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Short Report: Prevalence and Chloroquine Sensitivity of *Plasmodium malariae* in Madagascar

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Abstract. We report the results of clinical studies carried out at six sites in Madagascar, between January and October 2006. The aims were (i) to update our knowledge of the burden of *Plasmodium malariae* infection and (ii) to assess the therapeutic efficacy of chloroquine for uncomplicated quartan malaria. Our findings confirm that *P. malariae* is the third leading cause of malaria, accounting for 1.1% of all malarial infections. They also demonstrate that chloroquine—currently recommended for the home management of presumed malaria in children under the age of five years and commonly used by adults—remains highly effective in patients with uncomplicated *P. malariae* infection.

Plasmodium malariae, one of the four species of *Plasmodium* affecting humans, is found in tropical and subtropical regions, often in sympatry with other *Plasmodium* species, as in Madagascar. Its reported prevalence varies from less than 4% to more than 20% in endemic regions.[1–4] No accurate estimate of the prevalence of *P. malariae* infection worldwide is currently available, but it has been calculated that there are probably at least 60 million infections per year, based on the prevalence of *P. falciparum*[5,6] and known underestimation of the prevalence of *P. malariae*.[7,8] The clinical features associated with febrile bouts of *P. malariae* are generally milder than those caused by other species.[9] Fever displays quartan (4-day) periodicity, parasite density is usually considerably below 1000 parasites per ml of blood, and infection is rarely life-threatening in the absence of complications, such as nephrotic syndrome.[10–12]

All four of the main malaria parasites affecting humans are present in Madagascar, a crossroads of African, Indo-Asian, Middle Eastern, and European civilizations. Malaria remains a serious public health problem (e.g., official data reported 1,227,632 cases of suspected malaria in 2005) and the leading cause of morbidity and mortality, especially in children under 5 years of age.[13–15]

No recent data concerning the prevalence and chloroquine (CQ) sensitivity of *P. malariae* have been published. CQ is the antimalarial treatment recommended by the National Malaria Control Program (NMCP) for the home management of presumed malaria (HMM) in children under 5 years of age. *P. malariae* clearance from the blood is known to take longer than that of other species after chloroquine treatment[16] and two cases of resistance have been documented in south Sumatra, Indonesia.[17]

Recent WHO guidelines for the testing of therapeutic efficacy have not yet been applied to *P. malariae* infection. We therefore report here the results of a clinical study carried out at six sites in 2006. The aims of this study were to: (i) update our knowledge of the burden of *Plasmodium malariae* infections and (ii) assess the therapeutic efficacy of CQ for uncomplicated quartan malaria.

This clinical study, approved by the National Ethics Committee of the Ministry of Health and Family Planning of Madagascar (N° 102-SANPF-2006), was conducted in 2006, at six primary health centers and assessed the therapeutic efficacy of the main antimalarial drugs used in Madagascar. All patients with fever or a history of fever in the 48 h before their arrival at the health center were screened with the rapid diagnostic test (RDT) based on the detection of *Plasmodium*-specific lactate dehydrogenase (pLDH) (OptiMAL-IT™, DiaMed AG©, Cressier sur Morat, Switzerland). Giemsa-stained thin and thick blood films were prepared for each patient with a positive RDT result. A microbiologist differentiated between the different *Plasmodium species* and parasitemia was assessed. Patients with histologically confirmed *P. malariae* infection were enrolled according to inclusion criteria adapted from the WHO guidelines (2001)[18] for *P. vivax* therapeutic efficacy tests. Informed consent was obtained from all adults and from at least one parent for minors. Patients were then treated with the standard chloroquine regimen of 25 mg/kg over 3 days and followed for 28 days. Clinical examination, including axillary temperature recording and the screening of thick and thin films, was carried out on days 0, 1, 2, 3, 7, 14, 21, and 28. On these days, capillary blood was spotted onto filter paper for PCR confirmation. Hemoglobin concentration was measured on days 0 and 28.

DNA was extracted from blood spots with Instagene® Matrix resin (BioRad©, Marnes la Coquette, France), according to the manufacturer's instructions. The parasite species was confirmed by real-time PCR, using species-specific primers as described by de Monbrison,[19] with a protocol adapted to the RotorGene® 3000 thermocycler (Corbett Life Science®, Sydney, Australia).

From January to October 2006, 6,692 patients were screened: 66.8% tested negative for malaria. Of the patients whose test results indicated the presence of malaria, 91.9% tested positive for *P. falciparum* and 8.1% for other *Plasmodium* species.

