Plasmodium knowlesi: from Malaysia, a novel health care threat

Plasmodium knowlesi: dalla Malesia una nuova minaccia sanitaria

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Until recently there were only four Plasmodium parasites involved in causing human malaria: Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale probably evolved in ancient times alongside humans (so-called co-evolution), while Plasmodium falciparum is a proportionally more recent human parasite, which was passed by monkeys to humans, probably between the late Mesolithic and the early Neolithic era [1-7]. Monkeys are known to become infected by over 20 species of Plasmodium, although the primate-human transmission of these parasites has been considered since recently an infrequent event, with a reduced public health impact. The transmission of these Plasmodium species has been documented, although with a varied frequency, predominantly in South-East Asia, China, and Central America [8].

The expansion of human activities in former, poorly inhabited areas (i.e. historical rain forests which were turned into productive areas), and woodland regions, which are increasingly visited and explored by tourists on trekking holidays, represent objective elements which lead to an increased risk of plasmodial infection of simian origin. Moreover, the present demographic expansion of indigenous populations in different regions of Southern Asia and Southern America, also including woodland regions or areas close to rain forest, in small villages where primary agricultural and livestock breeding are performed, represent another, considerable epidemiological risk factor. In this context, there is an overlap of human and simian habitat, in the presence of mosquitoes belonging to the genus Anopheles, which are indispensable for human transmission of malaria infection.

Among the 20 Plasmodium species which are able to infect monkeys, five have been documented as agents which are infectious for humans: i.e. Plasmodium simium, Plasmodium braziliun (in Southern America), and Plasmodium cynomolgi, Plasmodium inui, and Plasmodium knowlesi (in South-Eastern Asia) [8]. Most strains of simian malaria are responsible for a mild to moderate, and frequently self-limiting, disease in humans; these infections infrequently require anti-malarial therapy, since they are commonly missed. The identification of P. knowlesi in the states of Sarawak (Malaysian Borneo), and Sabah (another state belonging to the Malaysia Confederation), between the end of the 20th century and the early 21st century, all of them confirmed by molecular biology techniques, has drawn the attention of researchers and clinicians who operate in South-East Asia, and more recently international health care authorities as well, focusing on patients who experienced typical malaria signs and symptoms, and received in particular a microbiological diagnosis of P. malariae infection [8-10]. A notable feature of these patients is given by frequent, elevated parasitaemia (Figure 1), and the severity of the clinical picture, should patients not receive appropriate and timely treatment [9, 10]. We recall that four fatalities have also been mentioned in the published reports [8-10].

After identifying bio-molecular primers specific for P. knowlesi, allowed to pose a definitive diagnosis also in subjects who were parasitized, and with their erythrocytes were morphologi-
cally indistinguishable from those of individuals infected by *P. malariae*, but when assessed with molecular biology primers specific for *P. malariae*, failed to test positive.

The most severe clinical consequence of *P. knowlesi* malaria is related to the evidence of its daily replication cycle and, when not countered by an adequate therapy, it may rapidly reach potentially lethal levels of parasitaemia, while we recall that the *P. malariae* cycle has its replication every three days (quartan fever), and never reaches very elevated levels of parasitized erythrocytes [9-11]. A particular feature of *P. knowlesi* is also a similar shape of early trophozoite forms to those of *Plasmodium falciparum*, whereas all other stages recall the aspect of *P. malariae* parasites.

Recent entomological studies show that the most efficient carrier of human transmission is the *Anopheles leucosphyrus*, and the most elevated sting rate is registered when humans are located close to rain forest (6.74%), while it is lower inside the forest (1.85%), and it is very low in longhouses (0.28%), which are the typical Malaysian dwellings (Figure 2) [12].

According to the reported data, it has been speculated that humans acquire the infection inside the forest, when going hunting, and/or when coming back to the farm at sunset, after work, while the carrier seems to acquire the Plasmodium from wild monkeys; this opinion is supported by the absence of evidence of epidemic clusters inside longhouses (until now), and the highest number of infected mosquitoes has been found in the rain forest, or at its margins [13].

After the early reports of Malaysian malaria foci, involving the native population, and identification of anecdotal imported cases in European or Northern American tourists who spent a short time inside the rain forest, or close to

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1 Professor Babir Singh of Malaysia-Sarawak University believes that the *Saimiri scirea* monkey may be used as an experimental model of infection rather than a natural model of infection (B. Singh, Personal Communication, 2010).

