1998

CHLOROQUINE-RESISTANT *PLASMODIUM VIVAX* IN TRANSMIGRATION SETTLEMENTS OF WEST KALIMANTAN, INDONESIA

David J. Fryauff
U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia

Sekar Tuti
National Institute for Health Research, Jakarta, Indonesia

Agus Mardi
Provincial Department of Health, Pontianak, West Kalimantan, Indonesia

Sofyan Masbar
U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia

Ricky Patipelohi
District Health Office, Ketapang, West Kalimantan, Indonesia

See next page for additional authors

Authors
David J. Fryauff, Sekar Tuti, Agus Mardi, Sofyan Masbar, Ricky Patipelohi, Budi Leksana, Kevin C. Kain, Michael J. Bangs, Thomas L. Richie, and J. Kevin Baird

Am. J. Trop. Med. Hyg., 59(4), 1998, pp. 513–518

CHLOROQUINE-RESISTANT *PLASMODIUM VIVAX* IN TRANSMIGRATION SETTLEMENTS OF WEST KALIMANTAN, INDONESIA

DAVID J. FRYAUFF, SEKAR TUTI, AGUS MARDI, SOFYAN MASBAR, RICKY PATIPELOHI, BUDI LEKSANA, KEVIN C. KAIN, MICHAEL J. BANGS, THOMAS L. RICHIE, AND J. KEVIN BAIRD

U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; National Institute for Health Research, Jakarta, Indonesia; Provincial Department of Health, Pontianak, West Kalimantan, Indonesia; District Health Office, Ketapang, West Kalimantan, Indonesia; Department of Medicine, University of Toronto, Toronto, Ontario, Canada; Naval Medical Research Institute, Rockville, Maryland

Abstract. Malariometric surveys were conducted during July 1996 in native Dayak villages and predominantly Javanese transmigration settlements in Ketapang district of West Kalimantan, Indonesia. Malaria prevalence ranged from 0.9% to 2.7% in Dayak villages and from 1% to 20% in the transmigration settlements. *Plasmodium falciparum* accounted for 67% of the cases among Dayaks but *P. vivax* was dominant among transmigrants, accounting for more than 72% of the infections. Chloroquine sensitivity/resistance was assessed by 28-day *in vivo* testing of uncomplicated malaria infections and measurement of chloroquine blood levels in cases where parasitemias reappeared within the 28-day test period. Resistance was based on the appearance of asexual parasites against chloroquine plus desethylchloroquine levels exceeding the minimally effective whole blood concentrations proposed for sensitive parasite strains (*P. vivax*, 100 ng/ml; *P. falciparum*, 200 ng/ml). All parasitemias cleared initially within four days of beginning supervised chloroquine therapy (25 mg base/kg over a 48-hr period), but asexual parasites reappeared within 28 days in 27 of 52 *P. vivax* and three of 12 *P. falciparum* cases. Chloroquine blood levels at the time of recurrent parasitemias revealed resistance in 12 of the 27 *P. vivax* cases and in one of the three *P. falciparum* cases. Genotypes of nine of the 12 recurrent *P. vivax* isolates matched with their primary isolates and ruled out reinfection. These findings establish the presence of chloroquine-resistant *P. vivax* on the island of Borneo. The pattern of malaria and the high frequency of chloroquine resistance by *P. vivax* at the West Kalimantan location may relate to demographic, ecologic, agricultural, and socioeconomic changes associated with transmigration.

Chloroquine has long been the world's standard effective drug for prevention and treatment of vivax malaria, but resistance appears to be an emerging problem in the Indo-Pacific region that may have already spread globally. The first report of clinical resistance came from Papua New Guinea in 1989[1] followed by successive reports of chloroquine-resistant vivax malaria from Irian Jaya,[2–5] Myanmar,[6,7] India,[8] Sumatra,[9] and Sulawesi.[10] In the New World, chloroquine-resistant *Plasmodium vivax* has been described in Guyana.[11]

Key to making a definitive pronouncement of *in vivo* resistance to therapeutic levels of chloroquine is strict supervision of the appropriate dose, careful follow-up to monitor parasitemia, and demonstration of chloroquine blood levels at the time of an unremitting or recurrent parasitemia in excess of the minimally effective concentrations (MECs) established for sensitive strains of the malaria parasites ($>$ 100 ng/ml for *P. vivax*; $>$ 200 ng/ml for *P. falciparum*). Genetic fingerprinting can also determine whether recurrent infections arise from recrudescence/relapse of the primary infection or from a separate inoculation. These stringent criteria and the methodologies they entail are not easily met and definitive reports of chloroquine-resistant *P. vivax* have been slowly forthcoming. A systematic effort has been underway to detect and measure the occurrence of this problem in Indonesia. This report describes studies conducted among native Pesaguan Dayaks and non-indigenous transmigrants in West Kalimantan (Borneo), Indonesia using an *in vivo* test format developed at this laboratory.[12] Results are presented of *in vivo* tests for chloroquine sensitivity/resistance, drug levels, and selective parasite genotyping from among the infected subjects that were treated.

