Unveiling The Enigmatic Intrigue Of Plasmodium Knowlesi, The Fifth Human Pathogen

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Abstract
Background: Plasmodium knowlesi was initially considered a simian malaria parasite but is now recognized as a significant human pathogen, prevalent in Africa and Southeast Asia. It is transmitted by Anopheles dirus and Anopheles crascens mosquitoes, primarily from the leucosphyrus group, and has infiltrated ten Southeast Asian nations.
Aim: The review article aims to unravel the enigmatic intrigue of Plasmodium knowlesi, highlighting its emergence as a predominant human malaria parasite. It also compares P. knowlesi with other parasitic players in malaria.
Methods: By focusing on studies examining the epidemiology, genetic profile, and clinical manifestations of Plasmodium knowlesi, emphasizing the importance of accurate diagnosis and prompt intervention. Proteins like Pk-fam-c and Pk-fam-e are identified as potential protein export signals in P. knowlesi.
Findings: Plasmodium knowlesi is a predominant and lethal parasite in humans, transmitted by mosquitoes that feed on infected macaques. Infection with P. knowlesi does not cause relapses, and it can be highly life-threatening, with common signs including pain in the abdomen, difficulty in breathing, and shock. Accurate diagnosis of P. knowlesi is crucial for distinguishing it from other Plasmodium species.
Practical Implication & Conclusion: Prompt intervention and treatment strategies are essential to control the increase in cases. WHO recommends Artemisinin-based combination therapy for uncomplicated knowlesi malaria. Surveillance and improved diagnostics are needed in Southeast Asian countries. The conclusion underscores the necessity for precise diagnostics and innovative therapies in combating Plasmodium knowlesi, emphasizing its unique characteristics and challenges, and calls for collective vigilance.

Keywords: Lehtal, parasite, Asia, predominate, emphasezing, intervention, enigmatic

Introduction

The emergence of Plasmodium knowlesi (the "kra" monkey), a zoonotic malarial strain once thought to be responsible for simian malaria, has revealed it to be the most common parasite infecting humans. Infecting rhesus macaques regularly with five human plasmodium parasites, even though the disease is not natural in humans. [1, 2] There are two main malaria-carrying species from Africa: Anopheles gambiae and Anopheles funestus, both of which are eukaryotic, unicellular parasites that cause malaria in mammalian hosts.[3-5] About 300 million years ago, an aquatic protozoan invertebrate had chloroplasts and a sexual life cycle. As time passed, it evolved into an asexual form, known as plasmodium, a parasite found in modern times. [6]

Unraveling the Historical Journey of Plasmodium

Over 25 distinct species of Plasmodium are identified out of four malarial parasites (P. falciparum, P. vivax, P. malariae, P. ovale curtisi) that are liable fo human sickness. In sub-Saharan Africa, 99.7% of infections are spawned by Plasmodium falciparum the most coincident and virulent specie. Plasmodium falciparum-caused malaria, also known as cerebral malaria, is most fatal due to its rapid blood cell replication and prevalence throughout Africa. Plasmodium vivax is almost completely absent from areas of West Africa, whereas P. vivax is highly prevalent throughout endemic America, South, Europe, and Asia. The most prevalent specie is Plasmodium malariae in America and Europe. It is responsible for about half of malaria cases during the low-dissemination timeline in regions with significant seasonal climate variation. P. malariae can cause chronic nephrotic syndrome, which is considered to be mild but clinically difficult to treat and has a high mortality rate once it is established.[7] Plasmodium ovale is mostly found in the East of Indonesia, West Africas, limited areas of tropical Africa, New Guinea, Central Africa to a lesser extent, the Middle East, Philippines, South east Asia, and is also reported in the Sub-Continent [India].[8] Surprisingly, both Plasmodium vivax and Plasmodium ovale can

lie quiescent in the liver, and hypnozoite relapses can occur months or even years after exposure. mysterious human malaria parasite called Plasmodium Knowlesi replaced Plasmodium ovale as the last human malaria parasite.

