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NON-HUMAN PRIMATE AND HUMAN MALARIA: PAST, PRESENT AND FUTURE

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ABSTRACT

Rationale

Studies of the malaria parasites infecting various non-human primates (NHPs) have increased our understanding of the origin, biology and pathogenesis of human *Plasmodium* parasites.

This review considers the major discoveries concerning NHP malaria parasites, highlights their relationships with human malaria, and considers the impact that this may have on attempts to eradicate the disease.

Key findings

The first description of NHP malaria parasites dates back to the early 20th century. Subsequently, experimental and fortuitous findings indicating that some NHP malaria parasites can be transmitted to humans have raised concerns about the possible impact of a zoonotic malaria reservoir on efforts to control human malaria.

Advances in molecular techniques over the last 15 years have contributed greatly to our knowledge of the existence and geographical distribution of numerous *Plasmodium* species infecting NHPs, and extended our understanding of their close phylogenetic relationships with human malaria parasites. The clinical application of such techniques has also made it possible to document ongoing spillovers of NHP malaria parasites (*Plasmodium knowlesi, P. cynomolgi, P. simium, P. brasilianum)* in humans living in or near the forests of Asia and South America, thus confirming that zoonotic malaria can undermine efforts to eradicate human malaria.

Conclusions

Increasing molecular research supports the prophetic intuition of the pioneers of modern malariology who saw zoonotic malaria as a potential obstacle to the full success of malaria eradication programmes. It is therefore important to continue surveillance and research based on one-health approaches in order to improve our understanding of the complex interactions between

NHPs, mosquito vectors, and humans during a period of ongoing changes in the climate and the use of land, monitor the evolution of zoonotic malaria, identify the populations most at risk, and implement appropriate preventive strategies.

KEY WORDS*: Plasmodium knowlesi, Plasmodium cynomolgi, Plasmodium simium, Plasmodium brasilianum, Plasmodium vivax-like, Plasmodium falciparum*, zoonotic malaria.

1. INTRODUCTION

The recent discovery that the autochthonous cases of human malaria observed in the Rio de Janeiro Atlantic Forest in 2015-2016 that were originally attributed to *Plasmodium vivax* were actually naturally acquired infections caused by *P. simium*, a parasite of howler monkeys, is reminiscent us of the emergence of *P. knowlesi* as a significant cause of human malaria in Malaysian Borneo.[1,2] These new findings once again raise the key question of mid-twentieth century malariologists: "What are the implications of zoonotic malaria for the ultimate success of programmes aimed at eliminating human malaria?" [3-13]

The aim of this review is to provide a brief history of major discoveries in the field (Table 1 and 2) and reappraise our knowledge of the close and complex relationships between monkey and human malaria.

2. SEARCH STRATEGY AND SELECTION CRITERIA

A PubMed search was made using the terms "Monkey malaria", "Non-human malaria parasites", 'Ape malaria', 'Simian malaria/s', 'Monkey malaria transmission', 'Zoonotic malaria', and 'Anthropozoonotic malaria' separately and combined with the name of each known monkey malaria parasite in order to identify articles published in English, French, Italian, German, Spanish, or Portuguese between January 1900 and January 2020. A total of 1287 non-duplicate citations were retrieved, of which 364 articles and 31 reviews specifically concerned the identification and distribution of monkey malaria parasites, their phylogenetic relationships with human malaria parasites, and their transmissibility to humans (Supplementary Figure 1). These were read and summarised in chronological order and the most relevant were used for the historical review. Other references provided by key publications but not in the PubMed archive were also reviewed in order to increase the possibility of finding all of the relevant literature.

3. NON-HUMAN PRIMATE MALARIA: FROM THE FORERUNNERS OF MODERN MALARIOLOGY TO THE DISCOVERIES MADE IN THE 1960s

3.1 *First description of non-human malaria parasites and experimental transmission to humans*

The first description of a monkey malaria parasite belonging to the genus *Plasmodium* (*P. cynomolgi)* dates back to 1907.[14] Over the next fifty years, other *Plasmodium* species infecting non-human primates (NHP) were identified and characterised (including *P. brasilianum*, *P. knowlesi*, and *P. simium)* (Table 1). [15-23]

The first successful attempt to transfer a monkey malaria parasite to humans must be attributed to Knowles and Das Gupta (Fig. 1),[24] who worked with a newly-discovered monkey malaria parasite (later classified as *P. knowlesi*) during the 1930s.[19,25-26] *P. knowlesi* proved to be transmissible to humans and capable of inducing severe clinical disease. Interestingly, an anonymous commentary published in *The Lancet* in 1957 suggested that *P. knowlesi* may be the "fifth malaria parasite of man" and forecast its importance as a human pathogen.[27]

4. RESEARCH INTO NON-HUMAN PRIMATE MALARIA: THE GOLDEN AGE (1960-1970)

4.1 Increasing evidence of the transmissibility of non-human primate malaria to humans

4.1.1 Accidental infections due to monkey malaria parasites

What was certainly the most fast-moving period of research into NHP malaria started in 1960 when Don Eyles, a parasitologist working at the Laboratory of Parasite Chemotherapy of the National Institute of Allergy and Infectious Diseases (LPC-NIAID) in Memphis, called the Head of the laboratory Robert Coatney and said: "Bob, I have monkey malaria".[28,29] Coatney and his collaborators had been investigating monkey malaria since 1944 and, as it was widely held among malariologists that malaria parasites were species-specific, took no precautions to avoid mosquito bites.[30-31] However, this all changed when, two days later, a technician working in the same laboratory also developed malaria symptoms and became the second recognised case of *Plasmodium cynomolgi* transmission from monkeys to humans.[32] In the same year, a third case of

accidental infection due to *P. cynomolgi* occurred in a technician working with infected *A. freeborni* in Cincinnati, Ohio.[33]

Additional experimental studies confirmed the transmissibility of *P. cynomolgi* from humans to humans and humans to monkey by means of the inoculation of infected blood, and from monkey to humans by means of mosquito bites.[34-36]

