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The drug sensitivity and transmission dynamics of human malaria on Nias Island, North Sumatra, Indonesia

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Nias Island, off the north-western coast of Sumatra, Indonesia, was one of the first locations in which chloroquine-resistant *Plasmodium vivax* malaria was reported. This resistance is of particular concern because its ancient megalithic culture and the outstanding surfing conditions make the island a popular tourist destination. International travel to and from the island could rapidly spread chloroquine-resistant strains of *P. vivax* across the planet. The threat posed by such strains, locally and internationally, has led to the routine and periodic re-assessment of the efficacy of antimalarial drugs and transmission potential on the island. Active case detection identified malaria in 124 (17%) of 710 local residents whereas passive case detection, at the central health clinic, confirmed malaria in 77 (44%) of 173 cases of presumed 'clinical malaria'. Informed consenting volunteers who had malarial parasitaemias were treated, according to the Indonesian Ministry of Health's recommendations, with sulfadoxine-pyrimethamine (SP) on day 0 (for *P. falciparum*) or with chloroquine (CQ) on days 0, 1 and 2 (for *P. vivax*). Each volunteer was then monitored for clinical and parasite response until day 28. Recurrent parasitaemia by day 28 treatment was seen in 29 (83%) of the 35 *P. falciparum* cases given SP (14, 11 and four cases showing RI, RII and RIII resistance, respectively). Recurrent parasitaemia was also observed, between day 11 and day 21, in six (21%) of the 28 *P. vivax* cases given CQ. Although the results of quantitative analysis confirmed only low prevalences of CQ-resistant *P. vivax* malaria, the prevalence of SP resistance among the *P. falciparum* cases was among the highest seen in Indonesia.

When the parasites present in the volunteers with *P. falciparum* infections were genotyped, mutations associated with pyrimethamine resistance were found at high frequency in the *dhfr* gene but there was no evidence of selection for sulfadoxine resistance in the *dhps* gene.

Night-biting mosquitoes were surveyed by human landing collections and tested for sporozoite infection. Among the five species of human-biting anophelines collected, *Anopheles sudaicus* was dominant (68%) and the only species found to be infective — two (1.2%) of 167 females being found carrying *P. vivax* sporozoites. The risk of malarial infection for humans on Nias was considered high because of the abundance of asymptomatic carriers, the reduced effectiveness of the available antimalarial drugs, and the biting and infection 'rates' of the local *An. sudaicus*.

Malaria caused by *Plasmodium vivax* occurs over a broader geographical range than that caused by *P. falciparum* and accounts for

an immense burden of human suffering and illness. Chloroquine (CQ) has long been a safe, inexpensive and reliable treatment for *P. vivax* malaria but there are accumulating reports of treatment failure from such diverse locations as Papua New Guinea (Rieckmann

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et al., 1989), Indonesia (Baird et al., 1991b; Schwartz et al., 1991), India (Garg et al., 1995), and Guyana (Craig and Kain, 1996). International travel, which has become much easier and more frequent over the last four decades, may figure more importantly in facilitating the geographical spread of CQ-resistant *P. vivax* (CQRPV) than it did in spreading CQ-resistant *P. falciparum* (CQRPF) in the 1960s.

Detailed, confirmative studies of CQRPV have been relatively few, and efforts to track the geographical spread of this problem have been largely confined to the Indonesian archipelago — a populous nation of some 140,000 islands. Among all of these islands, Nias, in north-western Sumatra, drew early attention as one of the first areas on the planet thought to be affected by CQRPV. As Nias draws large numbers of international tourists to its antiquities and surfers to its waves, it was apparent that there was a great risk of resistant strains of malarial parasite on the island being carried all over the globe. Following on the case report of CQRPV in a repatriated American tourist who acquired his infection on Nias (Schwartz et al., 1991) and confirmation of this CQRPV at the Centers for Disease Control and Prevention, in Atlanta (Collins et al., 1992), in-vivo field testing of CQ sensitivity/resistance was conducted in early 1995 (Baird et al., 1996). The results of this first survey revealed CQ resistance, to suppressive levels, in 33% of the *P. vivax* investigated, with parasitaemias recurring, within 19 days of supervised therapy with CQ of good quality, in 14% (Baird et al., 1996). The main aim of the present study, conducted 3 years later at the same site, was to re-assess the sensitivity of local *P. vivax* to CQ *in vivo*, to determine whether the frequency and degree of CQ resistance had measurably increased since the first survey. At the same time, the efficacy, *in vivo*, of sulfadoxine-pyrimethamine (SP) — the second-line therapy for uncomplicated *P. falciparum* infection — and the risk of malarial infection faced by a joint American-Indonesian military exercise planned for the area were evaluated.

MATERIALS AND METHODS

Study Sites and Subjects

All-age malaria screenings were conducted in five rural communities (Lagundri, Walondrawa, Botohilitano, Hilinifaoso and Hiliamaetaniha) around the port of Teluk Dalam, on the southern coast of Nias Island, between the July and September of 1998. Nias covers an area of 5625 km².

Passive case detection was also performed, on a daily basis for 3 weeks (1–21 August 1998), through the health clinic at Lagundri, which covers all five study communities. Most of the residents in the area have occupations that are agricultural (rice cultivation, patchouli cultivation and perfume-oil processing) or associated with tourism. The popularity of Lagundri as an international tourist/surfing destination has created an increasing demand for lodging, food and other services. Tourist lodgings, restaurants and housing for Indonesian families and workers have proliferated on the land between the rice paddies, mangrove forest and beach, with facilities ranging from extremely inexpensive/basic to western-style, 'five-star' luxury. Many young tourists appear to opt for very simple and affordable, longer-term lodging in traditional-style bungalows. Mosquito coils and untreated bednets are widely used by both local residents and tourists. CQ, SP and quinine are the respective first-, second- and third-line drugs for malaria treatment in the Indonesian National Health Service and all are locally available in the shops.