2 At present, the *Macaca mulatta* (Rhesus monkeys) is fairly common in Western Afghanistan, in India, in Northern Thailand; in addition, it was historically abundant present in Southern China and Tibet, although hunting caused its sharp decline.
South-East forests, the researchers’ attention shifted towards *P. knowlesi* [14-18]. In the year 2008, the journal *Nature* first published the nuclear genomic sequence of *P. knowlesi* [19]. It was the first complete “sequencing” of a simian plasmodium, which also makes a comparison possible with the genomic sequence of *Plasmodium vivax*, and that of other already sequenced plasmodia. As opposed to other plasmodium genomic patterns, familiar putative antigenic variants are scattered due to the genomic sequence, and they are associated to repeated intrachromosomal telomeres. Some of these genomic families, the so-called KIRs, contain sequences which are related to one half of the intracellular domain CD99 of the animal host, so that it was supposed that these sequences may lead to an unusual form of molecular mimicry [19, 20].

General, relevant information has been provided by the recent observations and by epidemiological surveys conducted in Malaysia. The human habitat is increasingly overlapping that of primates. This phenomenon is mainly due to the expansion of human-driven activities (agriculture, tree harvesting aiming to provide fine wood, and livestock farming), in areas where monkeys do live. The second emerging aspect is related to the increased presence and activity (i.e. trekking) of Western tourists, who after travelling in these inhospitable areas for a limited period of time, return to their countries of origin [11]. In this case, the poor knowledge of simian plasmodia and related diagnostic problems may disorientate the physicians, on the basis of a standard microbiological-parasitological examination, which does not allow *P. malariae* to be distinguished from *Plasmodium knowlesi*, thereby putting the patient’s life at risk [11]. Molecular biology techniques prove extremely useful in depicting the general epidemiology of malaria, and to completely clarify the role and importance of mixed infections which are still largely underestimated [11].

However, this testing is expensive, and not easily accessible to all countries, with special reference to developing nations.

When taking into account the epidemiological characteristics of the areas where epidemic foci and isolated imported cases of a “travel-related” *P. knowlesi* disease have been registered, it does not seem possible to establish definitively whether infection was acquired from an animal reservoir (i.e. Macacus monkeys), or whether cases of interhuman contagion occurred. Anyway, it is now clear that these animal pathologies are no longer the expression of episodic facts, but represent a true health care emergency in South-East Asia.

Figure 4 gives an overview of the co-evolutionary pathway of malaria Plasmodia together with that of the genus Homo. From hominids (group A), until contemporary humans (group C), with the appearance of *Plasmodium falciparum* which penetrated this scenario by infecting humans (group B) in the Mesolithic and early Neolithic: these humans live in simple huts at the edge of the rain forest, close to fields used for early agriculture. The early introduction of the first human activities helped the spread of *Plasmodium falciparum* malaria in the pathocenosis of prehistoric populations, unavoidably conditioning economic and anthropological development.

With regard to the appearance of a novel malaria parasite which is epidemiologically relevant to humans, the hope is that this novel pathocenosis remains circumscribed to regions in South-East Asia, without the involvement of further tropical and equatorial areas of other continents. In India, Thailand and Western Afghanistan, the diffuse presence of *Macaca*...
**mulatta** may represent a potential reservoir for *P. knowlesi*, opening the way to an epidemiological spread of the infection to regions which have not been involved by the problem until now. Should this occur, catastrophic effects are expected, not only for the native populations who lack a specific immune memory, but also for the ever growing number of tourists who, after making their excursions in the rain forest, will be exposed to the risk of a cumbersome and displacing microbiological examination, at the time of becoming sick and coming back to the country of origin.

Considerable complications related to the temporary lack of commercial, rapid and specific parasitological diagnostic tools, and possible subsequent delays in disease recognition and management, are expected in the immediate mean time, since the novel and rapid diagnostic kits will become available shortly and worldwide.

According to information updated at the end of November 2010, some commercial, rapid diagnostic kits including also *P. knowlesi* recombinant antigens, are under very advanced development, and are expected to add significantly to the prompt and timely recognition of the fifth human Plasmodium, in a route comparable to that already traced several years ago for the rapid laboratory search of other human malaria Plasmodia.

