University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Public Health Resources

2003

Primary infection by *Plasmodium falciparum* or *P. vivax* in a cohort of Javanese migrants to Indonesian Papua

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

United States Naval Medical Research Unit #2

I. R.F. Elyazar
United States Naval Medical Research Unit #2

H. Marwoto
IndonesianMinistry of Health Institute of Health Research and Development

T. L. Richie
United States Naval Medical Research Unit #2

See next page for additional authors

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

Authors

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

Primary infection by *Plasmodium falciparum* or *P. vivax* in a cohort of Javanese migrants to Indonesian Papua*

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

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

The clinical and parasitological characteristics of the first naturally acquired malarial infection have rarely been documented in humans. When 243 migrants from non-endemic Java were followed from the day of their arrival in Indonesian Papua, 217 (89%) were found to become infected with *Plasmodium falciparum* and/or *P. vivax* before they were lost to follow-up. The incidence of malarial infection in the children investigated (who were aged 6–10 years) was indistinguishable from that in the adults (aged >20 years), with 1.10 and 1.14 *P. falciparum* infections/person-year (relative risk = 0.97; 95% confidence interval = 0.72–1.29) and 1.47 and 1.49 *P. vivax* infections/person-year (relative risk = 0.99; 95% confidence interval = 0.72–1.29), respectively. During their first infections, the children had higher *P. falciparum* parasitaemias than the adults (with geometric means of 1318 and 759 parasites/μl, respectively; $P = 0.04$) but similar *P. vivax* parasitaemias (with geometric means of 355 and 331 parasites/μl, respectively; $P = 0.76$). At first infection, 56% of the subjects were febrile and 90% complained of symptoms. There were no differences between children and adults with respect to these two parameters, either for *P. falciparum* or *P. vivax*. These findings indicate that, with promptly diagnosed and treated uncomplicated malaria, migrant children and adults in north–eastern Indonesian Papua have an equal risk of malarial infection and of disease following their first infections with *P. falciparum* and *P. vivax*.

The results of therapeutic or experimental challenge of adult volunteers constitute most of the detailed body of clinical knowledge of acute malaria in non-immune people.

Malaria therapy early in the 20th century cured many thousands of neurosyphilis cases and studies of these infections profoundly improved our understanding of the clinical aspects of malaria (Von Juaregg, 1922). During World War II, the strategic urgency for new antimalarial drugs prompted clinical studies in soldiers and prison inmates (Collins and Jeffery, 1999). In more recent years, the results of a few experimental challenges have provided much more detailed information on the blood-chemistry, cell-profile and immunological aspects of malaria (Herrington *et al.*, 1991; Church *et al.*, 1997; Hoffman *et al.*, 2002). These studies have, however, shed no light on malarial infection and disease in

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.

children. In experimental challenge, moreover, it is difficult to mimic the effects of treatment-seeking behaviour or natural challenge by wild strains of the parasite. Although epidemics of the disease may offer opportunities for investigating primary malaria in human populations, there appear to have been no detailed clinical and parasitological observations published on primary infection during malaria epidemics.

The organized relocation of families from Java to Indonesian Papua (formerly known as Irian Jaya), at various times between 1965 and 1997, resulted in non-immune people of all ages being abruptly exposed to hyper- and holo-endemic malaria. An aggressive DDT-spraying campaign in the 1950s eradicated endemic malaria from East and West Java (Atmosoedjono, 1990; Arbani, 1992) and left only a few scattered foci of hypo-endemic risk in Central Java (Baird *et al.*, 1996). The unpublished records of the Indonesian Ministry of Health show that, between 1969 and 1996, the mean annual incidence of malaria throughout Java and Bali was only 0.03 case/1000 residents. Thus, the migrants investigated in the present study (with a mean age of 25 years of age and screened against residence on other islands or in a known focus of malaria risk on Java) arrived in Indonesian Papua having an estimated individual risk of just 0.075% for a single prior episode of malaria. In north–eastern Papua, in contrast, the risk of malarial infection within 3–6 months is 100% (Jones *et al.*, 1994; Fryauff *et al.*, 1995; Ohrt *et al.*, 1997; Taylor *et al.*, 1999; Baird *et al.*, 2001).

