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A Comprehensive Overview of Biological Aspects of *Plasmodium knowlesi*

Prakriti[1], Neha Zachariah[2], Kamakshi Srivastava[3], Varsha Mehra[4], Gauri Mishra [2*], Lokesh Chandra Mishra[5*]

[1]*Department of Life Sciences, Swami Shraddhanand College, University of Delhi, Delhi-110036, India*
[2]*Department of Zoology, Swami Shraddhanand College, University of Delhi, Delhi-110036, India*
[3]*Department of Computer Science, Jamia Millia Islamia, Delhi - 110025, India*
[4]*Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, Delhi-110096, India*
[5]*Department of Zoology, Hansraj College, University of Delhi, Delhi-110007, India*

***Corresponding Author:** *Lokesh Chandra Mishra & Gauri Mishra*

Abstract

Plasmodium knowlesi, originally known to cause simian malaria, is now recognized as the fifth human malarial parasite; in addition to *P. vivax, P. falciparum, P. malariae* & *P. ovale*. It is predominant in South East (SE) Asian Countries like Malaysia, Indonesia, Thailand, Cambodia, Vietnam, Myanmar, Singapore, Brunei, and Philippines. However, until recently, its prevalence was miniscule in India. A recent report from Andaman and Nicobar Islands, India, revealed presence of *P. knowlesi*-specific gene sequences in 53 out of 445 cases scanned for malarial parasites. The life cycle of the parasite, like its other counterparts, requires infection of both a mosquito and a warm-blooded host. The present review provides a detailed overview of the parasite, its life cycle, prevalence and its comparisons with other *Plasmodium* species. In addition, a comparison is drawn at the genomic and genetic level to provide an in-depth understanding of the parasite's unique characteristics. The cyto-adhering properties and antigenic variants of *Plasmodium knowlesi* are also discussed.

Keyword: Malaria; Plasmodium knowlesi; Erythrocytic; Cytoadherence; Antigenic Variations

Introduction

The genus *Plasmodium* comprises more than one hundred infectious species that can infect humans, birds, reptiles, rodents and non-human primates. Out of these, only four species, namely *P. vivax*, *P.*

falciparum, P. ovale, and P. malariae are infectious to humans. Despite of the belief that malaria parasite adapted to one vertebrate host does not cross into other hosts, a large focus of human *P. knowlesi* infections were first reported from the Sarawak region of Malaysian Borneo, followed by several reports from other South East (SE) Asian countries in the year 2004 [1] [2] [3]. Since it was a considerable number, *P. knowlesi* is now established as the fifth *Plasmodium* species (Figure 1) [4].

The vectors of *knowlesi* malaria are mosquitoes of *Anopheles* leucosphyrus group which are found widespread in the forest regions of many South East Asian countries like Malaysia, Thailand, Philippines and Vietnam. This region is especially endemic for vectors not only of *P. knowlesi*, but also for *P. falciparum* and *P. vivax*. The natural hosts of *P. knowlesi* are the long tailed (*Macaca fasciculate*) and pig-tailed (*Macaca nemestrina*) macaques which are predominant in South East Asia.

The life cycle of *P. knowlesi* is not very distinct from those of other malaria parasites. Like its other infectious counterparts, the pre-erythrocytic stage of *knowlesi* in liver is of the same duration (8-9 days) as that of *P. vivax* and *P. ovale*. However, its distinctive feature is that it has the shortest erythrocytic stage of only 24 hours compared to 48 hours for *P. falciparum* and *P. vivax*. Hyperparasitaemia is one of the important virulence features which *P. knowlesi* shares with *P. falciparum* and *P. vivax*. The emergence of *P. knowlesi* as a fatal pathogen in humans poses fresh challenges for the development of useful diagnostic tools as well as course of treatment especially in the areas where the infection is endemic. Misdiagnosis of *P. knowlesi* as *P. vivax* and *P. falciparum* and vice versa, is very common, and therefore might lead to inappropriate treatment, including chloroquine therapy for *P. falciparum* and a lack of anti-relapse therapy for *P. vivax* [5]. This warrants the application of uniform and stringent blood-stage treatment strategies for all *Plasmodium* species. Molecular methods like PCR and ELISA could help in diagnosis of *knowlesi* specific infections. A greater understanding of antigenic and genomic variations within the species can be helpful in correct diagnosis of the malaria. There is an urgent need for development of specific diagnostic tests for all species. Subsequent confirmation by nucleic acid amplification assays is crucial for appropriate treatment and surveillance.

