Plasmodium knowlesi infection in an Australian soldier following jungle warfare training in Malaysia

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Abstract:

An Australian Army soldier developed a fever after returning to Australia following a three-month deployment to Rifle Company Butterworth, Malaysia. Ten days prior to presentation he had participated in jungle warfare training at Burma Camp, Pulada in Johor, Malaysia. No known direct contact was made with monkeys, which were known to be in the area. Earlier he had stopped his doxycycline chemoprophylaxis for a few days during an episode thought to be due to gastroenteritis. Blood examination showed infrequent *Plasmodium* species ring-stage trophozoites on thick film. As the ring-stage trophozoites were morphologically different from *P falciparum*, blood was sent to the ADF Malaria and Infectious Disease Institute at Gallipoli Barracks for species confirmation. The soldier was treated with oral artemether-lumefantrine (Novartis, Coartem®) and became aparasitemic in two days. Subsequent nucleic acid studies confirmed the diagnosis as *Plasmodium knowlesi*, a parasite of macaques. Two weeks following treatment he was admitted to the hospital for glandular fever but was shown not to have had a recrudescence of his original infection.

Keywords: malaria, *Plasmodium knowlesi*, military training, chemoprophylaxis

Malaria risk to Australian soldiers in Southeast Asia is remarkably low compared to previous generations, but low risk is not equivalent to zero risk. Febrile episodes in soldiers returning from the tropics still need to be considered as possible malaria infections, as demonstrated in the following case report. A 21-year-old Australian soldier presented on 1 March 2018 to Edinburgh Health Centre at RAAF base, Edinburgh, having been recalled due to the results of a blood test from a week prior while in Malaysia. His original illness had been an episode of gastroenteritis following jungle warfare training at Burma Camp, Pulada, Johor, 26 January to 8 February 2018. On 16 February, the soldier had noted the onset of fatigue and gastroenteritis. On 20 February, he presented to sick parade at Butterworth Health Centre and was told to stop his doxycycline as it was likely making his gastroenteritis worse. He was reviewed on 23 February, with his gastroenteritis resolving but continuing fatigue; at this time, he was afebrile. Blood was drawn for investigations showing mild thrombocytopenia (73 000/mm3) and mild transaminase elevation (<2x upper limit of normal range). By 26 February, he was again reviewed, complaining of continued fatigue and nocturnal fever; his temperature was 37.5° in the clinic. The working diagnosis was a non-specific viral illness. As the soldier was unable to further participate in unit activities, he was scheduled to fly back to Australia on 28 February when further blood was drawn including a malarial smear. He had also resumed his doxycycline chemoprophylaxis at that time.

A blood film taken 28 February in Malaysia was subsequently reported to have contained rare malaria parasites thought to be *Plasmodium knowlesi*. When recalled on 1 March, the soldier was still taking his malaria chemoprophylaxis of doxycycline 100 mg daily. The eradication course of 30 mg primaquine daily for 14 days was not started until 2 March 2018. Since he was not ill and further blood tests were pending, it was decided to await either a confirmatory blood test or any fever prior to specific treatment. Blood taken on 2 March was subsequently shown to have rare malaria ring-stage trophozoites thought on morphological grounds not to be *P falciparum*. See Figure 1.

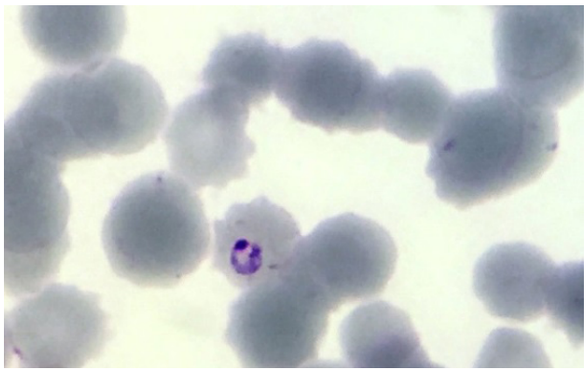

Figure 1: Giemsa stained blood film showing a ring form trophozoite of Plasmodium knowlesi *from the soldier taken on 2 March 2018.*