In a retrospective study of one transmigration community in Indonesian Papua, a high risk of evacuation with severe, *Plasmodium falciparum* malaria during the first 6 months was documented (Baird *et al.*, 1998). The risk of evacuation was markedly higher for the adult migrants than for their children [relative risk = 2.7; 95% confidence interval (CI) = 1.9–3.8], even though the risk of infection with *P. falciparum* appeared similar for all age-groups (Baird *et al.*, 1991, 1995).

Observations on travellers with malaria, on the infections occurring during malaria epidemics, and during a retrospective investigation of another transmigration settlement also indicated that malaria-naïve adults are at higher risk of severe disease than malaria-naïve children (Gill, 1936; Bastien, 1987; Greenberg and Lobel, 1990; Buck and Eichenlaub, 1994; Some, 1994; Baird *et al.*, 2003). It seems possible that intrinsic age-related differences in the immune response to primary infection form the basis of this adult susceptibility to severe disease. In the present study, to explore this possibility, the first smear-confirmed *P. falciparum* or *P. vivax* infections suffered by malaria-naïve Javanese transmigrants to Indonesian Papua, and the disease associated with each of these primary infections, were investigated.

SUBJECTS AND METHODS

Study Site

The study site was the transmigration village known as *Satuan Permukiman* (Settlement Unit) 2 (SP2), which lies, near the Pacific Ocean, in north–eastern Papua, Indonesia. The village, which has already been described in detail by Krisin *et al.* (2003), covers an area of about 4 km^2. It had 1428 residents in 1996, most (78.3%) of whom were migrants from Java. The Indonesian Department of Transmigration constructed SP2 in 1995 and 1996. The housing, of uniform woodplank and tin-roof construction, presented few obstacles to host-seeking mosquitoes. Hyper- to holo-endemic malaria, transmitted by the *Anopheles punctulatus* complex of mosquitoes, occurs throughout most of north–eastern Papua. Almost all of the malaria is caused by *P. falciparum* or *P. vivax*, with only occasional infections by *P. malariae* and very rare infections by *P. ovale* (Baird *et al.*, 1990, 1991). In compliance with the regulations of the Department of Transmigration, newcomers to SP2 received weekly chloroquine (5 mg/kg) for 12 weeks, as chemoprophylaxis.

Compliance with this regimen was supervised among the study subjects, to ensure uniform usage of chloroquine across the cohort. The results of previous studies in similar settings indicated, however, that chloroquine was no more effective than placebo in preventing infection by *P. falciparum* or *P. vivax* (Baird *et al.*, 1995; Fryauff *et al.*, 1995).

Follow-up

A research station and clinic were established in SP2 in July 1996, to enable a longitudinal study of adults (aged >20 years) and children (aged 6–12 years) who had migrated from non-malarious areas of Java. The study protocol was reviewed and approved by the relevant American and Indonesian committees for the protection of human subjects, and informed consent was obtained in accordance with the United States Navy's regulations governing the use of human subjects in medical research (SECNAVINST 3900.39B). A detailed description of the subjects and the methods of follow-up is available elsewhere (Krisin *et al.*, 2003). In brief, 243 subjects were enrolled on their day of arrival at SP2 (between 25 August and 3 October 1996) and within 48 h of their arrival in Indonesian Papua. This cohort was followed for 33 months. The regimen of follow-up included physical and laboratory examinations on enrolment and the production of routine bloodsmears and the recording of axillary temperatures every 2 weeks (or whenever the subject complained of symptoms consistent with malaria). A member of the research team visited each subject in his or her home every other day, to ascertain the subject's health status by a standardized procedure. The intention was to detect all episodes of clinical illness. Technicians certified as expert in the diagnosis of malaria by microscopical examination of Giemsa-stained thick bloodsmears provided diagnostic services. A thick smear was only considered negative if a technician checking 200 fields at a magnification of ×1000 failed to detect a single malarial parasite.

The analysis here is limited to each subject's first infection with *P. falciparum* and his or her first infection with *P. vivax*. Subjects parasitaemic first with *P. falciparum* were treated and still considered at risk for *P. vivax*, and *vice versa*. Owing to the lingering suppression of parasitaemia by the administered therapy, however, the 4 weeks following the first treatment for malarial infection were not counted as time at risk of infection by the other species. Individual subjects only ceased contributing 'person-time at risk' after having experienced infection by both *P. falciparum* and *P. vivax*. Subjects found smear-positive were given directly observed treatment with either chloroquine (25 mg/kg, in three doses over 48 h) or mefloquine (25 mg/kg, as a single dose) according to a randomized (1:1) assignment at enrolment. Non-pregnant subjects positive for *P. vivax* also received primaquine (30 mg daily for 14 days).