Geographical Distribution

Understanding the geographical distribution of any vector borne, zoonotic disease is very important as it helps to identify the regions where the disease predominates [6] [7]. *Plasmodium knowlesi* was originally found in long tailed macaques (*Macaca fascicularis*) and pig tailed macaques (*Macaca nemestrina*). Human infections of *P. knowlesi* were unknown until many natural human infections were reported in Sarawak, Malaysian Borneo in 2004 followed by reporting's from Malaysia, Indonesia, Thailand, Cambodia, Vietnam, Myanmar, Singapore, Brunei, Andaman & Nicobar islands of India, and Philippines. The regions with natural human *P. knowlesi* infections intersect with the distribution of long tailed and pig tailed macaques [8] [9] [10]. *P. knowlesi* is the most common cause of malaria in Malaysia [11] [12] [13]. *Macaca fascicularis* and *Macaca nemestrina* are more prevalent to *P. knowlesi* infection in Sarawak whereas these species are less prevalent to infection at Kuala Lumpur and Pahang States in Malaysia, Narathiwat and Ranong Provinces in Thailand and North Sulawesi Province in Indonesia [11] [14].

The *Anopheles latens* from the leucosphyrus complex and dirus complex, found in regions with open

canopy cover, vegetation mosaics, cropland, and closed canopy forests, transmits the *P. knowlesi* infection in human beings [11]. The vector species varies in different regions of South East Asia. *Anopheles balabacensis* acts as a vector in Indonesia, Philippines, and Eastern Malaysia whereas in Thailand, Indonesia and Western Malaysia, *Anopheles cracens* acts as a vector (Table 1). The most common vector found in Cambodia, China, Laos, Thailand and Vietnam are the *Anopheles dirus* [15] [16]. Both the hosts and vectors are widespread in certain regions of Indonesia, Cambodia, Philippines, Southern Thailand, Myanmar, and S. Vietnam. In Northern Myanmar, *M. fascicularis* and *M. nemestrina* are absent but *M. leonina*, *M. assamensis* and *T. phayrei* are present, which can harbour the parasite [11]. The vector distribution varies greatly due to extensive anthropogenic activities in forests [15] [16].

Table 1. Epidemiology of *Plasmodium*

	P. ovale	***P. vivax***	***P. malariae***	***P. falciparum***	***P. knowlesi***
Geographical distribution	Tropical Africa, Islands of the Western Pacific, Philippines, Eastern Indonesia, Papua New Guinea, Bangladesh, Cambodia, India, Myanmar, Thailand and Vietnam	Western Pacific regions, Brazil, India, Myanmar, Papua New Guinea	Sub- Saharan Africa, Southwest Pacific, Middle East, South America, Central America and Cambodian-Vietnamese border	Africa mainly in Nigeria, South-east Asia, Eastern Mediterranean, America, and the Western Pacific	Malaysia, Indonesia, Thailand, Cambodia, Vietnam, Myanmar, Singapore, Brunei, Andaman and Nicobar Islands and Philippines
Vectors	*A. gambiae*, *A. atroparvus*, *A. dirus*, *A. freeborni*, *A. albimanus*, *A. quadrimaculatus*, *A. stephensi*, *A. maculatus*, *A. subpictus*, *A. farauti* [17] [18]	*A. albimanus*, *A. darlingi*, *A. vestitipennis*, *A. punctimacula* [18] [19]	*A. freeborni*, *A. atroparvus* [18] [19]	*A. freeborni*, *A. albimanus*, *A. gambiae*, *A. maculatus*, *A. stephensi* [18] [19]	*A. freeborni*, *A. latens*, *A. balabacensis*, *A. quadrimaculatus*, *A. maculatus* [20]