SUBJECTS AND METHODS

Study site. Kalimantan is the Indonesian name given to the island widely known as Borneo. This study was focused in West Kalimantan province, Indonesia at Ketapang (2°S, 110°E), the largest and southernmost district of the province, with an area of 35,809 km^2 and an estimated population of 361,000. This district does not have abundant realized mineral and timber resources, but its climate and vast lowlands are suited to rubber and oil palm cultivation and large plantations; some in excess of one million hectares have been established. The development and production of these plantation industries have required more individuals than have been locally available and the Indonesian Ministry of Transmigration has settled thousands of landless families from crowded parts of Indonesia into Ketapang for this purpose. Government figures show that during fiscal year 1995 more transmigrants were settled into West Kalimantan than into any other province of Indonesia (33,012 of 112,069), and that the majority of these West Kalimantan transmigrants (16,081 of 33,012) were settled into Ketapang District (Indonesian Ministry of Transmigration and Resettlement, 1996, unpublished data). Most of these transmigrants (86.5%) were young, subsistence-level farmers from the relatively malaria-free provinces of Java. Families from the more malarious eastern provinces of West Nusa Tenggara and East Nusa Tenggara comprised the remaining 13.5%. Approximately half of the houses in Ketapang transmigration settlements are allocated to translocal Dayak families, but many of these indigenous people also maintain residence in their traditional villages.

Malaria is second only to respiratory infection as the most

frequently diagnosed acute illness in West Kalimantan province, accounting for 27% of all cases of infectious illness seen in health clinics during 1995. Annual incidence of clinical malaria ranged from three (Pontianak) to 44 (Sintang) cases/1,000 residents in the seven administrative districts of the province and averaged 15 cases/1,000 in Ketapang. Malariometric survey by active case detection during 1995 revealed that malaria point prevalence ranged from 0% (Pontianak) to 16% (Kapuas Hulu) and was 12.4% in Ketapang (West Kalimantan Department of Health, 1996, unpublished data). Entomologic surveys have identified 13 different anopheline species in West Kalimantan (*Anopheles letifer, An. lesteri, An. umbrosus, An. leucosphyrus, An. tesselatus, An. baezai, An. nigerrimus, An. sinensis, An. barbirostris, An. balabacensis, An. kochi, An. sundaicus,* and *An. peditaeniatus*) of which *An. letifer, An. sundaicus, An. balabacensis, An. barbirostris, An. nigerrimus,* and *An. leucosphyrus*[13–16] have been incriminated or reported as vectors of malaria on the island of Borneo. Human bait collections have reportedly captured *An. nigerrimus* and *An. sinensis* from within transmigration settlements (Ketapang Department of Health, unpublished data). Recent larval collections from rice paddies, streams, and weed-choked ditches at these sites have yielded only *An. nigerrimus* and *An. peditaeniatus* (Fryauff DJ, unpublished data).

Study subjects. All residents of four Dayak villages (Batu Tajam I, Batu Tajam II, Marau Sinar Bulan, Sei Melayu) and five transmigrant settlements (SP-4, -5, -6, -7, and Lalang Panjang) were invited to undergo parasitologic screening for malaria by submitting a drop of blood for thick and thin film preparation. The average residence time in West Kalimantan for inhabitants of the five transmigration settlements was three months, six months, three years, four months, and five years, respectively. Individuals from these screenings who were $\geq$ 5 years old, had uncomplicated malaria, were able to swallow chloroquine tablets, and who had used no antimalarial drug in the previous week were invited to volunteer for the 28-day *in vivo* test. Written informed consent was obtained from the subject/parent and a description of all complaints associated with the infection was verbally elicited.

This work was conducted in accordance with U.S. Navy and Republic of Indonesia regulations governing the protection of human subjects in medical research. American and Indonesian committees for the protection of human subjects reviewed and approved the procedures followed in this research.