Classification of Infections

First infections were classified as febrile or afebrile, a febrile case having a patent parasitaemia and a documented axillary temperature of >37.5°C. The first parasitaemias of 22 subjects were only detected when the routine bloodsmears produced biweekly were checked. The axillary temperature of a subject from whom a routine blood sample was being collected was not recorded if that subject did not complain of illness at that time. Any complaint of illness or notice of fever would have prompted the subject's referral for medical attention, the production and examination of a non-routine bloodsmear and the recording of the subject's axillary temperature. Each subject found parasitaemic only as the result of the examination of a routine blood sample was therefore assumed to have been afebrile and asymptomatic at the time that sample had been collected.

First infections were also classified as symptomatic or asymptomatic, on the basis of parasitaemia concurrent with a subject seeking medical attention or complaining of

symptoms consistent with malaria (i.e. fever, chills, headache, nausea, vomiting, myalgia, malaise and/or diarrhoea) during a home visit.

Statistical Analysis

Risk of infection was measured as both the mean time (the mean number of days from arrival) to the first slide-confirmed parasitaemia and the incidence of infection. Incidence was calculated as the number of infections divided by the number of person-years at risk. The results were expressed statistically as means or frequencies, and generally analysed using unpaired Student's *t*-tests or Mantel–Haenszel tests. A χ^2 test was used to assess differences in calculated proportions. A *P*-value of $\leqslant 0.05$ was considered indicative of statistical significance. Where appropriate, a relative risk (RR) or odds ratio (OR) and its 95% confidence interval (CI) were calculated. Kaplan–Meier survival analysis was used to calculate the cumulative probability curves, in weeks to the first malarial infection. All the analyses were carried out using version 8.0 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

Table 1 presents the demographic and parasitological data collected, stratified by the two age-groups. The overall ratio of males to females who participated in the study was approximately 2:1 (163:80). Almost all of the subjects — 93% (90/97) of the children and 87% (127/146) of the adults (RR = 1.07; CI = 0.98–1.16) — experienced at least one malarial infection over the observation period. Twenty-five of the 26 subjects who were never found parasitaemic were lost to follow-up after deciding to return to Java; the median times these 26 subjects were followed were 11 (range = 2–29) weeks for the seven children and 20 (range = 2–144)

weeks for the 19 adults. One adult subject never developed parasitaemia during all 33 months of follow-up.

The Figure illustrates the cumulative risk of infection by *P. falciparum* and *P. vivax* among children and adults. The mean time to first parasitaemia was essentially identical for *P. falciparum* (185 days, with a range 11–856 days) and *P. vivax* (190 days, with a range 14–901 days; *P* = 0.729). The times to first parasitaemia were essentially the same for the children and adults, both for *P. falciparum* (*P* = 0.57) and *P. vivax* (*P* = 0.29). Likewise, the incidence of infection was similar among the children and adults, both for *P. falciparum* (1.10 infections/child-year *v.* 1.14 infections/ adult-year; RR = 0.97; CI = 0.72–1.29) and for *P. vivax* (1.47 infections/child-year *v.* 1.49 infections/adult-year; RR = 0.99; CI = 0.75–1.30). Children and adults were at equal risk of infection with *P. falciparum* or *P. vivax*. Children, however, had generally higher first *P. falciparum* parasitaemias than the adults (with geometric mean counts of 1318 *v.* 759 parasites/μl; *P* = 0.04), although the levels of the first *P. vivax* parasitaemias were similar in the children and adults investigated (with geometric mean counts of 355 *v.* 331 parasites/μl; *P* = 0.76). In both age-groups, the *P. falciparum* counts were significantly higher than the *P. vivax* (*P* < 0.001).

At first infection with *P. falciparum* or *P. vivax*, 56% of the subjects were febrile and 90% complained of malaria-like symptoms (Table 1). With *P. falciparum*, there was no significant difference between the adults and children in the risk of fever (OR = 1.19; *P* = 0.60), or complaint of illness (OR = 0.55; *P* = 0.20). With *P. vivax*, similarly, there was no significant difference between adults and children in the frequency of associated fever (OR = 1.29; *P* = 0.40) or complaint of illness (OR = 0.77; *P* = 0.59). Between the two species, no differences appeared in the likelihood of fever (OR = 1.35; *P* = 0.16) or reported illness (OR = 0.84; *P* = 0.62).