As anticipated, diagnostic retardation may become fatal, should *P. knowlesi* infection not be identified in a reasonably short time. A recent prospective clinical study conducted in the Sarawak regions of Malaysian Borneo, disclosed that approximately 10% of patients infected with *P. knowlesi* had severe signs and symptoms already upon presentation of briefly later, and 1-2% of cases have a fatal outcome [21]. The complications occurred in patients who survived an acute, severe *P. knowlesi* malaria attack, include the acute respiratory distress syndrome (ARDS), liver and/or kidney end-organ dysfunction, hypotension, with or without a parasitaemia over 100,000 trophozoites/µL.

All lethal cases of *P. knowlesi* disease were characterized by prominent abdominal signs and symptoms, associated to a combined kidney-liver dysfunction, and the concurrent hyperparasitaemia [22].

From a histopathological point of view, the post mortem lesions observed in a 40-year-old man who rapidly deceased after 14 days with a “collapse”-like disease and quick death, was similar to that observed in the fatal *P. falciparum* malaria. It was the typical sequestration of pigment-ed, parasitized erythrocytes in the cerebellum, brain, heart, and lung vessels without any evidence of a chronic inflammatory reaction in the central nervous system or other examined or-

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**Figure 4** - The picture depicts the biological and phylogenetic evolutions of *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* (Group A), compared with the more recent evolution pathway of *Plasmodium falciparum* (Group B). *Plasmodium knowlesi* (belonging to Group C) is the last Plasmodium species which steadily entered the human pathology, and is related to productive and tourist human activities carried out in the forest regions of Malaysia and Borneo. The image was taken and modified from some studies published by Luigi Capasso (quoted among references), especially with Group C malaria parasites [1, 21].
organ, while an extensive tubular necrosis involved the kidneys.
The clinical differences, i.e. the absence of coma and other neurological complications notwithstanding the petechial haemorrhage, regardless of the parasite sequestration inside the brain, are characteristic features [22].
Until now, the therapeutic perspectives seem less worrying when prompt diagnosis is obtained during the initial disease phases. In a very recent clinical study performed at the Kapit Hospital (Sarawak, Malaysian Borneo), the authors assessed the response to a treatment with chloroquine at 10 mg/kg of body weight, followed by 5 mg/kg after 6, 24, and 48 hours, for a total of 25 mg/kg [23]. Twenty-four hours later, after excluding a deficiency of glucose 6-phosphate dehydrogenase, oral primaquine (at 15 mg/kg of body weight) was delivered for two consecutive days (as a gametocidal drug). All patients had a non-severe, non-complicated malaria, with a parasitaemia ≤100,000 trophozoites/micron. All treated subjects successfully benefited from therapy, and 96% of them had a frank improvement of signs and symptoms already after the first 24 hours of treatment [23]. The data presented in this Malaysian study demonstrate that conventional chloroquine dosages are associated to an initial parasite clearance which was more rapid, when compared to that of *P. vivax* (which acted as the control group in the Malaysian experience); furthermore, this therapeutic response proved to be the most rapid, amongst all malaria forms [24].
These last studies are very encouraging, since they demonstrate that a low-cost drug like chloroquine may be affordable in developing countries [23]. The authors, however, recommend to develop further rigorous studies, in order to assess whether this treatment may be used also in the severe forms of *P. knowlesi* malaria.