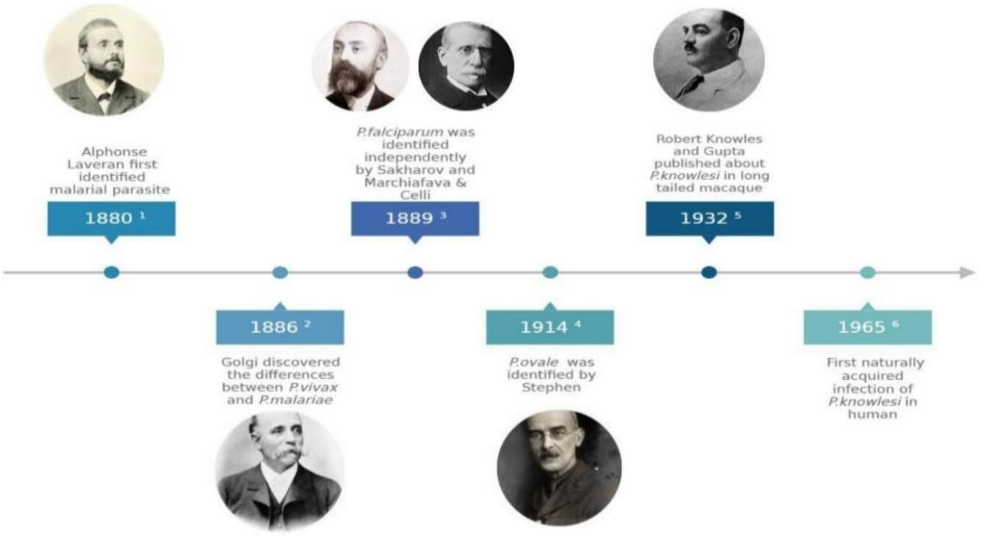


Figure 1. Time line of the genus *Plasmodium*.
1. [21], 2. [22], 3. [22], 4. [23], 5. [24], 6. [25]

Life Cycle of *Plasmodium knowlesi*

The *Plasmodium* parasite has an extraordinary complex life cycle. Because it is digenetic, it requires two hosts to complete its life cycle: mosquitos and particular vertebrate hosts [26]. All the human malaria parasites share a similar life cycle. For the majority of its development in the vertebrate, it is an obligate intracellular parasite that invades and performs asexual multiplication in hepatocytes at the start of its growth in the vertebrate, and subsequently it invades and undergoes several cycles of replication in red blood cells (RBCs) [27]. The parasite replicates inside an infected RBC to become schizonts, which are eventually segmented to form merozoites [28] [29]. *Plasmodium knowlesi* causes a zoonotic disease. It is a parasitic illness spread by animals. Zoonotic diseases are transmitted directly or indirectly from animals (the natural hosts) to humans via an insect vector, in this example a mosquito [30] [31]. Humans are routinely used as "dead-end" hosts, meaning that there is little to no transmission from one person to the next [32]. Humans are currently considered incompetent or dead-end hosts since direct transmission between humans through mosquito has not been demonstrated in nature (despite being demonstrated in experimental conditions) [33] [34]. *Plasmodium knowlesi* reproduces through multiple fission. It is a parasite that reproduces and completes a blood stage life cycle in 24 hours [35]. As a result, parasite densities rapidly reach considerable levels. If *P. knowlesi* is allowed to reproduce unchecked in the body, it can become a potentially fatal condition. If *P. knowlesi* is left untreated in the body, rapid reproduction makes it a potentially very serious condition. The life cycle of *Plasmodium knowlesi* is almost similar to any other species of *Plasmodium* (Figure 2). It involves three steps i.e., exoerythrocytic schizogony, erythrocytic schizogony and sporogony [36] [37] [38].

1. Exoerythrocytic schizogony

It initially begins when a female mosquito bites the host and injects sporozoites into it. After being introduced into the body by a mosquito, sporozoites, the infectious stages, migrate throughout the body until they infect liver hepatocytes, which is when an infection begins. The sporozoites go through exoerythrocytic schizogony, which is a period of asexual proliferation in the liver that results in a high number of uninucleate merozoites [38] [39] [40].