TABLE 1. *Summary of the demographic and parasitological data on the study cohort*

| Variable	Children	Adults	All subjects	Odds ratio and (95% CI)	RR and (95% CI)	P
No. of subjects enrolled	97	146	243			
Mean age and (range) (years)	9.2 (6–12)	31.8 (20–58)	22.8 (6–58)			
Median age (years)	9	31	26			
No. of males:females	63:34	100:46	163:80			
% OF SUBJECTS AND (NO. OF SUBJECTS/NO. INVESTIGATED)						
Lost to follow-up	7 (7/97)	12 (18/146)		0.55 (0.20–1.48)		
Primary infection with *P. falciparum*	44 (43/97)	44 (64/146)		1.02 (0.59–1.77)		
First *P. falciparum* infection after *P. vivax*	36 (35/97)	30 (45/146)		1.27 (0.71–2.26)		
Primary infection with *P. vivax*	49 (47/97)	49 (71/146)		0.99 (0.57–1.71)		
First *P. vivax* infection after *P. falciparum*	40 (39/97)	34 (50/146)		1.29 (0.73–2.27)		
Febrile on first *P. falciparum* infection	62.1 (41/66)	58.0 (58/100)		1.19 (0.60–2.36)		
Complaining of illness on first *P. falciparum* infection	85.9 (67/78)	91.7 (100/109)		0.55 (0.20–1.52)		
Febrile on first *P. vivax* infection	55.8 43/77)	49.5 (53/107)		1.29 (0.69–2.42)		
Complaining of illness on first *P. vivax* infection	89.6 (77/86)	91.7 (111/121)		0.77 (0.27–2.18)		
GEOMETRIC MEAN LEVEL OF PARASITAEMIA AND (95% CI) (parasites/µl)						
First *P. falciparum* infection	1348 (860–2114)	755 (539–1059)				0.41
First *P. vivax* infection	354 (255–491)	330 (250–438)				0.76
MEAN TIME AND (RANGE) TO FIRST INFECTION (days)						
With *P. falciparum*	192 (14–515)	180 (21–901)				0.57
With *P. vivax*	200 11–764)	182 (18–856)				0.29
INCIDENCE (infections/person-year)						
Plasmodium falciparum	1.1	1.14			0.97 (0.72–1.29)	
Plasmodium vivax	1.47	1.49			0.99 (0.75–1.30)	

CI, Confidence interval.
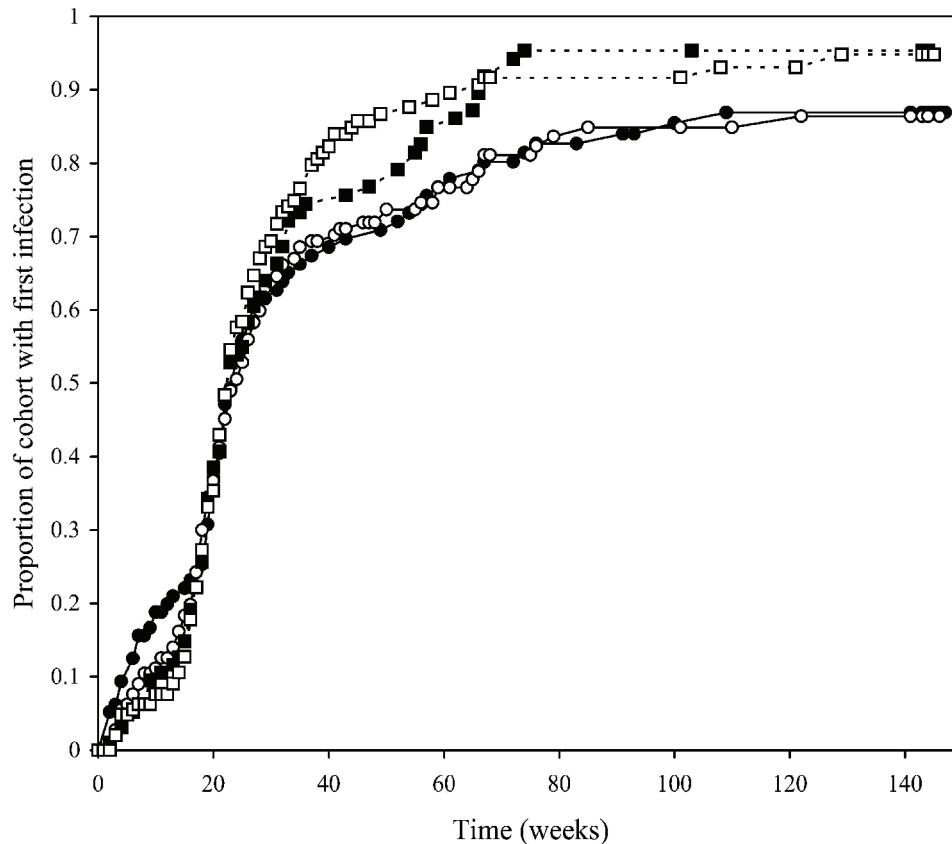
FIG. The weekly cumulative incidences of *Plasmodium falciparum* parasitaemia among adult (○) and child (●) migrants from Java, and the corresponding data for *P. vivax* parasitaemia among the same adults (□) and children (■), beginning on the day the migrants arrived at SP2 in Papua, Indonesia.