That same evening the soldier developed a fever to 38.9° and was started on oral artemether-lumefantrine (Novartis, Coartem®) to receive 6 doses over 60 hours. His symptoms rapidly resolved and his blood was free of parasites by day four post treatment. Two weeks following his treatment for malaria, he returned to the clinic with fever to 38° and feeling unwell as noted by fever and myalgia. Because of his history of tropical infection, he was admitted to the infectious disease service of the Royal Adelaide Hospital. Repeated blood films showed no parasites confirming that his malaria had not recrudesced after treatment. Serological evidence (IgM and IgG positive) of Epstein Barr virus was noted but no other diagnoses were confirmed despite extensive investigations. Polymerase chain reactions done at the ADF Malaria and Infectious Disease Institute from samples taken on both 2 and 3 March showed identity using *P knowlesi* primers as shown in Figure 2. To our knowledge, this is the first known case of *P knowlesi* to be confirmed in an ADF soldier.

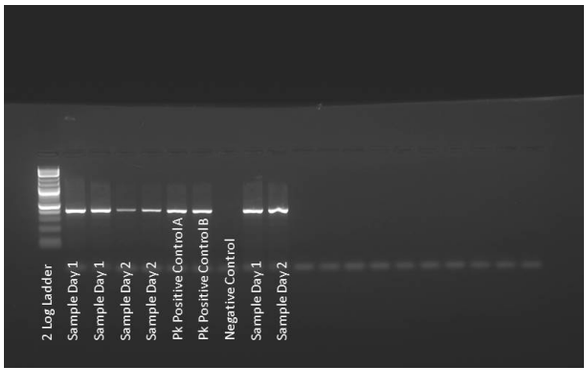

Figure 2: Photo of gel electrophoresis of products of polymerase chain reaction showing identity with Plasmodium knowlesi. *Lanes labelled as marked on photo with samples from 2 and 3 March.*

Figure 3: Photo of Macaca fascicularis, *the long-tailed or crab eating macaque, which is the most common reservoir host of* Plasmodium knowlesi. *Photo from Wikipedia 2007 by André Ueberbach under Creative Commons Attribution*

Plasmodium knowlesi is a species of primate malaria usually found in long-tailed and other macaques of South and Southeast Asia as shown in Figure 3.[1] Unlike *P falciparum*, *P knowlesi* has a 24-hour growth cycle that allows it to rapidly grow to high parasitaemia. This rarely happens in its natural host, the long-tailed macaque, but in the Indian or rhesus macaque, a lethal hyperparasitaemia rapidly occurs.[2] Although originally found to infect humans after natural exposure in Malaysia in 1960s, this was thought to be a very rare event.[3] In the 2000s, in other states of Malaysia (Sabah, Sarawak), numerous human malaria cases thought to be due to *P malariae* on morphological grounds, were subsequently shown by genomic analysis to be *P knowlesi.*[4] These infections were typically of adult men who worked in the jungle and if not rapidly treated could result in death. Unlike *P falciparum*, severe *P knowlesi* does not present as cerebral malaria; its most common severe forms being due to anaemia, acute kidney injury or pulmonary failure.[5] *P knowlesi* is uniformly drug sensitive as its primate reservoir hosts do not receive antimalarial drugs.[6] Chloroquine is a standard treatment, but artemisinin combination therapy is now preferred either as artemether-lumefantrine for oral use or intravenous artesunate if the patient cannot tolerate oral medication.[7]