2. Erythrocytic schizogony

Merozoites arise from the liver, move through the blood, and then attack red blood cells. Once within the cells, *P. knowlesi* initiates erythrocytic schizogony, a second stage of asexual reproduction that produces 8-16 merozoites that eventually infect new red blood cells. Malaria is the outcome of this phase. Cycles of erythrocytic schizogony can be seen seemingly indefinitely. As the infection progresses, some infant merozoites grow into male and female gametocytes, which circulate in the peripheral blood until female mosquitos pick them up during feeding [38] [40].

3. Sporogony

Gametocytes within the mosquito grow into male and female gametes, where fertilisation occurs, and a motile zygote is formed inside the mosquito gut, initiating a process known as sporogony. The motile zygote penetrates through the gut wall and develops into an oocyst, where another phase of multiplication occurs. As a result, sporozoites are formed, which migrate to a mosquito's salivary glands and are injected when the insect feeds on a new host [38] [40] [41].

The main difference of *Plasmodium knowlesi* from other species of *Plasmodium* is at two levels, first

transmission occurs at two levels- one from macaques (the most frequent one) and from macaques to humans (the least frequent); and second, *Plasmodium knowlesi* have a quotidian cycle in the RBCs, which means it has a cycle of 24 hours.

Comparison of different species of *Plasmodium*

P. knowlesi shares a lot of resemblance with *P. falciparum* and *P. malariae* which leads to difficulty in differentiating *P. knowlesi* infection from other *Plasmodium* infections. The erythrocytic cycle of *P. knowlesi* lasts for 24 hours, while it is 48 hours in *P. falciparum, P. vivax, P. ovale* and 72 hours for *P. malariae* [9] [42] [43]. The fever pattern observed in *P. malariae* was Quartan (fever occurred every 4th day), tertian pattern of fever was observed in *P. vivax* and *P. ovale* (fever occurred every 3rd day) and quotidian pattern was observed in *P. knowlesi* (fever occurred every day). Due to asynchronous release of merozoites by rupturing schizonts a particular fever pattern was not observed in *P. falciparum* [9] [44] [45]. The early trophozoites of *P. knowlesi* (Figure 3. Av.) [46] are similar to *P. falciparum* (Figure 3. Aiv.) [9] while other stages like schizonts and gametocytes are indistinguishable from *P. malariae* [46] [47].