4.1.2 Field investigations

As the *P. cynomolgi* strains involved in the first accidental cases came from a monkey captured in north-east Malaysia, researchers decided to go into the jungle in order to investigate whether and to what extent the cross-transmission of malaria parasites between monkeys and humans also occurs naturally. An American research team led by Eyles settled in Kuala Lumpur in 1960 to collaborate with the British Institute for Medical Research and, over a highly productive three-year period: 1) identified five new species of monkey malaria parasites[37-41] (Table 3); 2) identified *Anopheles leucosphyrus* mosquitoes as the natural vectors of local monkey malaria, with two species being equally attracted by monkeys and humans;[42-43] and 3) demonstrated the ability of indigenous mosquitoes to carry malaria parasites from monkey to humans.[44] However, the key question as to whether cross-transmission occurs naturally remained unanswered mainly because of the difficulties in distinguishing *P. vivax* and *P. cynomolgi* in blood smears, and the very low levels of parasitemia induced by *P. cynomolgi* in humans.[45]

4.1.3 Emerging evidence of the natural transmission of monkey malaria to humans, and progress in experimental transmission studies

In the early 1960s, a naturally-acquired *P. knowlesi* infection was diagnosed in the American surveyor in charge of the US Army Map Service who fell ill after returning home from the Malaysian jungle, and his blood samples were successfully used to inoculate rhesus monkeys and volunteer inmates at the Atlanta penitentiary.[46] Further experiments showed that *P. knowlesi* could also be transmitted to humans by means of mosquito bites.[47]

In 1963, Contacos *et al*. described successful experimental cross-transmissions of *P. brasilianum* between human volunteers and monkeys.[48] They failed to transfer *P. simium* to humans at the time but, a few years later, Deane *et al*. described the natural acquisition of *P. simium* malaria in a man acting as "mosquito bait" on the outskirts of the city of Sao Paulo in Brazil.[49]

Moreover, the LPC -NIAID team successfully infected human volunteers with *P. inui* and confirmed the 1950s hypothesis that *P. schwetzi* could also be transmitted to humans.[50-53]

In 1971, another probably natural infection by *P. knowlesi* was described in a man who had spent one week in western Malaysia.[54] It was initially misdiagnosed as being caused by *P. malariae* and even *P. coatney*, but indirect immunofluorescence testing of a convalescent serum sample showed the highest dilution for *P. knowlesi*. The authors describing this case concluded that "simian malaria in humans is probably not common but will almost surely be missed by routine examinations", a statement that proved to be prophetic 34 years later.[54]

5. RESEARCH INTO NON-HUMAN PRIMATE MALARIA: TRANSITION (1971-2003)

During this period, initial molecular analyses and phylogenetic reconstructions triggered intriguing hypotheses concerning the role of NHP malaria parasites in the evolution of the two major human malaria parasites *P. falciparum* and *P. vivax.*

5.1. *Hypotheses concerning the origin of P. falciparum*

Early phylogenetic reconstructions based on variations in DNA and small subunit (SSU) rRNA gene sequences generated the hypothesis that an avian-related *Plasmodium* species was the ancestor of *P. falciparum*.[55-57] However, this was refuted by Escalante and other researchers whose analyses of SSU rRNA genes and genes encoding the circumsporozoite (CS) protein of malaria parasites showed a closer relationship between *P. falciparum* and one *P. reichenowi* isolate retrieved from a captive chimpanzee that had not been included in previous studies.[58-60] Escalante *et al*. replicated their analyses using the gene encoding the cytochrome b protein of *Plasmodium* mitochondrial genome, and the results suggested that *P. falciparum* and *P. reichenowi* diverged

approximately 4-5 million years ago, the estimated time of the divergence of *Homo* (humans) and *Pan* (chimpanzees and bonobos) from their last common ancestor.[61] This led to the formulation of a "co-speciation hypothesis" in which *P. falciparum* and *P. reichenowi* originated from a common ancestor parasite and evolved in parallel with the divergence of their respective hosts.[61-62] However, Rich *et al*. challenged the proposed very early date of the origin of human *P. falciparum* by ascertaining the complete absence of neutral polymorphisms (i.e. genetic mutations expected to accumulate over time) in a large sample of *P. falciparum* strains from geographical areas worldwide.[63] This suggested a more recent world expansion that was estimated to have occurred a few thousand years ago and was probably due to a bottleneck event (malaria's "Eve hypothesis"), a hypothesis that was consistent with previous observations that the main mosquito vector of *P. falciparum* malaria in Africa underwent a similar recent expansion as a consequence of deforestation and agricultural activity.[63,64]

5.2. *Hypotheses concerning the origin of P. vivax*

The origin, age and spread of *P. vivax* were even more puzzling, and led to two prevalent theories. The first suggested an Asian origin (the 'out of Asia hypothesis') on the basis of the abundance of simian species similar to *P. vivax* in this region of the world, and initial phylogenetic data placing *P. vivax* in the radiation zone of Asian monkey malaria parasites.[61,65] However, this was not consistent with the very high (95-99%) prevalence of Duffy-negative blood types in western and central Africa (where *P. vivax* is almost non-existent) or the association between Duffy negativity and complete resistance to *P. vivax* malaria, both of which suggested that *P. vivax* had co-evolved with Africans longer than with any other human population (the 'out of Africa hypothesis').[66,67] The picture was made even more complicated when Li *et al*. found that *P. vivax* strains from different geographical regions express different patterns of genetic polymorphisms, and that the *P. simium* infecting New World monkeys is more genetically similar to Old World *P. vivax* strains than to those circulating in the Americas, which suggests that *P. vivax* entered the New World at least twice and from different geographical locations.[68]

6. RESEARCH INTO NON-HUMAN PRIMATE MALARIA: RENAISSANCE (2004-present)

Advances in molecular and genome sequencing techniques over the last 15 years have contributed greatly to a renaissance of research into monkey malaria by increasing our knowledge of the existence and geographical distribution of numerous *Plasmodium* species infecting NHPs, and improving our understanding of their close phylogenetic relationships with human malaria parasites. The development of molecular diagnostic techniques has also provided stronger evidence that some NHP malaria parasites can be naturally transmitted to humans (Figure 2).

6.1 New ideas concerning the origin of *P. falciparum*

In the early 2000s, an impressive series of papers described the discovery of new *Plasmodium* species infecting NHP that significantly contributed to changing previous assumptions concerning the origin of human *P. falciparum* malaria.

