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Plasmodium knowlesi Infection: Should Africa be Prepared for a New Human Malaria Threat?

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Authors' contributions

This work was carried out in collaboration between all authors. Author KIO designed the review, wrote the protocol and performed the initial literature search for the manuscript. Authors MOI, MOO, ETO, IOI, UAU and EOS managed the literature searches and selection of important literature for the review. Authors KIO, MOI and MOO wrote the first draft of the manuscript. Authors SEI and DZE reviewed the initial drafts and synchronized the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background: Human malaria was always believed to be caused by any of four species of Plasmodium namely; *Plasmodium vivax*, *P. falciparum*, *P. ovale* and *P. malariae*. However, a few years ago, it was observed that *Plasmodium knowlesi* could naturally infect humans especially in South East Asia. Can we have *P. knowlesi* infections in Africa? Are we prepared for a new human malaria threat? African institutions might not be searching for this parasite. There is no documented endemic transmission of this parasite in Africa despite many factors that could support its occurrence probably due to clinical and laboratory challenges in the diagnosis of *P. knowlesi* infections. A misdiagnosis may delay appropriate therapy leading to fatal consequences.
Methodology: This article reviewed in some details many issues surrounding *P. knowlesi* malaria diagnosis and infection in other parts of the world and the many factors that could promote transmission and occurrence of this infection in Africa through a database search (PubMed, Google Scholar, Cab direct, and African Journals Online) using terms related to the intended review. This is an area that has not been explored by authors in the past.
Conclusion: The possibility of transmission of *P. knowlesi* in tourist sites in Asia and spread through international travel to Africa is a reality. The Zika and Ebola experiences are clear examples of how international travel and interactions between man and vectors could lead to fatal consequences. Subsequently, these consequences can be averted when the scientific and clinical communities are primed to recognize and combat an emergence of *P. knowlesi* infections in non-endemic regions.

Keywords: Plasmodium knowlesi infections; Africa; threat; preparedness.

1. INTRODUCTION

Plasmodium knowlesi, a common malaria parasite of macaques which could naturally infect humans is currently the sixth major human malaria parasite following the division of *Plasmodium ovale* into 2 different species [1,2]. It may cause severe malaria as indicated by its asexual erythrocytic cycle about 24 hours, with an associated fever that typically occurs daily [1, 3,4]. This parasite is transmitted by the bite of *Anopheles* mosquitoes (*Anopheles Leucosphyrus* and *Anopheles latens*) [1]. *Plasmodiu knowlesi* has health, social, recreational and economic consequences in regions where infection is common [4].

Although first reported as a natural cause of human malaria in 2004, retrospective analysis of molecular and epidemiological data indicate that *P. knowlesi* is not a new parasite, but a previously known animal parasite whose zoonotic potential was not correctly identified by earlier researchers [1,3,4]. *P. knowlesi* is a species naturally found in long-tailed macaque (*Macaca fascicularis*) and pig-tailed macaque (*Macaca nemestrina*) monkeys and has recently been shown to be capable of causing human infection [1]. More than half of the world population live in malaria risk areas. The annual malaria associated mortality approaches 1 million globally, with 2 children dying from the disease every minute [5]. Malari is endemic in Africa. Speciation of the different *Plasmodium* species causing malaria is rarely carried ouas most diagnosis is usually made clinically or microscopically in the laboratories. *P. knowlesi* infections are potentially fatal, but readily treatable if detected early enough [6]. Despite the availability of effective antimalarial agents, failure to make the correct diagnosis has resulted in inappropriate therapy leading to preventable deaths [6,7].

Spread of *P. knowlesi* through international travel has become a major public health concern, and the possibility of *P. knowlesi* infection becoming one of the major causes of malaria in Africa is real. This article is aimed at reviewing the parasite *Plasmodium knowlesi* as a potential cause of human malaria infection in Africa, and to identify the risk factors and likely diagnostic challenges associated with this infection. We also intend to highlight the impact of international travel on *P. Knowlesi* malaria infections especially as regards increased trade and bilateral ties between Africa and South East Asia, and to suggest possible ways of overcoming these challenges.