Screening and Enrolment

Individuals with uncomplicated, slide-confirmed malaria who were aged >4 years, able to swallow pills, not pregnant and who had not consumed antimalarial drugs or antibiotics in the previous week were invited to volunteer for malaria treatment in the context of a 28-day, in-vivo test. Written, informed consent was obtained from each volunteer or the volunteer's parent/guardian.

This consent included an assurance that the volunteer agreed (1) to use no other drug or treatment but those provided and supervised by the researchers, (2) to submit to daily symptom evaluations and fingerpricks, and (3) to submit to venipuncture for cases of *P. vivax* that reappeared within 21 days of receiving CQ. An enrolment of 38 *P. vivax* cases was sought, based upon the proportion of CQRPV previously reported (14%; Baird *et al.*, 1996), and the aim to estimate the new proportion, with 90% confidence, to be within 10 percentage points of the true value (Lemeshow and Taber, 1991). With no previous measure of clinical or therapeutic resistance to SP in the local *P. falciparum*, it was arbitrarily assumed that treatment failure would occur in 25% of cases (the critical level above which an alternate treatment drug would be advised). With 90% confidence and 10% precision, the desired sample size for testing the sensitivity of local *P. falciparum* to SP was then estimated to be 51 infections (Lemeshow and Taber, 1991). Individuals with parasitaemia who declined to enrol or who were excluded from enrolment were provided with free treatment and follow-up, according to the local standard of care.

Chemotherapy and Follow-up

Each subject with *P. vivax* infection was given a total of 25 mg CQ base (ResochinTM; PT Bayer, Jakarta, Indonesia)/kg body weight, as three oral doses of 10, 10 and 5 mg/kg 0, 24 and 48 h after recruitment (i.e. days 0, 1 and 2), respectively. The subjects with *P. falciparum* infection were each given a single oral dose of SP (Fansidar[®]; Roche, Basel, Switzerland) — just one tablet (containing 500 mg sulfadoxine and 25 mg pyrimethamine) if they weighed 15–19.9 kg, and 1.5, 2.0, 2.5 and 3.0 tablets if they weighed 20–29, 30–39, 40–49 and >50 kg, respectively. All the tablets were consumed after a small snack of sweet biscuits and water and each treatment was witnessed by a member of the study team. Physical complaints and axillary temperature were

recorded and fingerprick blood samples (for thick and thin bloodsmears) were collected on days 0–4, 7, 11, 14, 18, 21 and 28 and also at any time of illness indicative of malaria. The bloodsmears were stained with Giemsa and examined by oil-immersion, light microscopy (at $\times 1000$) by a skilled microscopist. Each thick smear was examined for malarial parasites and only considered negative if no parasites were seen in 300 fields. Sexual- and asexual-stage parasites were separately counted against 200 leucocytes and each count was then multiplied by 40 to give an estimate of the number of parasites/ μ l blood (assuming each subject had 8000 leucocytes/ μ l).

Aliquots (100 μ l) of fingerprick blood obtained on day 0, 2 and any time of suspected treatment failure were collected onto filter papers, labelled and air dried for subsequent use in analysing blood concentrations of CQ or the parasite genotype (see below). Unremitting parasitaemia or reappearance of asexual parasites during the 28-day period of the in-vivo test constituted a treatment failure. The clinical symptoms of malaria, with parasitaemia that did not resolve following treatment or which recurred with parasitaemia, during the 28-day, follow-up period, constituted a clinical failure. In accordance with national health policy in Indonesia, any CQ and SP failures observed were given oral quinine for 7 days, as rescue treatment. Cumulative indices of therapeutic (parasitological) and clinical failure for each *Plasmodium* species and drug treatment were calculated using the life-table method. The parasitological responses of *P. falciparum* to SP were graded, according to the standard classification of the World Health Organization (1990), as S, RI, RII or RIII.

Measurement of Blood Concentrations of CQ

CQ and its principal metabolite, monodesethylchloroquine (DCQ), were extracted, from the filter-paper blots of the whole-blood samples of those treated with CQ, so

that their concentrations could be measured by HPLC (Patchen *et al.*, 1983; Fryauff *et al.*, 1998). The persistence or reappearance of asexual-stage *P. vivax* parasites, following full, supervised treatment and in the presence of whole-blood concentrations of CQ + DCQ that exceeded 100 ng/ml — the estimated minimal concentration effective against CQ-sensitive strains of this parasite (Baird *et al.*, 1997) — was taken as confirmation of CQ resistance. [Note that this definition may apply to post-treatment parasitaemias arising from recrudescence, relapse or re-infection (Baird *et al.*, 1997).]

Molecular Markers of SP Resistance in *Plasmodium falciparum*

Parasite DNA was extracted, from the filter-paper blots of the whole-blood samples of those treated with SP, using 5% Chelex-100 resin (Bio-Rad Laboratories, Hercules, CA) in distilled water and then purified through an A721C minicolumn (Promega, Madison WI). The DNA was then amplified using the nested-PCR method described by Nagesha *et al.* (2001), the appropriate positive and negative controls (to minimize the risk of contamination error in the first and second rounds of the PCR), and conventional primers and those specific for the resistance-associated mutations in the parasite's *dhfr* or *dhps* genes (Durasingsh *et al.*, 1998). The *dhfr* and *dhps* polymorphisms were identified by RFLP (restriction-fragment-length-polymorphism) analysis. The nested amplification products of *dhfr* were digested with *Nla*III, *Tai*I, *Tsp*5091, *Xmn*I and *Dra*I, for discriminating the alleles at codons 16, 50, 51 and 164, whereas codon 108 was selectively cleaved with *Alu*I, *Bsr*I and *Scr*FI. The *dhps* PCR products were digested with *Msp*AI, *Ava*II and *Fok*I for the identification of mutations at codons 436, 437 and 540, *Bst*UI and *Bsl*I for the identification of those at codon 581, and *Mwo*I, *Bsa*WI and *Age*I for the identification of those at codon 613. As a control to monitor the endo-

nuclease digestion, purified lambda DNA (Promega) was used in each RFLP experiment. Digested products were subjected to electrophoresis on 1.5%–3% agarose gel, or ultrapure agarose gel (Progen, Darra, Australia) for enhanced resolution. The PCR products of interest were sequenced according to published methodology (Wang *et al.*, 1997a, b), to verify the RFLP results and to identify any new mutation sites in the *dhfr* and *dhps* products.