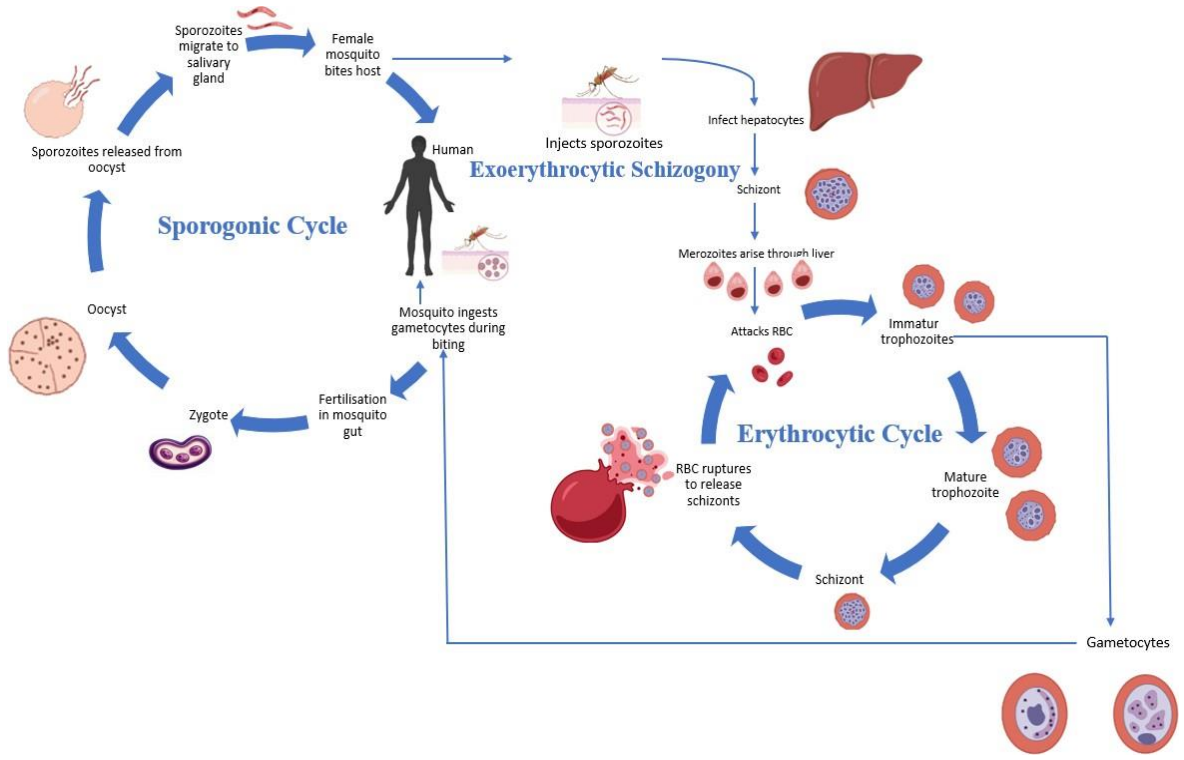


Figure 2. Life cycle of *Plasmodium knowlesi*

One of the major differences between *P. malariae* and *P. knowlesi* is that, in *P. malariae*, the merozoites are clustered around the dark brown malarial pigment [43] whereas in *P. knowlesi* the merozoites are scattered or arranged in grape-like clusters and the malarial pigment of *P. knowlesi* is scattered or can be seen as a single mass [46]. The schizont of *P. knowlesi* (Figure 3. Cv.) [46] has 16 merozoites, which are almost double the number of merozoites of *P. falciparum* (Figure 3. Civ.) [9] [48] whereas the schizont of *P. malariae* (Figure 3. Ciii.) [9] [46] has 6 to 12 merozoites [9] [49].
Comparative studies have found that the frequency and severity of thrombocytopenia was higher in *P.*

knowlesi than in *P. falciparum* and *P. vivax.* Deranged renal function and elevated levels of aminotransferase enzymes are found in *P. knowlesi* infections [50] whereas severe anaemia is found in *P. falciparum* infections [9].

Comparative study of *Plasmodium* species at the Genomic and genetic level

Genetic make-up

The *Plasmodium knowlesi* cells are haploid eukaryotic cells for most of their life span [40] [54][55]. The haploid nucleus contains 14 chromosomes. Along with the nuclear genome, circular plastid DNA and mitochondrial DNA are present in the cell. The presence of plastid DNA (35.5 kb) implies the presence of photosynthetic activity in cells during the divergence from ancestral specie which was eventually lost due to host-parasite interactions. The plastid DNA is functional and contains genes for fatty acid metabolism and isoprene metabolism [54] [56] [57]. There are about 5438 genes in the 24.4 MB genome of the malarial parasite [40] [54]. *Plasmodium knowlesi* genome sequencing and genome analysis suggests the presence of dimorphism in genes such as Pknbpxa. The interesting detail is that only one form out of two is infectious [58] [59]. It is assumed that the disease marker lost their virulence during evolution. The G-C percentage and A-T percentage in *P. knowlesi* is 38.7% and 62 % respectively [54] [59]. The A-T content varies among different *Plasmodium sp.* As shown in several experimental studies and genome sequencing studies *P. falciparum* contains the highest concentration of A-T rich regions [59], followed by *P. malariae, P. ovale, P. knowlesi & P. vivax* [40] [60] [61]. Higher concentration of the A-T rich regions results in relatively simpler DNA conformation.

