View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by DigitalCommons@University of Nebraska

University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Public Health Resources | Public Health Resources

2003

Malaria in a cohort of Javanese migrants to Indonesian Papua

United States Naval Medical Research Unit #2

H. Basri
United States Naval Medical Research Unit #2

D. J. Fryauff
United States Naval Medical Research Unit #2

M. J. Barcus
United States Naval Medical Research Unit #2

M. J. Bangs
United States Naval Medical Research Unit #2

See next page for additional authors

Follow this and additional works at: http://digitalcommons.unl.edu/publichealthresources

United States Naval Medical Research Unit #2; Basri, H.; Fryauff, D. J.; Barcus, M. J.; Bangs, M. J.; Ayomi, E.; Marwoto, H.; Elyazar, I. R.F.; Richie, T. L.; and Baird, J. Kevin, "Malaria in a cohort of Javanese migrants to Indonesian Papua" (2003). *Public Health Resources*. 388.
http://digitalcommons.unl.edu/publichealthresources/388

This Article is brought to you for free and open access by the Public Health Resources at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Public Health Resources by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors
United States Naval Medical Research Unit #2, H. Basri, D. J. Fryauff, M. J. Barcus, M. J. Bangs, E. Ayomi, H. Marwoto, I. R.F. Elyazar, T. L. Richie, and J. Kevin Baird

This article is available at DigitalCommons@University of Nebraska - Lincoln: http://digitalcommons.unl.edu/publichealthresources/388

This article is a U.S. government work, and is not subject to copyright in the United States.

Annals of Tropical Medicine & Parasitology, Vol. 97, No. 6, 543–556 (2003)

Malaria in a cohort of Javanese migrants to Indonesian Papua*

KRISIN[†], H. BASRI[†], D. J. FRYAUFF[†,‡], M. J. BARCUS[†], M. J. BANGS[†], E. AYOMI[§], H. MARWOTO[¶], I. R. F. ELYAZAR[†], T. L. RICHIE[†,‡] and J. K. BAIRD[†]

[†]*United States Naval Medical Research Unit #2, American Embassy Jakarta, FPO AP 96520-8132, U.S.A.*
[‡]*Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910, U.S.A.*
[§]*Papua Provincial Health Service, Jayapura, Papua, Indonesia*
[¶]*Indonesian Ministry of Health Institute of Health Research and Development (LITBANGKES), Departemen Kesehatan, Jalan Percetakan Negara No. 29, Jakarta 10560, Indonesia*

Received 31 March 2003, Revised 5 May 2003, Accepted 7 May 2003

The epidemiology of infection by *Plasmodium falciparum* and *P. vivax* was investigated among Javanese migrants to an endemic region of Papua, Indonesia. A cohort of 243 migrants from Java was followed for malaria in a new settlement village in the endemic Armopa area of north–eastern Papua, beginning on the day each migrant arrived in the village. The subjects were monitored during home visits (three/week) and by the twice-monthly production of bloodsmears that were checked for malarial parasites. At the end of 33 months, 159 (65%) of the subjects remained under follow-up. The prevalence of parasitaemia in the village declined from 16% among those already living there when the study began in August 1996, to 5% when the study finished in June 1999. Over this period, 596 infections by *P. falciparum* and 723 by *P. vivax* occurred in the cohort, 22 and 27 of the subjects each experiencing at least six infections by *P. falciparum* and *P. vivax*, respectively. The incidence of malarial infection was higher during the first and second years post-migration (3.2 and 2.7 infections/person-year) than during the third (1.2 infections/person-year). Although the geometric mean parasite counts for *P. falciparum* increased over time (1209, 1478, and 1830 parasites/µl in the first, second and third years, respectively), the corresponding values for *P. vivax* (497, 535 and 490 parasites/µl) showed no such trend. Only one of the nine subjects who developed severe malaria (requiring intravenous quinine therapy) was a child, giving an odds ratio for a case of severe malaria being in an adult of 6.1 ($P = 0.08$).

The published reports of numerous studies describe the epidemiology of parasitaemia and disease caused by *Plasmodium falciparum* and *P. vivax* in endemic areas. Such observations underpin hypotheses on the onset of clinical immunity to malaria. In areas of Africa where *P. falciparum* is holo-endemic, for example, the sharp declines seen in the prevalence and intensity of parasitaemia and the risk of symptomatic malaria with increasing age, beyond about 3 years, support the hypothesis that clinical immunity is the cumulative product of exposure to the antigenic repertoire of *P. falciparum* (McGregor, 1987; Newbold *et al.*, 1997; Saul, 1999), the slow onset of clinical immunity being attributed to the antigenic diversity of the parasite. In populations exposed to infection

Address enquiries to: J. K. Baird, U.S. Naval Medical Research Center Detachment, American Embassy Lima, APO AA 34031-3800, U.S.A.
E-mail: baird@nmrcd.med.navy.mil; fax: +51 1 561 3042.
*The opinions and assertions contained herein are those of the authors and do not purport to reflect those of the United States Navy and Department of Defense and the Ministry of Health of the Republic of Indonesia. Reprint requests should be sent to the Commanding Officer, United States Navy Medical Research Unit #2, American Embassy Jakarta, FPO AP 96520-8132, U.S.A. (for the attention of the Publication Office).

© 2003 The Liverpool School of Tropical Medicine
DOI: 10.1179/000349803225001454

since birth, however, any intrinsic age-dependent factors affecting the onset of clinical immunity are obscured. Only in previously unexposed populations of all ages that have been abruptly and permanently exposed to endemic malaria can the effects of age and cumulative exposure to infection be separated. When Baird *et al.* (1991*b*, 1993) conducted cross-sectional studies in such populations, they found evidence of the age-dependent onset of clinical immunity despite apparently equal cumulative exposure to infection among all age-groups.

The possibility that the onset of clinical immunity to *P. falciparum* is age-dependent, and not dependent on chronic, cumulative exposure (Baird, 1995, 1998), clearly has implications for those attempting to develop vaccines to diminish the burden of disease and death caused by *P. falciparum*. The conventional logic underlying vaccine development is that disease can be prevented by increasing exposure to protective antigens. The development of a malaria vaccine, for example, hinges largely upon strategies to provide sufficient exposure to the surface antigens of *P. falciparum* or other *Plasmodium* spp. (Good *et al.*, 1998; Plebanski and Hill, 2000). However, susceptibility to infection and disease may be a consequence not of insufficient exposure to antigen but of an inappropriate immune response driven predominantly by intrinsic factors related to age. If so, the induction of an immune response in infants and small children that provides adult-like protection from disease may require the manipulation of the intrinsic age-related character of the immune response to chronic exposure.