Assessment of Transmission by Mosquitoes

Mosquitoes were captured outdoors in each of the five study communities, between 18.00 and 23.00 hours, as they landed on the members of a two-man team equipped with flashlights and aspirators. Anophelines were sorted, identified to species and stored dried over silica gel until their heads and thoraces could be tested in an ELISA for *Plasmodium* sporozoites (Wirtz *et al.*, 1985, 1992). Monoclonal antibodies for *P. falciparum* and the circumsporozoite variants of *P. vivax* (VK247 and PV210) were employed in the ELISA. The entomological data were used to produce estimates of the man-biting rate (bites/person-night) and the entomological inoculation rate (infective bites/person-month) assuming that the local mosquitoes only fed during the collection period (18.00–23.00 hours) and that all the mosquitoes that landed on the human bait would have fed on the bait.

RESULTS

Malaria Prevalence by Location and Age-group

All-age active case detection (ACD), conducted in July–August 1998, identified malarial parasitaemia in 122 (17.2%) of the 710 people screened and revealed community-specific point prevalences ranging from 6% to 40% (Table 1). Malarial parasitaemias, all of which were low-density, were found in

TABLE 1. The results of malaria point-prevalence screening by active case detection on the southern coast of Nias Island, Sumatra, Indonesia, in July–September 1998

Location	Screened	No. and (%) of subjects:			
		Found positive for:			
		Malarial parasites	<i>Plasmodium falciparum</i> only	<i>Plasmodium vivax</i> only	<i>Plasmodium falciparum</i> and <i>P. vivax</i>
Hilinfaso	258	24 (9)	7 (3)	17 (7)	0 (0)
Lagundri	143	9 (6)	0 (0)	9 (6)	0 (0)
Walondrawa	104	26 (25)	13 (13)	13 (13)	0 (0)
Botohilitano	76	12 (16)	9 (12)	3 (4)	0 (0)
Hiliamaetaniha	129	51 (40)	29 (22)	20 (16)	2 (2)
All five study sites	710	122 (17.2)	58 (8.2)	62 (8.7)	2 (0.3)

27 (8.6%) of the 314 apparently asymptomatic schoolchildren (aged 6–13 years) screened; six (1.9%) appeared to have pure *P. falciparum* infections whereas the other 21 (6.7%) appeared to have pure *P. vivax* infections. Passive case detection (PCD), over 3 weeks in the Lagundri health clinic, confirmed malaria in 77 (44.8%) of the 173 cases judged to be of 'clinical malaria' by local healthcare providers. The confirmed cases appeared to be infected with *P. falciparum* only (47 cases), with *P. vivax* only (28 cases) or a mixture of these two species (two cases).

For both *P. vivax* and *P. falciparum*, the age distributions of those found positive by ACD differed markedly for those found positive by PCD (Fig. 1). Children aged <6 years, for example, accounted for 23% of the infections found by ACD screening but made up only 8% of the confirmed clinical malaria cases in the PCD ($P=0.007$). Despite the high frequency of infection seen in the children screened during the ACD and the predominance of children in the demography of the population (77% of the residents of the study communities were aged <16 years at the time of enrolment), adults (aged >15 years) made up 52% of the diagnosed cases of clinical malaria in the ACD and accounted for 43% of those confirmed by microscopy in the PCD. In the PCD, the older children and young adults (i.e. those aged 6–40 years) were twice as

likely to be confirmed positive by microscopy as those who were younger or older, with a relative risk (RR) of 2.14 and corresponding 95% confidence interval (CI) of 1.30–3.54.

Efficacy of the CQ Treatment of *Plasmodium vivax* Infection

Illness at enrolment — most commonly headache (76%), fever (68%), malaise (44%), nausea (40%) and muscle pain (24%) — was reported by 26 of the 36 subjects with *P. vivax* infection and eight were found to be febrile (i.e. with an axillary temperature $\geq 37.5^\circ\text{C}$). The characteristics of cases who received CQ treatment and remained available for all or most of follow-up period are presented in Table 2. The corresponding therapeutic and clinical responses are summarized in Figure 2. Baseline (pre-treatment) concentrations of CQ + DCQ in three of the 30 subjects tested ranged from 113–639 ng/ml, indicating recent previous treatment and/or potentially resistant infections. All parasitaemias declined rapidly in response to the CQ treatment given as part of the present study and cleared by 96 h. The mean parasite-clearance time was 36.7 h, with CI of ± 5.6 h. Illness abated in the majority of the subjects by 72 h, with only three of 35 still reporting headache or nausea at that