The clinical characteristics of the first infections with *P. falciparum* or *P. vivax* were also compared on the basis of prior exposure (in one or more infections) to the other species of parasite. Of all the 'first' infections considered, 55% of those in children and 59% of those in adults represented primary exposures to malaria. The remainder of the 'first' infections had been preceded by at least one infection with the other species and were therefore considered secondary exposures to malaria. The risk of fever or complaint of illness appeared the same for the primary and secondary exposures (Tables 2 and 3). For *P. falciparum* preceded by *P. vivax*, the OR for fever were 0.82 ($P = 0.71$) in children and 0.60 ($P = 0.25$) in adults, with corresponding OR for com-

plaint of illness of 0.66 ($P = 0.54$) and 1.15 ($P = 0.84$), respectively. For *P. vivax* preceded by *P. falciparum*, the OR for fever were 1.05 ($P = 0.92$) in children and 2.09 ($P = 0.06$) in adults whereas those for complaint of illness were 4.92 ($P = 0.07$) in children and 1.47 ($P = 0.56$) in adults.

DISCUSSION

The observations reported here demonstrate the high risk of disease with first exposure to *P. falciparum* or *P. vivax* among Javanese migrants to north–eastern Papua, Indonesia. As their first *P. falciparum* or *P. vivax* para-sitaemias became patent, more than half of the subjects were found febrile and >90%

TABLE 2.   *Effect of prior exposure to* Plasmodium vivax *upon risk of fever or illness with primary exposure to* P. falciparum

| | Prior *Plasmodium vivax* (no. of subjects)? | | |
	Yes	No	Odds ratio and (95% CI)
Febrile	41	58	1.96 (0.87–4.44)
Afebrile	13	36	
Complaint of illness	66	95	1.04 (0.37–2.98)
No complaint of illness	8	12	

CI, Confidence interval.

TABLE 3.   *Effect of prior exposure to* Plasmodium falciparum *upon risk of fever or illness with primary exposure to* P. vivax

| | Prior *Plasmodium falciparum* (no. of subjects)? | | |
	Yes	No	Odds ratio and (95% CI)
Febrile	39	57	0.65 (0.35–1.22)
Afebrile	45	43	
Complaint of illness	77	111	0.40 (0.14–1.17)
No complaint of illness	12	7	

CI, Confidence interval.

complained of being ill. The cyclical nature of fever caused by malarial parasites may explain why the prevalence of fever (detected at a single point in each infection) was lower than the frequency of reported illness. The possibility that the 12 weeks of chloroquine prophylaxis given to new migrants caused significant confounding of the risks of fever or complaint of illness was discounted, because 336 of the 394 first infections investigated occurred after this prophylaxis had ended. The slight lags in the development of the first detected *P. falciparum* and first detected *P. vivax* parasitaemias (see Figure) may, however, be attributed to the suppression of the parasitaemias by the chloroquine. The overall incidences recorded in SP2, of 1.1 *P. falciparum* infections and 1.5 *P. vivax* infections/person-year, are appreciably lower than the rates — of 2.5–3.3 *P. falciparum* infections and 2.3–2.5 *P. vivax* infections/person-year — measured in other studies in the Arso area of north–eastern Papua (Jones *et al.*, 1994; Ohrt *et al.*, 1997; Taylor *et al.*, 1999). They are, however, essentially the same as the rates measured in the nearby settlement villages of SP4, SP5 and SP6 during 1999 and 2000 (Baird *et al.*, 2001).