2. METHOD OF REVIEW

We conducted a database search (PubMed, GoogleScholar, Cabdirect, and African Journals Online) using broad terms related to the intended review such as *Plasmodium knowlesi* and

diagnosis, *Plasmodium knowlesi* and Africa and *Plasmodium knowlesi* and travelers. References in the identified articles were also screened. A total of 126 publications published in English were found, of which 26 were unobtainable. Articles found were screened for content and relevance. Seventy-four (74) of the 100 publications reviewed are referenced herein.

3. REVIEW AND DISCUSSION

3.1 Historical Background

Plasmodium knowlesi was first identified by the Italian scientist Giuseppe Franchiti in 1927 [8]. Dr. Robert Knowles and his assistant Das Gupta in 1932 described the blood forms of the parasite and demonstrated that the inoculation of infected monkey blood was able to produce a human infection [9,10]. This species was named *P. knowlesi* in honor of Dr Robert Knowles [11,12]. The first report of a case of naturally occurring infection of knowlesi malaria in humans was in an American man returning from Malaysia in 1965 [1,3]. He was a 37 year old US Army surveyor deployed for 5 days in the bush area of Malaysia [1,3]. He developed chills and fever on the way home to USA and was initially diagnosed as a case of an upper respiratory infection [1,3]. His family physician made a blood smear and saw ring forms similar to *P. falciparum* which he sent to the Clinical Centre of National Institute of Health (NIH) in Bethesda where a microscopic diagnosis of *P. malariae* was made [3,12]. The patient's blood sample was further sent to a group of malariologists studying *P. malariae* at the US penitentiary in Atlanta where it was finally recognized as *P. knowlesi,* following blood passage experiments into six human volunteers and three rhesus monkeys [3,12,13]. Three of the human volunteers required antimalarial therapy to terminate the infection whereas all the monkeys unfortunately died of overwhelming malaria infection. A few years later in 1971, there was another report of the natural infection of a man in Malaysia with *Plasmodium knowlesi* followed by the description of a large focus of human infections in the Kapit Division of Sarawak, Malaysian Borneo [7]. This was made possible due to the development of molecular detection techniques which could differentiate between *P. knowlesi* and the morphologically similar *P. malariae* [7,14]. Since 2004, there have been an increasing number of reports of the incidence of *P. knowlesi* among humans in various countries in South East Asia [14].

There are at least two subspecies of *P. knowlesi* known - *P. knowlesi edesoni* and *P. knowlesi knowlesi* [15]. The subspecies *P. knowlesi edesoni* was described by Garnham in 1963 [15]. It was named after the parasitologist J F B Edeson [15]. Clinical differences between these two subspecies are not yet known but researchers are studying their roles in clinical malaria infection.

3.2 Epidemiology of *Plasmodium knowlesi*

3.2.1 Geographic distribution

P. knowlesi is usually considered a parasite of macaques found in rainforest areas of parts of Asia [14,16]. With increasing deforestation and development efforts in South East Asia, many macaques are now coming in close and direct contact with humans [14]. Therefore, more and more people who live in semiurban areas are being found to be infected with knowlesi malaria. [14-16]. This parasite is mostly found in South East Asian countries particularly in Borneo, Cambodia, Malaysia, Myanmar, Philippines, Singapore, Thailand and neighboring countries, and it appears to occur more in regions that are reportedly free of the other four types of human malaria [17-19]. *Plasmodium knowlesi* is said to be absent in Africa for now as there are no studies that have proof of endemic transmission.

3.2.2 Morbidity and mortality

According to World Health Organization (WHO), malaria globally accounts for about 219 million cases and an estimated 1 million deaths per annum [5]. Children living in Africa are more vulnerable where a child dies every minute from malaria although of other plasmodium species different from knowlesi. In certain areas of South East Asia, *P. knowlesi* accounts for up to 70% of all malaria cases [14]. At least one fifth of the cases of malaria diagnosed in Sarawak and Malaysian Borneo are due to *P. knowlesi* [2]. Data is still very scanty on the specific morbidity and mortality of *P. knowlesi* infections in affected regions. However, researchers believe that *P. Knowlesi* contributes a large quota of morbidity and mortality attributable to malaria in South East Asia [14].