In 2009, Ollomo *et al*. described a new *P. falciparum*-like species (*P. gaboni*) isolated from wild chimpanzees kept as pets in Gabon villages, and estimated that it diverged from the *P. falciparum*/*P. reichenovi* lineage about 21 million years ago.[69] In the same year, Rich *et al*. identified eight new *P. reichenowi* isolates by amplifying three parasite gene fragments in blood and tissue samples taken from wild and wild-borne captive chimpanzees living in the Ivory Coast and Cameroon.[70] This addition to the only isolate previously available allowed them to show that *P. reichenowi* were much more genetically diverse than *P. falciparum*, a finding that contradicted the co-speciation hypothesis and suggested that *P. falciparum* evolved from a chimpanzee parasite between five and 50 thousand years ago.[70]

A real breakthrough in the field came with the 2010 publication of a paper by Prugnolle *et al*. This demonstrated the feasibility and efficiency of a non-invasive sampling method that allowed the the feces of free-living apes to be screened for malaria parasite DNA, thus circumventing the difficulty and ethical questions involved in obtaining blood samples from endangered wild animals.[71] This screening method revealed the unexpected diversity of *Plasmodium* species circulating in African

great apes. These included two new monophyletic lineages among gorillas (*P. GorA* and *P. GorB*) that clustered within the *P. falciparum* and *P. reichenowi* lineages, a finding that corroborated Rich's hypothesis that *P. falciparum* originated from *P. reichenowi* following a recent host transfer.[70] Moreover, the identification of *P. falciparum* in gorillas living in different geographical locations provided the first challenge to the view that the species is specific to humans.[71]

In the same year of 2010, Krief *et al*. described a further two *Plasmodium* lineages very close to *P. falciparum* (*P. billcollinsi* and *P. billbrayi*) isolated from chimpanzees in Uganda and the Democratic Republic of the Congo (DRC), as well as *P. vivax*-like and *P. malariae* strains isolated from bonobos in the DRC.[72] Moreover, Duval *et al*. confirmed the presence of *P. GorB* in gorillas and *P. gaboni* and *P. reichenowi* in chimpanzees in Cameroon,[73] and found *P. falciparum* in blood samples taken from two different chimpanzee sub-species, which suggested that they may act as a reservoir promoting human *P. falciparum* infection.[95]

This period of rapid discoveries culminated with a paper by Liu *et al*. that radically changed the hypothesis of the origin of human *P. falciparum* malaria.[4] Using single-genome amplification, they identified and characterised *Plasmodium* mitochondrial, apicoplast and nuclear gene sequences in nearly 3,000 fecal samples of wild-living apes in central Africa, and revealed the high prevalence and wide distribution of *Plasmodium* parasites among chimpanzees and western lowland gorillas, but not among other apes. Almost all of the retrieved sequences fell within six host-specific clades: three specific to western gorillas (*P. praefalciparum*, *P. blacklocki* and *P. adleri)* and three specific to chimpanzees (*P. reichenowi*, *P. billcollinsi* and *P. gaboni*), thus indicating the existence of at least six ape *Plasmodium* species in the *Laverania* sub-genus that also includes human *P. falciparum*. Phylogenetic trees showed that human *P. falciparum* formed a monophyletic lineage within the range of gorilla parasites that indicated the gorilla origin of human *P. falciparum*, although the calibrations did not fully clarify when the cross-species transmission had taken place.[4] However, a very recent study by Otto *et al*. that provides the full genomes of the members of *Laverania* sub-genus infecting great apes allows it to be estimated that *P. falciparum* emerged 40-

60 thousand years ago from the *P. praefalciparum* harboured by gorillas as a result of multiple transmission events and a possible bottleneck about 5,000 years ago, at the same time as advances in agriculture were leading to the rapid expansion of the human population.[75]

Although these new acquisitions concerning the evolutionary history of malaria highlight the potential transfer of monkey malaria parasites to humans, the currently available evidence seems to indicate that monkey *Laverania* parasites are strictly host-specific, although this may not be true of primates living in captivity.[71,76-79] It has been suggested that host selection by mosquitoes may play a key role in the frequency and efficiency of the cross-species transmission of *Plasmodium* parasites, although two studies carried out in Gabon have shown that at least three species of *Anopheles* are susceptible to ape malaria parasites carrying *Plasmodium* sporozoites in their salivary glands and may act as a bridge between different primate species.[80-83] On the other hand, the findings of two other studies conducted in Gabon are conflicting: one failed to demonstrate human infection due to ape *Plasmodium* species among the inhabitants of local villages, whereas the other showed a surprising 11% of cross-species *Laverania* transmission in infrastructures hosting captive primates (from humans to chimpanzees, from gorillas to chimpanzees, and from chimpanzees to gorillas), thus suggesting that the host specificity of *Laverania* parasites is not completely impermeable.[84-85]

6.2 Alternative hypothesis concerning the origin of *P. vivax*

Although the new data provided by Escalante *et al*. were in line with the "out of Asia" hypothesis[86], more recent studies have shed a different light[5, 87-90]. Liu *et al*. have found that African apes are endemically infected with *P. vivax*-like parasites, and their single-gene amplification analysis has shown that human-derived *P. vivax* sequences form a monophyletic lineage within the radiation of ape parasites, whereas sequences from ape parasites are more genetically diverse and lack host specificity[5]. These findings have led to the alternative hypothesis that all human *P. vivax* derive from a single ancestor that escaped from Africa (where it was eliminated from humans but not from apes) when selective pressure started the spread of the Duffy-negative mutation.[5] Moreover,

Loy *et al*. have examined two nearly complete and various partial genome sequences of African ape *P.vivax* parasites, and found that they are 10-fold more diverse than human *P. vivax* and show no signs of species specificity, thus suggesting that human *P. vivax* is a bottlenecked lineage that emerged from within the group of ape parasites.[89]

The evidence indicating the very close genetic similarity of human *P.vivax* and *P.simium* has led to the hypothesis of a recent lateral host switch between humans and monkeys in the New World, although the direction of the transfer is still unclear.[86,90-92] However, one recent study of the mitochondrial genomes (mitogenomes) of a large sample of *P. vivax* and *P. simium* parasites currently circulating in three separate location in southern and south-eastern Brazil found less genetic diversity among *P. simium* lineages harboured by monkeys than among *P. vivax* strains recovered from humans, which strongly supports a recent human-to-monkey transfer.[93]