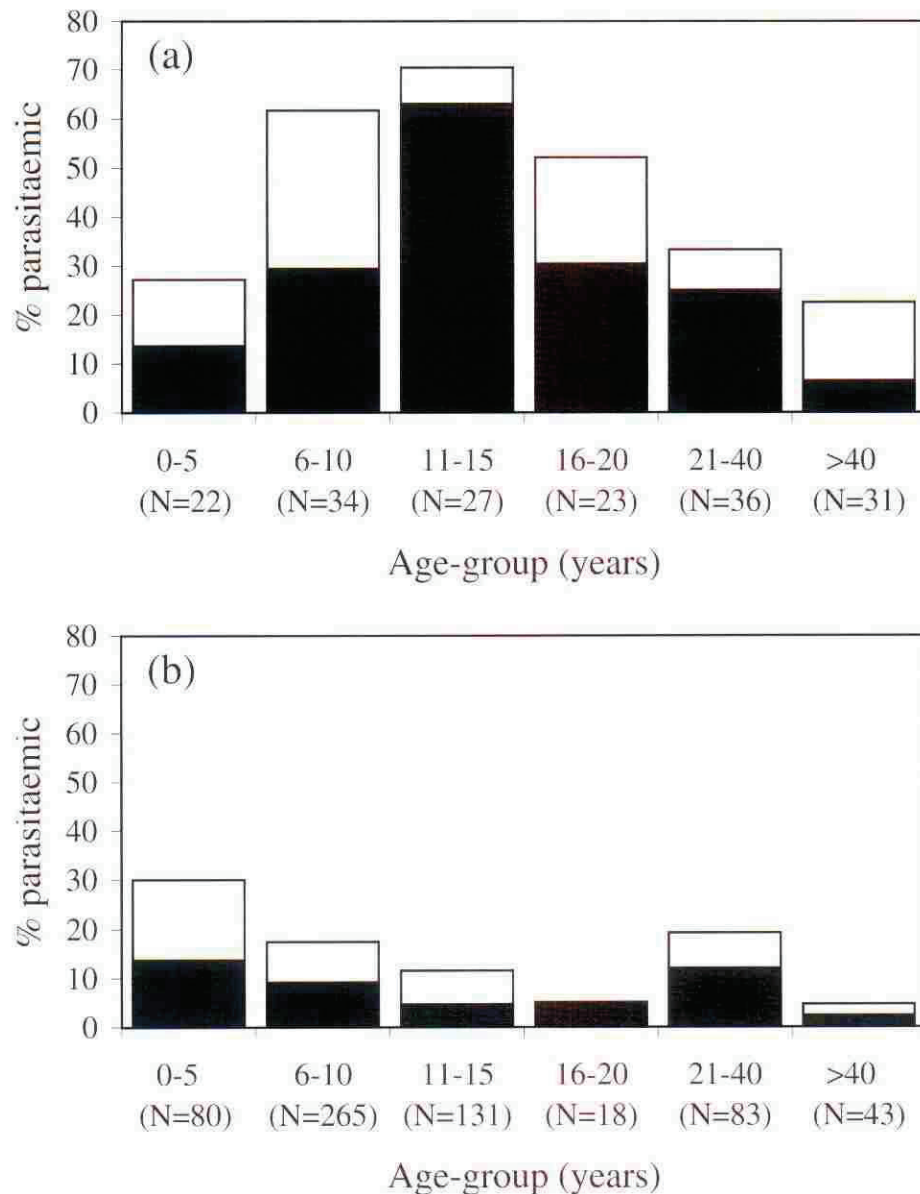


FIG. 1. The proportions of each age-group, screened by passive (a) and active (b) case detection, found to be infected with *Plasmodium falciparum* (■) or *P. vivax* (□).

time. The first reappearance of asexual parasites following therapy and clearance was on day 11, in two asymptomatic children. These two children, who had had 7280 and >40,000 trophozoites/ μ l and corresponding CQ + DCQ concentrations of 235 and 57 ng/ml on day 0 (pre-treatment), both

had 160 trophozoites/ μ l on day 11 (Table 2) and CQ + DCQ concentrations of 0 and 270 ng/ml, respectively, on day 12. The only other recurrent parasitaemias detected, on day 18 in another four subjects, were all found when those affected had low or undetectable concentrations of CQ + DCQ in their blood.

TABLE 2. Summary of the results of the 28-day, in-vivo tests of the sensitivities of *Plasmodium falciparum* to sulfadoxine-pyrimethamine (SP) and *P. vivax* to chloroquine (CQ)

Characteristic	<i>Plasmodium falciparum</i>	<i>Plasmodium vivax</i>
No. of subjects	39	36
No. of males : females	22:17	22:14
DAY-0 (PRE-TREATMENT) VALUES		
Mean age, (median) and [range] (years)	15.6 (12) [6-55]	14 (11) [4-50]
Mean weight, (median) and [range] (kg)	33.8 (35) [15-57]	29.3 (27.5) [12-49]
Mean haemoglobin concentration and (95% confidence interval) (g/dl)	9.7 (9.2-12.2)	9.5 (9.2-9.8)
Geometric mean parasitaemia and (range) (asexual parasites/ μ l)	4093 (40-80,000)	483 (40-40,000)
CUMULATIVE INCIDENCE OF PARASITAEMIA		
Day 0	0.00	0.00
Day 4	0.23	0.00
Day 7	0.34	0.00
Day 11	0.59	0.06
Day 14	0.69	0.20
Day 18	0.69	0.20
Day 21	0.73	0.20
Day 28	0.76	0.20
Mean time to recurrence and (range) (days)	11.6 (4-28)	17.0 (12-19)
MEAN CQ + DCQ CONCENTRATIONS AND/OR (RANGE) (ng/ml)		
Day 0	—	— (0-633)*
Day 2	—	568 (216-1169)
Day 28	—	38 (0-315) [†]
Day of recurrence	—	— (0-270) [‡]

*Blood samples collected from 30 of the subjects at enrolment revealed that seven of the 30 then had detectable blood concentrations (22-633 ng/ml) of CQ plus its metabolite monodesethylchloroquine (DCQ), and that three of the seven had >100 ng CQ + DCQ/ml.

[†]Seven of the 22 subjects checked had detectable blood concentrations of CQ + DCQ on day 28, three having >100 ng/ml at that time.

[‡]One of the six subjects checked on the day of recurrence had >100 ng CQ + DCQ/ml.