Into the Wild: Knowlesi's Transmission Tale

Mosquitoes are the main vectors for the transmission of P. knowlesi and P. cynomolgi, two parasite species that can be transmitted from macaques to humans. [2] While there is no conclusive evidence of human-to-mosquito-human transmission, P. knowlesi is spread to people by mosquitoes that have fed on infected macaques. [9] In 1931, P. knowlesi was first identified by the Italian scientist (a malariologist) in the blood of a long-tailed macaque (Macaca fascicularis) afterwards further studies were been done by Das Gupta Napier, Campbell, and Knowles. [1, 10] [11] Plasmodium infection in humans has been documented since the 1960s. Using Plasmodium cynomolgi, an experiment was conducted in 1960 to test interspecies transmission. The first simian malaria parasite was successfully transmitted by a mosquito to humans. In 1967, P. knowlesi was marked as zoonotic malariae being spread from monkeys to people, although being uncommon malaria for time.[11, 12] The risk of zoonotic malaria from both human and nonhuman primate parasites was associated with nights spent in a forest area where natural vertebrates host the macaque, and the leucosphyrus vectors overlap. [13]

There are heterogeneous vector groups that can proliferate between Homo sapiens and non-humans. The pigtail macaque (Macaca nemestrina) and long-tailed macaque (Macaca fascicularis) are common hosts of P. knowlesi in Southern Asia. Moreover, stump-tailed macaques (Macaca arctoides) play a crucial role as reservoirs, as well as developing severe illness. [1, 2, 14] P. knowlesi is well-vectored by Anopheles dirus and Anopheles crascens which belong to the leucosphyrus group[1, 15]. Taking in view for sustenance mode of Leucosphyrus group of Anopheles mosquitoes', the apex period which is immediately after murk is continual in them. [16, 17] The goal of transmission is served only by the group found in Southeast Asian forests; research on biting behavior is yet in its early stages. [9] Nowadays, it is generally acknowledged that P. knowlesi plays a significant function in human malaria. [14]

Mapping Prevalence Patterns

Human knowlesi infections have been reported in ten of the eleven Southeast Asian nations over the past ten years. [14, 18, 19] As a result widespread distribution of tropical forests in Southeast Asian countries, particularly in east Malaysia two States, Sabah and Sarawak Indonesia, Thailand, Myanmar, Singapore, the Philippines, Brunei, Vietnam, and Cambodia, have recorded cases of P. knowlesi malaria. In the Kapit Division of Sarawak, a US military surveyor spent the night working in a forested region of Malaysia's Pahang and became the first person to naturally contract P. knowlesi. In 2004, considering it a rare illness more than 1000 cases were reported. [15] Since then, incidences of knowlesi malaria have been reported across different Southeast Asia and remain a public health problem, especially an epidemic in Malaysia. [18] After the incidence of Sarawak, a rapid surge of cases received from different areas including Peninsular Malaysia, Malaysian Borneo, and most recently, Indonesia. Although, its spread between humans is not reported but it comprises upto 75-80% of cases in different states of Malaysian such as Sarawak and Sabah in Malaysian Borneo. [19] The epidemic of malaria in Malaysia involved over 16,500 cases during the timeline of 2013-2017 while P. knowlesi was solely responsible for 69 % of them. Thus it will be incautious to anticipate the reduction in this zoonotic parasitic infection [20]. Over and above that, 10,968 cases of knowlesi malaria in total have been reported in Malaysia between 2017 and 2019. The hypothesized conclusion proposed that increased deforestation and anthropogenic land use would bring vectors, primary hosts, and potential host humans into close proximity [18]. The etiological factors suggest the zoonotic relation and also depict the severity of infection in different people in an exposure.