6.3 Evidence of current non-human primate malaria spillovers into humans

6.3.1 *P. knowlesi*

In 2004, Singh *et al*. reported an increase in the number of human malaria cases in the Kapit Division of Sarawak (Malaysian Borneo) that were microscopically attributable to *P. malariae*, but characterised by atypically severe clinical and parasitological features.[2] Suspecting a variant form of *P. malariae* or a newly emergent *Plasmodium* species, they used a nested polymerase chain reaction (PCR) to test blood samples taken from 208 cases and failed to detect the DNA of any known human malaria parasites, but parasite DNA sequencing demonstrated that *P. knowlesi* was responsible.[2] The large number of human *P. knowlesi* infections described by Singh *et al*. and subsequent case reports from other countries in south-east Asia or involving travellers to Malaysian Borneo indicate that *P. knowlesi* is more widespread among humans than previously thought.[94-98]

Although cases of *P. knowlesi* malaria have been reported throughout south-east Asia, Malaysian Borneo currently has the greatest burden.[99] Malaysia is nearing the elimination of the indigenous transmission of human *Plasmodium* species, but cases of *P. knowlesi* malaria have recently increased alarmingly: [98] in Sabah, North Borneo, *P. knowlesi* accounted for 817 of 1018 cases of

malaria in 2015 (80%), 677 of 771 cases in 2016, (88%), and 2030 of 2078 cases in 2017 (98%) respectively.[100]

The wider use of molecular diagnostics in health facilities has certainly contributed to our increased ability to identify *P. knowlesi* malaria, but current research indicates that ongoing environmental and ecological changes (i.e. deforestation, less biodiversity, the displacement of monkeys to forest fringes) and the consequently greater contact between humans, mosquito vectors and macaques are the real causes of the increase in *P. knowlesi* malaria in Malaysian Borneo.[101-106] Grigg *et al*. have found that farmers, palm oil plantation workers, and people recently clearing vegetation were all at increased risk of acquiring *P. knowlesi* infection,[107] and similar risk factors were detected by Fornace *et al*. in a study conducted in Northern Sabah (Malaysia) and Palawan (the Philippines).[108] The seroprevalence rates of *P. knowlesi* infection in these studies vary from 5.1% to 11.7% , with the highest rates among older people.[103,106] Moreover, the possibility of the peri-domestic transmission of zoonotic malaria in such areas suggested by Manin *et al*., seems to be confirmed by the findings of a study in which GPS tracking devices provided evidence of high rates of infected mosquito bites in household areas surrounding forest edges.[109]

Interestingly, whole genome sequencing analyses made by Divis *et al*. have demonstrated the existence of three divergent *P. knowlesi* sub-populations in Malaysia depending on their geographical origin (Borneo or Peninsular) and, in Borneo, their macaque host (*M. fascicularis* or *M. nemestrina*).[110,111] Moreover, Benavente *et al*. have detected the existence of genetic exchanges between these different sub-populations, with fragments of the *M. nemestrina*-associated sub-population genotype (including genes involved in the process of erythrocyte invasion) in the *M. fascicularis*-associated sub-population genotype and in clinical isolates from Peninsular Malaysia,[112] and suggested that the recombination of partially differentiated parasite genomes may increase opportunities for new parasite adaptations, including further host transitions, that facilitate transmission.

Another study by Benavente *et al*. has shown that nearly half of the *P. knowlesi* isolates from the Betong region in Sarawak carry a recombinant gene in chromosome 12 that is orthologous to the *P. falciparum* gene involved in infecting *Anopheles gambiae*, thus indicating the potential adaptation of *P. knowlesi* to other mosquito vectors and a consequent increase in its transmission range.[113]

6.2.2 *P. cynomolgi*

P. cynomolgi, a parasite sharing 90% of orthologous genes with *P. vivax*, was long considered to be restricted to macaques but, in 2014, a naturally acquired *P. cynomolgi* infection was detected in a Malaysian man by means of PCR and genome sequencing.[114,115] The authors describing this case suggested that there may be other undiagnosed or misdiagnosed cases because routine diagnostic methods were incapable of distinguishing *P. cynomolgi* from *P. vivax*, a view that is supported by a subsequent report of five naturally acquired cases of *P. cynomolgi* infection in Sarawak.[116] Furthermore, a molecular malariometric survey of 23 villages in western Cambodia carried out in 2015-16 identified asymptomatic infections due to NHP malaria parasites in 21 people (1.9%) living close to forested areas, with *P. cynomolgi* accounting for 11 cases, *P. knowlesi* for eight, and *P. vivax* and *P. cynomolgi* co-infection for two.[117] Interestingly, two of the subjects with *P. cynomolgi* infection experienced recurrent parasitaemia after an interval of approximately three months, thus suggesting a tendency to persist or relapse. In another cross-sectional study conducted in Sabah (Malaysia), two asymptomatic infections due to *P. cynomolgi* were detected among subjects reporting a history of forest-based activities and contact with macaques.[118]

Finally, a case of *P. cynomolgi* malaria was diagnosed in a Danish tourist, although it was not possible ascertain whether the infection was acquired in peninsular Malaysia or Thailand.[119]

6.2.3 *P. simium*

Between 2006 and 2016, increasing numbers of cases of autochthonous malaria microscopically attributable to *P. vivax* were observed among the indigenous population living in the Atlantic Forest in the state of Rio de Janeiro, an area in which it was thought that malaria had been eradicated 50 years before.[120] This unexpected finding led Brasil *et al*. to conduct a molecular epidemiological

investigation using DNA sequencing that eventually identified *P. simium* as the cause.[1] There is also a 2007 narrative report of a tertian malaria attack suffered by a biochemist who had visited the municipality of Novo Friburgo (also in the state of Rio de Janeiro) that is a further probable case of human *P. simium* malaria.[121,122] In order to help identify *P. simium* without using genomic sequencing, Madureira de Alvarenga *et al*. have recently developed a nested PCR test followed by enzyme digestion that is capable of recognising the two unique single nucleotide polymorphisms (3535T>C and 3869A>G) differentiating *P. simium* and *P. vivax.*[123]