Chemotherapy and follow-up. The first-line treatment for all clinical malaria cases in Indonesia is standard chloroquine therapy combined with a 15 mg dose of primaquine on the first day. Chloroquine therapy is combined with a five-day regimen of primaquine for confirmed *P. vivax* infections. If there is no clinical improvement within the first 2–3 days, chloroquine therapy is repeated with a 14-day regimen of primaquine.[17] Primaquine was omitted from the malaria treatments described here to yield valid *in vivo* assessments of *P. vivax* sensitivity/resistance to chloroquine alone. Combined chloroquine and primaquine have been shown to work additively or synergistically against chloroquine-resistant strains of malaria parasites.[18,19]

Enrolled study subjects were administered 25 mg of chloroquine (Resochin®, P. T. Bayer, Jakarta, Indonesia) base/kg of body weight as 10 + 10 + 5 mg/kg doses at 24-hr intervals. Consumption of each dose was witnessed by a member of the study team and physical complaints were recorded at the time of each visit. Study subjects were advised to take no other drugs but those provided to them by the study team and to immediately report any incident of drug reaction or heightened/recurrent malaria symptoms to an on-site team member. Thick and thin blood films were made on days 0, 2, 4, 7, 11, 14, 18, 21, and 28, or on any occasion of malaria-like illness. Giemsa-stained blood films were examined by light microscopy (oil-immersion 1,000× magnification) and considered negative if no asexual parasites were detected in 300 ocular fields of the thick film. Parasite counts/200 white blood cells were multiplied by a factor of 40 to estimate the parasite count/μl of blood. Standard sulfadoxine plus pyrimethamine or quinine therapy was provided for all unremitting or recurrent symptomatic infections.

Chloroquine levels in whole blood. Whole blood aliquots of 100 μl were collected by a heparinized capillary from fingersticks on days 0, 2, and 28, or on the day of a recurrent asexual stage parasitemia. This blood was blotted onto a disk of Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, United Kingdom) and air-dried, with care taken to prevent contamination with chloroquine residues in the air or on fingers. In our Jakarta laboratory, the parent compound chloroquine and its major active metabolite desethylchloroquine were extracted with polar solvent according to published methods.[20] Quantitative analysis of extracts was performed by means of high-performance liquid chromatography (HPLC) using a Bio-Rad model 2700 solvent delivery system, a Bio-Sil Polyol 90-10 column (Bio-Rad Laboratories, Inc., Melville, NY), and a model 1311 flouroMonitor™ III analyzer (LDC Analytical, Riviera Beach, FL) interfaced with an IBM (Yorktown Heights, NY) compatible desktop computer via Microsoft (Redmond, WA) Windows 3.0 and version 2.3 HPLC software (Bio-Rad) software. Clean mobile phase recycling was integrated into the system via an IRS 1000 integrated recovery system for liquid chromatography (Jones Chromatography USA, Inc., Lakewood, CO), and an internal standard of isopropyl chloroquine was used for calibration. In accordance with current survey methods, resistance to chloroquine therapy was based upon the appearance of a post-therapeutic asexual stage parasitemia against whole blood levels of chloroquine/desethylchloroquine $>$ 100 ng/ml for *P. vivax* or $>$ 200 ng/ml for *P. falciparum.*[12]

Genetic fingerprinting of primary and failure isolates of *P. vivax.* The DNA was extracted from whole blood samples of paired primary and failure infections. Portions of the genes coding for the merozoite surface protein-1 and the circumsporozoite protein were amplified by polymerase chain reaction (PCR) using oligonucleotide primers synthesized to conserved sequences flanking the variable regions. The paired primary and failure PCR products were analyzed by single-stranded conformational polymorphism (SSCP) according to published methodology.[11] Isolates from treatment failures were considered to be clones of the primary infection if the SSCP pattern of the failure isolate was the same as or contained within the SSCP pattern of the primary isolate.

TABLE 1

Malaria point prevalence by species and age group among transmigrants and native Dayaks in Ketapang District, West Kalimantan, Indonesia, July–August 1996*

Age group (years)	Transmigrants			Native Dayaks		
	No. tested	No. *P.f.*	No. *P.v.*	No. tested	No. *P.f.*	No. *P.v.*
<2	124	0	0	35	1	0
2–5	270	3 (1g)†	6 (1g)	114	3 (2g)	1
6–10	212	5 (3g)	13 (1g)	109	2 (2g)	1
11–15	85	1	9 (4g)	67	1	2
16–25	314	6 (4g)	8 (3g)	113	0	0
25–40	405	5 (3g)	13 (5g)	186	2 (1g)	0
>40	40	0	3 (3g)	134	1	2‡ (1g)
Total	1,450	20 (1.4%)	52 (3.6%)	758	10 (1.3%)	6 (0.8%)

* *P.f. = Plasmodium falciparum; P.v. = P. vivax.*
† (g) denotes number of cases with gametocytes.
‡ One mixed *P. falciparum* + *P. vivax* infection.