The WHO now recognizes P. knowlesi as one of the agents responsible for causing human malaria. Since the initial revelation, great progress has been made in studies examining the parasite's epidemiology and disease control relies heavily on the identification of vector species involved in the transmission. [9]

Although older human red blood cells can eventually adapt to being infected by the parasites, but in vitro research has shown that the parasites prefer youthful red blood cells. This characteristic now prevents the infection from spreading quickly outside of the human: monkey. In addition to the typical symptoms of fever, chills, and headache associated with malaria, this illness also has unusual symptoms including nausea, vomiting, myalgia, arthralgia, upper respiratory symptoms, and jaundice. [21]

Unlocking Knowlesi's DNA Secrets

The phylum Apicomplexa has been thought to include Plasmodia. Three genomes are present in Plasmodium:

1) a Genetic material made up of 14 linear-shaped chromosomes
2) one of the shortest mitochondrial genomes
3) a red-algal-derived 35 kb circular plastid genome stored in the apicoplast. [22]

Plasmodium knowlesi have an overall 23.5 megabase (Mb) nuclear genome size, has a substantial number of unique structured ncRNA candidate genes in addition to the expected number of non-coding RNA (ncRNA) genes with known functions. The number of genes in the genome is 5,188 the simple sequence repeat SSR content is 4.92%. [18] The mitochondrion and the apicoplast are two separate organelles found outside of the nucleus in the Plasmodium cell, each of which has its own genomic DNA. A total of 37.5% of the G+C base is present, for Plasmodium species, it is unusual to find (G1C)-rich repeat regions with intrachromosomal telomeric sequences positioned specifically or as parts of larger repeat units at numerous internal sites in the chromosomes of P. knowlesi. Whereas the overall A+T content is 63%. Evolutionary genes have been identified from both species in P. knowlesi having unique ssrRNA and the mitochondrial

gene cox [19]. Presence of specific binding protein genes termed as Normocyte binding protein genes (nbpxa and nbpxb), preferably involved in P. knowlesi invasion. This parameter has become the motif of genetic association studies. All Plasmodium species under investigation have members of the reticulocyte binding-like protein (RBP) family, which is involved in erythrocyte invasion and selection. A connection exists between certain haplotypes of the Plasmodium knowlesi normocyte binding protein (Pknbp)xa and the Pknbpxa on chromosome[12], characterized by a minor polymorphic segment spanning 885 base pairs (bp) and 9578 base pairs (bp)..

P. knowlesi RBP gene family alleles (nbpxa and nbpxb) revealed that certain SNPs were associated with significant parasitemia and severe illness in human infection ns. These families have a two-exon gene structure with a signal peptide and a carboxy-terminal transmembrane domain, but they don't include the typical export motifs. Pk-fam-c and Pk-fam-e members are two new families of proteins with potential protein export signals. Surface protein-1 located on merozoite (MSP1) is a particular antigen involving blood during its transformational stage. Its C-terminal 19 kDa domain has been discovered to bind to the host erythrocyte, and antigenicity against the 19 kDa domain has been noted. It is produced in P. knowlesi during asexual stages as a progenitor to the 200 kDa protein, and after undergoing the process called proteolytic cleavage, it yields protein structures that are four polypeptides of about the same size. Only the 19 kDa fragment of the C-terminal 42 kDa protein remains on the surface of the merozoite after further processing into two shreds of 33 kDa (MSP133) and 19 kDa (MSP-119) during the invasion phase.[23]

The greatest groupings of P. knowlesi-specific expansions are composed of the SICAvar genes 13 and kir genes9, which are P. knowlesi-specific variant antigen gene families. Schizont-Infected Cell Agglutination Antigens (SICA) are the name given to the surface proteins produced by Plasmodium knowlesi, and they are encoded by the SICAvar gene family. Although they are not closely related, PfEMP1 proteins and SICAvar proteins both have binding signature motifs.[24] The SICA antigen encoded by the SICAvar gene family was the name given to the variant antigen that was expressed on the surface of P. knowlesi-infected erythrocytes and is linked to parasite virulence. P. knowlesi only contains five separate gene families with functions yet to be known [9, 21]