6.2.4 *P. brasilianum*

Lalremruata *et al*. have recently found that *P. brasilianum*, a parasite whose geographical distribution in South America is wider than that of *P. simium* (it includes the Amazon forest of Panamà, Venezuela, Peru and Brazil, as well as Brazil's Atlantic Forest), can naturally infect the Yanomami people living in the Venezuelan Amazon, which provides further evidence that humans and non-human primates can share quartan parasites without any host specificity under conditions of close contact.[124] The authors suggested that *P. brasilianum* and *P. malariae* should be considered a single anthropozoonotic species, and that the African *P. rhodaini* and American *P. brasilianum* should be included under the name of *P. malariae*. The recently available reference genome of *P. malariae* and a draft *P. brasilianum* reference genome seem to confirm the appropriateness of this suggestion. [125,126]

6.2.5 Ape *P. vivax*

P. vivax malaria has long been considered extremely rare among the native populations of western and central Africa because of the high prevalence of the protective Duffy-negative phenotype, although cases in travellers returning from Africa suggest that the disease exists at a low level of endemicity throughout the continent.[127] However, there has recently been an increase in the number of reports of *P. vivax* infection among Africans, including some Duffy-negative subjects.[128,129] The finding of widely distributed *P.vivax*,-like parasites in wild-living African apes has suggested that they may be a reservoir for *P.vivax* transmission. There are currently no data regarding the

relevance of zoonotic malaria in Africa, but the DNA sequencing analysis of a case of ape *P.vivax* malaria in a European traveller to the Central African Republic[130] suggests that the risk exists.

It is worth mentioning that malaria is still one of the most frequent causes of fever in returning travellers,[131] and the possibility of zoonotic malaria should not be overlooked in the case of travellers visiting wildlife areas with NHP populations.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is a growing body of evidence supporting old concerns that zoonotic malaria transmission is a potential obstacle to the full success of malaria eradication programmes that only consider human malaria parasites. Effective surveillance of zoonotic malaria in tropical forested areas where humans and NHP live in close proximity to each other should therefore be encouraged and supported by local and global health authorities responsible for malaria control. Many of the regions exposed to the risk of zoonotic malaria still have difficulties in correctly distinguishing human and non-human malaria parasites in blood smears, thus leading to the under-diagnosis of cases of zoonotic malaria cases. It is therefore important to ensure that physicians in these regions have access to the latest diagnostic techniques that can identify multiple malaria species. The development of molecular techniques that do not require costly machines or expertise, such as the loop-mediated isothermal amplification (LAMP) of DNA, will be particularly helpful.

Recent species-specific analyses of malaria have shown that *P. knowlesi* has become dominant in Malaysian Borneo and that the dynamics of its transmission is probably due to local ecological disruption and reduced biodiversity. As the mechanisms underlying the relationship between such changes and the increased risk of zoonotic transmission are complex and multi-factorial, it is important to continue multidisciplinary research using 'one-health' approaches in order to clarify the interactions between NHPs, mosquito vectors, and humans, and monitor the evolution of zoonotic malaria, identify the populations at highest risk and implement appropriate preventive strategies.

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Authors' contributions

Spinello Antinori conceptualised the paper, searched for and analysed the literature, and drafted the manuscript. Anna Lisa Ridolfo and Carlo Parravicini analysed the literature and drafted the manuscript. Cecilia Bonazzetti, Mario Corbellino and Andrea Giacomelli searched for and analysed the literature, and organised the scientific content of each section of the manuscript.

Massimo Galli critically reviewed the intellectual content of the manuscript.

Conflict of interests

None declared

Table 1– Milestones in monkey malaria discoveries and research

Year	Main findings
1899	Laveran and Kossel described *Plasmodium kochi*, the first plasmodium-like parasite observed in non-human primates, now classified in the genus *Hepatocystis**
1907	Mayer working in Hamburg described *P. cynomolgi* in the blood of a monkey (*Macaca cynomolgus*) imported from Java[14]
1907	Halberstaedter and von Prowazek described a parasite (probably previously observed by Laveran in 1905) in the blood of a Javan oraguntan which they named *P. pitheci* and another parasite named *P. inui*[15]
1908	Gonder and von Beremberg-Gossler found a parasite in the blood of a cacajo (*Brachyrus calvus*) imported from Brazil to Germany, and named it *P. brasilianum*[16]
1920	Reichenow working in Cameroon found parasites resembling human malaria parasites *P.vivax/ovale*, *P.malariae* and *P.falciparum* in the blood of chimpanzees and gorillas[17]
1922	Blacklock and Adler in Sierra Leone described a parasite resembling *P. falciparum* in a chimpanzee[18]
1932	Knowles and Das Gupta in Calcutta first experimentally transmitted a newly identified monkey malaria parasite to humans by means of blood inoculation[19,24]
1932-1933	Sinton and Mulligan provided the first taxonomic revision of monkey malaria parasites and elevated the parasite identified by Knowles and Das Gupta to the rank of a species named *P. knowlesi* [26]
1935	*P. knowlesi* was used for the malariotherapy of neurosyphilis**
1939	Brumpt described and named *P. schwetzi* and *P. rhodaini*, the simian counterparts of *P. vivax* and *P. malariae* first observed by Reichenow[20]
1940	Rhodain transferred *P. malariae* to chimpanzees by means of blood inoculation[21]
1948	Shortt and Garnham demonstrated the pre-erythrocytic stages of *P. cynomolgi*[22]
1951	Fonseca described and named *P. simium*, a parasite isolated from the blood of a howler monkey (*Alouatta fusca*) that he had misidentified as *P. brasilianum* in 1939[23]
1960	Eyles and Schmidt described the first accidental case of malaria due to *Plasmodium cynomolgi* in a laboratory worker bitten by infected mosquitoes[31]
1965	Description of a natural human infection caused by *P. knowlesi* acquired by an American who travelled to Malaysian Borneo, the first demonstration of the existence of zoonotic malaria[46]
1971	Coatney, Collins, Warren and Contacos published the book "The Primate Malarias"
2004	Singh *et al.* identified *P. knowlesi* as the cause of a large number of cases of human malaria in Malaysian Borneo[2]
2010	Prugnolle *et al.* developed a non-invasive method of identifying and characterising ape *Plasmodium* species from fecal samples[71]
2010	A series of studies revealed new *Plasmodium* species belonging to the *Laverania* sub-genus, and indicated the high prevalence and widespread distribution of these parasites among African apes[71,72,73]
2010	Liu *et al.* identified the *P. praefalciparum* infecting gorillas as the ancestor of human *P. falciparum*[45]
2014	Description of the first case of naturally acquired human infection with *Plasmodium cynomolgi* in Peninsular Malaysia[116]
2017	Brasil *et al.* identified *P. simium* as the cause of an outbreak of human malaria in the Atlantic Forest of Rio de Janeiro state[1]
2018	Asymptomatic natural human infections due to *Plasmodium cynomolgi* described in Sarawak (Malaysia) and western Cambodia[117,118]

* Laveran A: Un nouveau parasite trouvé dans le sang de malades atteints de fièvre palustre. Origine parasitaire des accidents de l'impaludisme. *Bull Mém Soc Méd Hôpitaux* Paris 1881, 17:158-164.

