with expected efficacy of 50%, a p-value (α) of .05, and power (1- β) of 0.8. It can be seen that the attack rates measured in Arso are sufficient for a vaccine trial with a range of 50 to 100 individuals in each arm of the study, using six months of surveillance (the period of follow-up after the last vaccine dose).

CONCLUSIONS

Indonesia provides excellent opportunities for malaria vaccine evaluation. Key features:

- 1) Potential health benefits for vaccine recipients.
- 2) Well characterized study sites in Irian Jaya. Extensive epidemiological data available, including multiple incidence measurements.
- 3) Malaria incidence sufficient for vaccine trials involving just a few hundred people.
- 4) Malaria risk uniform among age, sex and occupation groups.
- 5) Cooperative study populations (both transmigrants and military) with low drop-out rates.

Table 2. Sample Size as a Function of Attack Rate
(Assuming efficacy 50%, $\alpha = .05$, power (1- β) = .8 or .95)

Measured Attack Rate (incidence)*	Sample Size Each Group	
	power = .8	power = .95
5%	904	1494
10%	343	716
20%	199	332
30%	120	198
40%	81	133
50%	58	94
60%	42	68
70%	31	50
80%	22	36
90%	16	25

* the incidence (attack rate) is measured as the number of new *P. falciparum* infections experienced by the placebo group during the period of surveillance.

- 6) Range of previous malaria exposure from none to ten years.
- 7) Ability to vaccinate naive individuals in malaria free area prior to deployment to study site (military).
- 8) Geographic layout permitting randomization of whole villages to vaccine or placebo to evaluate the efficacy of transmission blocking vaccine.
- 9) Proximity to field site of both GLP certified clinical laboratories and research facilities.

If successful, the eradication of malaria through the use of vaccines will be one of the premiere health achievements on this century. Indonesia has the potential to play a pivotal role in this enterprise. It is likely that once an effective vaccine is developed, sponsoring institutions such as the World Health Organization will wish to target island nations for the first efforts at eradication. This is because island geography allows step-wise consolidation of control efforts. The experience gained thereby can then be brought to bear on the much more difficult task of eradicating malaria from large land masses such as Africa or mainland southeast Asia.

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