3.2.3 Vectors and modes of transmission

Theoretically there are four modes of transmission: from an infected monkey to

another monkey, from an infected monkey to a human, from an infected human to another human and from an infected human back to a monkey [1,20,21]. The known vectors belong to the genus *Anopheles*, subgenus *Cellia*, series *Neomyzomyia* and group *Leucosphyrus* [1]. Mosquitoes of this group are typically found in forest areas in South East Asia but with a greater clearing of forest areas for farmland, humans are increasingly becoming exposed to these vectors. These vectors are also found in African rain forest and savannah regions [20].

3.2.4 Risk factors for transmission

Plasmodium knowlesi infection is normally considered a parasite of monkeys. Humans who work at the forest fringe or enter the rainforest to work are at risk of infection [1]. Following massive deforestation and development efforts in Asia in the past few decades, many macaques are now coming in close contact with humans who work close to them or keep them as pets. Currently, massive infrastructure development has also been embarked upon by many African countries. In addition, hunting of apes for food is still a common practice in some parts of Africa**.** Taking a practical lesson from several Ebola disease outbreaks in Africa which were results of close interactions between humans and African apes through hunting, consumption as bush meat, keeping as pets or for market shows, one can assume that a high risk of transmission exists in Africa just as in Asia.

3.3 Pathogenesis and Life Cycle of *Plasmodium knowlesi*

Plasmodium knowlesi replicates and completes its blood stage cycle in humans within 24 hours resulting in fairly high loads of parasite densities in a very short period of time which makes it a potentially severe disease if it remains untreated in humans [1]. Incubation period for *P. knowlesi* is 8 to 12 days [1,3]. This life cycle duration is the shortest of all the known malarias that infect humans and primates [1,21]. In man, the life cycle begins with the exoerythrocytic stage in the liver similar to the typical life cycle of other *Plasmodium* species [21]. These stages of *P. knowlesi* are microscopically indistinguishable from *P. malariae* and the early trophozoites are identical to those of *P. falciparum* [21]. This is what accounts for the diagnostic inaccuracy by microscopy [22]. In the mosquito vector, the stages in the life cycle are also identical to those of other species of *Plasmodium* [21].

3.4 Clinical Manifestations of *Plasmodium knowlesi* Infection

Patients experimentally infected with *P. knowlesi* either by intravenous or intramuscular injection of infected blood, developed clinical manifestations generally after an average of eight days (3-14 days) [3]. Temperature rise presented a quotidian behavior with the highest peak at 40 °C [3,7,22]. Many studies have shown that there are no distinctive presenting symptoms or signs that may help the clinician separate *P. knowlesi* malaria from malaria caused by the other human species [7,22-35]. Singh et al. [7] showed that clinical symptoms in 94 patients with single species *P. knowlesi* infection were fever, chills, and rigor in 100% of patients, headache in 32%, cough in 18%, vomiting in 16%, nausea in 6%, and diarrhea in 4%. Respiratory distress, renal failure, jaundice and hyperparasitemia were the most frequently observed manifestations of severe malaria in another study [25]. We may not yet know if partial immunity from transmission of other *Plasmodium* species can alter presentation in sub-Saharan Africa.

3.5 Treatment

Uncomplicated *P. knowlesi* malaria seems to be responsive to all schizonticidal antimalarial drugs currently employed in clinical practice [1,7,23, 25,31,36,37]. Chloroquine at standard doses plus primaquine (15 mg/base for 2 days) has been shown to be effective in cohort studies [7,38]. The experience with the use of artemisinin derivatives for the treatment of uncomplicated *P. knowlesi* malaria is limited but shows a faster parasite clearance [36]. It remains to be determined what would be the best treatment to be employed in case of severe *P. knowlesi* malaria. In a non-randomized study, artesunate was associated with faster parasite clearance and a better outcome (17% case fatality rate versus 31%) when compared to quinine [36]. *P. knowlesi* infection can be treated using already existing anti-malaria therapy [7,36,38].

3.6 Prevention and Control

Public health control may be challenging in areas where zoonotic human malaria is endemic. Insecticide treated nets (ITNs), indoor residual spraying, and intermittent preventive treatment in the reservoir population are likely to be less effective than for typical forms of human malaria because of its outdoor transmission potentials [39]. Current indoor control measures for malaria

do not prevent zoonotic transmission of malaria by vectors that mainly feed in the forest [14,33]. Zoonotic *P. knowlesi* infection can continue to be a problem for malaria control, and also poses a significant threat to the renewed efforts aimed at fully eradicating malaria [39]. Individuals at risk and travelers to endemic areas are encouraged to imbibe mosquito bite protection measures and chemoprophylaxis as practiced for other types of human malaria. Immediate diagnosis and treatment if a fever develops within 3 months of departure from a risk area may help in control efforts.