Table 1: List of key proteins empowering plasmodium to influence the host

Proteins	Chromosome number	Importance	Reference
Circumsporozoite protein	13	Mediation of sporozoites development and assisting sporozoites migration from mosquito guts to host liver play a crucial past in sporozoite development and mammalian hepatocyte invasion	(25, 26)
Gamma protein region II	---	Mediates the invasion of Plasmodium knowlesi into erythrocytes Increased adaption in humans The essential motifs for adhesion to the erythrocyte Duffy antigen are found in region II.	(27)
Rhoptry-associated protein 1	5	Formation of the parasitophorous vacuole plays a role in the latter process	(28) (29) (30)
Normocyte binding protein Xa/Xb	14	Invasion into host erythrocytes was analyzed by using the technique of erythrocyte-binding-assay (EBA)	(31)
Duffy binding protein alpha Region II	6	Erythrocytes binding domain Duffy blood group antigen is used as a receptor for invasion into human and monkey erythrocytes chemokines-receptors (DARC) on the surface of RBCs	(27, 32)
Apical membrane antigen-1	11	Essential for parasite survival Host cell reorganization Immune responses to AMA1 appear to be a component of the adaptive immune response that partially protects older individuals living in malaria endemic areas	(33, 34)
Merozoite surface protein 1	9	Invade as a ligand reorganization on the red cell receptor Have an essential role in blood-stage growth	(35) (36)
Merozoite surface protein 3	10	Protect the parasite against haem that is released during parasite aggression	(37)

Life Cycle Dynamics

All Plasmodium species share a similar life cycle. It comprises of two phases: the first occurs when a person or vertebrate host contracts the parasite, and the second occurs when an insect vector ingests the blood-carrying gametocytes from an infected vertebrate host.[11] An exogenous sexual phase (known as sporogony), in which multiplication takes place in various species of Anopheles mosquitoes, and an endogenous asexual phase (known as schizogony), which takes place in the vertebrate host, define the life cycle of all species of human malaria parasite.[23] Mosquitoes of the genus Anopheles act as the vectors that spread the Plasmodium species that infect humans, it often discharges tens to hundreds of sporozoites into the skin because the haploid genome of Plasmodium cells allows them to develop in their vertebrate hosts.[38]

The inoculum of sporozoites is extracellular, mobile parasites that move through the dermis and finally reach the liver through the lymphatic system. After being injected into the bloodstream by the mosquito bite, sporozoites journey through various cellular barriers within the body. They traverse dermal fibroblasts, endothelial cells, liver sinusoidal endothelial cells (LSECs), and Kupffer cells (KCs), which are the liver's resident macrophages. Eventually, they invade hepatocytes,

where they proliferate and develop without causing any symptoms. A key mechanism underlying sporozoite movement and host cell penetration is microneme secretion. According to intravital imaging, sporozoites can pass through KCs, LSECs, or use a paracellular route to reach the underlying hepatocytes in the liver, where they infect hepatocytes and multiply by thousands. [39] The pre-erythrocytic stage lasts for 8-9 days then schizogony is the term for the process through which sporozoites, after being inoculated by a mosquito, penetrate the parenchymal cells of the liver and begin to mature and multiply. For one to two weeks, the activated sporozoites reproduce in hepatocytes via mitosis, producing up to 40,000 parasites per hepatocyte. The asexual lifecycle lasts just about 24 hours afterwards, parasite forms are released into the bloodstream, resulting in the clinical symptoms of malaria. [23] The sporozoites replicate in the liver, where they develop into tissue schizonts that are home to merozoites. Primarily erythrocytes are infected by adult merozoites which are initially present inside a hepatocyte, multiplying vigorously and eventually resulting in rupturing the cell. [39] One merozoite in an erythrocyte replicates asexually through schizogony to produce between 8 and 64 additional merozoites. To advance to a further ring stage, it replicates again in rbc. [23, 39] The "prepatent phase" last for 9-12 days and is the interval between the trophozoites' first appearance in the erythrocytes. The catabolism of hemoglobin inside the plasmodium results in a brown crystalline form term as Hemozoin (hz), which is fundamentally used for nutritional requirements.[40]