In the present study, Javanese subjects were followed for 33 months after they migrated to a malaria-endemic region of Indonesian Papua. The main aim was to explore the age- and exposure-related determinants of the onset of clinical immunity to *P. falciparum* malaria. This report, the first in a series from this cohort, describes the methods of recruitment, enrolment and follow-up, along with the demographic features of the study population and the epidemiology of the malarial infections observed throughout the study period.

SUBJECTS AND METHODS

Study Site

A newly constructed village, designated *Satuan Permukiman* (Settlement Unit) 2 (SP2) by the Indonesian Department of Transmigration, served as the study site. SP2 was located at Armopa (139°32′E, 21°6′S) in the Bonggo sub-district of Jayapura district in north–eastern Papua (formerly known as Irian Jaya), Indonesia. Housing was of uniform, wood-plank and tin-roof construction, and laid out in a grid pattern. The village was completed, in an area of about 4 km^2 cleared of dense, lowland tropical rainforest and located within 2 km of the Pacific Ocean, shortly before the first migrants arrived in August 1996. It was fully occupied, with 1551 residents living in 450 homes, by October 1996. The resident families had mostly migrated from East (180 families), Central (97) or West Java (128), although 45 families were native to Papua. The new villagers soon established cash and subsistence crops, primarily of soy, peanut, cassava, melon, papaya and maize.

Perennial transmission of malaria — primarily *P. falciparum* and *P. vivax* but also *P. malariae* and *P. ovale* — occurs in north–eastern Papua (Baird *et al.*, 1990; Jones *et al.*, 1994). Members of the *Anopheles punctulatus* group are the overwhelmingly dominant vectors of the region (Metselaar, 1961). Most species in this group are widely dispersed and show niche-specific habitat preferences, their distributions often being increased by forest clearing and the establishment of new settlements (Slooff, 1964). The likely sources of malaria in new Papuan villages settled entirely by malaria-naïve migrants are, initially, infected workers constructing the villages and, after occupation, residents arriving from endemic areas and/or visitors from neighbouring Papuan villages.

There is no known occupational risk factor for exposure to the vector mosquitoes in the Armopa area, and such mosquitoes exhibit no marked preference for adults (compared with children) when blood-seeking, beyond that attributable to the greater surface area of adults (Burkot, 1988; Burkot *et al.*, 1989, 1998).

Subjects

Groups of approximately 25 families each arrived at SP2 *en masse* within a day or two of having been flown, on chartered airplanes, from Java to Papua (arriving at Sentani airport, near Jayapura). On the day of their arrival, the newcomers were screened for their suitability for enrolment in the present study. Most were screened at Sentani airport, and the others were screened within a day of their arrival at SP2. To be enrolled, a migrant had to be aged 6–12 or $\geqslant 20$ years, to have no history of residence in a malarious area in the previous 5 years, to be bloodsmear-negative for malarial parasites, to have no splenomegaly, to be in good general health, and give a normal result in a test for glucose-6-phosphate-dehydrogenase (G6PD) deficiency. All of the eligible volunteers or their guardians provided written informed consent and all of those enrolled were assigned study-subject identification codes.

To ensure the treatment of the subjects was ethical, a protocol detailing the conduct of this study was reviewed and approved by the institutional review boards of the United States Navy and Indonesian Ministry of Health. Moreover, all of the work was performed in accordance with the relevant regulations governing the use of such subjects — those of the Indonesian Ministry of Health, and United States government (code 32 of Federal Regulation, Part 219, Protection of Human Subjects; U.S. Navy, SECNAVINST 3900.39B).

Local authorities provided bednets (without insecticide) to all of the residents as they arrived. The nets were plentiful, freely available and very often used by all members of the household, net use being encouraged by an abundance of pest insects, rodents and venomous snakes. Apart from the bed nets, the housing offered no barriers to mosquitoes, and insect-repellent lotions were not widely available. Each evening, most residents of SP2 put on clothing that covered their arms and legs, to minimize mosquito biting.

Follow-Up

The research team established a clinic in SP2 staffed with a physician, nurse and a team of laboratory specialists and assistants. The clinic was the primary source of health care for all residents of SP2. All residents had free access to the clinic without regard to enrolment status. Full-time paid assistants were hired from SP2 and trained to conduct the routine follow-up procedures and prepare bloodsmears. These 'health workers' visited each subject in his or her home three times/week, to see if the subjects were in good health. Subjects appearing ill or complaining of illness in response to a standardized question (i.e. 'Do you have any symptoms?') were referred to the study physician for evaluation and bloodsmear examination. Any complaint of illness prompted a bloodsmear examination, and the subject was considered to have symptomatic malaria if found smear-positive. Smear-positive subjects with at least one symptom attributable to malaria were treated promptly (see below) and began a post-infection follow-up consisting of the collection of a sample of venous blood on the first day of treatment (day 0) and day 28, and the preparation of bloodsmears on days 0, 2, 4, 11, 14, 18, 21, 28, 35 and 42.

Routine bloodsmears prepared every 2 weeks were stained, stored and later examined in a laboratory in Jakarta. Blood and plasma were collected from all subjects every 2 months. Twice a year, each subject was given a physical examination that included

measurement of spleen size, body weight, height and middle-upper-arm circumference and a complete blood cell count on a QBC™ counter (BD, Franklin Lakes, NJ). This regimen of follow-up began on the day of arrival and enrolment in August–October 1996 and ended in July 1999, when the study ceased.

Treatment of Malaria

As prophylaxis, all those who had just moved to SP2 received a supervised weekly dose of 5 mg chloroquine base/kg for a period of 90 days, in accordance with the policy of the Indonesian Ministries of Health and Transmigration. Although this regimen is ineffective in preventing parasitaemias caused by *P. falciparum* or *P. vivax* (Baird *et al.*, 1995; Fryauff *et al.*, 1995), it may reduce the risk of severe disease and death during the 90 days of prophylaxis. On the day of enrolment, the subjects were randomized to either chloroquine- or mefloquine-treatment groups. Treatment of uncomplicated, slide-confirmed *P. falciparum* malaria during the 33 months of follow-up was consistent according to this assignment for each subject: directly observed chloroquine (Resochin™; P.T. Bayer Indonesia, Jakarta), as doses of 10, 10 and 5 mg base/kg at 24-h intervals, or mefloquine (Lariam™; Hoffman–La Roche, Basel), as a single dose of 15 mg base/kg. This approach addressed the hypothesis that a curative drug (mefloquine) would inhibit or delay the onset of acquired immunity more than a primarily suppressive drug (chloroquine).