3.7 Challenges in the Diagnosis of *Plasmodium knowlesi* Infections

There are both clinical and laboratory challenges in the diagnosis of *P. knowlesi* infections especially in resource-limited settings in Africa.

Similar to other human malaria, *P. knowlesi* infection presents with fever accompanied by constitutional symptoms such as headache, rigors, malaise, myalgia, abdominal pain and breathlessness [22]. This poses a major challenge to clinicians who are unable to differentiate knowlesi malaria infection from other human malaria on clinical grounds alone. This is aside several other common causes of febrile illnesses such as typhoid fever and viral infections. However, cerebral involvement which occurs in complicated falciparum malaria and occasionally in severe vivax malaria has not yet been reported in knowlesi malaria [25]. Moreover, just as in *vivax* and *falciparum* malaria*, knowlesi* malaria also causes thrombocytopenia [25,40,41].

Microscopic examination of thick and thin blood smears in the laboratory fails to correctly identify *P. knowlesi* because its early trophozoites resemble the ring forms of *P. falciparum*, and its later stages mimic those of *P. malariae* (Fig. 1) [22]. This explains why *P. knowlesi* infection is usually misidentified as *P. falciparum* and/or *P. malariae* infection [22,42,43]. Microscopy is considered as the gold standard malaria investigation and can establish the diagnosis in parasitemia of >50 parasites/µl. However, researchers have now established that microscopy alone cannot reliably distinguish *P. knowlesi* from *P. falciparum*, *P. malariae* and *P. vivax* [44,45]. The early trophozoites of *P. knowlesi* have features such as double chromatin dots, multiply-infected erythrocytes and appliqué forms which are also found in that of *P. falciparum* [44,46]. Late and mature trophozoites, schizonts and mature gametocytes of *P. knowlesi*are generally indistinguishable from those of *P. malariae*. “Band form trophozoites,” a characteristic feature of *P. malariae* is also seen in *P. knowlesi* infection [44].

Rapid diagnostic immunochromatographic tests (RDTs) detecting malarial antigens has become widely used in most countries as an alternative to microscopic differentiation of the *Plasmodium* species [42]. Due to the predominance of *vivax* and *falciparum* malaria, almost all of these RDTs are designed to detect either or both of these antigens in a single kit [42]. RDTs with monoclonal antibodies against the *P. vivax* specific lactate dehydrogenase (pvLDH) exclusively identify *P. vivax* while those against histidine rich protein-2 or *P. falciparum* specific lactate dehydrogenase (pfLDH) specifically detect *P. falciparum* [42]. In addition to this, certain RDTs also detect but not differentiate the antigens of other *Plasmodium*species using monoclonal antibodies against pan-malarial markers such as the pan-*Plasmodium* lactate dehydrogenase (pLDH) or *Plasmodium* aldolase (pALD) [42].

Introduction of RDTs have revolutionized the diagnosis of human malaria. The advantages range from being rapid, user friendly to its cost effectiveness [47]. The RDTs have the capability to detect parasitemia of >100 parasites/µl of the four well-known *Plasmodium* species [47]. However, *P. knowlesi* specific RDTs are not commercially available and the existing formats of RDTs are found to perform suboptimally when used in *P. knowlesi* endemic regions [48]. The sensitivity of the pan malarial marker pALD, has been found to be the lowest while detecting *P. knowlesi* antigens when compared with the detection of *P. falciparum* and *P. vivax* antigens. The overall sensitivity of pALD for detecting *P. knowlesi* is reported to be as low as 23% [48,49,50]. *P. knowlesi* infections also pose a hindrance in the specific diagnosis of *P. vivax* and *P. falciparum* using RDTs. The protein expression and characterization of the *P. knowlesi* lactate dehydrogenase (pkLDH) has demonstrated 96.8% homology to pvLDH and over 90% homology to pfLDH [50]. This antigenic similarity is held responsible for the cross reactions observed in the *P. vivax* and *P. falciparum* specific RDTs designed to detect the parasite specific LDH [48,49]. This takes us back to *’status quo’* with microscopy and the challenges of diagnosing *P. knowlesi* infections