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Table 2. Characteristics of monkey *Plasmodium* species and their links with human malaria

Region	Simian *Plasmodium* species, year of discovery	Geographical distribution	Natural host(s)	Vectors	Human *Plasmodium* species they resemble	Documented transmission to humans?
Asia						
	P. cynomolgi, 1907	Cambodia, South-west India, Indonesia, Malaysia, Sri Lanka, Taiwan	*Macaca arctoides*, *M. cyclopis*, *M. fascicularis*, *M. nemestrina*, *M. mulatta*, *M. radiata*, *M. sinica*; *Presbytis cristatus*, *P. entellus;* *Trachypithecus cristatus*	*Anopheles dirus*, *A. introlatus*, *A. elegans*, *A. hackeri*	*Plasmodium vivax*	Yes: experimental and accidental laboratory infections and a few natural infections * [31,32,34-36,44,115-119]
	P. inui, 1907	Bangladesh; China; India; Indonesia; Malaysia; Philippines; Sri Lanka; Taiwan	*Macaca cyclopis*, *M. fascicularis*, *M. mulatta*, *M. nemestrina*, *M. nigra*, *M. radiata*, *M. sinica*; *Presbytis melalophos*, *Trachypithecus cristatus*, *T. obscurus*	*A. balabacensis*, *A. dirus*, *A. maculates*, *A. stephensi*	*P. malariae*	Yes: experimental infections [50]
	P. pitheci, 1907	Malaysia (Borneo)	*Pongo pygmaeus*	Unknown	*P. vivax*	No
	P. knowlesi, 1932	Indonesia; Malaysia; Philippines; Singapore; Taiwan; Thailand; Vietnam	*Macaca fascicularis*, *M. nemestrina*; *Presbytis melalophos*; *Trachypithecus obscurus*	*A. hackeri*, *A. dirus*, *A.latens*	*P. falciparum*; *P. malariae*	Yes: experimental laboratory infections and natural infections [2,24,25,27,46,47,54,94-96,100,103,107,108]
	P. hylobati, 1941	Indonesia (Borneo)	*Hylobates moloch*, *H. muelleri*	*A. balabacensis*, *A. stephensi*	*P. vivax*	No
	P.coatney, 1962	Malaysia, Philippines	*Macaca arctoides*, *M. fascicularis;* *Trachypithecus cristatus*	*A. hackeri*, *A. farauti*	*P. falciparum*	No
	P. fieldi, 1962	Malaysia	*Macaca fascicularis*, *M. mulatta*, *M. nemestrina*, *M. radiate;* Baboon: *Papio doguera*	*A. argiropus*, *A. atroparvus*, *A. balabacensis*, *A. dirus*, *A. freeborni*; *A. hackeri*, *A. kochi*, *A. letifer*, *A. philippinensis*, *A. quadrimaculatus*,	*P. ovale*	No

				A. sinensis, *A. stephensi*, *A. vagus*		
	P. youngi, 1964	Malaysia	*Hylobates lar*	*A. leukosphyrus* group	*P. vivax*	No
	P. eylesi, 1965	Malaysia	Gibbon*: Hylobates lar*	*A. introlatus*, *A. kochi*, *A. elegans*, *A. hackeri*, *A. letifer*; *A. lesteri*, *A. leukosphyrus*, *A, maculates*, *A. macarthuri*, *A. roperi*, *A. sinensis*, *A. umbrosus*, *A. vagus*	*P. vivax*	Yes: experimental laboratory infections[40]
	P. fragile, 1965	India; Sri Lanka	*Macaca radiata*, *M. fascicularis*, *M. sinica*	*A. elegans*	*P. falciparum*	No
	P. jefferyi, 1966	Indonesia; Malaysia	*Hylobates lar*	*A. balabacensis*, *A. freeborni*	*P. vivax*	No
	P. simiovale,1965	Sri Lanka	*Macaca sinica*	*A. atroparvus*, *A. balabacensis*, *A. stephensi*	*P. ovale*	No
	P. silvaticum, 1972	Malaysia (Borneo)	*Pongo pygmaeus*	*A. balabacensis*, *A. maculates*, *A. sundaicus*	*P. vivax*	No
Africa						
	P. reichenowi, 1922	Cameroon; Democratic Republic of the Congo; Sierra Leone; Uganda	*Pan troglodytes*; *Gorilla gorilla*	Unknown	*P. falciparum*	No
	P. rhodaini, 1939	Central Africa	*Pan stayrus verus*	Unknown	*P. malariae*	No
	P. schwetzi, 1939	Cameroon; Democratic Republic of the Congo; Liberia; Sierra Leone	*Pan troglodytes*; *Gorilla gorilla*	*A. balabacensis*	*P. vivax*/*P. ovale*	Yes: experimental infections [51,52]
	P. gaboni (*P. billbray*), 2009	Cameroon, Democratic Republic of the Congo; Gabon	*Pan troglodytes*; *Gorilla gorilla*	Unknown	No morphologic observation	No
	P. gor A (*P. adleri*) and *P. gor* B (*P. blacklocki*), 2011	Cameroon, Democratic Republic of the Congo; Gabon	*Gorilla gorilla*	Unknown	No morphologic observation *(P. falciparum)*	No
	P. praefalciparum , 2011	Cameroon; Central African Republic; Democratic Republic of the Congo	*Gorilla gorilla; Pan paniscus*; *Pan troglodytes*	Unknown	*P. falciparum*	No
	P. lomamiensis,	Democratic	*Pan paniscus*	Unknown	No	No

