



Emergence of the Fifth Human Malaria Parasite, *Plasmodium knowlesi*: A New Threat to Public Health?

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Authors' contributions

This work was carried out in collaboration among all authors. Author AB designed the study. Authors SKM and NB managed the analyses of the study and the literature searches. All authors wrote, read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2020/v5i430176

Editor(s):

(1) Dr. Win Myint Oo, SEGi University, Malaysia.

Reviewers:

(1) Anthony Godswill Imolele, Ambrose Alli University, Nigeria.

(2) Parameshwar Hanwathe, Osmania University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63548>

Mini-review Article

Received 28 September 2020

Accepted 03 December 2020

Published 18 December 2020

ABSTRACT

Malaria, a mosquito-borne, protozoan disease is caused by a protozoan parasite of genus *Plasmodium* (Phylum: Apicomplexan). Four species of malaria parasites are recognized to infect humans. However, a fifth species, *Plasmodium knowlesi*, has been reported to show potential zoonotic infection in humans as several cases have been testified throughout South East Asia and on the Nicobar and Andaman Islands of India. The most widespread species of macaque in Southeast Asia i.e. long-tailed macaque (*Macaca fascicularis*) is the natural host for this zoonotic malaria species. Recent case reports have suggested knowlesi malaria are associated with comorbidities which leads to poor treatment outcome. In this review, we have searched the literature from PubMed and Google Scholar and tried to highlight the epidemiology, parasite biology and future challenges regarding this emerging zoonotic infection.

Keywords: *Malaria; plasmodium; P. knowlesi; anopheles.*

1. INTRODUCTION

1.1 The Malaria Parasite: *Plasmodium*

The malaria parasite life cycle involves two hosts: Human and Anopheles mosquito (female). The parasites are transmitted to humans by mosquitoes of the genus *Anopheles*. There are four types of human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. Of all, *Plasmodium falciparum* and *Plasmodium vivax* are the most commonly prevalent parasites. While *P. falciparum* causes a large majority of the clinical cases and mortalities of the four species and if untreated it can lead to fatal cerebral malaria. In cerebral malaria, the parasite-infected red blood cells block the small blood vessels to the patient brain leading to swelling of brain and brain damage. In the 1930s, RM Knowles and B Das Gupta worked extensively on monkey malaria (*P. knowlesi*) and reported that the species of *Plasmodium* which exists as a safe commensal in the blood and tissues of the common Malayan monkey, *Silenus irus*, when introduced into the common Indian monkey, *Silenus rhesus*, produces a virulent infection [1]. Professor R Knowles and Assistant Surgeon BM Das Gupta were working at Protozoology Laboratory, Calcutta School of Tropical Medicine and Hygiene, India. They subsequently worked on its experimental transmission to human subjects [2]. JA Sinton and HW Mulligan (1932-1933) noted the

distinctive features of this parasite and the 24-h schizogonic cycle, were convinced that this parasite represented a new species of malaria. They called it *Plasmodium knowlesi* in honor of Dr. R. Knowles, who was the first to infect humans with this malaria parasite [3,4]. This simian malaria parasite *Plasmodium knowlesi* is now recognized as the fifth human malaria parasite [5].

The *Plasmodium* species have a very complex life cycle. When a parasite-infected female anopheline mosquito bites a human host, it injects the sporozoites (parasites) into the bloodstream released from its salivary glands (mosquito). Through the bloodstream they travel and invade liver cells (hepatocytes), where the exo-erythrocytic schizogony takes place. Subsequently, the ruptured schizonts enter the erythrocytic cycle to infect the human red blood cells (RBCs). In the human blood stages, it passes through various stages namely ring stage (immature trophozoite), mature trophozoite and schizont (Fig. 1). Some parasites differentiate into sexual erythrocytic stages (gametocytes, male and female). During a blood meal, *Anopheles* mosquito consumes the male gametocytes i.e. microgametocytes and female macrogametocytes. The microgametes enter the macrogametes producing zygotes in the mosquito's stomach, the microgametes. Further parasites' multiplication takes place in the mosquito and is known as the sporogonic cycle (Ookinete, oocyst, sporozoites).