Efficacy of the SP Treatment of *Plasmodium falciparum* Infection

Symptoms — most commonly headache (94%), fever (81%), malaise (72%), nausea (56%), and vomiting (37%) — were reported at enrolment by 33 of the 39 subjects with *P. falciparum* infection although only 14 (36%) were found febrile at that time. The geometric mean (GM) *P. falciparum* parasitaemia before treatment (on day 0) was 10 times higher than the corresponding *P. vivax* parasitaemia (Table 2). In general, the clinical and therapeutic responses to SP were poor. At the day-3 (72 h) follow-up, fever had resolved in 13 of the 14, initially febrile cases but 47% and 24% of all those given

SP were still complaining of headache and malaise, respectively. Asexual parasitaemias declined by >25% within 48 h of treatment in 34 cases (of the 38 providing the relevant data) and had cleared by day 4 (96 h) in 28 of the 39 cases. Unremitting RIII- or RII-type responses within 7 days of treatment characterized, respectively, four (11%) and 11 (29%) of the SP treatments. Two of the subjects treated with SP withdrew from follow-up and three others were dis-enrolled between days 7 and 11 because of intercurrent *P. vivax* infection. Figure 2 illustrates the cumulative incidence of clinical and therapeutic failures over the 28 days following SP treatment. The majority (24/29) of the

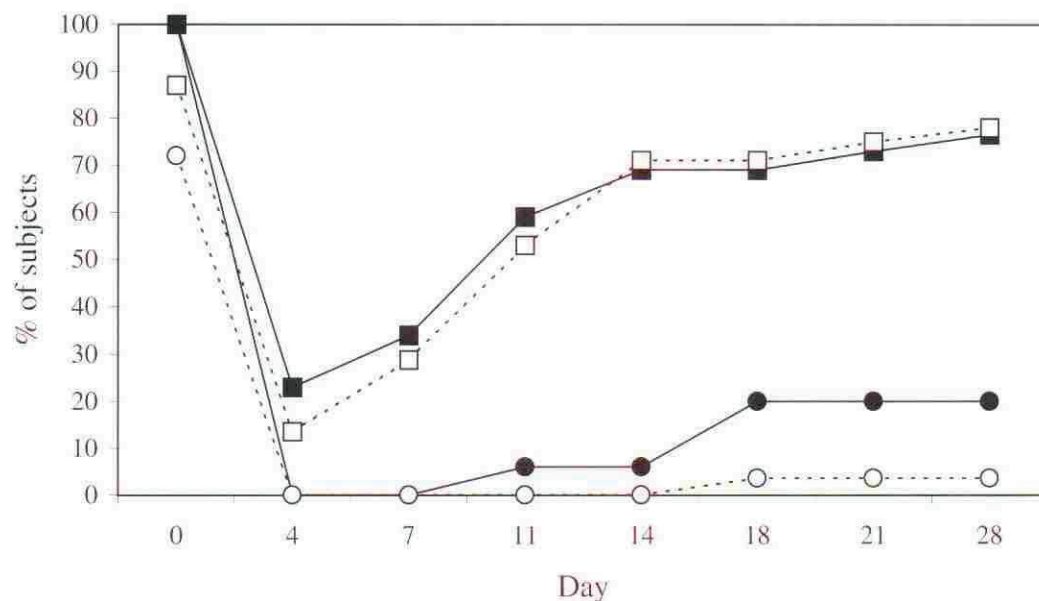


FIG. 2. Profiles of the clinical and parasitological responses observed in the subjects post-treatment. The lines plotted show the proportions of subjects who were symptomatic (□) or parasitaemic (■) after their *Plasmodium falciparum* infections were treated with sulfadoxine-pyrimethamine (on day 0) and the corresponding proportions of subjects who were symptomatic (●) or parasitaemic (○) after their *P. vivax* infections were treated with chloroquine.

therapeutic failures occurred during the first 11 days, with treatment success achieved in only six of the 35 cases who provided sufficient data. The frequency of gametocyte carriage and the GM density of the gametocytaemias observed increased progressively over the first 7 days following SP treatment (Fig. 3). Retrospective molecular analyses, to determine whether allelic mutations in the *dhps* and *dhfr* genes of the infecting parasites could have predicted SP treatment outcome, were performed on 27 cases who had been graded S, RI, RII or RIII. Sequencing of the PCR products revealed that *dhps* codons 436, 437, 581, and 613 were all normal wild-type and that mutations in this gene had not arisen or been selected for. Although sequencing of *dhfr* identified point mutations only at codons 108 and 59, these mutations were detected in 26 (96%) and 21 (78%) of the 27 cases, respectively, with 20 cases each having the double mutation. There was no apparent association between the frequency

or pattern of these *dhfr* mutations and the in-vivo response (Table 3); the double mutation, for example, was seen in each of three sensitive (S) infections as well as in three RIII treatment failures. The single infection that was wild-type at all *dhfr* codons appeared to recrudesce on day 11 post-treatment. A comparison of the day-0 and day-7 *dhfr* profiles of each of nine cases demonstrating RII- or RIII-type resistance showed the Arg59 and Asn108 double mutation at both time-points. Quinine rescue therapy was successful for all of the SP treatment failures.

Malaria Transmission Potential

Human-biting *Anopheles* accounted for 251 (12.5%) of the 2003 night-biting mosquitoes captured during 216 person-hours of collection. Five anopheline species, including three known to be vectors of *P. falciparum* and/or *P. vivax* — *Anopheles sundaicus* (Rodenwalt),