Risk of infection may be confounded by differential risk of exposure to biting anophelines. The degree of this confounding largely depends upon behaviours that put mosquitoes in contact with humans. People who are particularly likely to enter forests, such as loggers, are more likely to be bitten by species of anopheline mosquito that 'prefer' forest habitats. Children who tend to remain in the village setting are at relatively low risk of being bitten by these mosquito species. In earlier cross-sectional studies of Javanese migrants to Indonesian Papua, the risk of parasitaemia and disease appeared to fall for adults, but not children, after several years (Baird *et al.*, 1991, 1993). In these studies, the possibility that the adults were simply at higher risk of exposure than the children, because adult-specific behaviours, such as logging, increased the risk of being bitten by a potential vector, was discounted. The local vectors — mosquitoes of the *An. punctulatus* complex — avoid forest and prefer the open,

sun-lit areas to be found in transmigration villages. It was therefore assumed that transmigrant infection with *P. falciparum* or *P. vivax* occurred predominantly in the village setting, where children and adults were at equal risk. Past (Baird *et al.*, 1991, 1993) and present observations (Table 1), particularly the similar prevalences of parasitaemia and incidences of infection in adult and child transmigrants during the first few months of their exposure, support this view.

In the present study, children and adults appeared uniformly susceptible to disease with primary *P. falciparum* or *P. vivax* infection, the associated risks of fever or complaints of illness being essentially identical for the two age-groups. This observation appears to conflict with that made in an earlier retrospective analysis of the risks of severe disease among migrants at Arso PIR IV (Baird *et al.*, 1998), but essential differences in methods almost certainly account for this discrepancy. In the earlier study in Arso PIR IV, adults appeared much more likely to experience severe disease, during the first few months of exposure to infection, than children. This observation, however, was made in the absence of readily available diagnosis and treatment. In SP2, subjects were followed closely and treated at the first sign of disease with parasitaemia. In Arso PIR IV, no active follow-up took place and residents lacked immediate access to diagnosis or to medical care provided by a physician. The availability of these services in SP2 virtually eliminated the risk of severe disease (Krisin *et al.*, 2003). Children and adults appear to be equally susceptible to mild disease caused by primary exposure to *P. falciparum*, even though, in other studies, adults appeared initially much more likely than children to experience severe and complicated disease.

The concept of immunological cross-protection between species of *Plasmodium* in humans remains controversial (McKenzie and Bossert, 1999; Bruce *et al.*, 2000; Taylor-Robinson, 2000). Exposure to the antigens of one species may prime a protective immune response to infection by another species. In the present SP2 cohort, however, the risk of fever or complaint of illness when first infected with *P. falciparum* was similar whether there had been a prior infection with *P. vivax* or not, and *vice versa*. This was true among both children and adults. The SP2 cohort, however, would not allow the detection of functional cross-protection from severe disease.

In summary, fever and complaints of illness occurred among most Javanese migrants to Papua experiencing first exposure to *P. falciparum* or *P. vivax*. For each *Plasmodium* species, neither the host's age nor prior exposure to the other species confounded the risk of fever or illness. The finding of equal incidences of infection in children and adults supports the assumption, of similar exposure to infection across all age-groups, made in earlier studies on the patterns of parasitaemia and disease in migrants to Indonesian Papua.

REFERENCES

Arbani, P. R. (1992). Malaria control program in Indonesia. *Advanced Knowledge on Malaria in Southeast Asia*, **23** (Suppl. 4), 29–38.

Atmosoedjono, S. (1990). Malaria control in Indonesia since World War II. *Wageningen Agricultural University Papers*, **90**, 141–154.

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, Purnomo, Bangs, M. J., Subianto, B., Patchen, L. C. & Hoffman, S. L. (1991). Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, **44,** 547–552.

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., Richie, T. L., Marwoto, H. & Gunawan, S. (1995). Epidemic malaria among transmigrants in Irian Jaya. *Buletin Penelitian Kesehatan*, **23,** 18–34.

Baird, J. K., Sismadi, P., Masbar, S., Ramzan, A., Purnomo, Sekartuti, Tjitra, E., Rumoko, B. W. & Arbani, P. R. (1996). A focus of endemic malaria in central Java. *American Journal of Tropical Medicine and Hygiene*, **54,** 98–104.

