2002

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Oral chloroquine is the treatment of choice for uncomplicated *Plasmodium malariae* infections worldwide. We did a prospective 28-day in-vivo assessment of the efficacy of chloroquine for treatment of *P malariae* on Legundi Island in Lampung Bay, Sumatra, Indonesia. Of 28 patients, one had recurrent parasitaemia on day 28, and two had persistent parasitaemia to day 8. Whole-blood chloroquine and desethylchloroquine concentrations were at ordinarily effective levels (≥100 μg/L) on day 8 in both cases of persistent parasitaemia. These findings suggest that clinical resistance to chloroquine by *P malariae* occurs in the Indonesian archipelago of southeast Asia.

Lancet 2002; **360:** 58–60

Plasmodium malariae causes quartan malaria—an important re-emerging parasitic disease around the globe. Although well described in eastern Indonesia and common in Java and Sumatra during the early 20th century, we have found no recent record of *P malariae* in the western Indonesian archipelago. Oral chloroquine remains the treatment of choice for uncomplicated quartan malaria, and although chloroquine therapy for *Plasmodium falciparum* and *Plasmodium vivax* infections in Indonesia often fails, *P malariae* is presumed sensitive to chloroquine, and resistance has not been documented.

In August, 2000, we identified a focus of *P malariae* on the island of Legundi in Lampung Bay near the southernmost tip of Sumatra at the Sunda Strait. Since chloroquine use is heavy in this area, we aimed to find out whether chloroquine resistance could develop in *P malariae*. Infected individuals were identified by a cross-sectional prevalence survey for malaria, and were enrolled in a 28-day in-vivo test of resistance to chloroquine. Patients were treated with the standard chloroquine regimen, in accordance with a protocol approved by the United States Naval Medical Research Unit Institutional

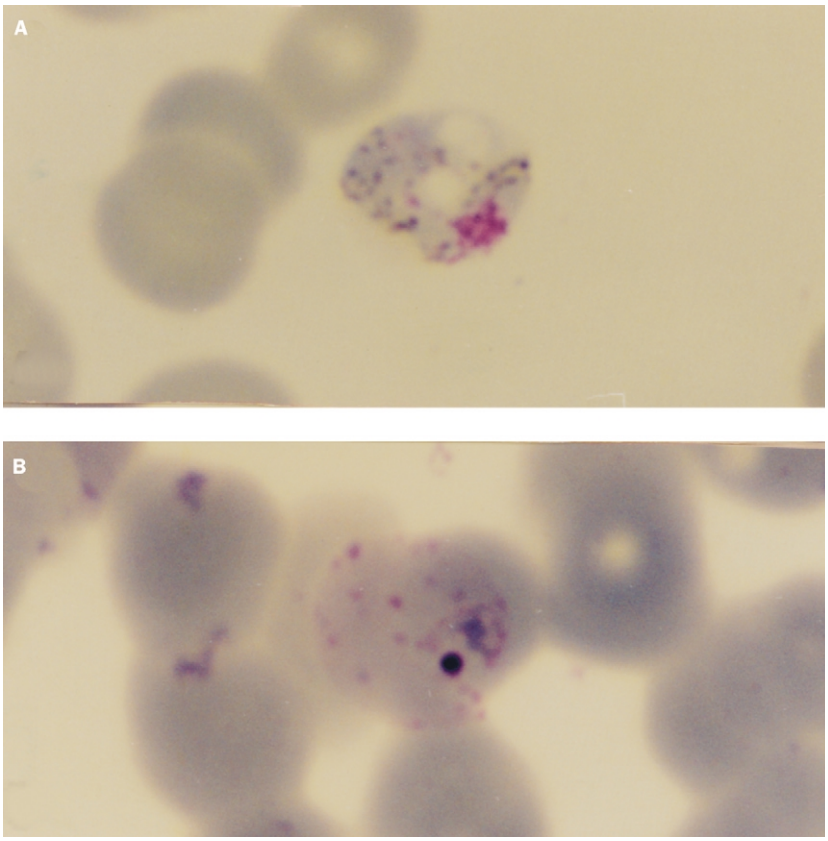

Figure 1: **Giemsa-stained peripheral blood in patients with *Plasmodium malariae* infection**

A: Day-7 sample from patient with persistent *P malariae* parasitaemia. On day 8, this patient's whole-blood chloroquine and desethylchloroquine concentration was 170 μg/L and was positive for *P malariae* and *P falciparum*. Note characteristic course, scattered, golden pigment and vacuolated late trophozoite form in normal-sized red blood cell. B: Day-8 sample from patient whose whole-blood chloroquine and desethylchloroquine concentration was 100 μg/L and was positive for *P malariae* and negative for *P falciparum*. Note ring stage in normal-sized red blood cell with prominent Ziemann's stippling.

Review Board and the ethical review committee of the host country. All patients provided informed consent. Patients received directly observed doses of chloroquine diphosphate (Resochin, P T Bayer Indonesia, Jakarta): 10 mg base/kg on day 0, 10 mg base/kg on day 1, and 5 mg base/kg on day 2, and were followed up for 28 days.