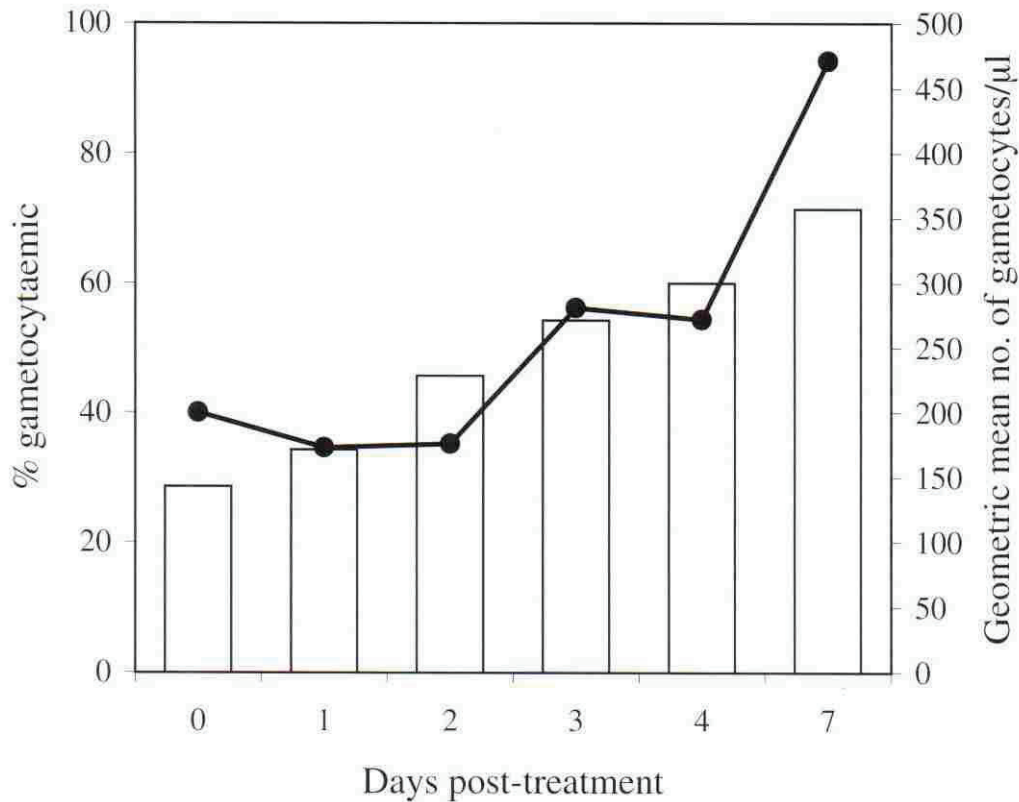


FIG. 3. Effect of sulfadoxine-pyrimethamine treatment of *Plasmodium falciparum* infections on the prevalence of (detectable) gametocytaemia (□) and the geometric mean densities of *P. falciparum* gametocytes (●) among the gametocytaemics.

An. sinensis (Wiedemann) and *An. tessellatus* (Theobald) — were identified. *Anopheles sundaicus* accounted for 68% of the 245 *Anopheles* identified and tested and *An. sinensis* for 18%. Collectively, *Anopheles* biting rates ranged from 1–20/person-night, with *An. sundaicus* predominant in 11 of the 18 locations surveyed and *An. sinensis* predominant in six inland locations.

Only two mosquitoes — both *An. sundaicus* infected with the Pv210 variant of *P. vivax* — were found to be carrying sporozoites. The sporozoite 'rate' was therefore 0.8% for all of the *Anopheles* tested and 1.2% for the *An. sundaicus*. Since the mean biting rate for all *Anopheles* was 6.8 bites/person-night, the entomological inoculation rate was (conservatively) estimated at 1.6 infective bites/person-month (Table 4).

DISCUSSION

The principal aim of the present study was to initiate periodic, in-vivo re-testing, to detect any change in the frequency of CQRPV at an Indonesian site where this trait had been first recognized and confirmed (Schwartz *et al.*, 1991; Collins *et al.*, 1992). The present investigation, conducted 3 years after the first survey for CQRPV on Nias (Baird *et al.*, 1996), does not provide any evidence of such a change. Although the present results include clear indications of *P. vivax* resistance to both suppressive and therapeutic concentrations of CQ, they show no greater frequency of such resistance than that reported for the same site in 1995 (Baird *et al.*, 1996). The CQ-tolerant or CQ-resistant *P. vivax* trophozoites observed in the present

TABLE 3. The wild-type alleles (○) and allelic mutations (●) detected in the genes for dihydrofolate reductase (Pfdhfr) and dihydropteroate synthetase (Pfdhps) in *Plasmodium falciparum* isolates from the subjects treated with sulfadoxine-pyrimethamine

Patient no.	Clinical response	Pfdhps codon				Pfdhfr codon					
		436	437	581	613	16	50	51	59	108	164
PLG 28	RI (day 14)	○	○	○	○	○	○	○	●	●	○
PLG 47	RI (day 14)	○	○	○	○	○	○	○	●	●	○
PLG 51	RI (day 11)	○	○	○	○	○	○	○	●	●	○
PLG 63	RI (day 11)	○	○	○	○	○	○	○	●	●	○
PLG 109	RIII	○	○	○	○	○	○	○	●	●	○
PLG 139	RI (day 21)	○	○	○	○	○	○	○	○	○	○
PLG 193	RI (day 11)	○	○	○	○	○	○	○	●	●	○
PLG 199	RII	○	○	○	○	○	○	○	●	●	○
2154	RI (day 14)	○	○	○	○	○	○	○	●	●	○
2156	S	○	○	○	○	○	○	○	●	●	○
2161	RI (day 11)	○	○	○	○	○	○	○	●	●	○
3050	S	○	○	○	○	○	○	○	●	●	○
3057	S	○	○	○	○	○	○	○	●	●	○
4004	RI (day 11)	○	○	○	○	○	○	○	○	○	○
4006	RI (day 11)	○	○	○	○	○	○	○	○	○	○
PLG 124*	RIII	○	○	○	○	○	○	○	●	●	○
PLG 143*	RII	○	○	○	○	○	○	○	○	○	○
PLG 197*	RII	○	○	○	○	○	○	○	●	●	○
PLG 198*	RIII	○	○	○	○	○	○	○	●	●	○
6007*	RII	○	○	○	○	○	○	○	●	●	○
9071*	RII	○	○	○	○	○	○	○	●	●	○
5003*	RII	○	○	○	○	○	○	○	●	●	○
6051*	RII	○	○	○	○	○	○	○	●	●	○
6004*	RII	○	○	○	○	○	○	○	○	○	○
6060*	RII	○	○	○	○	○	○	○	●	●	○
203*	RII	○	○	○	○	○	○	○	○	○	○
6071*	RII	○	○	○	○	○	○	○	●	●	○

*The codons of isolates collected from this patient on day 0 and the day of recurrence were compared (and found to be identical).