The occurrence of a recurrent *P. falciparum* parasitaemia within 28 days of the first treatment dose (on day 0) was considered a therapeutic failure and prompted the supervised administration of a single dose of sulfadoxine–pyrimethamine (Kimia Farma, Bandung, Indonesia) equivalent to 25 mg sulfadoxine and 1.25 mg pyrimethamine/kg, and a second round of 42 days of post-therapeutic monitoring. Recurrence during this period prompted therapy with generic quinine (Kimia Farma), given at 10 mg/kg three times daily for 7 days.

Subjects meeting the World Health Organization's criteria for severe *P. falciparum* malaria (WHO, 2000) were treated with intravenous quinine in a standard protocol: generic quinine dihydrochloride (Kimia Farma) at 20 mg salt/kg in 5% dextrose saline as a 4-h loading dose, followed 4 h later, with 10 mg salt/kg over another 4 h, repeated every 8 h until the subject was capable of taking oral medication.

Subjects diagnosed with *P. vivax* malaria were not only treated with chloroquine or mefloquine according to assignment (as above) but also received 15 mg primaquine daily for 14 days.

Pre-treatment, all women with symptomatic *P. falciparum* malaria were tested for pregnancy using a urine dipstick for human chorionic gonadotropin; those found positive received mefloquine regardless of their treatment assignment.

A physician evaluated each subject who required therapy and recorded axillary temperature and all the physical complaints reported by the subject, at presentation in the clinic. The results of clinical trials in the region demonstrated a 28-day cumulative risk of chloroquine-treatment failure of approximately 80% for *P. falciparum* and 70% for *P. vivax* (Baird *et al.*, 1991*a*, 1997).

At 6-month intervals, as a crude gauge of risk of infection among the study subjects, homes in SP2 were visited at random and occupants aged >5 years who were not already enrolled in the present study were asked if fingerprick samples of blood might be collected from them. Smears of the blood samples so collected allowed a cross-sectional exploration of the prevalence and density of parasitaemia among the SP2 residents.

The availability of antimalarial drugs outside of the clinic operated by the research team was very limited. The village was extremely isolated from market places by distance, a poor road, and an incomplete

bridge over a large river. In other words, drugs through the research clinic were nearby and free, whereas those in the market places were far away and relatively expensive. Unsupervised therapy was therefore unlikely to have been a serious potential confounder in the study cohort.

Classification of Infection

Each parasitaemia detected was classified as a new infection or a recrudescence simply on the basis of the length of time since the patient had last been parasitaemic, only a parasitaemia that occurred within 28 days of a previous parasitaemia being considered a recrudescence. The results of examining the bloodsmears prepared routinely every 2 weeks sometimes revealed a parasitaemia in a subject who, though not on chemotherapy, had not complained of any symptoms and was afebrile when (and remained afebrile for $\geqslant 24$ h after) the blood sample had been collected. Such parasitaemias were included in the process of assigning infection sequence during the 33 months of follow-up. An ordered sequence of species-specific (i.e. *P. falciparum* or *P. vivax*) new infections was therefore determined for each subject. New infections were further classified as febrile or afebrile, according to the clinical records for the day of therapy and/or blood collection.

Microscopy

The microscopical demonstration of asexual malarial parasites in Giemsa-stained thick smears supported all diagnoses of malaria. The locally hired, field-based microscopists, all of whom had more than 7 years' experience of checking bloodsmears, were examined and certified competent. Only if no parasites had been detected in 200 fields at a magnification of $\times 1000$ was a thick smear considered negative. Levels of parasitaemia (asexual parasites/μl) were estimated by counting asexual parasites against 200 leucocytes in thick smear and assuming each subject had 8000 leucocytes/μl.

Entomology

Mosquito and climate surveillance began in late August 1996 and lasted until June 1999. During the study period, the local health authorities conducted three spray rounds with a residual insecticide: two (in August 1996 and March 1997) with bendiocarb and one (in February 1998) with lambda-cyhalothrin. Routine, all-night, human-landing collections (HLC) were conducted weekly to estimate human contact rates, vector activity patterns and sporozoite infection 'rates'. Four sentinel HLC houses were selected to derive human-landing rates (HLR; i.e. the number of mosquitoes captured/person-evening). The HLR were based on the results of twice-weekly, 12-h collections (from approximately 18.00–06.00 hours). Mosquitoes were collected, for 50 min in each collection hour, from the exposed legs of a trained technician, using a mouth aspirator and flashlight. Captured mosquitoes were placed in collection cups each hour. At the study site, *Anopheles* mosquitoes were sorted, identified by species, and stored dry, over silica gel, until assayed for *Plasmodium* infection in the laboratory in Jakarta. Individual mosquitoes were tested, in an ELISA (Wirtz *et al.*, 1987), for the circumsporozoite proteins (CSP) of *P. falciparum* and two *P. vivax* variants (Pv210 and Pv247). Daily rainfall and maximum/minimum ambient temperatures were recorded on site throughout the study.

Statistical Analysis

Results were expressed as means or frequencies and analysed using unpaired Student's *t*-tests, or Mantel–Haenszel tests. Fisher's exact tests were used when expected cell values were below 5.0. A *P*-value of $\leqslant 0.05$ was considered indicative of statistical significance. All the analyses were carried out using version 9.0 of the SPSS (SPSS Inc., Chicago, IL) or version 6.0 of the Epi Info (Centers for Disease Control and Prevention, Atlanta, GA) software packages.

RESULTS

Screening, Enrolment, and Withdrawal

Screening and enrolment typically occurred as groups of 10–40 families arrived *en masse*. Eleven such groups were screened between 25 August 1996 and 3 October 1996. Screening against people likely to have a history of exposure to malaria produced 16 exclusions among the 322 interviewed and examined. Prior residence in a malarious area (16) was the commonest reason among the total of 61 exclusions. Overall, 159 adults (aged $\geqslant$20 years) and 102 children (aged 6–12 years) were enrolled in the study; 131 randomized to the chloroquine- and 130 to the mefloquine-treatment group. Figure 1 illustrates the flow of subjects at screening, enrolment and study conclusion, and Table 1 lists the demographic features of the cohort at enrolment.