RESULTS

Malaria prevalence. A total of 758 ethnic Dayaks and 1,450 predominantly Javanese transmigrants were screened for malaria infection. Screening prevalence ranged from 0.9% to 2.7% in the four Dayak villages (Sei Melayu, 0.9%; Batu Tajam II, 1.2%; Marau Sinar Bulan, 2.0%; and Batu Tajam I, 2.7%) and falciparum malaria accounted for 10 of the 15 cases identified in this population (67%). Malaria prevalence ranged from 1.0% to 20.0% in the five transmigrant settlements (SP-7, 1.0%; SP-5, 1.2%; SP-4, 1.7%; SP-6, 5.8%; and Lalang Panjang, 20.0%), and vivax malaria accounted for 72.2% of the 72 cases identified. The highest prevalence occurred in the oldest transmigrant settlement where numerous rice paddies and fish ponds had been established. Table 1 shows the proportions of malaria infection by species and age group in the two populations. In both populations, the majority of infections were found in children 2–15 years old (Dayaks, 10 of 15 = 67%; transmigrants, 37 of 72 = 51.4%). The prevalence of malaria in transmigrant children 2–15 years old (37 of 567 = 6.5%) and adults > 15 years old (35 of 759 = 4.6%) was comparable ($P = 0.16$); however, malaria prevalence in Dayak children 2–15 years old (10 of 290 = 3.4%) was significantly greater ($P = 0.03$) than that of Dayak adults >15 years old (4 of 433 = 0.9%). Overall, malaria prevalence in transmigrants (72 of 1,450 = 5.0%) was significantly greater ($P < 0.001$) than in Dayaks (15 of 758 = 2.0%).

***In vivo* sensitivity/resistance.** Table 2 shows the results of 28-day *in vivo* tests for chloroquine sensitivity/resistance conducted with 12 cases of falciparum and 52 cases of vivax malaria from the surveyed populations of Batu Tanjam (n = 2), SP-5 (n = 3), SP-6 (n = 20), SP-7 (n = 1), and Lalang Panjang (n = 39). Three subjects, one with *P. falciparum*, and two with *P. vivax*, were disenrolled after the first day of therapy because of presumed allergic reaction to chloroquine (urticaria) and were provided alternative treatment with sulfadoxine/pyrimethamine. All 12 *P. falciparum* parasitemias decreased more than 75% by the last day of therapy (day 2) and asexual stages were absent by day 4. Gametocytes appeared unaffected and consistently present through follow-up in five cases. Trophozoites reappeared in two of the 12 cases on days 11 and 18. Whole blood levels of chloroquine plus desethylchloroquine on these days were 186 ng/ml and 205 ng/ml, respectively. One asymptomatic parasitemia appeared on day 28 with an undetectable low blood level of chloroquine.

All 52 parasitemias by *P. vivax* decreased by more than 75% within the first 48 hr of receiving chloroquine. Parasite

TABLE 2

Summary of 28-day *in vivo* test results for chloroquine sensitive/resistant malaria in transmigration settlements of Ketapang, West Kalimantan, Indonesia, July–August 1996 (95% confidence intervals are given in parentheses)

Characteristic	*Plasmodium falciparum*	*Plasmodium vivax*
Number tested	12	52
Age (years)	25.2 (18.3–32.2)	24.7 (21.1–28.3)
Hemoglobin (mg/dL of blood)	10.4 (9.2–11.5)	9.6 (9.3–10.0)
GM* parasitemia (/μl of blood)	440 (139–1,390)	803 (525–1,228)
CI† of therapeutic failure		
Day 7	0	1.9% (+1)
Day 14	8.3% (+1)	5.8% (+2)
Day 21	16.5% (+1)	34.6% (+15)
Day 28	25% (+1)	51.9% (+9)
Drug‡ levels (ng/ml of blood)		
Day 0	24§	17¶
Day 2	911 (687–1,133)	940 (807–1,073)
Day of recurrence	205	203 (156–251)

* Geometric mean parasitemia.
† Cumulative incidence of recurrent parasitemias.
‡ Mean ng/ml of chloroquine (CQ) + desethylchloroquine (DCQ).
§ CQ only detected in 5 of 12 subjects at enrollment.
¶ CQ only detected in 14 of 52 subjects; CQ + DCQ detected in 3 of 52 subjects at enrollment.