Because of the success of malaria control and the near elimination of ordinary malaria from Malaysia, there are very few human malaria infections remaining. Most plasmodial infections in Malaysia are now due to *P knowlesi.* There are 1000–3000 reported infections each year of *P knowlesi* in East Malaysia, but in Peninsular Malaysia it remains a relatively unusual infection.[8] The changing ecology of Malaysia with extensive conversion of the natural jungle habitat of the monkeys into palm oil plantations has caused macaques to move into areas

where they may be more frequently in proximity to humans. The anopheline vectors are not the usual ones associated with human malaria in Southeast Asia such as *An minimus* and *An dirus* which are usually forest fringe mosquitoes. *An leucosphyrus* and related species are thought to be the main vector of *Plasmodium knowlesi.* These mosquitoes are more frequently found inside the jungle rather than the forest fringe which likely explains why this zoonotic infection is associated with jungle warfare training.[9] Epidemiological risk factors for *P knowlesi* infection include males >15 years of age, plantation workers who sleep outside buildings or whose houses have open eaves, as well as living in areas where monkeys are known to reside.[10] The soldier's particular exposure was very likely during night-time jungle warfare training operations in Johor, Malaysia, where the reservoir monkeys are known to exist.

Prevention of *P knowlesi* is not different from preventing other less exotic malarial infections. Mosquito avoidance measures such as repellents and nets are often not practical during jungle warfare training. Reliance has to be placed on malaria chemoprophylaxis, typically consisting of either daily doxycycline or atovaquone-proguanil. Doxycycline has the added advantage of effectiveness against scrub typhus and leptospirosis which are both infectious disease threats during jungle warfare training. Gastrointestinal disturbances or inability to eat during survival training does not favour doxycycline and thus there are times when atovaquone-proguanil will be preferred. The recent registration of tafenoquine, a long acting 8-aminoquinoline similar to primaquine, has now provided the ADF with an additional chemoprophylaxis option. Compliance with chemoprophylaxis in areas thought to have low malaria risk is often suboptimal. ADF Medical Officers need to be aware that deployments of soldiers to Malaysia, especially those conducting jungle warfare training in areas known to have resident macaque populations have the occasional risk of *P knowlesi* infection which should reinforce the need for compliance with force health protection measures.

Contributors:

KR and AT were the medical officers responsible for the clinical case, GDS was responsible for initiating this report as well as the writing of the first draft of the manuscript. KL was responsible for malaria blood film diagnosis and SD did the polymerase chain reactions. All authors participated in writing the final manuscript.

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References:

1. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet. 2004;363(9414):1017–24.
2. Assefa S, Lim C, Preston MD, Duffy CW, Nair MB, Adroub SA, et al. Population genomic structure and adaptation in the zoonotic malaria parasite Plasmodium knowlesi. Proc Natl Acad Sci USA. 2015;112(42):13027–32.
3. Fong YL, Cadigan FC, Coatney GR. A presumptive case of naturally occurring Plasmodium knowlesi malaria in man in Malaysia. Trans R Soc Trop Med Hyg. 1971;65(6):839–40.
4. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46(2):165–71.
5. Rajahram GS, Barber BE, William T, Grigg MJ, Menon J, Yeo TW, et al. Falling Plasmodium knowlesi Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010-2014. Emerg Infect Dis. 2016;22(1):41–8.

6. Barber BE, Grigg MJ, William T, Yeo TW, Anstey NM. The Treatment of Plasmodium knowlesi Malaria. Trends Parasitol. 2017;33(3):242–53.
7. Grigg MJ, William T, Barber BE, Rajahram GS, Menon J, Schimann E, et al. Artemether-Lumefantrine Versus Chloroquine for the Treatment of Uncomplicated Plasmodium knowlesi Malaria: An Open-Label Randomized Controlled Trial CAN KNOW. Clin Infect Dis. 2018;66(2):229–36.
8. William T, Rahman HA, Jelip J, Ibrahim MY, Menon J, Grigg MJ, et al. Increasing incidence of Plasmodium knowlesi malaria following control of P. falciparum and P. vivax Malaria in Sabah, Malaysia. PLoS Negl Trop Dis. 2013;7(1):e2026.
9. Vythilingam I, Wong ML, Wan-Yussof WS. Current status of Plasmodium knowlesi vectors: a public health concern? Parasitology. 2018;145(1):32–40.
10. Grigg MJ, Cox J, William T, Jelip J, Fornace KM, Brock PM, et al. Individual-level factors associated with the risk of acquiring human Plasmodium knowlesi malaria in Malaysia: a case-control study. Lancet Planet Health. 2017;1(3):e97–e104.