Malaria smears were prepared on days 0, 1, 2, 3, 4, 7, 11, 14, 18, 21, 28, or any day a patient complained of illness. Blood-blot specimens were obtained for measurement of whole-blood chloroquine concentrations by high-performance liquid chromatography (Model 2700, Bio-Rad, Richmond, CA, USA) on day 0 (pretreatment), day 2, day 28, or the day of recurrent parasitaemia. Blood-blot specimens were also obtained for plasmodial DNA analysis on days 0, 7, 14, 21, 28, or the day of recurrent parasitaemia. Patients with persistent or recurrent parasitaemia during follow-up were treated with pyrimethamine-sulfadoxine according to Indonesian Ministry of Health guidelines. Studies done elsewhere show that, after standard chloroquine therapy, *P malariae* parasitaemia clears within 24–72 h and does not recur.[1,2] We therefore considered *P malariae* parasitaemia persisting beyond 72 h as evidence of an inadequate therapeutic response consistent with chloroquine resistance.

Active screening of 41% (752/1819) of the population revealed an overall 17% (127/752) prevalence of parasitaemia by plasmodia (31·5% *P falciparum*, 30% *P vivax*, 37% *P malariae*, 1·5% both *P falciparum and P vivax*). At the two primary villages of Selesung and Keramat, prevalence was 15% (59/408) and 23% (56/247), respectively. *P malariae* was the most common species at both locations, accounting for 41% (24/59) in Selesung and 39% (22/56) of positive smears in Keramat. Spleen rates and average enlarged spleen (AES) indices among children (2–9 years) were 68% (59/87; AES 1·8) in Selesung and 81% (43/53; AES 2·7) in Keramat.

Among 47 patients with *P malariae* infection in the prevalence survey, we enrolled 28 in the test of resistance to chloroquine. 15 were male and 13 were female, and ages ranged from 6 to 65 years (mean 13). Four patients had mixed *P malariae* and *P vivax* infections. One declined further participation after day 2, which left 27 individuals who successfully completed the test. The mean asexual parasite count was 220/μL (range 40–1120) at baseline. Only one patient had detectable *P malariae* gametocytes. The blood of 18 individuals had evidence of chloroquine and its major metabolite desethylchloroquine before treatment (mean concentration 142·5 μg/L [SD 116·5]), which suggested that island residents practised routine self-administration of chloroquine.

All but two of the 28 enrolled patients cleared *P malariae* parasitaemia after treatment. The mean time to asexual-stage parasite clearance in 24 of 26 individuals who initially cleared parasitaemia, irrespective of eventual outcome, was 2 days (range 1–4). The times to clearance could not be determined in the other two patients. In both cases, parasitaemia was still present on day 4, but the patients did not undergo another malaria smear until day 7 in 1 case and day 13 in the other, at which time malaria smears were negative for parasites.

18 of the 27 individuals who completed the test remained parasite-free on day 28. During follow-up, six patients developed asexual-stage parasitaemia with a species other than *P malariae*: three with *P falciparum* on day 18, one with *P vivax* on day 21, and two with *P vivax* on day 28. These cases represented intercurrent infections with a different species or possibly relapse in cases of *P vivax* infection.

Of the three remaining patients, two had persistent *P malariae* parasitaemia to day 8, and one had recurrent parasitaemia on day 28. The 28-day cumulative incidence of therapeutic failure was 12% by life-table (actuarial) analysis. Thick-smear asexual parasite counts (per μL) for the two patients with persistent parasitaemia on days 0, 1, 2, 3, 4, 7, and 8 were 520, 120, not done, not done, 80, 80, 40; and 520, 240, 40, 80, 80, 80, 80, respectively. In the same two individuals, day 2 chloroquine and desethylchloroquine concentrations were 205 μg/L and 680 μg/L, respectively. Just before rescue therapy with pyrimethamine-sulfadoxine on day 8, their chloroquine and desethylchloroquine concentrations were 100 μg/L and

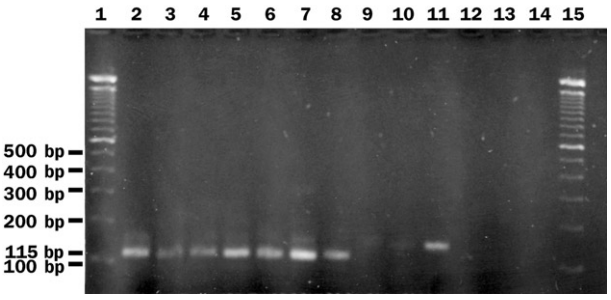

Figure 2: **Electrophoresis after *Plasmodium-malariae*-specific PCR amplification**

Lanes 1 and 15: bp ladder; lane 2: patient LG004 day 0; lane 3: patient LG004 day 28; lane 4: patient LG033 day 0; lane 5: patient LG033 day 8; lane 6: patient LG046 day 0; lane 7: patient LG046 day 8; lane 8: *P malariae* positive control; lane 9: *P falciparum* control; lane 10: *P vivax* control; lane 11: *P ovale* control; lanes 12, 13, and 14: negative controls.