clearance was achieved by day 2 in 72% of the cases and by day 4 in 98%. Trophozoites in steadily reducing numbers remained through day 4 in one case but cleared by day 7 and remained absent through the remaining three weeks of follow-up. Trophozoites reappeared between seven and 28 days post-therapy in 52% (27) of the *P. vivax* cases (Table 1). Gametocytes, present at the start of treatment in 31.4% of the *P. vivax* cases, disappeared with treatment and did not reappear within the 28 days of follow-up in any subject. Whole blood levels of chloroquine measured at the time of each recurrence (reinfection/recrudescence/relapse) ranged from 0 to 411 ng/ml. Fifteen parasitemias appeared between test days 18 and 28 against whole blood levels of chloroquine plus desethychloroquine less than 100 ng/ml. Drug levels in 12 cases that recurred from seven to 21 days post-treatment (mean = 17 days) were above this MEC. Genotypic fingerprints of primary and failure isolates, which matched in eight of these cases, ruled out reinfection and indicated true treatment failure. The other four pairs were mismatches as a result of either insufficient DNA from the drug-resistant clone in the primary infection or reinfection. Whole blood chloroquine plus desethychloroquine levels in vivax malaria subjects measured on day 2, after the first two doses of chloroquine, ranged from 187 to 2,095 ng/ml (mean = 940 ng/ml), with a 95% confidence interval around this mean of 807–1,072 ng/ml. There was no significant correlation between low day 2 drug levels and early recurrence of parasitemia ($r = 0.16$, $P = 0.22$). Low drug absorption, indicated by day 2 levels of chloroquine plus desethychloroquine less than 500 ng/ml, was seen in seven subjects and parasitemias appeared within the 28-day follow-up period (days 14, 18, 18, 18, and 28) in five of these cases.

DISCUSSION

These survey results revealed distinct demographic patterns of malaria occurring in Ketapang District of West Kalimantan, Indonesia, and an unexpectedly high frequency of chloroquine resistance in *P. vivax.* The overall higher prevalence rate of malaria among transmigrants was not surprising. Most of these people came from the crowded provinces of Java where the incidence of malaria has been estimated to be less than two cases per 10,000 person-years since 1965,[21] and were thus highly vulnerable to infection and clinical symptoms. Indonesia's transmigration settlements are sited by design in the underpopulated and underdeveloped frontier provinces of the nation, and plans for each location allocate up to half of the new houses for members of the local ethnic population. This cross-cultural mixing is intended to introduce transmigrants to appropriate cultural practices and agricultural techniques, while benefiting the indigenous people with jobs, schools, places of worship, and a variety of government services that were previously beyond their reach. This blending inadvertently brings gametocyte carriers into proximity with the malaria naive newcomers and may be partly responsible for explosive outbreaks that occur each year.[22] However, this scenario was not evident in Ketapang. Although half of the houses in each Ketapang transmigration settlement were allocated to local Dayak families, many of these houses were vacant and Dayaks, if present, did not participate in our open malaria screenings. Moreover, survey within Dayak communities showed low malaria prevalence. It seems possible that the nuclei of infection in Ketapang transmigration settlements may have arisen from among the transmigrants themselves; possibly from among the hundreds of families that immigrated from the more malarious provinces of eastern Indonesia. Recent screening of 260 apparently healthy schoolchildren from a populous district in East Nusa Tenggara (Sikka, Flores Island) showed the malaria prevalence to be 40% (Fryauff DJ, unpublished data). While chloroquine resistance characterizes more than 70% of *P. vivax* cases among transmigration settlements in Irian Jaya,[18,22–24] it is not yet known whether there are also significant pools of chloroquine-resistant *P. vivax* circulating in the nearby provinces of East Nusa Tenggara and West Nusa Tenggara.

Environmental and cultural factors are believed to have also strongly determined the different patterns of malaria transmission at our survey sites. Dayak communities were long-established, traditional villages situated within or at the fringe of secondary forest. Their houses were more densely clustered than those of the transmigrant settlement, domestic animals were abundant, and their villages were well-shaded by diverse species of mature trees. Dayak villages also appeared to be well drained, due to their placement along swiftly flowing streams and rivers. A highly conspicuous, and perhaps critical difference between transmigrant and native communities was the Dayak's tendency to distance their rice paddies from their homes and to grow rice by shifting cultivation in naturally occurring swamp areas. In contrast, the radically altered environment of the young palm plantation and its new settlements eliminated the original diverse populations of flora and fauna and opened the land to almost unchecked colonization by a relative few mosquito species. In the absence of either wild or domestic animals, humans are virtually the only source of blood feeding by mosquitoes in the new settlements. *Anopheles nigerrimus,* which was collected as larvae from rice paddies and irrigation ditches within the Lalang Panjang settlement, has been responsible for localized malaria outbreaks when it proliferates in close proximity to large human populations and when domestic animal blood sources are scarce.[13] The vectorial status of *An. peditaeniatus,* found in the same breeding habitats as *An. nigerrimus,* is unknown, but both of these species are considered predominantly zoophilic and breed preferentially in rice paddies and open freshwater habitats exposed to the sun.[14]