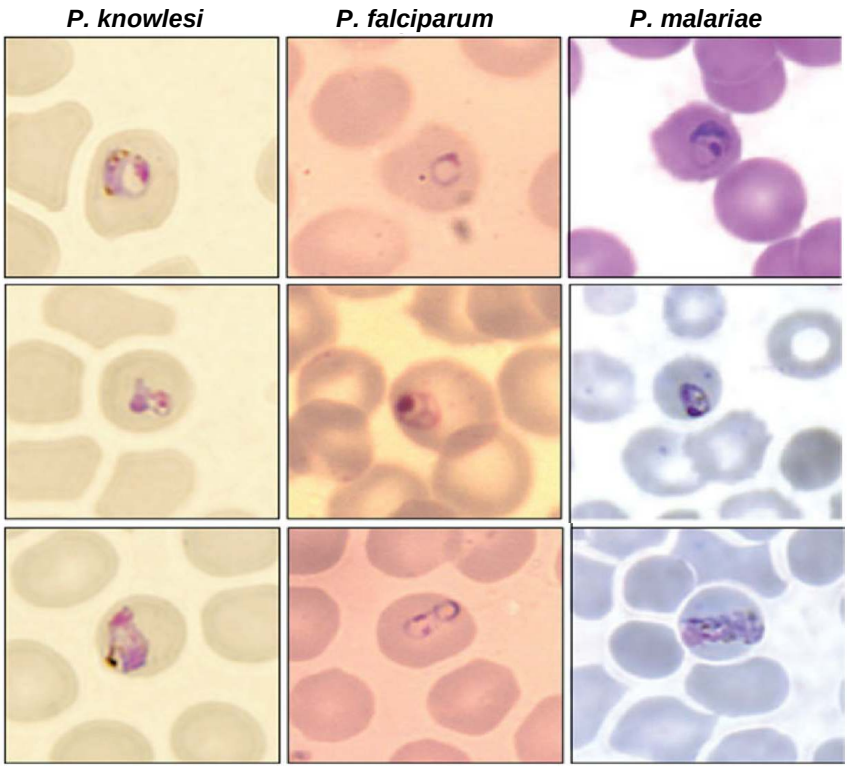

Fig. 1. Morphologic forms of different types of Plasmodium with similar features as seen during microscopic examination

This is an example of the challenge in species identification using microscopy. On the left are photos from thin smears from a patient with a Plasmodium knowlesi infection after a visit to Malaysia. In the middle are thin smears from patients with Plasmodium falciparum infection, and on the right are thin smears from patients with Plasmodium malariae infection (Giemsa or May-Grünwald-Giemsa-staining; original magnification, ×1000).
Adapted from Kantele et al. [22]

as different from the other known human plasmodia infections.

Using recombinant deoxyribonucleic acid (DNA) technology, researchers have developed a 34 kDa pkLDH and have proposed that it has a role in RDTs for the specific diagnosis of P. knowlesi [51]. However, due to the cross reactivity exhibited among the LDH antigens of the different Plasmodia, its potential use is also questionable. Subsequently, novel antigenic targets with increased specificity in species discrimination are being evaluated. Some of the targets studied include the *P. knowlesi* merozoite surface protein (pkMSP1-[33]), *P. knowlesi* merozoite surface protein-142 (pkMSP-142), *P. knowlesi* 1Cys peroxiredoxin and the *P. knowlesi* surface protein containing an altered thrombospondin repeat domain [52-55]. However, these molecules too are not devoid of inter-species cross reactivity and an ideal antigenic target is yet to be identified [55].

Considering the unprecedented challenges in microscopy and antigen detection tests, molecular methods are currently the only reliable option for the definitive diagnosis of *P. knowlesi* malaria infections [7]. In fact, the very discovery of human knowlesi malaria was made possible only due to molecular techniques [7]. The 18S small-subunit rRNA (ssu-rRNA), the circumsporozoite surface protein gene, a nuclear gene encoding a cysteine protease and the cytochrome b gene are some of the targets currently studied [14]. Among the aforementioned targets, the genetic variations in the 18S ssu-rRNA are the most studied and widely used for the species discrimination of the different Plasmodia [56-58].