170 μg/L. The patient with recurrent *P malariae* parasitaemia on day 28 cleared parasitaemia 1 day after initiation of therapy and had a chloroquine and desethylchloroquine concentration of 450 μg/L on day 2, which we deemed consistent with adequate absorption of drug. The chloroquine and desethylchloroquine concentration on the day of recurrence was 35 μg/L.

Diagnoses were established by standard light microscopy and PCR. The microscopic diagnosis of *P malariae* was based on the presence of characteristic pigmented band forms and scattered, course pigment granules in prominently vacuolated late trophozoites in normal-sized red blood cells (figure 1). Diagnoses of recurrent or persistent *P malariae* infection were confirmed by species-specific single-stranded ribosomal RNA PCR methods.[3] In all three cases of therapeutic failure, blood-blot specimens obtained on day 0 and the day of rescue therapy were positive for *P malariae* by PCR (figure 2). Neither *P vivax* nor *P ovale* were detected in these specimens. One of the patients with persistent *P malariae* parasitaemia on day 8 was also PCR-positive for *P falciparum* on day 0 and day 8. We believe that this individual might have had a mixed infection with subpatent *P falciparum* parasitaemia. *P malariae* can suppress parasitaemia of subsequent *P falciparum* superinfections.[4]

The case of recurrent *P malariae* parasitaemia on day 28 could represent either new infection or late recrudescence of a chloroquine-resistant strain, but the two cases of *P malariae* persisting for 8 days after directly observed chloroquine therapy with adequate absorption constitute evidence of resistance. The time to parasite clearance seen in this study and others[1,2] supports our view that these two cases represent treatment failures due to inadequate therapeutic response by the parasite, rather than delayed clearance. Although *P malariae* has a longer cycle of schizogony than other human plasmodia, we have seen no evidence to suggest that chloroquine-sensitive *P malariae* survives beyond 72 h after the patient starts standard chloroquine treatment. Figure 1 shows that these patients still carried developing late trophozoites 7 days after initiation of chloroquine therapy. In previous reports of natural infection,[2] time to parasite clearance did not exceed 2 days. Collins and colleagues[1] reported 13 chloroquine-treated experimental *P malariae* infections with parasite clearance times ranging from 1 to 7 days. However, these were experimental infections, many of which were manipulated by low-dose quinine sulphate for several months before definitive treatment with chloroquine, and in most cases, patients received only a single dose of 600 mg chloroquine base rather than a standard 3-day regimen. Those parasite clearance times cannot therefore be compared with those in naturally infected patients treated with the standard 3-day course of chloroquine. The presence of early ring-form parasites as late as day 8 (figure 1) also suggests ongoing schizogony. The persistence of healthy asexual-stage parasitaemia to day 8 after adequate chloroquine absorption and in the presence of ordinarily effective chloroquine and desethylchloroquine concentrations[5] is highly suggestive of resistance to chloroquine by *P malariae*.

Health-care providers could encounter patients with uncomplicated *P malariae* infection that proves unresponsive to the recommended chloroquine regimen. Further assessment of chloroquine-resistant *P malariae* at other locations around the world is needed, particularly in areas where indiscriminate chloroquine use is common and could lead to inadequate dosing and selection for resistant organisms.

Contributors

J Maguire was the principal investigator, project coordinator, and main author, and contributed to data interpretation; I W Sumawinata was a team field physician; S Masbar was a microscopist and field logistical coordinator; B Laksana was the high-performance liquid chromatography technologist and laboratory supervisor; P Prodjodipuro was the senior expert microscopist; A I Susanti was the principal PCR technologist; P Sismadi was the Indonesian Ministry of Health coordinator and a team field physician; N Mahmud was the district health office project coordinator; M J Bangs was the team field supervisor; and K Baird coordinated the implementation of the technology at NAMRU-2 and contributed to data interpretation.

Conflict of interest statement

None declared.

Acknowledgments

We thank the members of the NAMRU-2 Parasitic Diseases Program Clinical Trials Team for technical assistance, especially Krisin, Mark Lacy, Mochamad Awalludin Sutamihardja, Suradi, Sunardi, Bimo Wicaksana, and Dwiko Susapto. We also acknowledge the support of the Legundi Island health-worker team: Dicky Soehardiman, Sri Nurhayadi, Pinnur, Wayan Warso, Edi Trimulyono, Fahruji, Gofir, Mamun, Amrulloh, and M Taher.

This work was supported by the Republic of Indonesia Ministry of Health and Social Welfare, especially Ingerani and D Djamal. This research was funded by the US Department of Defense Global Emerging Infections Surveillance (GEIS) Program. The assertions herein are the views of the authors and do not reflect official policy of the US Department of the Navy, the US Department of Defense, or the US government.

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