Bed nets, chloroquine prophylaxis for the first 90 days, and insecticide (bendiocarb) spraying of the interior walls of each house are important government efforts that are routinely made to protect the new transmigrants.[18,25,26] While these measures were insufficient to prevent the early and explosive malaria outbreaks seen in transmigration settlements of Irian Jaya,[22] they may be effective during the first year in Ketapang where different transmission dynamics prevail and are only overcome in subsequent years when extensive rice cultivation and irrigation systems increase and stabilize the anopheline vector populations. This phenomenon of delayed malaria outbreak has also been observed and reported for the Satai transmigration settlement of West Kalimantan.[26] In the more established transmigration settlements (SP-4, 3 years; Lalang Panjang, 5 years), night time electri-

cal supply facilitates social gatherings during the peak hours of vector activity. The growing popularity of open air, night time television viewing in these communities serves to inform and educate the people but also plays an unfortunate role in heightening malaria transmission. It is repeatedly observed that privately owned televisions, when offered for community viewing each night, become potent lures that draw people, mainly children, out into the night and increase their risks of acquiring malaria.

In vitro test results have identified chloroquine-resistant falciparum malaria in each of Indonesia's 27 provinces,[27] but this drug remains the first line of treatment for clinical malaria at each of the four levels of the national health service. Full appropriate chloroquine treatment dosages can be provided at each level when the drug is in stock, but outside the clinic strict compliance with the treatment regimen cannot be ensured, and it is probable that many people who feel improvement after the first 600-mg dose do not take the second and third, choosing to save the pills for another occasion. In Ketapang transmigration settlements, the local kiosks are a reliable source of chloroquine, which is sold in packaged units of four pills, each pill containing 150 mg base, for US $0.44/package. An adult transmigrant must purchase three packages at an equivalent cost of US $1.32 to obtain sufficient pills for a full standard therapy of 1,500 mg base; a cost equal to half of his or her daily wage in the oil palm plantation. These socioeconomic details suggest that self-treatment with locally purchased chloroquine might typically involve the use of only one packet of pills, or less than half of the standard treatment dose. If the drug were packaged in units of 10 pills, corresponding to the full adult treatment regimen, packaging costs could be reduced and savings potentially realized at the user level. Self-treatment would then at least start with purchase of the correct or adequate dosage of chloroquine. Compliance may be better when one has no choice but to purchase the full regimen.

In the context of our study, compliance was absolute, and unambiguous chloroquine resistance accounted for 12 of the 27 *P. vivax* parasitemias that appeared within the 28-day test period. Abnormal drug absorption from the start of therapy is believed to have accounted for an additional five recurrences. The remaining 10 parasitemias appeared opportunistically between test days 18 and 28 against low or undetectable chloroquine levels. There is marked individual variation in the absorption and metabolism of chloroquine.[28] Studies in Indonesia have found that the majority of people given a standard therapeutic dose achieve and then maintain whole blood levels of chloroquine plus desethychloroquine greater than the MEC of 100 ng/ml through the 28-day test period.[23,24,29] This was not apparent in our study, in which the mean day 28 level of chloroquine plus desethychloroquine (26.8 ng/ml) following standard treatment was significantly lower than that of five other ethnic populations in the region (Fryauff DJ, unpublished data). It seems possible that variant patterns of chloroquine absorption, metabolism, and elimination may characterize different racial/ethnic groups. Similarly, unique dietary or cultural practices, as well as concurrent enteric infections that reduce intestinal absorption may alter the usual kinetics of this drug in some populations and open an earlier window of opportunity for reinfection, relapse, and/or recrudescence.

In summary, the results indicate that important differences characterize malaria in native Dayak and transmigrant communities of Ketapang District in West Kalimantan. Demographic, ecologic, agricultural, and socioeconomic factors may be responsible for the patterns observed. Insufficient cases of *P. falciparum* were found to enable a useful or conclusive analysis of chloroquine sensitivity or resistance for this location. However, *P. vivax* cases were abundant within transmigration settlements and our unexpected *in vivo* test results confirmed chloroquine resistance in 23% of the cases treated. Within Indonesia, this *in vivo* testing methodology has only identified sites in Irian Jaya with higher frequencies of chloroquine resistance by *P. vivax.*[23,24] It is an interesting coincidence that in both Irian Jaya and Kalimantan, where high frequencies of *P. vivax* resistance to chloroquine have been documented, the *in vivo* test subjects at each site have been predominantly Javanese transmigrants. These results encourage additional surveys for chloroquine-resistant vivax malaria among transmigrant communities in other provinces of Indonesia.