Unfortunately, PCR techniques used for the diagnosis of malaria that is not specifically designed for detection of *P. knowlesi* fail to detect it [37,59]. This parasite has been detected only with *P. knowlesi*– specific PCR assays that

use nested PCR [60], real-time PCR [61], or PCR combined with sequencing [23]. The initial molecular test deployed for the detection of *P. knowlesi* was the conventional nested multiplex PCR targeting the 18 S ssu-rRNA gene developed by Singh et al. [7]. Although, nested PCR is considered the "molecular gold standard" in the diagnosis of malaria, it has various drawbacks. As the technique involves five to six separate PCR reactions to detect the five *Plasmodium* species, it requires a large number of reagents and consumables and also involves more handling and possible cross contamination. Moreover, the assay is capital intensive, cumbersome, time consuming, labor intensive and not available in most African institutions [57].

Another consideration is the real time multiplex PCR which have the advantages of rapidity, direct quantification without post amplification analysis and minimal cross contamination over the conventional ones [62]. Currently, real time PCR is one of the most advanced molecular tests available for the combined detection of *P. knowlesi* along with the other human Plasmodia [62]. It can be introduced in any of the existing platforms in previously equipped molecular laboratories [62]. However, expensive reagents and high installation costs are the major drawbacks facing the introduction of these tests in many laboratories [57]. These factors are even more important in most parts of South East Asia and Africa where resources are highly limited [57]. Considering the limitations of conventional nested PCR and the real time PCR techniques, some researchers have developed a straightforward single-step hexaplex PCR system targeting the 18S ssu-rRNA of the five human Plasmodia [56]. The procedure eliminates the need of time consuming multiple rounds of amplification as in nested multiplex PCR and the necessity of expensive equipment and reagents as with real time PCR [63].

Another molecular method that has been considered for *P. knowlesi* diagnosis is the Loop mediated isothermal amplification (LAMP) [64,65]. This molecular method rapidly amplifies nucleic acids at a constant temperature [64,65]. The technique has certain advantages over the PCR such as rapidity, minimal user training and lower installation cost as it does not require a thermal cycler [64]. These assaysare capable of detecting extremely low level parasitemia, can be performed directly on the blood sample and are claimed to be 10-100 times more sensitive than the currently available PCR formats [65]. Because *P. knowlesi* malaria is life threatening and difficult to diagnose in the laboratory, this parasite should always be suspected in cases in which microscopic examination suggests *P. malariae* or a recent history of woods/forest visitation or travel to South East Asia.

3.8 *Plasmodium knowlesi* as Cause of Human Malaria in Africa

As of the time of this review, *P. knowlesi* infections have been reported only from Indonesia, Thailand, Myanmar, Malaysia (peninsular and Borneo), Singapore, Cambodia, Vietnam, Brunei, the Palawan Island in the Philippines and the Nicobar and Andaman Islands [1,3,17-19,22-24]. There have been imported cases from South East Asia to many other countries [25,26]. There are no known cases of *P. knowlesi* in Africa. This may be because there are no long-tailed and pig-tailed macaques (the reservoir hosts of *P. knowlesi*) in Africa or that it is possibly being misdiagnosed as other forms of malaria or other febrile illnesses. In the light of these uncertainties, the question is: Is Africa really devoid of *P. knowlesi*?

Interestingly, similar to *P. vivax*, *P. knowlesi* uses the Duffy blood group antigen as a receptor to invade human erythrocytes as they both share close phylogenetic relationships [66-68]. Many West Africans lack the Duffy antigen - a protein on the surface of the red blood cell [69]. However, the paradigm that erythrocytes of Duffy negative individuals are always refractory to *P. vivax* infection has been recently challenged by reports from East and Central Africa [69,70] and the Brazilian Amazon [71]. Therefore, it is presently unknown if *P. knowlesi* may have started exploiting alternative invasion mechanisms. It has been previously shown that *P. knowlesi* merozoites are able to interact with Duffy negative human erythrocytes and that apical orientation take place normally but invasion is aborted since a junction does not develop [72]. In 1975, Miller and his colleagues showed that *P. knowlesi* merozoites would not invade Duffy negative human erythrocytes as they did successfully in Duffy positive cells. In 1976, these researchers proved the refractoriness of *P. vivax* in Duffy negative black patients [73,74]. More studies are required to evaluate the possibilities of *P. knowlesi* invading Duffy negative erythrocytes. The Duffy antigen absence is only amongst blacks of West African descent. It does not explain the absence of *P. knowlesi* infection amongst other Africans who

possess the Duffy antigen nor does it explain the absence of infections in Northern Africa where we have individuals of Arabian descent or in Southern Africa where we have numerous Caucasians and Asians.