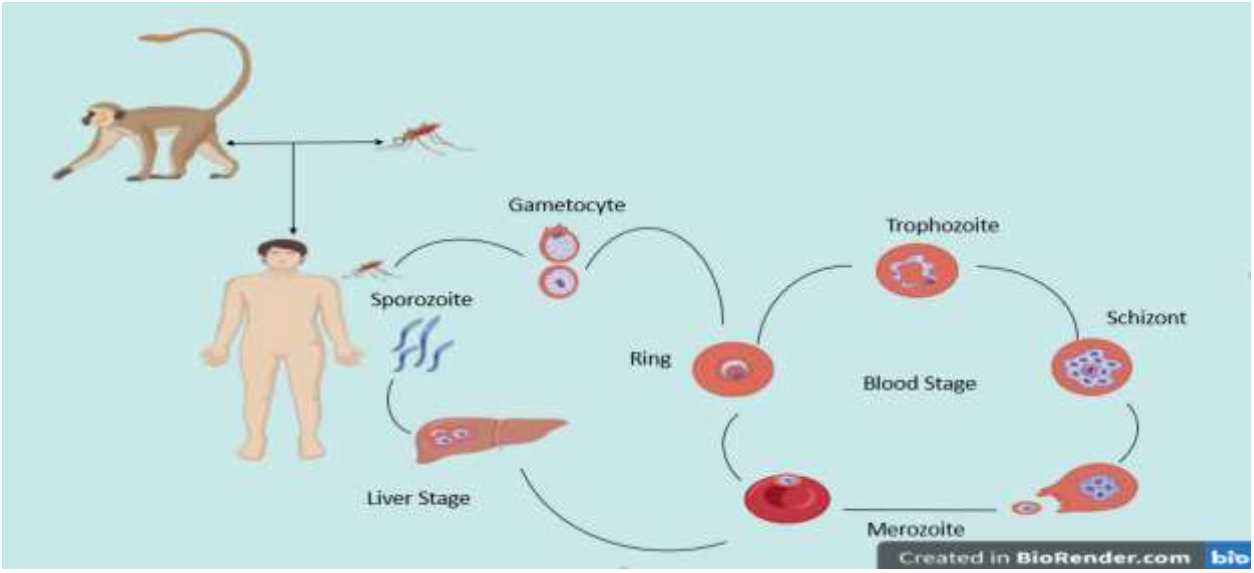


Figure 1 Life cycle of Plasmodium knowlesi mosquito from the Leucosphyrus group transmit the plasmodium to macaque the intermediate host which is further transmitted to humans during contact at the night. A number of sporozoites are injected they then move to the liver for replication and form merozoites. These merozoites rupture liver cells and are released which are then penetrated into RBCs. The ring is then formed which forms gametes that are further consumed by mosquitoes during a blood meal. Or the ring in RBCs can form trophozoite which then forms schizont to form merozoites to advance the ring stage.

The sexual forms mature more slowly than the asexual forms, usually taking 48 hours to complete. The macrogametocyte, which fills the host cell at the conclusion of maturation, has a blue-stained cytoplasm, while the microgametocyte is occasionally smaller and has a pink-stained cytoplasm.[41]

When an insect vector consumes the blood containing gametocytes from an infected vertebrate host, the second phase of the insect's life cycle starts. It involves the differentiation of gametes into male and female ones and principally inseminates to form a zygote in the mid gut of the vector.

The microgamete fertilizes the macrogamete to produce the parasite's fertilized egg, which is the only embryonic stage with a diploid chromosome. Soon after entering meiosis, the zygote transforms into a motile ookinete with four haploid chromosomes in its nucleus. The zygote matures into mobile ookinetes, which develop into oocysts. To create mature oocysts, sporozoites move to endocrine glands (Salivary glands). [39]

The oocyst undergoes multiple rounds of mitosis, and sporogony generates a large number of sporozoites. A mature oocyst bursts when it is ready to release sporozoites into the lymphatic fluid. Following their transfer to the salivary glands, these sporozoites begin their life cycle by developing the ability to spread when introduced into the skin of the host organism by mosquito bites. The Plasmodium species that invade humans finish the second stage of their life cycle, from germ cells to sporozoites preparing to infect the next host, in 10 to 18 days on average. [42]