Microscopy results confirmed by species-specific real-time PCR showed that 86.9% of the patients infected with non-*falciparum* species were infected with *P. vivax* and 13.1% were infected with *P. malariae*. None of the patients tested positive for *P. ovale*. *Plasmodium malariae* infection accounted for 0% (Ihosy) to 2.0% (Maevatanana) of all malaria cases, depending on the site considered (Figure 1).

Clinical and parasitological monitoring was complete for all (16 of 16) *P. malariae*-infected patients until day 28. There

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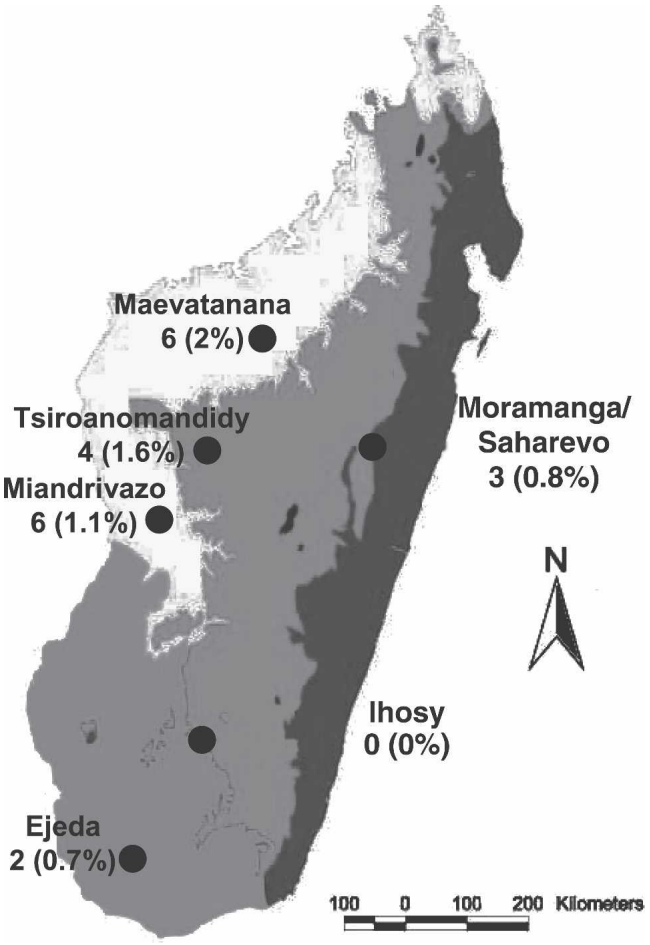

FIGURE 1. Distribution and prevalence of *P. malariae* infections at six sites in Madagascar from January to October 2006.

were six male and 10 female patients (62.5%), aged from 1 to 25 years (median 4.5), with a median weight of 10.5 kg (range 5–63). All patients had had fever during the past 48 hours and none declared having taken antimalarial drugs. Neither microscopy nor real-time PCR showed mixed infections (*P. malariae* with other species). The geometric mean of asexual parasite count was 947/μL (range 250–14,000) at baseline. None of the patients had detectable gametocytes on microscopy at day 0 or during the time of follow-up. Mean hemoglobin concentration was 9.1 g/dL on day 0, and the mean increase in hemoglobin concentration observed on day 28 (mean of individual increases in Hb) was 1.0 g/dL.

Fifteen patients successfully cleared *P. malariae* parasitemia after CQ treatment. One case was excluded on day 28 because *P. falciparum* parasites were detected on a blood smear (confirmed by real-time PCR). Microscopy showed parasite clearance before day 7 in all patients. However, real-time PCR showed parasites to be present in the blood, as described for diagnosis in one patient, and this patient displayed clearance between days 7 and 14 (Figure 2).

Our data confirm that *P. malariae* is the third leading cause of malaria in Madagascar after *P. falciparum* and *P. vivax*. No *P. ovale* parasites were observed due to the very low prevalence of this specie in Madagascar.[20] The prevalence of *P. malariae*, estimated at 1.1% of all malaria infections, was higher in the Western and the Central Highlands areas. This prevalence was most probably underestimated by using RDT based on pLDH detection for malaria diagnosis. According to our previous study,[21] its sensibility was estimated at 88.9–100% for parasitemias between 500 to 5000 non-*P. falciparum* parasites/μL and at 50.0% when the number of parasites detected with microscopy falls below 100 parasites/μL. Previous studies also reported a heterogeneous distribution of this specie according to season, transmission rate, and location: 1% of malaria infections in Analamiranga (western foothill area of the Highlands)[20] to 8.5% in Andasibe (eastern foothill area of the Highlands) and 12.3% at Sainte Marie (island east of the main island of Madagascar).[22,23]

