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Two cases of recurring ovale malaria in Sarawak, Malaysia after successful treatment of imported *P. falciparum* infection

Running head: Recurrent ovale malaria after falciparum malaria

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Abstract

Here are two cases of recurring ovale malaria in Sarawak, Malaysia, that are likely relapses which occurred 1 – 2 months after successful treatment of the initial imported falciparum malaria with artemisinin-based combined therapy (ACT). The patients have no history or recollection of previous malaria episodes. These cases add to the little evidence of the relapsing nature of *Plasmodium ovale*, after a febrile episode. In regions where *P. ovale* is not known to be autochthonous, active follow-up of treated imported malaria patients is highly recommended following their return, particularly to areas nearing or having achieved elimination.

Case Report

A 51-year-old Chinese male presented with fever, headache, rigor and nausea 3 days after returning to Sarawak, Malaysia from Liberia in June 2017 (Case 1). He had been working in the timber industry for one and a half years in Liberia. Blood films for malaria parasites (BFMP) indicated falciparum infection with 1294 asexual parasites/μL of blood. Riamet® (artemether/lumefantrine) was prescribed for 3 days followed by a dose of primaquine for one day. Treatment with ACT reduced the parasite density to 80 parasites/μL of blood one day later. Parasites were not found during follow-ups until he experienced fever on Day 26 after ACT. On Day 28 of follow-up, BFMP was positive for *P. ovale* with 5822 asexual parasites/μL and 118 gametocytes/μL of blood. He was subsequently treated with Riamet® for 3 days and primaquine for 14 days. The patient was cured, with no parasites observed in the blood films for up to 5 months after treatment. During his stay in Sarawak, he had only travelled to non-receptive areas.

Another case (Case 2) was recorded on November 29, 2017 as an imported falciparum malaria in a patient returning from Equatorial Guinea with parasite density of 4123 asexual parasites/μL of blood. The patient was treated as above in Case 1. On January 24, 2018, approximately 2 months after the first malaria episode, *P. ovale* was detected in the blood smears of this patient. The parasite density was 515 asexual parasites/ and 68 gametocytes/μL of blood. Treatment with Riamet® and primaquine was similarly given as in Case 1. Within this period of time, the patient resided in a non-receptive area in Sarawak and had no travel history to any receptive area.

Microscopic examination of Giemsa-stained blood smears, yielded a diagnosis of imported *Plasmodium falciparum* infection and relapse of *P. ovale* for both cases. A retrospective nested polymerase chain reaction (PCR) assay targeting the malaria parasites' 18S small-subunit rRNA gene,[1, 2] were performed on DNA extracted from bloodspots collected on admission (on the day following ACT administration) and recurrence episodes for both cases. These confirmed falciparum malaria on admission and infection with *P. ovale wallikeri* in the recurrence episode for Case 1, though that of Case 2 was negative. This is the second report of an imported *P. ovale wallikeri* infection in Malaysia.[3]

Discussion

There are increasing reports of ovale malaria, caused by *P. ovale wallikeri* and *P. ovale curtisi*, among travellers to sub-Saharan Africa, Indonesia, Papua New Guinea and India.[4] *Plasmodium ovale* is believed to be capable of causing relapses (due to liver hypnozoites) months to years after the primary infection.[5] Furthermore, co-infections of *P. ovale* with other malaria species, especially *P. falciparum* are

frequent in malaria endemic areas.[6] Two cases similar to those presented here were recently reported.[7, 8] The patients were admitted in Brazil and Canada, respectively, where there is no ovale malaria transmission. Recurrent ovale infections following successful treatment of falciparum malaria, could be a new infection, a recrudescence, a relapse or a delayed primary attack (the last two derived from hypnozoites).

A new infection of *P. ovale* is highly unlikely in the two cases reported here because *P. ovale* is not known to be autochthonous in Malaysia, including Sarawak.[3] Furthermore, a retrospective PCR screening of 44 bloodspots collected between 2015 – 2017 from microscopically determined *Plasmodium vivax*-infected patients in Sarawak (a parasite species that is morphologically similar to *P. ovale*), showed only 1 misdiagnosed imported ovale malaria case from Gabon (Y.L. Lau, unpublished data). Therefore, the possibility of misdiagnosing ovale malaria as vivax malaria in Sarawak is low.

Co-infections are frequent in endemic areas though *Plasmodium malariae* and *P. ovale* whose, parasitemias are often very low or below the detection limit are easily overlooked microscopically,[6, 9, 10] and are often found with *P. falciparum* following PCR detection.[11, 12] For Case 1, a patent co-infection on admission is unlikely because only *P. falciparum* was detected by the PCR assay, and only *P. ovale wallikeri* in the recurrent episode sample. It is unfortunate that the sample from Case 2 failed to amplify, most likely due to degradation of the DNA or maybe because of sequence polymorphism at specific primer binding site. A co-infection on admission would imply that ACT administration, which has effectively eliminated *P. falciparum*, failed to clear *P. ovale*. This is unlikely as ACT is considered highly

efficacious for non-falciparum malaria,[13] moreover the recurrent *P. ovale* episodes in the two cases presented here were rapidly cleared by the ACT administered.

Thus, the recurring ovale malaria in both cases are most likely relapses from hypnozoites even though the patients did not recall any previous clinical malaria episodes. The timing of these relapses is consistent with previously observed median times between a primary attack of *P. ovale* and first relapse of 17 weeks (range 2 – 60 weeks).[4] Our observations are also consistent with the suggestion that hypnozoite activation to cause relapse may be induced by fever due to a bacterial, viral or malarial infection.[7, 14, 15] Despite the paucity of observations, as compared to those of *P. vivax* malaria, suggestions that *P. ovale* does not produce hypnozoites are likely to be incorrect.[4, 16] In a recent report, convincing evidence for relapse in *P. ovale* infection showed that the re-appearing *P. ovale* parasites following supervised treatment of a patient in a non-endemic region were of the same genotype as that of the parasites found in the primary episode.[17] A delayed primary attack originating from hypnozoites, as described for some *P. vivax* malaria strains from temperate regions cannot be formally discounted.[14] However, in previous reports of such delayed attacks, anti-malarial prophylaxis during initial exposure was noted, and a clinically mild primary episode could not be excluded.

Clinical, parasitological and biological investigations on hypnozoites and relapses are restricted by logistical and ethical considerations, yet this phenomenon is of importance in the context of malaria control and elimination strategies. The two cases reported here add to the small number of such observations on *P. ovale* and provide evidence to support the relapsing nature of this parasite species. For patients returning from ovale endemic areas, and in particular to regions where malaria transmission is possible, clinicians should keep in mind the possibilities of

co-infection or pre-patent infection with *P. ovale*, especially as prophylactic medications have little effect on the hypnozoite.[18] Provided adequate screening for glucose-6-phosphate dehydrogenase, primaquine could be considered for routine administration to those presenting with malaria on their return from endemic areas, though cases of primaquine failure to prevent ovale relapses have been reported.[4] Ultimately, it will be important to maintain an active follow-up for at least three months for patients returning from areas endemic for relapsing malaria species.[8]

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