Clinical Drama of Plasmodium Knowlesi

It is currently believed that both host and parasite factors can affect whether an infection becomes symptomatic and potentially fatal, or if it remains asymptomatic. [43] The amount of parasitized erythrocytes present in the infected host at the time of sampling is known as parasitemia. Infection with Plasmodium knowlesi can manifest as asymptomatic to severe/fatal depending on the parasitemia level. In 10 - 12% of cases, P. knowlesi malaria is accompanied by a severe

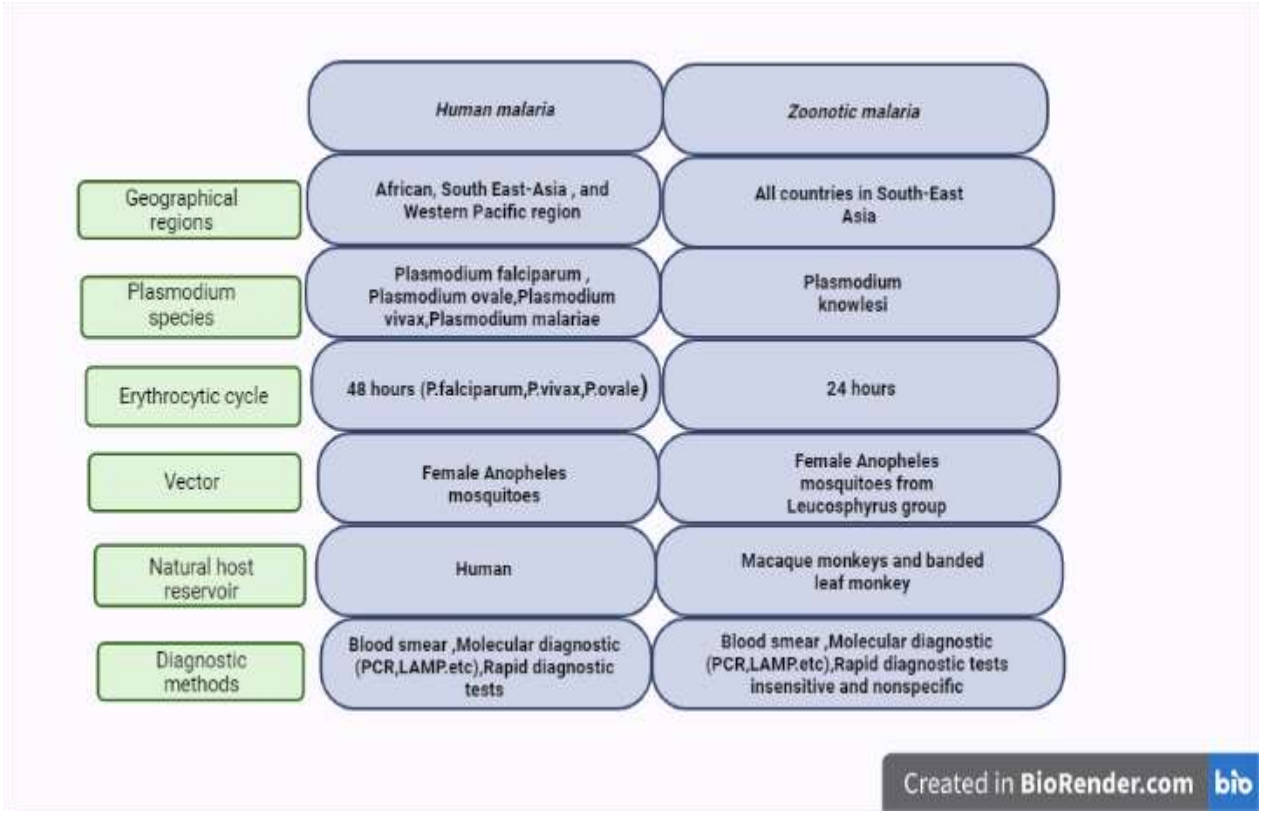
illness that can be fatal. Patients began to show clinical symptoms after eight days, with the smallest time being three days and the longest being fourteen days. Severe cases of P. knowlesi malaria can be highly life-threatening. Patients reported with pain in upper and lower abdomen, difficulty in breathing, cough, thrombocytopenia, anaemia, and shock common signs of knowlesi malaria.[44] A majority of literature reports thrombocytopenia associated with knowlesi malaria infection. Although severe malaria, including hyper parasitaemia, can cause brain pathology, as demonstrated by one recorded autopsy case of P. knowlesi.