Our study provides the first demonstration that CQ is highly effective against uncomplicated *P. malariae* infection in Madagascar. Three days of CQ treatment gave a clinical and parasitological cure rate of 100% by day 28 and a mean increase in hemoglobin concentration of 1 g/dl over the study period, whereas CQ is only effective in 55.6% of cases of uncomplicated *P. falciparum* malaria and in 89.7% of *P. vivax* malaria cases.[24] The NMCP has revised its treatment policy since December 2005, replacing CQ with artemisinin-based combination therapy (AQ+AS, artesunate plus amodiaquine), with the support of the Global Fund.[25] However, ACT treatment is used in only 31 of the 111 health districts in

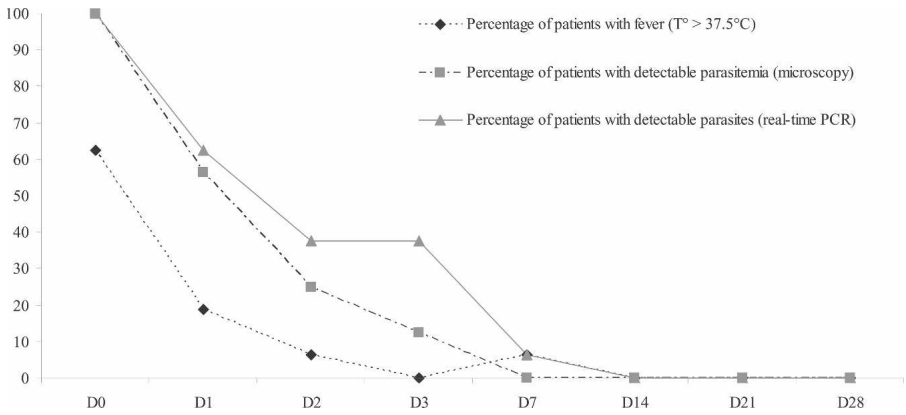

FIGURE 2. Parasites and fever clearance during the 28 days of patient follow-up. Parasite species were distinguished by microscopy and real-time PCR.

Eastern Madagascar. CQ remains the drug most widely available (distribution and financial criteria) and is the first drug used in most of areas in Madagascar, particularly in the prepackaged PaluStop® form, sold at an affordable price (US $0.025), or as Ody Tazomoka®, distributed free at primary public health facilities.[26] Obviously, with the progressive implementation of the artemisinin-based combination therapy, which is effective in treating malaria infections,[27] an approach that does not consider *Plasmodium* species seems to be the most suitable politic treatment at the primary healthcare facilities level in a country where material and human resources to perform a *species*-specific diagnosis are lacking.

In conclusion, our findings demonstrate that oral CQ, which is commonly used, remains highly effective in patients with uncomplicated *P. malariae* infection, the third leading cause of malaria in Madagascar.