The presence and adaptability of the macaque reservoir is another factor to be considered in the absence of *P. knowlesi* in Africa. The absence of the reservoir Macaque monkeys in Africa may be a plausible explanation for this phenomenon. This only means that we still have tremendous risk of *knowlesi* malaria infection in Africa when these monkeys migrate to Africa or are imported into Africa as pets or as zoo animals. The Zika and Ebola experiences which started as a result of close proximity of man and vectors in certain environments only means that the possibility of cross transmission of *knowlesi* malaria infection is real. Studies already show that the *Anopheles* vectors of *P. knowlesi* are present in our environment [19]. Therefore, we must all be on the lookout with a high index of suspicion as the African environment can support the existence and transmission of the malaria parasite *P. knowlesi*.

3.9 Impact of International Travel on *Plasmodium knowlesi* Infection

International travel has made the world a global village. One can hop on an airplane from Malaysia at dusk and be in Nigeria at dawn. The first reported case of *P. knowlesi* infection was in a traveler from Malaysia in 1965 [3]. The next case was reported years later in a Finnish man who was infected during a four week visit to Peninsular Malaysia, where he stayed in a jungle area for 5 days [23]. Many more cases of *P. knowlesi* have been reported as a result of the increased frequency of international travel. The Finnish traveller did not take any malaria prophylaxis, and his illness was initially misdiagnosed by microscopy as co-infection due to *P. falciparum* and *P. malariae* [23]. This and the other cases were only confirmed using PCR and genetic sequencing.

Two cases have been reported from non-endemic countries in Asia (Japan and Taiwan) in people with a history of travel to Malaysia and the Philippines [23]. As of the time of this review, twelve cases were imported to their home countries by travelers from other continents: two cases reported from the USA [22], two from the Netherlands, two from Germany, and one each from Spain, France, Sweden, Finland, Australia, and New Zealand. In most cases, the infection was associated with a trip to or near forested areas. The common symptoms exhibited by the patients who imported these cases were fever (n=12), headache (n=6), chills (n=6), nausea (n=4), myalgia (n=3) and back pain (n=3). All these are symptoms of almost any febrile illness and these patients were initially misdiagnosed as having any other febrile illness but *knowlesi* malaria infection.

P. knowlesi appears to be a threat not only to the local population in Malaysia, but also to the estimated 25 million annual tourists [73], students and occupational travelers to Malaysia, especially those who visit rural, forested areas of the country many of whom are from Africa. Therefore, the *P. knowlesi* risk is not limited to Malaysia. Travelers from South East Asia presenting with any febrile illness should be considered for a diagnostic work-up that includes *P. knowlesi*. This includes both foreigners and Africans returning from South East Asia.

4. CONCLUSION

Currently, human *P. knowlesi* infections have so far been limited to South East Asian countries except for a few cases imported into other countries by travelers. As *knowlesi* malaria has a greater risk in development of complications than *falciparum* malaria, an accurate and timely diagnosis can be lifesaving. Consequently, Africans need to be educated that there may be a fifth human malaria parasite and there is a need to challenge our scientists, researchers and clinicians to search for this parasite and study its infectivity. The possibility of transmission of *P. knowlesi* in tourist sites is well documented and can easily spread through international travel. In Africa, clinicians may have to consider this diagnosis in patients who had visited and stayed in forested areas of Southeast Asia. A confirmed diagnosis of *P. knowlesi* malaria requires the use of specific molecular facilities which are lacking in many African institutions. In the light of the above conclusions, screening of African captive or forest monkeys for the presence of *P. knowlesi* should be looked into, equally, an experimental demonstration of *knowlesi* malaria in African monkey species should be considered. We also propose a review of malaria diagnostic protocols to include *knowlesi* malaria. In addition, adequate training of clinicians and laboratory workers on case identification, diagnostic microscopy and specific *P. knowlesi* PCR techniques should henceforth take priority.

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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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