Within a few days of enrolment, 13 of the adult subjects and five of the children admitted a history of residence in malarious areas and were dropped from the study. The remaining 146 adults and 97 children were considered the complete cohort. Of these 243 subjects, 213 (88%) remained in the study after the first year, 168 (69%) after the second year, and 159 (65%) when the study terminated in July 1999 (Table 2). Virtually all of the withdrawals (68 of 84) were the result of families electing to return to Java.

Prevalence of Parasitaemia in SP2

Although the first of the 6-monthly, cross-sectional examinations of bloodsmears covered just 13% of the population (samples collected from 163 villagers), all the subsequent prevalence samples consisted of 20%–23% of the population of SP2 (i.e. 200–268 villagers). Figure 2 illustrates the prevalences of *P. falciparum* and *P. vivax* infection among children (i.e. the subjects aged 6–12 years) and adults in SP2 over the period of study. Each age-specific point represents a sample of between 18 and

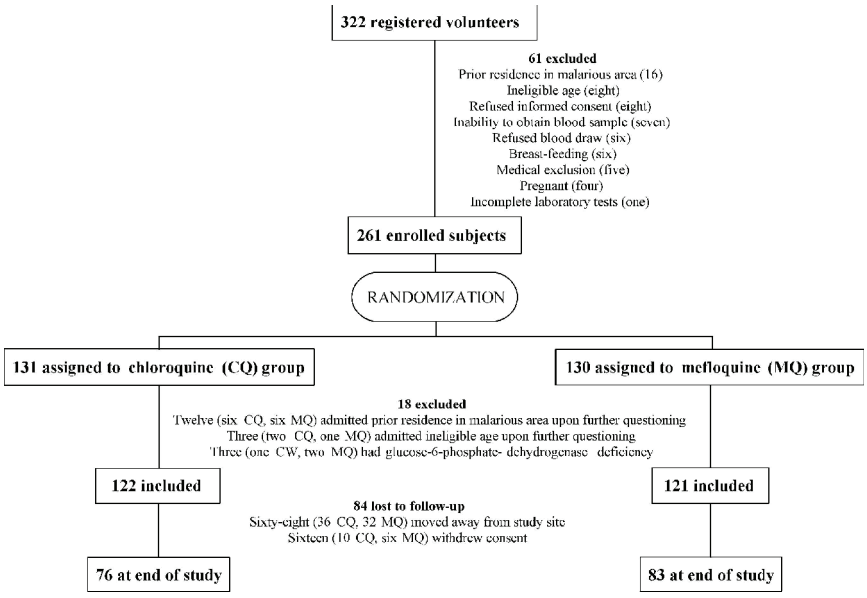

FIG. 1. Flowchart showing the number of subjects available for screening, at enrolment and up to the study conclusion. Those with glucose-6-phosphate-dehydrogenase deficiency could not be excluded prior to randomization because it took several days to test for this deficiency.

TABLE 1. *Demographic characteristics of the cohort of Javanese migrants taking up residence at SP2 in Armopa, Papua, Indonesia*

	Children			Adults			
	Routine therapy*			Routine therapy*			
	CQ	MQ	All	CQ	MQ	All	All subjects
No. enrolled	50	47	97	72	74	146	243
No. of males:females	32:18	31:16	63:34	47:25	53:21	90:46	163:80
Mean age and (range) (years)	9.1 (6–12)	9.3 (6–12)	9.2 (6–12)	31.9 (20–58)	31.7 (20–50)	31.8 (20–58)	22.8 (6–58)
Mean weight and (range) (kg)	22.6 (15.4–38.2)	22.8 (13–34.7)	22.7 (13–38.2)	51.1 (35.5–64)	52.1 (37.8–83.3)	51.6 (35.5–83.3)	40.1 (13–83.3)

*The subjects were randomly assigned to routine treatment of symptomatic malaria with chloroquine (CQ) or mefloquine (MQ).

TABLE 2. *The changing size of the cohort of Javanese migrants investigated at SP2 in Armopa, Papua, Indonesia, through the 33 months of post-migration follow-up*

	No. of children			No. of adults			
	Routine therapy*			Routine therapy*			
Time	CQ	MQ	All	CQ	MQ	All	No. of subjects
Enrolment	50	47	97	72	74	146	243
End of Year 1	45	45	90	59	64	123	213
End of Year 2	34	37	71	46	51	97	168
End of Year 3	32	37	69	44	46	90	159

*The subjects were randomly assigned to routine treatment of symptomatic malaria with chloroquine (CQ) or mefloquine (MQ).

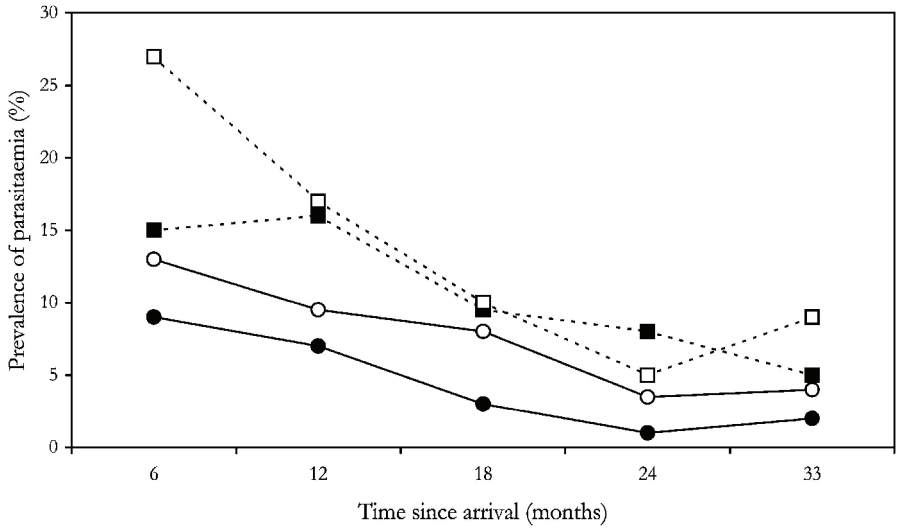

FIG. 2. The prevalences of *Plasmodium falciparum* parasitaemias in adults (○) and children (●) and of *P. vivax* parasitaemias in adults (□) and children (■), recorded, over the course of the study, among a random sample of residents of SP2 village who were not enrolled in the longitudinal cohort.