TABLE 4. The prevalences of infection with malarial sporozoite, man-biting rates (MBR), and estimated entomological inoculation rates (EIR) for the *Anopheles* mosquitoes collected on Nias Island, Sumatra

Species	Sporozoite ELISA		MBR* (bites/person-night)	EIR* (infective bites/person-month)
	No. tested	No. and (%) positive		
<i>Anopheles tessellatus</i> †	6	0 (0)	0.2	0
<i>An. kochi</i>	13	0 (0)	0.4	0
<i>An. craxfordi</i>	14	0 (0)	0.4	0
<i>An. sinensis</i> ‡	45	0 (0)	1.2	0
<i>An. sundaicus</i> ‡	167	2‡ (1.2)	4.6	1.6

*Assuming all bites occur each night only during the 5-h period (18.00–23.00 hours) when the landing catches were made and that all landing mosquitoes would have bitten the human bait. For the MBR, a month was taken to be 28 days.

†Confirmed vector of at least one *Plasmodium* species that infects humans.

‡Both infected with *Plasmodium vivax*.

study were also found in the presence of CQ + DCQ concentrations similar to those detected in 1995 (Baird *et al.* 1996).

It is unclear why, given the apparently equal selective pressure applied world-wide to *P. vivax* and *P. falciparum* by CQ use and mis-use, CQRPV is much less common than CQRPF. This may be related to the observation that CQ-sensitive *P. vivax* can be killed by a dose of CQ that is only about a quarter of the dose needed to kill CQ-sensitive *P. falciparum* (Berliner *et al.*, 1948). The CQ resistance in *P. falciparum* has been shown to be centrally dependent upon a point mutation (K76T) in a single gene (*pfert*) coding for a transport protein in membrane of the parasite's digestive vacuole (Fidock *et al.*, 2000). This point mutation was present in every clinical case of CQRPF studied in Mali by Djimde *et al.* (2001) and in 93% of the CQRPF cases studied in Papua, Indonesia, by Maguire *et al.* (2001). Although a gene ortholog present in *P. vivax* (*pvcg10*) is remarkably similar to *pfert* in terms of its position, composition and structure, no point mutations that distinguish CQ-sensitive and CQ-resistant phenotypes of *P. vivax* have been found in *pvcg10* (Nomura *et al.*, 2001). The apparent molecular divergence in the CQ-resistance strategies of *P. falciparum* and *P. vivax* is in contrast to the common mechanism of antifolate resistance seen in all the malarial parasites of rodents and humans investigated (De Pecoulas *et al.*, 1998; Carlton *et al.*, 2001).

The present results do not warrant increased concern about CQRPV on Nias or more frequent monitoring of CQRPV on the island. However, the poor clinical and therapeutic effects of SP on local *P. falciparum* and the almost ubiquitous occurrence of *dhfr* mutations conferring pyrimethamine resistance in these parasites are worrying. The frequency of SP treatment failure observed on Nias is among the highest recorded in Indonesia — even exceeding those recorded on Papuan Indonesia (Baird *et al.*, 1991a; Tjitra *et al.*, 1997; Nagesha *et al.*, 2001) — and meets the World Health Organization's

criterion for a change in the antimalarial drug used (WHO, 1994). All 74 cases from central and northern Sumatra who were tested *in vivo* between 1981 and 1985 demonstrated sensitivity to SP, although in-vitro testing over the same period and in the same locations showed SP resistance in 28 of 38 isolates (Tjitra *et al.*, 1997). A rapid change in the sensitivity of *P. falciparum* to pyrimethamine and SP was observed in north-eastern Papuan Indonesia (Hoffman *et al.*, 1985, 1987; Baird *et al.*, 1991a), although resistance to pyrimethamine and cross-resistance to proguanil evolved early and progressed rapidly there, as a result of the mass distribution of pyrimethaminized salt in 1959–1960 (Meuwissen, 1961). Interestingly, the campaign to distribute and promote the use of this medicated salt markedly reduced the prevalence of *P. vivax* and *P. malariae* malaria, with pyrimethamine remaining effective as both a treatment and prophylactic against *P. vivax* at a time of increasing resistance in *P. falciparum* (Meuwissen, 1964). There appears to be no record of the use of pyrimethaminized salt on Nias. Resistance to CQ in *P. falciparum* and high frequencies of gametocyte carriage in children prompted health officials and researchers to adopt SP plus primaquine for the routine treatment of malaria in coastal north Sumatra during a community health project in 1985–1989. Although this co-operative (Indonesian–Japanese) programme led to a marked reduction in the prevalence of *P. falciparum* infection, there was little change in *P. vivax*, despite the combined use of chemotherapy, bednets and larviciding (Doi *et al.*, 1989).

The results of the present analyses of *dhfr* and *dhps* in *P. falciparum* from Nias are singular in demonstrating no evidence of any mutation in the *dhps* gene associated with SP resistance. The non-involvement of *dhps* point mutations appears not to have been reported before in any area where there were such high frequencies of SP treatment failure. The *dhps* mutation at position 437 is a commonly reported marker of sulfadoxine pressure

and was detected in all the *P. falciparum* isolates, from nearby Malaysia, tested by Cox-Singh *et al.* (2001). Analysis of *dhfr* from *P. falciparum* collected in Papuan Indonesia identified the Asn108 mutation in 81% of malaria-naïve immigrants (Nagesha *et al.*, 2001) and 62% of malaria-immune native Papuans (H. S. Nagesha, unpubl. obs.); both significantly lower proportions than the frequency of Asn108 in the present samples from Nias (96%). In contrast to the present results from Nias, the *P. falciparum* in the symptomatic, non-immune immigrants from Papuan Indonesia commonly expressed point mutations in the *dhps* gene at codons 437 (83%) and 540 (47%), although *dhps* mutations (at position 437 in 24% and at position 540 in 5%) were seen far less frequently in the parasites from the asymptomatic infections in the native Papuans (H. S. Nagesha, unpubl. obs.). It seems possible that *dhps* mutations play a relatively small role in SP resistance (or confer a relatively small advantage in terms of the parasite's survival) under conditions of low transmission intensity.

