OPEN ACCESS Freely available online

PLoS PATHOGENS

Plasmodium knowlesi: Reservoir Hosts and Tracking the Emergence in Humans and Macaques

Kim-Sung Lee[1], Paul C. S. Divis[1], Siti Khatijah Zakaria[1], Asmad Matusop[2], Roynston A. Julin[1], David J. Conway[3], Janet Cox-Singh[1,4], Balbir Singh[1]*

1 Malaria Research Centre, Faculty of Medicine and Health Sciences, University Malaysia Sarawak, Kuching, Sarawak, Malaysia, **2** Sarawak State Health Department, Kuching, Sarawak, Malaysia, **3** Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom, **4** Centre for Infection, St. George's University of London, London, United Kingdom

Abstract

Plasmodium knowlesi, a malaria parasite originally thought to be restricted to macaques in Southeast Asia, has recently been recognized as a significant cause of human malaria. Unlike the benign and morphologically similar *P. malariae*, these parasites can lead to fatal infections. Malaria parasites, including *P. knowlesi*, have not yet been detected in macaques of the Kapit Division of Malaysian Borneo, where the majority of human knowlesi malaria cases have been reported. In order to extend our understanding of the epidemiology and evolutionary history of *P. knowlesi*, we examined 108 wild macaques for malaria parasites and sequenced the circumsporozoite protein (*csp*) gene and mitochondrial (mt) DNA of *P. knowlesi* isolates derived from macaques and humans. We detected five species of *Plasmodium* (*P. knowlesi*, *P. inui*, *P. cynomolgi*, *P. fieldi* and *P. coatneyi*) in the long-tailed and pig-tailed macaques, and an extremely high prevalence of *P. inui* and *P. knowlesi*. Macaques had a higher number of *P. knowlesi* genotypes per infection than humans, and some diverse alleles of the *P. knowlesi csp* gene and certain mtDNA haplotypes were shared between both hosts. Analyses of DNA sequence data indicate that there are no mtDNA lineages associated exclusively with either host. Furthermore, our analyses of the mtDNA data reveal that *P. knowlesi* is derived from an ancestral parasite population that existed prior to human settlement in Southeast Asia, and underwent significant population expansion approximately 30,000–40,000 years ago. Our results indicate that human infections with *P. knowlesi* are not newly emergent in Southeast Asia and that knowlesi malaria is primarily a zoonosis with wild macaques as the reservoir hosts. However, ongoing ecological changes resulting from deforestation, with an associated increase in the human population, could enable this pathogenic species of *Plasmodium* to switch to humans as the preferred host.

Citation: Lee K-S, Divis PCS, Zakaria SK, Matusop A, Julin RA, et al. (2011) *Plasmodium knowlesi*: Reservoir Hosts and Tracking the Emergence in Humans and Macaques. PLoS Pathog 7(4): e1002015. doi:10.1371/journal.ppat.1002015

Editor: James W. Kazura, Case Western Reserve University, United States of America

Received September 27, 2010; **Accepted** February 8, 2011; **Published** April 7, 2011

Funding: This research was supported by grants from the Wellcome Trust (grant number: 078538/Z/05/Z) (URL: http://www.wellcome.ac.uk/), the Malaysian Ministry of Science, Technolgy and Innovation (URL: http://ernd.mosti.gov.my/escience/) and Universiti Malaysia Sarawak (URL: http://www.unimas.my/). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: bskhaira55@gmail.com

Introduction

Until recently, it was believed that malaria in humans was caused by only four species of parasite (*Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*). However, this perception changed when we discovered a large focus of human infections with *P. knowlesi* in the Kapit Division of Sarawak, Malaysian Borneo [1]. These infections had predominantly been mistakenly identified as *P. malariae* by microscopy, since both species have similar morphological characteristics [1,2]. With subsequent reports of human infections in other parts of Malaysia [3,4], and in Thailand [5,6], Myanmar [7], Singapore [8,9], the Philippines [10], Vietnam [11] and Indonesia [12,13], *P. knowlesi* is now recognized as the fifth species of *Plasmodium* responsible for human malaria. It causes a wide spectrum of disease and can lead to high parasite counts, severe complications and death [3,14]. In a recent study, we found that approximately 1 in 10 knowlesi malaria patients at Kapit Hospital developed potentially fatal complications, comparable to *P. falciparum* malaria, which is considered to be the most virulent type of malaria in humans [14].

P. knowlesi is primarily a simian malaria parasite and was first isolated from a long- tailed macaque (*Macaca fascicularis*) imported to India from Singapore in 1931 [15]. Subsequently, *P. knowlesi* has been detected in wild long-tailed macaques of Peninsular Malaysia [4,16] and the Philippines [17], in pig-tailed macaques (*M. nemestrina*) of Peninsular Malaysia [16] and in banded leaf monkeys (*Presbytis melalophus*) in Peninsular Malaysia [16]. There has been no documented evidence of *P. knowlesi* or any other malaria parasites in monkeys in Malaysian Borneo, and although a monkey source for the hundreds of human *P. knowlesi* infections that have been described in the Kapit Division of Sarawak [1,3,14] appeared likely, it remained to be proven.

Prior to our report in 2004 of the large focus of human infections in Sarawak, Malaysian Borneo, when we utilized molecular methods for characterisation and PCR assays for detection of *P. knowlesi* [1], there had been only one confirmed case of a naturally-acquired *P. knowlesi* infection in a human [18]. That person got infected with *P. knowlesi* while spending a few weeks in the forest of Pahang, Peninsular Malaysia in 1965. It is not known whether the large focus in Malaysian Borneo and subsequent