Acknowledgments: We gratefully acknowledge critical review of the manuscript provided by Dr. Aftab Ansari. Special thanks are extended to Ali Thalib, Agus Mudhafar, and Fransiskus Sugio (Tumbangtiti, West Kalimantan) for valuable field assistance, and to Dr. Emiliana Tjitra (National Institute for Health Research, Jakarta) for providing malaria survey records for Indonesian provinces.

Financial support: This work was supported by the U.S. Naval Medical Research and Development Command work unit number 623002A810.00101.HFX.1433.

Disclaimer: The views of the authors expressed herein do not purport to reflect those of the U.S. Navy, The U.S. Department of Defense, or the Indonesian Ministry of Health.

Authors' addresses: David J. Fryauff, Sofyan Masbar, Budi Leksana, Michael J. Bangs, and Thomas L. Ritchie, U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia. Sekar Tuti, National Institute for Health Research, Jakarta, Indonesia. Agus Mardi, Provincial Department of Health, Pontianak, West Kalimantan, Indonesia. Ricky Patipelohi, District Health Office, Ketapang, West Kalimantan, Indonesia. Kevin C. Kain, Tropical Diseases Unit, EN G-224, Department of Medicine, The Toronto Hospital, 200 Elizabeth Street, University of Toronto, Toronto, Ontario, Canada M5G 2C4. J. Kevin Baird, Malaria Program, Naval Medical Research Institute, 12300 Washington Avenue, Rockville, MD 20852.

REFERENCES

1. Reickmann KH, Davis DR, Hutton DC, 1989. *Plasmodium vivax* resistant to chloroquine? *Lancet ii:* 1183–1184.
2. Baird JK, Basri H, Purnomo, Bangs MJ, Subianto B, Patchen L, Hoffman SL, 1991. Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. *Am J Trop Med Hyg 44:* 547–552.
3. Schwartz IK, Lackritz EM, Patchen LC, 1991. Chloroquine resistant *Plasmodium vivax* from Indonesia (letter). *N Engl J Med 324:* 927.
4. Schuurkamp GJ, Spicer PE, Kereu RK, Bulongol PK, Rieckmann KH, 1992. Chloroquine-resistant *Plasmodium vivax* in Papua New Guinea. *Trans R Soc Trop Med Hyg 86:* 121–122.
5. Murphy GS, Basri H, Purnomo, Andersen EM, Bangs MJ, Mount DL, Gorden J, Lal AA, Purwokusumo AR, Harjosuwarno S, Sorensen K, Hoffman SL, 1993. Vivax malaria resistant to treatment and prophylaxis with chloroquine. *Lancet 341:* 96–100.