	2017	Republic of the Congo (Lomani National Park)			morphologic observation	
South America						
	P. brasilianum, 1908	Brazil; Colombia; French Guiana; Panama; Peru; Venezuela	*Alouatta belzebul*, *A. caraya*, *A. guariba*, *A. palliata*, *A. seniculus*; *Ateles fusciceps*, *A. geoffroyi*, *A. paniscus*; *Brachyteles arachnoides*; *Cacajao calvus*; *Callicebus brunneus*, *C. cupreus*, *C. moloch*, *C. ornatus*, *C. torquatus*; *Cebus albifrons*, *C. apella*, *C. capucinus*; *Chiroptes albinatus*, *C. chiropotes*, *C. satana*; *Lagotrix lagotricha*, *L. poeppigii*; *Pithecia irrorata*, *P. monachus*, *P. pithecia;* *Saguinus geoffroyi*, *S. midas*; *Saimiri boliviensis*, *S.sciureus*, *S. ustus*	*A. kruzii*	*P. malariae*	Yes: experimental and natural infections [48,124]
	P. simium, 1951	Brazil	*Cercocebus atys*; *Alouatta fusca*, *A. guariba*; *Brachyteles arachnoides*	*A. kruzii*	*P. vivax*	Yes: natural infections [1,49,121,122] **

* Kuvin SF, Beye HK, Stohlman F Jr, Contacos PG, Coatney GR. Malaria in man. Infection by *Plasmodium vivax* and the B strain of *Plasmodium cynomolgi*. *JAMA* 1963 ; 184 :84-86. Most H. *Plasmodium cynomolgi* : accidental human infection. *Am J Trop Med Hyg* 1973 ;22 :157-158. Druilhe P, Trape JF, Leroy JP, Godard C, Gentilini M. Deux cas d'infection humaine accidentelle par *Plasmodium cynomolgi bastianellii*. Etude clinique et serologique. Ann Soc belge Med Trop 1980 ; 60 :349-54. ** Deane LM. Simian malaria in Brazil. *Mem Inst Oswaldo Cruz* 1992 ;87 Suppl.3 :1-20.

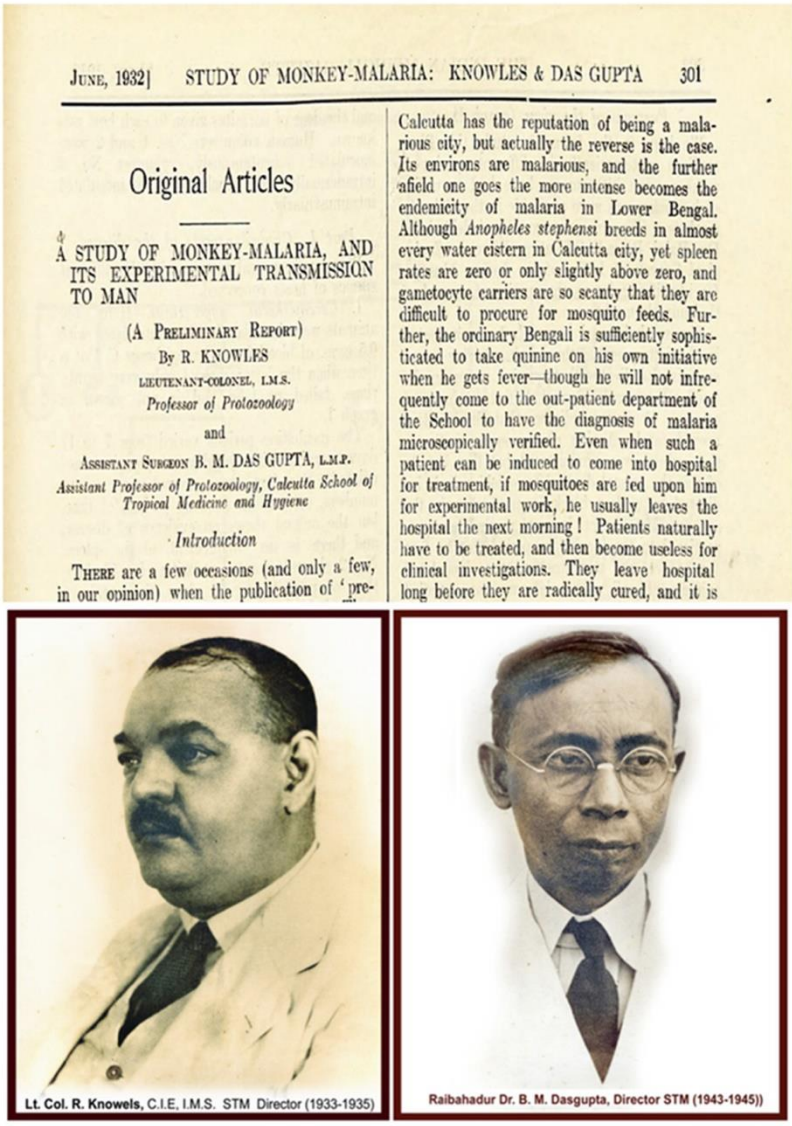
Table 3. American parasitologists most involved in research into monkey malaria in the 1960s and 1970s

Name of researcher	No. of articles on malaria (years of publication)	No. of articles on monkey malaria (years of publication)	Main achievements
Robert G Coatney (1902-1990)	111 (1947-1976)	24 (1960-1971)	Responsible for monkey malaria research at the NIH. Principal editor of the superb monograph "The Primate Malarias". Description of the first naturally acquired human case of *P. knowlesi* malaria. Co-discoverer of two new species of malaria parasites of Malaysian monkeys (*P. eylesi*, *P. jefferyi*). Description of the transmission of the monkey parasite *P. inui* to humans by means of mosquito bites
Don E Eyles (1915-1963)	45 (1948-1966)	17 (1960-1964)	Description of the first natural (accidental) transmission of *Plasmodium cynomolgi* monkey malaria to a human. Leader of the monkey malaria research unit in Kuala Lumpur. Co-discoverer of three new species of malaria parasites in Malaysian monkeys (*P. coatneyi*, *P. fieldi*, *P. youngi*); Studies identifying the vectors of monkey malaria in Asia (*Anopheles hackeri, A. balabacensis introlatus, A. leucosphyrus*)
William E Collins (1929-2013)	398 (1963-2014)	197 (1965-2013)	The most prolific researcher of the group. Co-editor of the monograph "The Primate Malarias"
Peter G Contacos	100 (1961-1982)	59 (1961-1982)	Description of the first naturally acquired human case of *P. knowlesi* malaria. Description of transmission of simian malaria to humans by means of mosquito bites (*P. brasilianum*; *P. schwetzi*). Description of the transmission of the monkey parasite *P. inui* to humans by means of mosquito bites.
William Chin	43 (1964-1984)	16 (1965-1984)	Description of the first naturally