**Key words:** malaria, *Plasmodium knowlesi*, emerging parasite diseases.

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**SUMMARY**

Epidemic foci of *Plasmodium knowlesi* malaria have been identified during the past ten years in Malaysia, in particular in the States of Sarawak and Sabah (Malaysia Borneo), and in the Pahang region (peninsular Malaysia). Based on a review of the available recent international literature, the authors underline the importance of molecular biology examinations, polymerase chain reactions (PCR), performed with primers specific for *P. knowlesi*, since the current microscopic examination (haemoscope) may fail to distinguish *P. knowlesi* from *Plasmodium malariae*, due to the very similar appearance of the two parasites. *P. knowlesi* has been described as the causal agent of life-threatening and lethal forms of malaria: its clinical picture is more severe when compared with that of *P. malariae*, since the disease is characterized by greater parasitaemia, as opposed to that documented in the course of *P. malariae* disease. The most effective carrier is *Anopheles leucosphyrus*: this mosquito is attracted by both humans and monkeys. Among primates, the natural hosts of *P. knowlesi* are *Macaca fascicularis* and *Macaca nemestrina*, while *Saimiri sciurea* and *Macaca mulatta*, which cannot become infected in nature, may be useful in experimental models. When underlining the potentially severe evolution, we note the key role played by prompt disease recognition, which is expected to be more straightforward in patients monitored in endemic countries at high risk, but should be carefully implemented for subjects being admitted to hospital in Western countries suffering from the typical signs and symptoms of malaria, after travelling in South-East Asia where they were engaged in excursions in the tropical forest (trekking, and similar outdoor activities). In these cases, the diagnosis should be prompt, and suitable treatment should follow. According to data in the literature, in non-severe cases chloroquine proves very effective against *P. knowlesi*, achieving the disappearance of signs and symptoms in 96% of cases after only 24 hours after treatment start. In the light of the emerging epidemiological data, *P. knowlesi* should be added to *Plasmodium vivax*, *Plasmodium ovale*, *P. malariae*, and *Plasmodium falciparum*, as the fifth aetiological agent of malaria. During the next few years, it will become mandatory to plan an appropriate surveillance program of the epidemiological evolution, paying also great attention to the clinical features of patients affected by *P. knowlesi* malaria, which are expected to worsen according to the time elapsed; some studies seem to point out greater severity according to increased parasitaemia, paralleling the increased interhuman infectious passages of the plasmodium.
Foci epidemic di malaria da Plasmodium knowlesi sono stati individuati negli ultimi dieci anni in Malesia, in particolar negli stati di Sarawak e di Sabah (Borneo Malese) e nella regione di Pahang (Malesia peninsulare). Sulla scorta della disamina della letteratura recente gli Autori sottolineano l’importanza dell’esame diagnostico di biologia molecolare (PCR, polymerase chain reaction), eseguiti con primer specifici per P. knowlesi, in quanto si è osservato che all’esame microscopico tale plasmodio non ha caratteristiche peculiari ed è difficilmente distinguibile dal Plasmodium malariae. Si è osservato che in alcuni casi il P. knowlesi può portare al decesso, presentando un quadro clinico più grave di quello osservato nelle infezioni da P. malariae e presentata, come caratteristica laboratoristica differenziale, la tendenza a determinare parasitemie più elevate rispetto a quelle documentate nella malaria da P. malariae. Il veettore più efficace è l’Anopheles leucosphyrus; questa zanzara viene attirata sia dall’uomo che dalla scimmia. I primati ospiti naturali sono la Macaca fascicularis e la Macaca nemestina, mentre la Saimiri sciurea e la Macaca mulatta, che non si infettano in natura, possono essere utili come modelli sperimentali. Vista la possibile evoluzione clinica grave, si sottolinea l’importanza del riconoscimento precoce non solo nei pazienti ricoverati nelle zone a rischio ma anche nei pazienti che si presentano negli ospedali occidentali, dopo viaggi nel Sud-Est asiatico ove hanno compiuto escursioni nella foresta tropicale (trekking), accusando i sintomi tipici della malaria. In questi casi la diagnosi deve essere precoce e si deve subito trattare adeguatamente il paziente. Secondo dati della letteratura, nei casi non gravi, la clorochina ha dimostrato essere farmaco molto efficace, determinando la scomparsa dei sintomi dopo 24 ore dall’inizio del trattamento nel 96% dei casi. Alla luce dell’emergente quadro epidemiologico, il P. knowlesi si è aggiunto ai plasmodi ovoale, ovale, malariae, e falciparum, come quinto agente etiologico dalla malaria. Nei prossimi anni sarà necessario sorvegliare adeguatamente l’evoluzione epidemiologica, valutando anche se le caratteristiche cliniche dei pazienti con infezione da P. knowlesi evolvono con il tempo verso un peggioramento; alcuni studi sembrano indicare un aumento di gravità, con aumento dell’indice di parasitemia, aumentando il numero dei passaggi infettivi interumani.

**REFERENCES**


**RIASSUNTO**