150 residents (median sample = 48). The prevalence of malarial infection diminished sharply between the first cross-sectional survey (at month 6) and the last (at month 33), among both children and adults. There was no significant difference in the prevalences of *P. falciparum* and *P. vivax* infection except among the adults in the first sample, who were much more likely to be infected with *P. vivax* than *P. falciparum* (27.5% *v.* 14.5%; $P = 0.043$).

Number of Infections

Figure 3 illustrates the numbers of new infections by *P. falciparum* or *P. vivax* detected among the child and adult subjects over the 33 months of the study. A total of 596 infections by *P. falciparum* occurred in the cohort (255 among children and 341 among adults), and 22 subjects (nine children and 13 adults) each experienced at least six infections by *P. falciparum*. A total of 723 infections by *P. vivax* occurred in

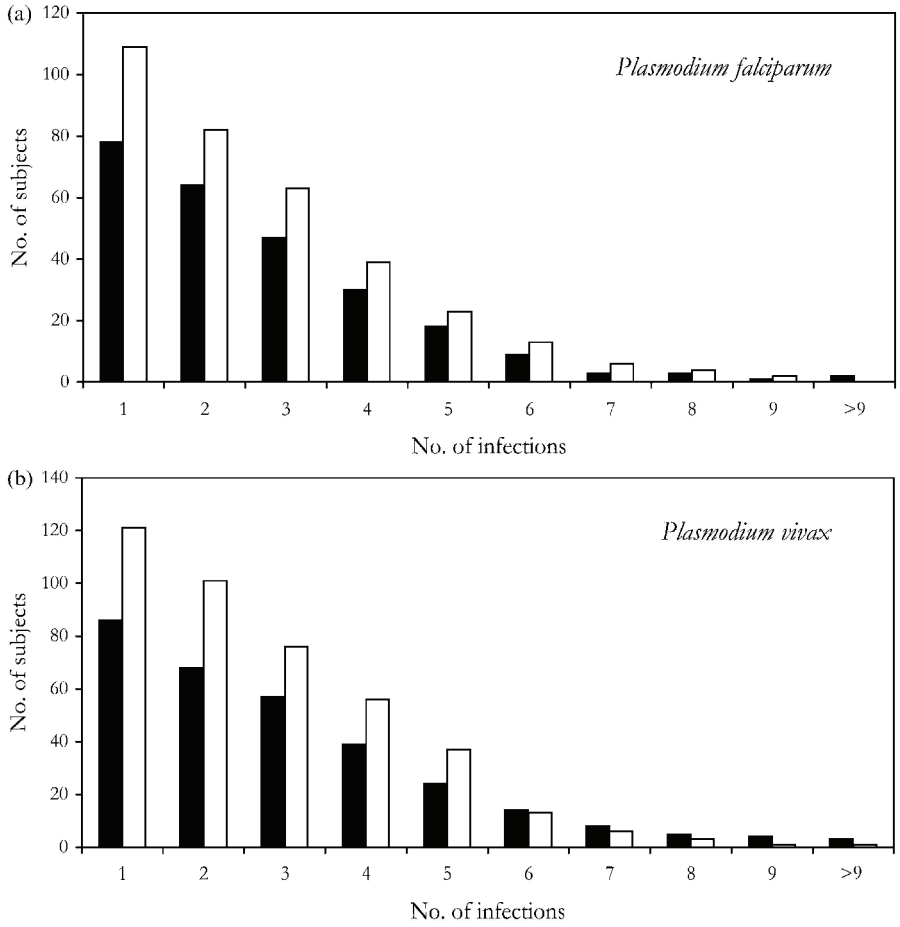

FIG. 3. The numbers of adult (□) and child (■) subjects in the SP2 longitudinal cohort experiencing one to more than nine infections with *Plasmodium falciparum* or *P. vivax*.

the cohort (308 among children and 415 among adults), 27 subjects (14 children and 13 adults) each experiencing at least six infections with this species.

Incidence of Patent Infection

Figure 4 illustrates the annual incidences of new infections with *P. falciparum* and *P. vivax* among the subjects enrolled in the study. There were approximately 1.5 *P. falciparum* and 1.8 *P. vivax* infections/subject (among both children and adults) in the first year. In the second year, there were 1.2 *P. falciparum* and 1.4 *P. vivax* infections/child, and 1.3 *P. falciparum* and 1.7 *P. vivax* infections/adult. In the third year, the annual incidence was 0.5 *P. falciparum* and 0.5 *P. vivax* infection/child and 0.7 *P. falciparum* and 0.7 *P. vivax* infection/adult. Overall, the annual incidences of malarial infection (either *P. falciparum* or *P. vivax*) during the first, second and third years were 3.2, 2.7 and 1.2 infections/subject-year, respectively.

Mean Parasite Counts

Figure 5 illustrates the geometric mean parasite counts (GMPC) for *P. falciparum* and *P. vivax* for children and adults. Between the first and third years, the annual GMPC

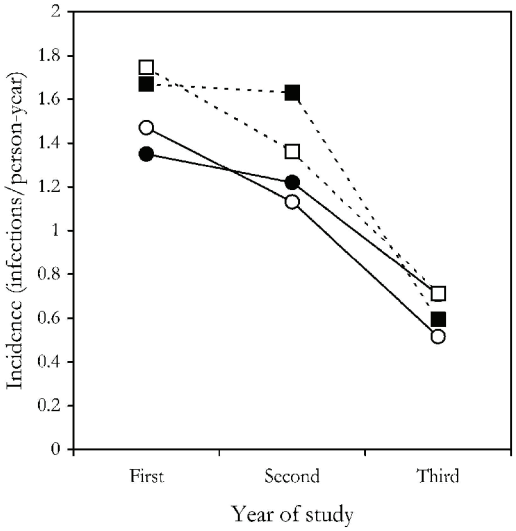

FIG. 4. The annual incidences of infection by *Plasmodium falciparum* among the adults (○) and children (●) enrolled in the SP2 cohort and by *P. vivax* among the same adults (□) and children (■).