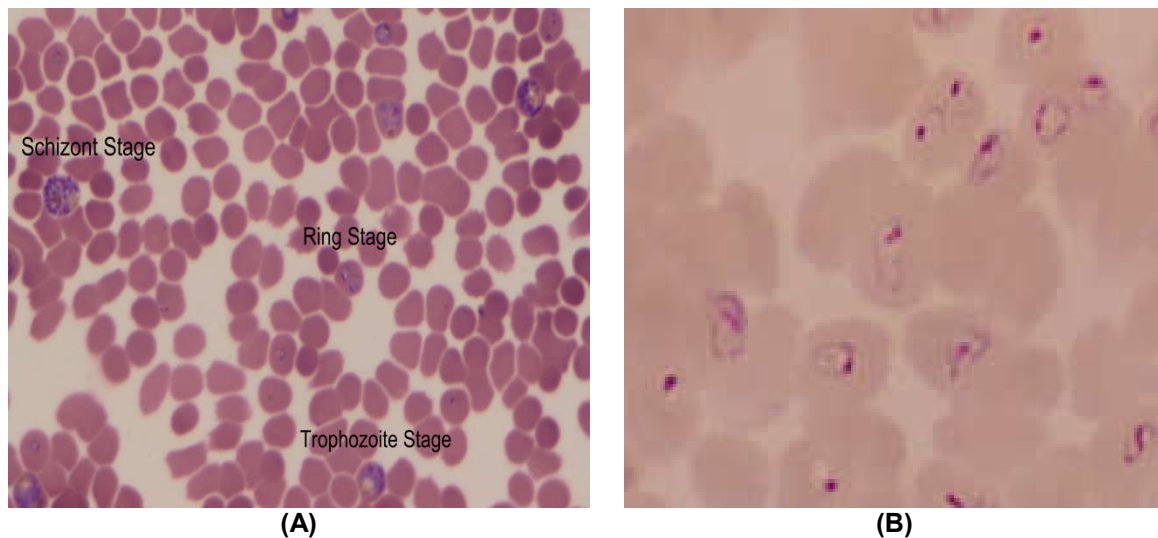


Fig. 1. The lab grown *in vitro* culture of *Plasmodium falciparum* showing (A) asynchronous culture (ring, trophozoite and schizont stages) and (B) synchronous culture showing ring stages only (Image Source: Amit Bhattacharya, 2011)

1.2 Distinguished Researches on Malaria: Nobel Prizes in Physiology or Medicine

Few eminent researchers on malaria or malarial parasites have been awarded Nobel Prizes till now. These are as follows:

Nobel Prize in 1902: In 1897 Ronald Ross, an army surgeon working in India, discovered that culicine mosquitoes (the most extensive subfamily of mosquitoes and vector for several diseases) transmitted the avian malaria parasite (*Plasmodium relictum*) and proposed that the anopheles mosquito was involved in causing human malaria. Subsequently in 1899 while working in Sierra Leone, he established that the human malaria parasites were indeed spread by anopheline mosquitoes. Ronald Ross succeeded in showing the life-cycle of the malarial parasites in mosquitoes, thus establishing the hypothesis of Laveran and Manson. However, between 1898 and 1900, several Italian scientists (like Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava) were also working on understanding the malaria disease. Thus, Ronald Ross was awarded the Nobel Prize in Physiology or Medicine in 1902 “for his work on malaria, by which he has explained its entry into the organism and placed the base for successful research on this disease with methods to combat it” [6].

Nobel Prize in 1907: The French physician Charles Louis Alphonse Laveran identified a protozoan of *Plasmodium* family as a causative agent of malaria after examining blood from malaria-infected people under the microscope. He published his breakthrough work on these parasites in 1884 [7]. Charles Louis Alphonse Laveran was awarded the Nobel Prize in Physiology or Medicine in 1907 “in recognition of his work on the role played by protozoa in causing diseases” [8].

Nobel Prize in 2015: Tu Youyou studied the Chinese medical texts from the Zhou, Qing, and Han Dynasties to find a traditional herbal cure for malaria that has been used for over thousand years for fever resolution. She ultimately extracted a compound called ‘Artemisinin’, the key ingredient obtained from the aerial parts of a plant *Artemisia annua* (also known as sweet wormwood). Artemisinin and Artemisinin Combination Therapy (ACTs) is one of the most

potent antimalarial drugs till date that has led to the survival and improved health of millions of malaria-infected people [9]. The Nobel Prize in Physiology or Medicine in 2015 was divided. The one half was shared by William C. Campbell and Satoshi Ōmura “for their discoveries regarding a novel therapy against roundworm parasites infections” and the other half of the prize to Tu Youyou “for her findings of a novel therapy against malaria” [10].