6. Myat-Phone-Kyaw, Myint-Oo, Myint-Lwin, Thaw-Zin, Kyin-Hla-Aye, Nwe-Nwe-Yin, 1993. Emergence of chloroquine-resistant *Plasmodium vivax* in Myanmar (Burma). *Trans R Soc Trop Med Hyg 87:* 687.
7. Marlar-Than, Myat-Phone-Kyaw, Aye-Yu-Soe, Khaing-Khaing-Gyi, Ma-Sabai, Myint-Oo, 1995. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. *Trans R Soc Trop Med Hyg 89:* 307–308.
8. Garg M, Gopinathan N, Bodhe P, Kshirsagar NA, 1995. Vivax malaria resistant to chloroquine: case reports from Bombay. *Trans R Soc Trop Med Hyg 89:* 656–657.
9. Baird JK, Sustriayu Nalim MF, Basri H, Masbar S, Leksana B, Tjitra E, Dewi RM, Khairani M, Wignall FS, 1996. Survey of resistance to chloroquine by *Plasmodium vivax* in Indonesia. *Trans R Soc Trop Med Hyg 90:* 409–411.
10. Fryauff DJ, Soekartono, Sekartuti, Leksana B, Suradi, Baird JK, 1998. Survey of *in vivo* resistance to chloroquine by *Plasmodium falciparum* and *P. vivax* in north Sulawesi, Indonesia. *Trans R Soc Trop Med Hyg 92:* 82–83.
11. Craig AA, Kain KC, 1996. Molecular analysis of strains of *Plasmodium vivax* from paired primary and relapse infections. *J Infect Dis 174:* 373–379.
12. Baird JK, Leksana B, Masbar S, Fryauff DJ, Sutamihardja MA, Suradi, Wignall FS, Hoffman SL, 1997. Diagnosis of resistance to chloroquine by *Plasmodium vivax*: timing of recurrence and whole blood chloroquine levels. *Am J Trop Med Hyg 56:* 621–626.
13. Reid JA, 1968. *Anopheline Mosquitoes of Malaya and Borneo.* No. 31. Studies from the Institute for Medical Research, Malaysia. Kuala Lumpur, Malaysia.
14. O'Connor CT, 1980. The *Anopheles hyrcanus* group in Indonesia. *Mosq Syst 12:* 293–301.
15. Kirnowardoyo S, 1985. Status of *Anopheles* malaria vectors in Indonesia. *Southeast Asian J Trop Med Public Health 16:* 129–132.
16. Harbach RE, Baimai V, Sukowati S, 1987. Some observations on sympatric populations of the malaria vectors *Anopheles leucosphyrus* and *Anopheles balabacensis* in a village-forest setting in South Kalimantan. *Southeast Asian J Trop Med Public Health 18:* 241–247.
17. Department of Health, Republic of Indonesia, 1991. *Malaria.* Jakarta: Direktorat Jenderal Pencegehan Pemberantasan Penyakit Menular & Penyehatan Lingkungan Pemukiman. 614.532.
18. Baird JK, Basri H, Subianto B, Fryauff DJ, McElroy PD, Leksana B, Richie TL, Masbar S, Wignall SF, Hoffman SL, 1995. Treatment of chloroquine-resistant *Plasmodium vivax* with chloroquine and primaquine or halofantrine. *J Infect Dis 171:* 1678–1682.
19. Peters W, Robinson BL, Milhous WK, 1993. The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline. *Ann Trop Med Parasitol 87:* 547–552.
20. Patchen LC, Mount DL, Schwartz IK, Churchill FC, 1983. Analysis of filter paper absorbed, fingerstick blood samples for chloroquine and its major metabolite using high performance liquid chromatography with fluorescence detection. *J Chromatogr 278:* 81–89.
21. Baird JK, Sismadi P, Masbar S, Romzan A, Purnomo BW, Sekartuti, Tjitra E, Rumoko BW, Arbani PR, 1995. A focus of hyperendemic malaria in Central Java. *Am J Trop Med Hyg 54:* 98–104.
22. Baird JK, Gunawan S, 1995. Epidemic malaria among transmigrants in Irian Jaya. *Bull Health Studies (Indonesia) 23:* 18–34.
23. Baird JK, Leksana B, Masbar S, Suradi, Sutamihardja MA, Fryauff DJ, Subianto B, 1997. Whole blood chloroquine concentrations with *Plasmodium vivax* in Irain Jaya, Indonesia. *Am J Trop Med Hyg 56:* 618–620.
24. Baird JK, Sumawinata IW, Fryauff DJ, Sutamihardja MA, Leksana B, Widjaja H, Kysdarmanto, Subianto B, 1997. Resistance to chloroquine by *Plasmodium vivax* and *Plasmodium falciparum* at Nabire, Southwestern Irian Jaaya, Indonesia. *Am J Trop Med Hyg 56:* 627–631.
25. Church CJ, Atmosoedjono S, Bangs MJ, 1995. A review of anopheline mosquitoes and malaria control strategies in Irian Jaya, Indonesia. *Bull Health Studies (Indonesia) 23:* 3–17.
26. Abisudjak B, Kotanegara R, 1989. Transmigration and vector-borne diseases in Indonesia. Service MW, ed. *Demography and Vector-borne Diseases.* Boca Raton, FL: CRC Press Inc., 207–224.
27. WHO, 1990. *The Clinical Management of Acute Malaria.* New Delhi: WHO Regional Publications, South-East Asia Series No. 9, Third Edition.
28. Rombo L, Bjorkman A, Sego E, Ericsson O, 1986. Whole blood concentrations of chloroquine and desethylchloroquine during and after treatment of adult patients infected with *Plasmodium vivax, P. ovale* or *P. malariae. Trans R Soc Trop Med Hyg 80:* 763–766.
29. Fryauff DJ, Baird JK, Candradikusuma D, Masbar S, Sutamihardja MA, Leksana B, Marwoto H, Richie TL, Romzan A, 1997. Survey of *in vivo* sensitivity to chloroquine by *Plasmodium falciparum* and *P. vivax* at Lombok, Indonesia. *Am J Trop Med Hyg 56:* 241–244.