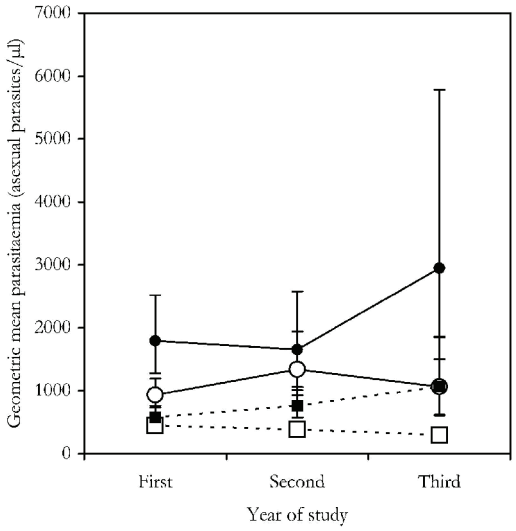

FIG. 5. The levels of *Plasmodium falciparum* parasitaemia (annual geometric mean parasite counts) among the adults (○) and children (●) enrolled in the SP2 longitudinal cohort and the corresponding values for *P. vivax* among the same adults (□) and children (■). The vertical lines indicate 95% confidence intervals.

for *P. falciparum* among children (1796 *v.* 2949 parasites/μl; $P = 0.20$) and adults (935 *v.* 1066 parasites/μl; $P = 0.66$) showed no significant change. The GMPC for *P. falciparum* were higher in the children than in the adults over the first (1796 *v.* 935 parasites/μl; $P = 0.002$) and third years (2949 *v.* 1066 parasites/μl; $P = 0.03$) but not over the second year (1655 *v.* 1345 parasites/μl; $P > 0.05$). The corresponding counts for *P. vivax* were similar among the children and adults during the first year (581 *v.* 448 parasites/μl; $P = 0.14$) but significantly higher in the children than in the adults during both the second (763 *v.* 384 parasites/μl; $P < 0.001$) and third years (1067 *v.* 299 parasites/μl; $P < 0.001$).

Severe Malaria

During the study, 31 residents of SP2 developed severe malaria requiring intravenous-quinine management. All 22 of these cases who were not enrolled in the study had slide-proven *P. falciparum* malaria. Six of these 22 cases were children and three (all children) died.

Among the other nine cases of severe malaria — eight adults and one child, who were all enrolled on the study and all of whom survived — one appeared smear-positive for *P. vivax* only and the rest smear-positive for *P. falciparum*. The severe episode of *P. vivax* malaria occurred with the first infection whereas the severe attacks of *P. falciparum* malaria occurred with the first (two cases), third (four) or fourth infections (two). The odds ratio for a *P. falciparum* infection developing into severe malaria in an adult subject, compared with that in a child, was 6.1 ($P = 0.08$).

Entomology

Members of the *Anopheles punctulatus* group were collected year-round over the course of the study. Based on both morphological and molecular analysis, *Anopheles punctulatus* Doentz, *Anopheles koliensis* Owen, *Anopheles farauti* Laveran and *Anopheles farauti* No. 4 (a sibling species) were identified in collections from inside and outside the sentinel homes.

For the purpose of data analysis and the ELISA, both sibling species of *An. farauti* were grouped together (as *An. farauti* s.l.). The predominant species encountered was *An. punctulatus*, followed by *An. farauti* s.l. Vector biting rates typically increased during and immediately after the wet months of the year (November–March) and decreased to far lower levels during the drier months (May–October; see Figure 6). In early 1998, indoor and outdoor anopheline biting densities declined markedly, and remained relatively low until the end of the study.

Between April and May 1997, a 97% reduction in the HLR was recorded. This was attributed to the second round of indoor spraying with bendiocarb, in late March 1997. The total number of anophelines captured in 1998–1999 was >84% lower than the number caught in 1996–1997. A sustained, general suppression in mosquito population densities continued into 1998 and 1999, with only small adult-activity peaks seen during the wet months of September–October 1998 and February–April 1999. The ELISA-based estimates of the combined indoor/outdoor sporozoite 'rates' remained around 0.2% throughout the study period.

DISCUSSION

The incidence of infection by *P. falciparum* in the study cohort was approximately 1.5 infections/person-year during the first 2 years, and just 0.6 infection/person-year in the third year. Most subjects experienced only three or four infections with this parasite during the study. The incidence of *P. falciparum* infection in the Armopa region was also relatively low (Baird *et al.*, 2001) but that observed in the Arso region, where the original cross-sectional studies of Javanese transmigrants were conducted (Baird *et al.*, 1991*b*, 1993), was much higher. The results of longitudinal studies of malaria attack in the Arso region typically indicated an annual incidence of two to four *P. falciparum* infections/person (Jones *et al.*, 1994; Ohrt *et al.*, 1997; Taylor *et al.*, 1999). Infection incidence is a critical issue to those exploring the onset of clinical immunity to *P. falciparum*, because the attack rates in Arso and Armopa and the results of repeated challenge of neurosyphilis patients with *P. falciparum* (Ciuca *et al.*, 1934) indicate that such immunity may require at least four infections within 12–24 months (Baird *et al.*, 2003). Unlike

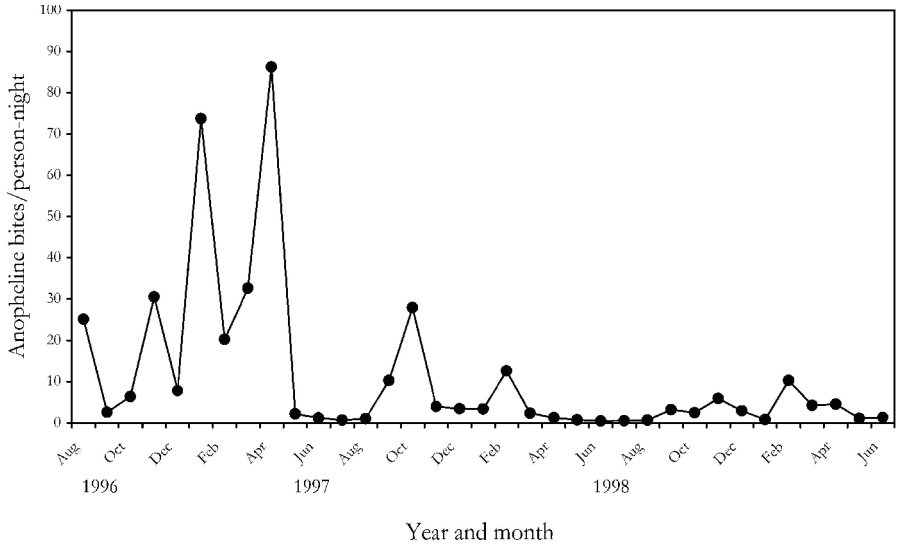

FIG. 6. The monthly mean levels of anopheline-mosquito biting in SP2 over the course of the longitudinal study.

those who live in the Arso region, relatively few residents of SP2 would have experienced such exposure during the period of the present study. This difference may explain why, after more than 2 years of exposure, the prevalence of *P. falciparum* parasitaemia among the children investigated in SP2 was similar to that in the adults (Fig. 2) whereas in Arso the adults were much less likely to be parasitaemic than the children (Baird *et al.*, 1991*b*, 1993).