1.3 Discovery and Early Studies of *Plasmodium knowlesi*

In early 1930, *Plasmodium knowlesi* was first isolated from long-tailed macaque monkey (*Macaca fascicularis*) imported from Singapore to Calcutta School of Tropical Medicine in India [2,3,11]. On analysis, it was observed that *P. knowlesi* shows mild infection in *Macaca fascicularis* but very high infection in *Macaca mulatta* i.e. rhesus macaques and showed potential to infect human beings. Microscopic analysis showed similar morphology of *P. knowlesi* with that of *P. malariae*. In 1965, the first case of malaria caused by *P. knowlesi* was observed and described as a naturally acquired case in American army surveyor, who returned from the jungle of Pahang, Peninsular, Malaysia. Later, in 1971, another case of *P. knowlesi* infection in a human was reported in Peninsular Malaysia. To identify the extent of *P. knowlesi* infection rate in the population of humans living close to the Pahang state, Peninsular Malaysia, the blood samples of 1,117 villagers were taken and found that 28 of them had malaria. The blood of 28 individuals having malaria was pooled and injected into rhesus macaques were for their surprise none of the monkeys showed malarial infection. At the same time, 2 out of 4 long-tailed macaques was identified having *P. knowlesi* infection in the same area and it was concluded that the risk of natural infection to humans from *P. knowlesi* and other simian parasite are very rare [12,13].

1.4 Epidemiology, Reservoir Hosts and Vectors

The infection of *P. knowlesi* has been observed in the Southeast Asia. From 2004 onwards, there are reports of malaria cases in humans due to *P. knowlesi* in Malaysian Borneo, Peninsular Malaysia, Thailand, Philippines, Myanmar, Singapore, Vietnam, Indonesia, Brunei, and Cambodia. Some of the hospitals in Malaysian

Borneo has reported *P. knowlesi* malaria in the majority of malarial infection cases in humans. It has been identified as a cause of severe and fatal malaria with associated cardiovascular comorbidities increasing at a rapid rate in Southeast Asia [14].

The reservoir host of *P. knowlesi* identified in different monkey species starting from *M. fascicularis*, *Macaca nemestrina* from Singapore and Peninsular Malaysia, macaques from Cebu, Palawan Island, Philippines and leaf monkey from Peninsular Malaysia. The two most common macaque species i.e. long-tailed macaques and wild pig-tailed macaques are the most common non-human primates distributed throughout Southeast Asia including Sarawak, Southern Thailand, and Singapore also showed *P. knowlesi* infections.

Further, the vector for *P. knowlesi* belongs to *Anopheles leucosphyrus* groups of forest dwelling mosquitoes whose distribution overlaps in Southeast Asia with long-tailed and pig-tailed macaques. The *Anopheles hackeri*, a predominantly zoophagic mosquito found in forest of Peninsular Malaysia identified as a first vector followed by *A. balabacensis*, *A. phensi*, *A. maculatus* and *A. freeborni* [12,13]. Recent research studies (2019) reported *Plasmodium knowlesi* parasites in the *Anopheles sundaicus* mosquitoes, which serves as a malaria vector, collected from Katchal Island in the Andaman and Nicobar Islands, India [15].

1.5 Human Infections and Detection of *Plasmodium knowlesi*

Laboratory findings have suggested a wide range of clinical complication and symptoms in *P. knowlesi* infected patients, which includes tachypnea, fever, tachycardia as most common; palpable liver and spleen in few cases; and low oxygen saturations, chest crackles, hypotension and jaundice in severe disease conditions. Due to its morphological similarity and minor differences in morphology with other *Plasmodium* species, most of the malaria infection due to *P. knowlesi* has been misdiagnosed as being caused by *P. malariae* via microscopic analysis in 1996 (Sarawak region). Mostly, technicians working on microscopic identification of human malaria (using blood film slides) are often trained in identification of three main species of *Plasmodium*, mainly *P. falciparum*, *P. vivax* and *P. malariae*. Over the last decade, there is a major advancement in diagnosis techniques which includes the emergence of novel molecular

detection and characterization techniques like PCR using *P. knowlesi* specific gene primers for detection in the DNA isolated from the blood samples of infected individuals. Such techniques decisively help to identify species-specific malaria infection in the reported cases. There are no specific treatments or pharmacological strategies available to date for complicated or uncomplicated *P. knowlesi* infections [13]. The more precise picture on the prevalence of *P. knowlesi* can be provided by the correct use of specific Rapid Diagnostic Tests (RDTs) and microscopic screening method especially in inaccessible areas [15,16].

2. CONCLUSION

P. knowlesi has been reported as a potentially lethal malarial parasite. Hence, an extensive entomologic and parasitologic survey (in humans and macaques) along with the molecular characterization of the parasite in India (regions such as Andaman and Nicobar Islands) and abroad are urgently required [15]. *P. knowlesi* has shown differently *in vitro* drug susceptibility profiles from *P. falciparum* parasites, which points out a serious concern [17]. Also, the emergence of antimalarial drug resistance and low parasite clearance by using standard antimalarials is another mounting problem. Hence, critical analysis of approved and experimental antimalarial agents along with novel drug strategies need to be revisited to combat emerging *P. knowlesi* infection in the human population.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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