A recently reconsidered problem is the potential for SP treatment to exacerbate community levels of *P. falciparum* transmission, by elevating the prevalence and intensity of gametocytaemias. This disadvantage of SP has already been reported in Asia (Tin and Nyunt Hlaing, 1984; Marwoto *et al.*, 1986) and much debated (Ittiravivongs *et al.*, 1984; Strickland *et al.*, 1986; Baird *et al.*, 1991c). Among the non-immunes in north-eastern Papuan Indonesia who were given SP in 1996–1998, as a rescue treatment following CQ failure, the prevalence of gametocytaemia rose from 0% to 43% within 7 days, with the GM intensity (among those who were gametocytaemic) peaking, on day 14, at 199 gametocytes/ μ l (CI = 153–277/ μ l; unpubl. obs.). However, the gametocyte response observed in Nias, where the prevalence of gametocytaemia rose from 26% to 73% within 7 days and the peak GM intensity was 445 gametocytes/ μ l (CI = 282–700/ μ l) on day 7, was significantly

higher. In comparison, on an isolated island off north-western Papuan Indonesia, where all the *P. falciparum* appeared SP-sensitive, gametocytes were seen in only four of 40 cases between days 0 and 7 and in only 12 of 288 smears prepared later in the follow-up, and then always at low densities (Fryauff *et al.*, 1999). No attempt was made to determine the viability and infectivity of the gametocytes in the elevated gametocytaemias apparently induced by SP treatment on Nias, but these gametocytes may well be viable and similarly resistant to the sporonticidal activity of SP (Butcher, 1997; Robert *et al.*, 2000). The early appearance of the gametocytaemias, soon after SP treatment, has been taken as an indication that the gametocytes seen, many of which are immature and non-infective, have not been induced by the treatment but merely mobilized or re-distributed from sites where they have been sequestered (Targett *et al.*, 2001). While the pyrimethamine-mediated mobilization or development of gametocytes (Chutmongkonkul *et al.*, 1992) on Nias may have been enhanced by mutations in the *dhfr* gene, it also seems possible that the absence of mutations associated with sulfadoxine resistance in *dhps* may have somehow stimulated gametocytogenesis (Butcher, 1997; Hogg *et al.*, 1998).

The present demonstration of *P. vivax* sporozoites in *An. sundaicus* and the dominance of this mosquito species among the man-biting anophelines collected were not unexpected in the coastal study area. *Anopheles sundaicus* is considered to be the dominant vector of both *P. vivax* and *P. falciparum* over much of coastal Indonesia (Imai *et al.*, 1988) and is responsible for the persistence of malaria along the southern coast of Java despite the successful elimination of malaria from most of this island (Atmoesodjono *et al.*, 1992). *Anopheles tessellatus* is a confirmed vector of *P. vivax* and *P. falciparum* in southern Asia (Gamage-Mendis *et al.*, 1993) and was previously the only confirmed vector of malarial parasites on Nias (Indonesian Ministry of Health, unpubl. obs.). *Anopheles sinensis* is a common, primarily zoophilic/exophilic

anopheline of eastern Asia that is reportedly refractory to most *P. falciparum* infections (Somboon *et al.*, 1994; Rongsriyam *et al.*, 1998). The potential for *P. vivax* transmission by *An. sinensis* derives mainly from the high densities that populations of this species can attain (Ree *et al.*, 2001). The present collections of mosquitoes on Nias were limited in sampling time, season and location and therefore probably biased. *Anopheles tessellatus*, *An. sinensis* and other freshwater-breeding anophelines undetected in the brief survey on coastal Nias may be more abundant inland, where they may play a more important role in malarial transmission.

In summary, active case detection identified large numbers of asymptomatic infections and gametocytaemias, predominantly among young children, in the rural population of southern, coastal Nias. No evidence was found of increasing resistance to CQ in the local *P. vivax* at this confirmed focus of CQRPV but the results of in-vivo testing demonstrated poor therapeutic and clinical responses to SP in *P. falciparum*. There was a high frequency of mutations in the *dhfr* gene that are associated with pyrimethamine resistance in *P. falciparum*, but no sign of selection pressure by sulfadoxine in the *dhps* gene. In addition to its poor clinical performance, SP appeared to increase the frequency and intensity of gametocytaemias, potentially heightening malaria transmission and disease risk in the area. Although CQ still affords good treatment efficacy against *P. vivax* on Nias, the use of SP alone in the treatment of *P. falciparum* on the island should be strongly discouraged. SP should only be used in combination with a rapidly acting and gametocytocidal artemisinin derivative (Kumar and Zheng, 1990; Price *et al.*, 1996). In Africa, the cost of treatment with a combination of SP and artesunate is currently reported to be U.S.\$1.30 more than the cost of SP alone (which is below U.S.\$0.20) and beyond the reach of most of those with malaria (Vick, 2002). In Indonesia, however, the costs of SP plus

artesunate may be manageable, especially if they could be reduced greatly by in-country production of *Artemisia annua*. Even though most isolates of *P. falciparum* from Nias appear resistant to treatment with CQ and SP, the use of a combination of CQ and SP might offer some advantage over the use of either CQ or SP as a first-line strategy against uncomplicated, *P. falciparum* malaria (Winstanley, 2001). Tourists and travellers to Nias are advised always to use bednets, topical mosquito repellents and effective chemoprophylaxis, to reduce their risk of acquiring malaria on the island (and of carrying multi-resistant strains to other regions).

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