Several key factors probably explain the relatively low risk of infection in SP2 compared with that in the Arso region. There is clearly an ethical responsibility to execute a programme of close surveillance and prompt treatment among a cohort of essentially non-immune subjects exposed to a potentially life-threatening infection. Application of such a strategy in SP2 almost certainly curtailed the pool of infectious people and thereby artificially reduced the incidence of infection. Indeed, over the course of the study, sharp drops in the prevalence of parasitaemia in the village and in the incidence of infection among the study subjects were observed. Also, the numbers of biting anophelines sharply declined following a routine, indoor application of a residual insecticide throughout the village. Finally, an important distinction between the habitat of the Arso study sites and those in Armopa is the amount of cleared *versus* forested land; a new palm-oil plantation dominated the habitat surrounding the Arso sites whereas virgin forest surrounded the Armopa study sites. This may be the most important factor because only at the one study site in the Arso region that was surrounded by forest rather than palm-oil plantation was the annual incidence of *P. falciparum* infection found to be as low (1.1 infections/person; Fryauff *et al.*, 1995) as those recorded in the Armopa region, either in SP2 (1.1/person; present study) or elsewhere (0.9/person; Baird *et al.*, 2001).

The present results indicate that, in SP2 over the study period, the risk of patent infection was similar for the children and adults; the incidence of infection by *P. falciparum* in the SP2 cohort over 33 months was 1.11 infections/child-year and 1.10/adult-year. A similar analysis restricted to first infections also showed virtually identical risk for the children and adults (Barcus *et al.*, 2003). Baird *et al.* (1991*b*) thought that the risk of infection (i.e. sporozoite inoculation) in Arso was probably similar for children and adults because the main local vectors tend to breed near to houses and to blood-feed predominantly around homes at night (Slooff, 1964; Burkot, 1988; Burkot *et al.*, 1989). That the prevalence of parasitaemia during early exposure to risk appeared to be unaffected by subject age supported that view (Baird *et al.*, 1993). The differences in susceptibility to infection and disease observed between children and adult migrants have therefore been attributed to age-related differences in immune response rather than to differences in cumulative experience with infection (Baird *et al.*, 1991*b*, 1993; Baird, 1995, 1998). The results of the research in SP2 confirm that, in rural, north–eastern Indonesian Papua, the risk of exposure to infection in children is practically the same as that in adults.

Severe disease rarely occurred in the SP2 cohort. Only nine of the study subjects required intravenous quinine over the 33-month study period. This contrasts with the situation in villages lacking the active surveillance and prompt therapy afforded to the study subjects. During the first 2 years at Arso PIR IV, for example, the monthly incidence of severe malaria requiring evacuation to hospital, among the adult Javanese migrants, was typically 0.5 case/person-year and peaked at 1.4 cases/person-year during the first 6 months (Baird *et al.*, 1998). In SP2, the eight cases of *P. falciparum* malaria who required intravenous quinine (also predominantly adult subjects) represented a corresponding incidence of only 0.01 case/person-year. In other words, subjects in the cohort at SP2 were at least 50 times less likely to develop severe disease than similar

migrants in the Arso region. This is almost certainly the product of prompt diagnosis and treatment in SP2, and the consequent reductions in the prevalence of parasitaemia and therefore risk of severe disease. The SP2 cohort does not represent patterns of risk of severe malaria among most non-immune migrants to Indonesian Papua, who live in villages where the facilities for surveillance, diagnosis and treatment are far more limited.

In summary, the incidence of *P. falciparum* infection, or *P. vivax* infection, among both children and adults during their first 33 months at SP2 was about 1.1 infections/person-year. That this rate was relatively low compared with rates measured earlier, in the Arso region, was probably the result of prompt diagnosis and treatment, along with diminished vector abundance. The onset of clinical immunity, observed in most residents in the Arso study sites, was therefore anticipated to be limited to the minority of the cohort experiencing at least four infections within 1 or 2 years.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge the advice and direct assistance of many officers of the Ministry of Health in Jakarta and of the Provincial Health Service of Papua in Jayapura. We are especially indebted to Drs Ingerani, A. Soemarjati and Sri Astuti in Jakarta, and Drs W. Kalalo and B. Subianto in Jayapura, for their continuous support of this 3-year project. Our site leaders, technicians and assistants at SP2, whose uninterrupted vigour made this project possible, include Dr H. Wijaya, Soegoto, S. Wangsamuda, M. Sutamiharja, T. Karubuy, Supriyanto, F. Sanggamele, Sunardi, J. Anis, W. Burdam and the Papuan health workers. The mosquito collections were supervised and processed by S. Rusmiarto, N. Hamzah, B. Trenggono, D. Susapto and S. Atmosudjono. Dr N. Punjabi served as the medical monitor and never wavered in his duty to the safety and fair treatment of the study subjects. This work was supported by the U.S. Department of Defense, Military Infectious Diseases Research Program under STO F, malaria vaccine development. Finally, we thank all of the residents of SP2 who welcomed us into their village and homes.

REFERENCES

Baird, J. K. (1995). Host age as a determinant of naturally acquired immunity to *Plasmodium falciparum*. *Parasitology Today*, **11,** 105–111.

Baird, J. K. (1998). Age-dependent characteristics of protection versus susceptibility to *Plasmodium falciparum*. *Annals of Tropical Medicine and Parasitology*, **92,** 367–390.

Baird, J. K., Purnomo & Masbar, S. (1990). *Plasmodium ovale* in Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, **21,** 541–544.

Baird, J. K., Basri, H., Jones, T. R., Bangs, M. J. & Ritonga, A. (1991*a*). Resistance to antimalarials in northeastern Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, **44,** 640–644.

Baird, J. K., Jones, T. R., Danudirgo, E. W., Basri, H., Annis, B. A., Bangs, M. J., Wiady, I. & Masbar, S. (1991*b*). Age-dependent acquired immunity against *Plasmodium falciparum* among people with 2 years exposure to hyperendemic malaria. *American Journal of Tropical Medicine and Hygiene*, **45,** 65–76.

Baird, J. K., Purnomo, Basri, H., Bangs, M. J., Andersen, E. M., Jones, T. R., Masbar, S., Harjosuwarno, S., Subianto, B. & Arbani, P. R. (1993). Age-specific prevalence of *Plasmodium falciparum* among six populations with limited histories of exposure to endemic malaria. *American Journal of Tropical Medicine and Hygiene*, **49,** 707–719.

