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Molecular Epidemiological Investigation of *Plasmodium knowlesi* in Humans and Macaques in Singapore

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Abstract

Singapore reported its first locally acquired human *Plasmodium knowlesi* infection in 2007, involving a soldier who had undergone training in a forested area where long-tailed macaques are frequently seen. Comprehensive disease surveillance and monitoring system that was set up after the initial case detected four additional human *P. knowlesi* cases in 2007 and one in 2008. All involved military personnel who had undergone training in the forested area, and none had traveled out of Singapore 1 month before the onset of symptoms. Screening for malaria parasites on blood obtained from long-tailed macaques revealed that wild monkeys ($n = 3$) caught from the forested area were infected with *P. knowlesi*, whereas peri-domestic monkeys ($n = 10$) caught from a nature reserve park were not infected with any malaria parasites. Phylogenetic analysis of the nonrepeat region of the *P. knowlesi csp* genes showed that the sequences obtained from the human cases were not distinct from those obtained from wild monkeys. Further, certain genotypes were shared between samples from humans and macaques. Our findings provide evidence that long-tailed macaques are the natural hosts of *P. knowlesi* in Singapore and the human cases acquired their infection in the same vicinity where these monkeys are found. Further, the risk of acquiring *P. knowlesi* infection among the general population of Singapore is small as evident from the absence of *P. knowlesi* in peri-domestic monkeys.

Key Words: Singapore—*Plasmodium Knowlesi*—long-tailed macaques—Circumsporozoite genes.

Introduction

Plasmodium knowlesi was first identified in India in 1931 from a long-tailed macaque (*Macaca fascicularis*) imported from Singapore (Knowles and Das Gupta 1932). Its ability to infect humans was first described in 1932, when Knowles and Das Gupta successfully transmitted the parasite to two human volunteers by blood passages from infected macaques. However, the first natural human infection of *P. knowlesi* was only reported in 1965 in an American army surveyor who had acquired the disease while working in the jungle in the state of Pahang, Malaysia (about 300 km north of Singapore) (Chin et al. 1965). This was followed by a presumptive case reported from the state of Johor, Malaysia, which is adjacent to the island of Singapore (Yap et al. 1971). Human infections were thought to be rare until a large focus of humans infected with *P. knowlesi* were identified by nested polymerase chain reaction (PCR) detection assays in Sarawak, Malaysian Borneo, in 2004 (Singh et al. 2004). Since then, cases of *P. knowlesi* infections in humans have been reported in other parts of Malaysia, China, Thailand, Singapore, and Philippines (Jongwutiwes et al. 2004, Zhu et al. 2006, Cox-Singh et al. 2008, Luchavez et al. 2008, Ng et al. 2008, Vythilingam et al. 2008), resulting in knowlesi being recognized as the first *Plasmodium* sp. implicated in zoonotic disease. *P. knowlesi* infections have also been reported from European travellers returning from endemic countries (Kantele et al. 2008, Bronner et al. 2009).

In Singapore, the first reported locally acquired human *P. knowlesi* infection occurred in 2007 and involved a soldier in the Singapore military who had no significant travel history and had trained in a restricted-access forested area in Singapore (Ng et al. 2008). Long-tailed macaques, the natural hosts for *P. knowlesi*, are found in this forested area and also in various nature reserve parks in Singapore that are open to the general public. Comprehensive fever surveillance and monitoring was started among military personnel who had taken part in training exercises in the affected forested area. Five

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