Also noted is a delayed clearance of parasites. The maximum temperature peak was at 40 degrees Celsius, and parasite density increased. The shorter 24-hour asexual blood-stage cycle of P. knowlesi malaria parasites results in a substantially faster parasite clearance time than other malaria parasites.[44]

There is a 24-hour period between when the parasite manifests itself, although it has been reported that some imported cases took longer to manifest. Infection with P. knowlesi does not cause relapses. A majority of literature reports thrombocytopenia associated with knowlesi malaria infection. A febrile illness is most typically the initial symptom of infection with P. knowlesi, as there are no specific clinical features or symptoms that can distinguish it from other malaria species.[45]

Diagnosis Innovation

The most important and crucial step is the correct identification of the parasite and Its predominant form in the blood for intime treatment of the patient. The rapid test available in almost every laboratory is a peripheral smear examination. But it can impart crucial effect in case of misidentification. The P. knowlesi can be misidentified as P. falciparum due ring form present in both.[46] The mature trophozoites and schizonts of plasmodium in malariae and knowlesi have quite a resemblance. In Malaysia, the results of 178 positive samples of malaria were compared with using Hexaplex PCR system. Remarkable, it identified 35 positive P. knowlesi, initially misidentified as P. malariae. Rapid immunological devices are malaria detection except P. knowlesi till date. According to study on rapid testing, it is due to least sensitive markers on it. [47] Rapid diagnosis can be made in certain areas with high prevalence of specific parasite or when it becomes difficult to pin down one on microscopic level. For such cases, the ultimate solution is PCR testing.



The Game-Changer in Malaria's Lineup – A Riveting Comparison with Other Parasitic Players

Future Knowlesi Treatment Strategies

A plethora of drugs are under study to find the effective one against malaria, especially P. knowlesi. Some of these are artemisinin, artemether, artesunate, dihydroartemisinin, chloroquine, and mefloquine. By comparison, artemisinin is considered more effective in treatment than mefloquine. Artemisinin-based combination therapy (ACT) is recommended for both adults and children suffering from uncomplicated knowlesi malaria by the World Health Organization (WHO). A parenteral artesunate therapy followed by ACT for at least 24 hours is recommended in severe cases. It is imperative to improve diagnostics, surveillance, and treatment strategies to control the rapid increase in knowlesi malaria cases. The

CDC recommends chloroquine or hydroxychloroquine treatment for adults or pediatric patients with uncomplicated P. knowlesi malaria. Treatment should be based on the patient's clinical condition, the Plasmodium species infecting him, and the parasite's suspected drug susceptibility. Previous antimalarial treatments, including those for malaria chemoprophylaxis, should also be considered. [1, 48]

Despite being antimalarial drugs, macrolides, doxycycline and antifolate antibiotics have also shown positive effects against malaria in non-human animal studies.[1]

As we conclude our exploration of Plasmodium knowlesi, we unveil a once mysterious pathogen, now revealing its intricate dance between hosts, mosquitoes, and molecular realms. This fifth human parasite, originating from animals, challenges our comprehension with swift replication in youthful blood cells. With a distinctive genetic profile and a concerning prevalence in Southeast Asia, it emerges as a formidable foe. Our journey underscores a pressing need for precise diagnostics and innovative therapies. Armed with newfound knowledge, we stand resilient against this stealthy adversary. This conclusion marks not an end but a beginning—a call for collective vigilance and unwavering pursuit in the ongoing battle against infectious complexities.

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