Baird, J. K., Fryauff, D. J., Basri, H., Bangs, M. J., Subianto, B., Wiady, I., Purnomo, Leksana, B., Masbar, S., Richie, T. L., Jones, T. R., Tjitra, E., Wignall, F. S. & Hoffman, S. L. (1995). Primaquine for prophylaxis against malaria among nonimmune transmigrants in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, **52,** 479–484.

Baird, J. K, Wiady, I., Fryauff, D. J., Sutamihardja, M. A., Leksana, B., Widjaya, H., Kysdarmanto & Subianto, B. (1997). In vivo resistance to chloroquine by *Plasmodium vivax* and *Plasmodium falciparum* at Nabire, Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, **56,** 627–631.

Baird, J. K., Masbar, S., Basri, H., Tirtokusumo, S., Subianto, B. & Hoffman, S. L. (1998). Age-dependent susceptibility to severe disease with primary exposure to *Plasmodium falciparum*. *Journal of Infectious Diseases*, **178,** 592–595.

Baird, J. K., Lacy, M. D., Basri, H., Barcus, M. J., Maguire, J. D., Bangs, M. J., Gramzinski, R., Sismadi, P., Krisin, Ling, J., Wiady, I., Kusumaningsih, M., Jones, T. R., Fryauff, D. J., Hoffman, S. L. & The NAMRU-2 Clinical Trials Team (2001). Randomized, parallel placebo-controlled trial of primaquine for malaria prophylaxis in Papua, Indonesia. *Clinical Infectious Diseases*, **33,** 1990–1997.

Baird, J. K., Krisin, Barcus, M. J., Elyazar, I. R. F., Bangs, M. J., Maguire, J. D., Fryauff, D. J., Richie, T. L., Sekartuti & Kalalo, W. (2003). Onset of clinical immunity to *Plasmodium falciparum* among Javanese migrants to Indonesian Papua. *Annals of Tropical Medicine and Parasitology*, **97,** 557–564.

Barcus, M. J., Krisin, Elyazar, I. R. F., Marwoto, H., Richie, T. L., Basri, H., Wiady, I., Fryauff, D. J., Maguire, J. D., Bangs, M. J. & Baird, J. K. (2003). Primary infection by *Plasmodium falciparum* or *Plasmodium vivax* in a cohort of Javanese migrants to Indonesian Papua. *Annals of Tropical Medicine and Parasitology*, **97,** 565–574.

Burkot, T. R. (1988). Non-random host selection by anopheline mosquitoes. *Parasitology Today*, **4,** 156–162.

Burkot, T. R., Dye, C. & Graves, P. M. (1989). An analysis of some factors determining the sporozoite rates, human blood indexes, and biting rates of members of the *Anopheles punctulatus* complex in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene*, **40,** 229–234.

Burkot, T. R., Graves, P. M., Paru, R. & Lagog, M. (1998). Mixed blood feeding by the malaria vectors in the *Anopheles punctulatus* complex (Diptera: Culicidae). *Journal of Medical Entomology*, **25,** 205–213.

Ciuca, M., Ballif, L. & Chelarescu-Vieru, M. (1934). Immunity in malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **27,** 619–622.

Fryauff, D. J., Baird, J. K., Basri, H., Sumawinata, I., Purnomo, Richie, T. L., Ohrt, C. K., Mouzin, E., Church, C. J., Richards, A. L., Subianto, B., Sandjaya, B., Wignall, F. S. & Hoffman, S. L. (1995). Randomized, placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria in Indonesia. *Lancet*, **346,** 1190–1193.

Good, M. F., Kaslow, D. C.& Miller, L. H. (1998). Pathways and strategies for developing a malaria blood-stage vaccine. *Annual Review of Immunology*, **16,** 57–87.

Jones, T. R., Baird, J. K., Bangs, M. J., Annis, B. A., Purnomo, Basri, H., Gunawan, S., Harjosuwarno, S., McElroy, P. D. & Hoffman, S. L. (1994). Malaria vaccine study site in Irian Jaya, Indonesia: *Plasmodium falciparum* incidence measurements and epidemiologic considerations in sample size estimation. *American Journal of Tropical Medicine and Hygiene*, **50,** 210–218.

McGregor, I. A. (1987). Malarial immunity: current trends and prospects. *Annals of Tropical Medicine and Parasitology*, **81,** 647–656.

Metselaar, D. (1961). Seven years' malaria research and residual house spraying in Netherlands New Guinea. *American Journal of Tropical Medicine and Hygiene*, **10,** 327–334.

Newbold, C. I., Craig, A. G., Kyes, S., Berendt, A. R., Snow, R. W., Peshu, N. & Marsh, K. (1997). PfEMP1, polymorphism and pathogenesis. *Annals of Tropical Medicine and Parasitology*, **91,** 551–557.

Ohrt, C., Richie, T. L., Widjaja, H., Shanks, G. D., Fitriadi, J., Fryauff, D. J., Handschin, J., Tang, D., Sandjaja, B., Tjitra, E., Hadiarso, L., Watt, G. & Wignall, F. S. (1997). Mefloquine compared with doxycycline for the prophylaxis of malaria in Indonesian soldiers. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*, **126,** 963–972.

Plebanski, M. & Hill, A. V. S. (2000). The immunology of malaria infection. *Current Opinion in Immunology*, **12,** 437–441.

Saul, A. (1999). The role of variant surface antigens on malaria-infected red blood cells. *Parasitology Today*, **15,** 455–457.

Slooff, R. (1964). *Observations on the Effect of Residual DDT House Spraying on Behaviour and Mortality in Species of the* Anopheles punctulatus *Group*. Leyden: A. W. Sythoff.

Taylor, W. R., Richie, T. L., Fryauff, D. J., Picarima, H., Ohrt, C., Tan, D., Braitman, D., Murphy, G. S., Widjaja, H., Tjitra, E., Ganja, A., Jones, T. R., Basri, H. & Berman, J. (1999). Malaria prophylaxis using azithromycin: a double-blind, placebo-controlled trial in Irian Jaya, Indonesia. *Clinical Infectious Diseases*, **28,** 74–81.

Wirtz, R. A., Burkot, T. R., Graves, P. M. & Andre, R. G. (1987). Field evaluation of the enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *P. vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *Journal of Medical Entomology*, **24,** 433–437.

World Health Organization (2000). Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94** (Suppl. 1), 1–31.