Cytoadherence

Plasmodium parasites are known for their varied severity in different stages of disease progression. If not diagnosed early, it can lead to kidney failure, coma or death. This is due to the property of cytoadherence of *Plasmodium*-infected RBC. Cytoadherence is the phenomenon where infected RBCs adhere to the surface of endothelial cells. This is resulted due to the expression of the proteins such as ICAM1, VCAM1 and CD36 on the membrane of iRBCs that in turn interact with the membrane proteins present on the nerve cells [62] [63]. Not all *Plasmodium* parasites have this property. The two major species exhibiting cytoadherence are *P. falciparum* and *P. knowlesi. P. falciparum* infected RBCs have high affinity for the surface of endothelial cells with the above-mentioned genes whereas *P. knowlesi* shows a wide range of affinity with ICAM1 and VCAM1 gene but no interaction is reported for CD36. The clinical and pathological implications are not known yet [62]. However, cytoadherence is not reported in either *P. vivax* or *P. ovale*.

Antigenic variation

Over time parasite-host relationship between *Plasmodium* and mammals respectively has evolved to enable both species a better chance at defence. *Plasmodium* parasite evolved to escape the immune response mediated by the host by continuously varying its antigenic molecules over the time in different populations [64]. This is called antigenic variation. Antigenic variation of *Plasmodium* cells is due to the presence of *Plasmodium* Interspersed Repeats (PIR). These repeats give rise to new antigenic variants in *Plasmodium*, resulting in successful infection in the host organism. PIR gene families are abundant in their genome.

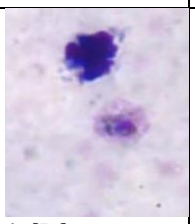
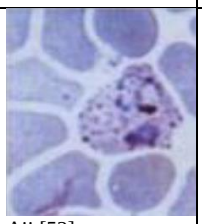
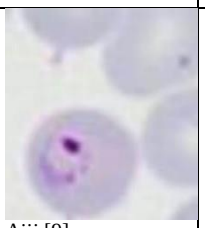
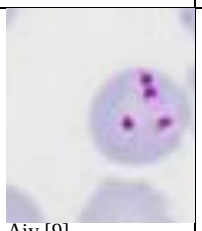
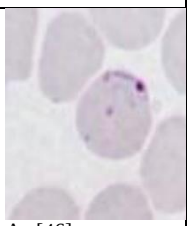
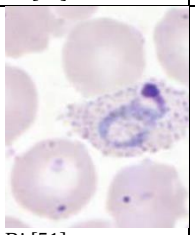
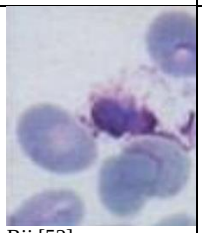
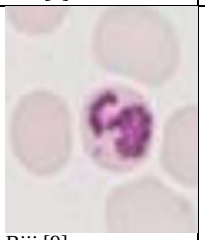
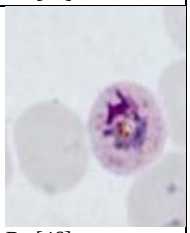
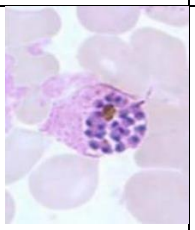
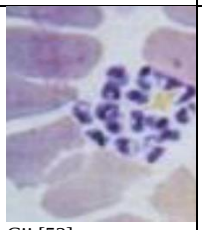
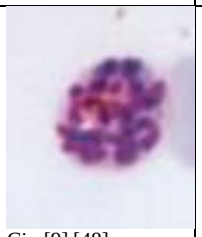
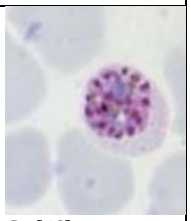
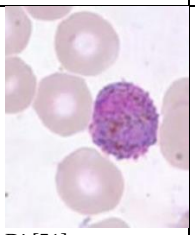
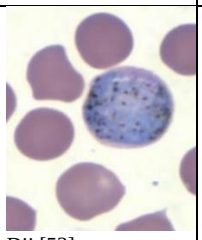
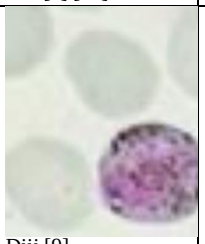
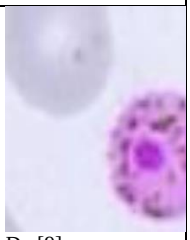
Stages/Species	*P. ovale*	*P. vivax*	*P. malariae*	*P. falciparum*	*P. knowlesi*
Early Trophozoite **A.**	 Ai [51]	 Aii [52]	 Aiii [9]	 Aiv [9]	 Av [46]
Late Trophozoite **B.**	 Bi [51]	 Bii [52]	 Biii [9]	 Biv [9]	 Bv [46]
Schizonts **C.**	 Ci [51]	 Cii [52]	 Ciii [9] [46]	 Civ [9] [48]	 Cv [46]
Gametocytes **D.**	 Di [51]	 Dii [53]	 Diii [9]	 Div [9]	 Dv [9]