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., Kusmaningsih, 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., Basri, H., Weina, P., Maguire, J. D., Barcus, M. J., Picarima, H., Elyazar, I. R. F., Ayomi, E. & Sekartuti (2003). Adult Javanese migrants to Indonesian Papua at high risk of severe disease caused by malaria. *Epidemiology and Infection*, in press.

Bastien, P. (1987). Particularités épidémiologiques des accès pernicieux à *Plasmodium falciparum* dan un contexe d'épidémie palustre. Vanuatu, 1975–1985. *Médecine Tropicale*, **47,** 125–131.

Bruce, M. C., Donnelly, C. A., Alpers, M. P., Galinski, M. R., Barnwell, J. W., Walliker, D. & Day, K. P. (2000). Cross-species interactions between malaria parasites in humans. *Science*, **287,** 845–848.

Buck, R. A. & Eichenlaub, D. (1994). Prognostiche faktoren der malaria tropica — ergebnisse einer evaluationsstudie in der Bundesrepublik Deutschland 1963–1988. *Gesundheitswesen*, **56,** 29–32.

Church, L. W., Le, T. P., Bryan, J. P., Gordon, D. M., Edelman, R., Fries, L., Davis, J. R., Herrington, D. A., Clyde, D. F., Shmuklarsky, M. J., Schneider, I., McGovern, T. W., Chulay, J. D., Ballou, W. R. & Hoffman, S. L. (1997). Clinical manifestations of *Plasmodium falciparum* malaria experimentally induced by mosquito challenge. *Journal of Infectious Diseases*, **175,** 915–920.

Collins, W. E. & Jeffery, G. M. (1999). A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. *American Journal of Tropical Medicine and Hygiene*, **61** (Suppl. 1), 4–19.

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., Sandjaja, B., Wignall, F. S. & Hoffman, S. L. (1995). Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria. *Lancet*, **346,** 1190–1193.

Gill, C. A. (1936). Some points in the epidemiology of malaria arising out of the study of the malaria epidemic in Ceylon in 1934–35. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **29,** 429–480.

Greenberg, A. E. & Lobel, H. O. (1990). Mortality from *Plasmodium falciparum* malaria in travelers from the United States, 1959 to 1987. *Annals of Internal Medicine*, **113,** 326–327.

Herrington, D., Davis, J., Nardin, E., Beier, M., Cortese, J., Eddy, H., Lonsonsky, G., Hollingdale, M., Sztein, M., Levine, M., Nussenzweig, R. S., Clyde, D. & Edelman, R. (1991). Successful immunization of humans with malaria sporozoites: humoral and cellular responses of the protected individuals. *American Journal of Tropical Medicine and Hygiene*, **45,** 539–547.

Hoffman, S. L., Goh, L. M., Luke, T. C., Schneider, I., Le, T. P., Doolan, D. L., Sacci, J., de la Vega, P., Dowler, M., Paul, C., Gordon, D. M., Stoute, J. A., Church, L. W., Sedegah, M., Heppner, D. G., Ballou, W. R. & Richie, T. L. (2002). Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. *Journal of Infectious Diseases*, **185,** 1155–1164.

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.

Krisin, Basri, H., Fryauff, D. J., Barcus, M. J., Bangs, M. J., Ayomi, E., Marwoto, H., Elyazar, I. R. F., Richie, T. L. & Baird, J. K. (2003). Malaria in a cohort of Javanese migrants to Indonesian Papua. *Annals of Tropical Medicine and Parasitology*, **97,** 543–556.

McKenzie, F. E. & Bossert, W. H. (1999). Multispecies *Plasmodium* infections of humans. *Journal of Parasitology*, **85,** 12–18.

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.

Some, E. S. (1994). Effects and control of highland malaria epidemic in Uasin Gishu district, Kenya. *East African Medical Journal*, **71,** 2–8.

Taylor, W. R., Richie, T. L., Fryauff, D. J., Picarima, H., Ohrt, C., Tang, D., Braitman, D., Murphy, G. S., Widjaja, H., Tjitra, E., Ganjar, 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.

Taylor-Robinson, A. W. (2000). Species-transcending regulation of malaria parasitaemia. *Parasitology Today*, **16,** 460–461.

Von Juaregg, W. (1922). The treatment of general paresis by inoculation of malaria. *Journal of Nervous and Mental Disease*, **55,** 369–375.