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Frequent and complex relapses confound assessment of chloroquine resistance in Cambodian Plasmodium vivax

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Background: *Plasmodium vivax* resistance to chloroquine has been observed in several endemic countries. In Cambodia, up to 17% clinical treatment failure following 3-days standard chloroquine treatment was reported in vivax malaria patients in 2009. The loss of chloroquine efficacy was solely described in northeast area of Cambodia while chloroquine seemed to remain fully effective in other provinces. This led to the withdrawn of chloroquine and its replacement by dihydroartemisinin-piperaquine in 2012. To rigorously assess the extent of *P. vivax* chloroquine-resistance in Cambodia, we conducted a comprehensive therapeutic efficacy study with extensive genotyping of the parasites.

Methods & Materials: The study was conducted in Rattanakiri, in northeastern Cambodia in 2014. 40 enrolled patients were treated with chloroquine (30 mg/kg) for three days and followed for two months. Reinfection was controlled for half of the patients by relocating them to a no-transmission area. The 2-months follow-up consisted in frequent clinical examination and capillary blood collection for microscopic, molecular parasite detection and drug concentration measure. The entire genomes of the initial and recurrent parasites were sequenced and complemented by genotyping of more than 100 SNPs for each PCR-positive blood samples collected during follow-up.

Results: Recurrences occurred in 24/40 (60%) patients within the follow-up. No difference was observed between relocated and non-relocated patients. Recurrent parasites were always detected when chloroquine concentration in blood was below therapeutic level. Genotyping revealed that all *P. vivax* clones, within a given infection, responded similarly to CQ. In addition, whole genome sequencing unambiguously showed that most relapsing parasites were different from those in the initial infections.

Conclusion: Recurrences within two months are frequent among Cambodian vivax malaria patients and originate from relapsing parasites from the liver. Pharmacological and genetic analyses revealed no evidence of CQ resistance and suggest that CQ is fully effective against *P. vivax* episodes in Rattanakiri Cambodia. Our results suggest that CQ resistance might be over-diagnosed and confounded with relapses from liver parasites. Our clinical and analytical framework has the potential to differentiate between relapse and resistance and should be implemented in vivax malaria endemic areas with suspected drug resistance.

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Novel vectors of the zoonotic malaria parasite, Plasmodium knowlesi, in two districts of Sarawak, Malaysian Borneo

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Background: The zoonotic malaria parasite, *Plasmodium knowlesi* (*P. knowlesi*), is the most common cause of human malaria in Sarawak, Malaysian Borneo. It accounted for over 80% of hospitalised malaria cases from 2014-2016. Previously identified vectors of the parasite in nature in Malaysia and Vietnam all belong to the *Anopheles* Leucosphyrus Group. Only one study on vectors of *P. knowlesi* has been conducted in Sarawak that incriminated *Anopheles latens* (*An. latens*) as the vector in the Kapit District. This project was therefore undertaken to identify malaria vectors in other districts of Sarawak.

Methods & Materials: Human landing catches were conducted in forested sites of the Betong and Lawas Districts. The salivary glands of anophelines were removed, DNA was extracted and screened with nested PCR assays for *Plasmodium* and species of *Plasmodium*. The sequences of the small sub-unit ribosomal RNA (SSUrRNA) genes of *Plasmodium* spp. and the internal transcribed spacer 2 (ITS2) region and mitochondrial cytochrome c oxidase subunit 1 (CO1) gene of the mosquitoes were derived from the *Plasmodium*-positive samples.

Results: Collectively, 237 anophelines and 2,128 culicines were caught. *An. letifer* (44.8%, n = 172) and *An. balabacensis* (47.7%, n = 65) were found to be the predominant anophelines in Betong and Lawas districts, respectively. By PCR, 26 anophelines were found to be infected with *P. knowlesi* and other simian *Plasmodium* species (*P. coatneyi, P. cynomolgi, P. fieldi* and *P. inui*) while phylogenetic analysis of the SSUrRNA genes confirmed the presence of those malaria parasites in 3 *An. barbirostris*, 6 *An. balabacensis*, 6 *An. latens* and 6 *An. letifer.* Phylogenies inferred from the ITS2 and CO1 sequences of *An. balabacensis* and *An. barbirostris* indicate that the former is genetically indistinguishable from *An. balabacensis* in Borneo while the latter is a sibling species of the Barbirostris Subgroup. Preliminary phylogenetic analysis of the CO1 gene of *An. letifer* suggests that they are distinct, but closely related to the *An. letifer* from Singapore.

Conclusion: New vectors of *P. knowlesi* were identified in Betong (*An. latens* and *An. letifer*) and Lawas (*An. barbirostris* and *An. balabacensis*), including 2 species (An. *barbirostris* and *An. letifer*) which are not from the *Anopheles* Leucosphyrus Group.

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