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REFERENCES

1. Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, Tulloch S, Oldfield FS, Hayes R, 1987. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Trans R Soc Trop Med Hyg 81:* 478–486.
2. Molineaux L, Storey J, Cohen JE, Thomas A, 1980. A longitudinal study of human malaria in the West African Savanna in the absence of control measures: relationships between different *Plasmodium species*, in particular *P. falciparum* and *P. malariae. Am J Trop Med Hyg 29:* 725–737.
3. Smith T, Charlwood JD, Kihonda J, Mwankusye S, Billingsley P, Meuwissen J, Lyimo E, Takken W, Teuscher T, Tanner M, 1993. Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Trop 54:* 55–72.
4. Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, Pereira Da Silva L, 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg 51:* 123–137.
5. Breman JG, Egan A, Keusch GT, 2001. The intolerable burden of malaria: a new look at the numbers. *Am J Trop Med Hyg 64:* iv–vii.
6. Greenwood B, Marsh K, Snow R, 1991. Why do some African children develop severe malaria? *Parasitol Today 7:* 277–281.
7. Kawamoto F, Liu Q, Ferreira MU, Tantular IS, 1999. How prevalent are *Plasmodium ovale* and *P. malariae* in East Asia? *Parasitol Today 15:* 422–426.
8. Zhou M, Liu Q, Wongsrichanalai C, Suwonkerd W, Panart K, Prajakwong S, Pensiri A, Kimura M, Matsuoka H, Ferreira MU, Isomura S, Kawamoto F, 1998. High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in malaria patients along the Thai-Myanmar border, as revealed by acridine orange staining and PCR-based diagnoses. *Trop Med Int Health 3:* 304–312.
9. Werndorfer WH, MacGregor I, 1998. *Malaria: Principles and Practice of Malariology.* London: Churchill Livingstone.
10. Abdurrahman MB, Aikhionbare HA, Babaoye FA, Sathiakumar N, Narayana PT, 1990. Clinicopathological features of childhood nephrotic syndrome in northern Nigeria. *Q J Med 75:* 563–576.
11. Kibukamusoke JW, 1986. The hazard of malarial nephropathy. *Parasitol Today 2:* 119–121.
12. Otieno LS, Mc'Ligeyo SO, 1988. Review article: immune nephritides due to malaria. *East Afr Med J 65:* 402–406.
13. Institut National de la Statistique de Madagascar, 2002. *Enquête Auprès des Ménages*. Antananarivo: Institut National de la Statistique de Madagascar, 71–83.
14. Jeremiah M, Sulhuan A, 2004. Mortalité des enfants de moins de 5 ans à Madagascar. *Enquête Démographique et de la Santé III à Madagascar 2004:* 191–201.
15. UNICEF. Multiple Indicator Cluster Survey (MICS), 2000. Available at: http://www.childinfo.org/MICS2/newreports/madagascar/madagascar.pdf.
16. Collins WE, Jeffery GM, 2002. Extended clearance time after treatment of infections with *Plasmodium malariae* may not be indicative of resistance to chloroquine. *Am J Trop Med Hyg 67:* 406–410.
17. Maguire JD, Sumawinata IW, Masbar S, Laksana B, Prodjodipuro P, Susanti I, Sismadi P, Mahmud N, Bangs MJ, Baird JK, 2002. Chloroquine-resistant *Plasmodium malariae* in south Sumatra, Indonesia. *Lancet 360:* 58–60.
18. World Health Organization, 2001. Monitoring Antimalarial Drug Resistance. Report of a WHO consultation (WHO/CDS/CSR/EPH/2002.17; WHO/CDS/RBM/2002.39). December 3–5, 2001; Geneva, Switzerland.
19. de Monbrison F, Angei C, Staal A, Kaiser K, Picot S, 2003. Simultaneous identification of the four human *Plasmodium species* and quantification of *Plasmodium* DNA load in human blood by real-time polymerase chain reaction. *Trans R Soc Trop Med Hyg 97:* 387–390.
20. Robert V, Le Goff G, Andrianaivolambo L, Randimby FM, Domarle O, Randrianarivelojosia M, Raharimanga V, Raveloson A, Ravaonjanahary C, Ariey F, 2006. Moderate transmission but high prevalence of malaria in Madagascar. *Int J Parasitol 36:* 1273–1281.
21. Ratsimbasoa A, Randriamanantena A, Raherinjafy R, Rasoarilalao N, Menard D, 2007. Which malaria rapid test for Madagascar? Field and laboratory evaluation of three tests and expert microscopy of samples from suspected malaria patients in Madagascar. *Am J Trop Med Hyg 76:* 481–485.
22. Lepers JP, Fontenille D, Rason MD, Raharimalala L, Coulanges P, 1990. Malaria in 1989 in a village in the Highland Plateaux of Madagascar. Parasitologic and clinical data obtained in a longitudinal study of a population representative of this region. *Arch Inst Pasteur Madagascar 57:* 11–52.
23. Lepers JP, Rason MD, Raharimalala L, Rabarison P, Rene JP, Coulanges P, 1990. Malaria in the island of Sainte Marie in 1989. Epidemiologic, parasitologic, serologic and clinical data. *Arch Inst Pasteur Madagascar 57:* 53–74.
24. Menard D, Nina Harimanana Andrianina N, Ramiandrasoa Z, Randriamanantena A, Rasoarilalao N, Jahevitra M, Tuseo L,

Raveloson A, 2007. Randomized clinical trial to assess artemisinin versus non-artemisinin combination therapy for uncomplicated falciparum malaria in Madagascar. *Malar J 6:* 65.
25. Service de Lutte contre le Paludisme, 2005. Politique Nationale de Lutte contre le Paludisme. Ministère de la Santé et du Planning Familial: Antananarivo, Madagascar.
26. Ratsimbasoa A, Randrianarivelojosia M, Millet P, Soares JL, Rabarijaona L, Rakotoson B, Malvy D, Menard D, 2006. Use of pre-packaged chloroquine for the home management of presumed malaria in Malagasy children. *Malar J 5:* 79.
27. Tangpukdee N, Thanachartwet V, Krudsood S, Luplertlop N, Pornpininworakij K, Chalermrut K, Phokham S, Kano S, Looareesuwan S, Wilairatana P, 2006. Minor liver profile dysfunctions in *Plasmodium vivax, P. malaria* and *P. ovale* patients and normalization after treatment. *Korean J Parasitol 44:* 295–302.