Figure 3. Comparison of erythrocytic stages of *Plasmodium knowlesi* with other species

The name for the PIR gene changes according to the *Plasmodium* species genome to which it belongs, hence PIR genes present in *knowlesi* are referred to as the KIR gene family. Similarly, those for *Plasmodium ovale* and *Plasmodium vivax* are respectively referred as the OIR gene family and the VIR family. The of KIR gene family comprises least 71 copies in *knowlesi* genome, followed by PfEMP1 family found in *Plasmodium falciparum* (189), *Plasmodium malariae* (255), *Plasmodium vivax* (1212) and *Plasmodium ovale* (more than 2100) in increasing order [54] [59] [65] [66].The schizont-infected cell agglutination (SICA) and KIR gene family are the analogues of the PfEMP1 family present in *P. falciparum* [66]. The abundance of the copies shows the evolutionary importance of the PIR genes for the survival of the *Plasmodium* parasite in the host organism and points to the fact that it is a result of gene duplication, natural selection, and mutational variations [64] [66].

A de-novo genome is constructed for *Plasmodium knowlesi* to modify the previous reference genome using two strains, i.e., KH273 and KH195. These strains are genetically different clusters isolated from different patients. The new genome constructed is a better representation of the genomic sequence of *P. knowlesi*, as it was generated using advanced next-generation sequencing and incorporating different methods to screen and polish the quality of the sequence. This effort is promising to shed light on the important genetic differences in *Plasmodium* species. Such insights will be helpful in making better discoveries for vaccines, targeted delivery systems, and treatment methods [66].

Conclusion

Despite the fact that *P. knowlesi* malaria was extremely rare in other South East Asian nations, it is quite common in Malaysia, particularly in east Malaysia, Indonesia, and to a lesser extent in west Malaysia. Our current understanding of the clinical course, treatment, pathogenesis, and epidemiology of *P. knowlesi* malaria is based on a number of case reports, modest retrospective and prospective investigations, and relatively small field research. According to the data collected, *P. knowlesi* malaria is largely a zoonotic disease, with long-tailed and pig-tailed macaques serving as the primary reservoir hosts. Further research reveals that the life cycle of *Plasmodium knowlesi* and *Plasmodium falciparum* are fairly similar. A quotidian cycle is present in RBCs for *Plasmodium knowlesi*. Due to the digenetic nature of *Plasmodium knowlesi*, it has two different kinds of hosts.

Additionally, *Plasmodium knowlesi* resembles *Plasmodium falciparum* and *Plasmodium malariae* greatly, despite having a number of variations from other *Plasmodium* species based on a variety of conditions. The presence of photosynthetic activity in cells during the divergence from the ancestral specie, which was ultimately lost as a result of host-parasite interactions, was found by comparative genomic analysis of the *Plasmodium* species. Furthermore, *Plasmodium knowlesi* is extremely harmful; if not treated promptly, it can result in kidney failure, a coma, or even death.

The primary focus of the paper is a thorough biological analysis of the *Plasmodium knowlesi*, which is covered in all of its dimensions. The suggested idea can direct future investigation into human-related aspects of *P. knowlesi* malaria infection. More importantly, it will be helpful in the design of studies that are pertinent to the evaluation of *P. knowlesi* and in advising policymakers on the development and execution of focused measures to lower the disease burden in the future.

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