(1929-			acquired human case of *P. knowlesi* malaria. Description of the transmission of the monkey parasite *P. inui* to humans by means of mosquito bites
McWilson Warren	65 (1962-2009)	37	Co-discoverer of two new species of malaria parasites of Malaysian monkeys (*P. eylesi*, *P. jefferyi*). Co-editor of the monograph “The Primate Malarias”

NIH, National Institute of Health

FIGURE LEGENDS



JUNE, 1932] STUDY OF MONKEY-MALARIA: KNOWLES & DAS GUPTA 301

Original Articles

A STUDY OF MONKEY-MALARIA, AND ITS EXPERIMENTAL TRANSMISSION TO MAN

(A PRELIMINARY REPORT)

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and

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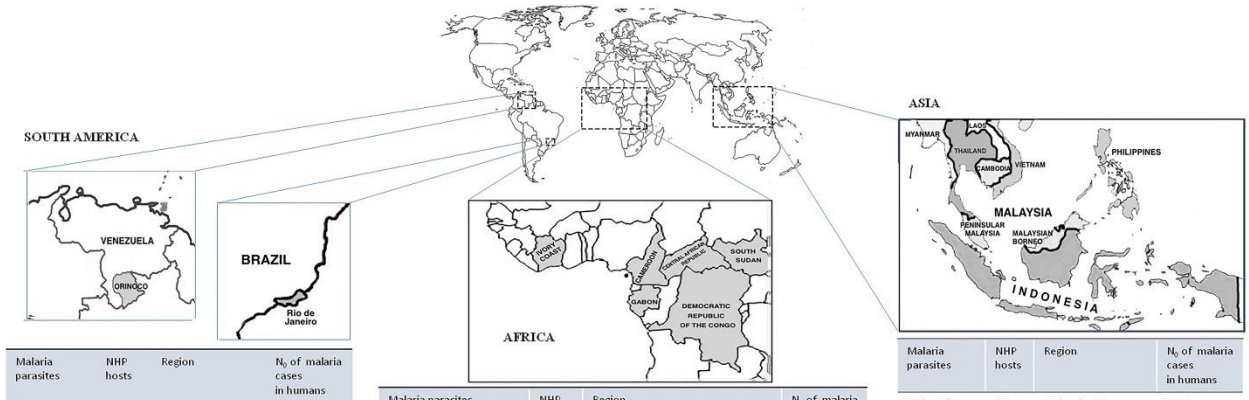
Introduction

THERE are a few occasions (and only a few, in our opinion) when the publication of 'pre-

Calcutta has the reputation of being a malarious city, but actually the reverse is the case. Its environs are malarious, and the further afield one goes the more intense becomes the endemicity of malaria in Lower Bengal. Although *Anopheles stephensi* breeds in almost every water cistern in Calcutta city, yet spleen rates are zero or only slightly above zero, and gametocyte carriers are so scanty that they are difficult to procure for mosquito feeds. Further, the ordinary Bengali is sufficiently sophisticated to take quinine on his own initiative when he gets fever—though he will not infrequently come to the out-patient department of the School to have the diagnosis of malaria microscopically verified. Even when such a patient can be induced to come into hospital for treatment, if mosquitoes are fed upon him for experimental work, he usually leaves the hospital the next morning! Patients naturally have to be treated, and then become useless for clinical investigations. They leave hospital long before they are radically cured, and it is

Figure 1: Original article published in the Indian Medical Gazette describing the first experimental transmission of monkey malaria to humans. Inset: The authors of the article: Lt-Colonel Robert Knowles left) and Dr Biraj Moahn Das Gupta (right). Courtesy of Prof. Krishnangshu Roy, Director of the Calcutta School of Tropical Medicine.



Malaria parasites	NHP hosts	Region	N_0 of malaria cases in humans
P. brasilianum	NWM	Venezuela (Casiquiare Canal-Orinoco River)	12
P. simium	NWM	Brazil (Rio de Janeiro-Atlantic forest)	35

Malaria parasites	NHP hosts	Region	N_0 of malaria cases in humans
P. malariae-like	C	Ivory Coast	-
P. billcollinsi	C	Ivory coast, Gabon, DRC	-
P.billbrayi (P. gaboni)	C	Ivory coast, Gabon, DRC, Cameroon	-
P. reichenowi	C	Ivory coast, Gabon, DRC, Cameroon	-
P. ovale	C	Cameroon	-
P. carteri/vivax	C	Cameroon	-
P. falciparum	C, B	Cameroon	-
P. ovale-like	C,G	Ivory coast, Gabon, DRC	-
P. vivax-like	C,G	Ivory coast, Gabon, DRC, Cameroon	1
P. schwetzi	C,G	Cameroon	-
P. vivax	C, G	Central African Republic	-
P. prefalciparum	G	Gabon	-
P. adleri (P. Gor A)	G	Gabon	-
P. blacklocki (P.Gor B)	G	Gabon, South Sudan	-
P. lomamiensis	B	DRC	-

Malaria parasites	NHP hosts	Region	N_0 of malaria cases in humans
P. knowlesi	M	Malaysian Borneo (Sarawak, Sabah)	6588
		Peninsular Malaysia	627
		Indonesia,	480
		Thailand	59
		Myanmar	49
		Vietnam	35
		Laos	15
		Cambodia	10
		Philippines	7
		Singapore	6
P. cynomolgi	M	Cambodia	12
		Malaysian Borneo (Sarawak, Sabah)	7
		Peninsular Malaysia	1
		Malaysia/Thailand border	1

Figure 2: Geographical distribution of the *Plasmodium* species known to parasitise non-human primates and the number of human malaria cases due to NHP malaria parasites reported in the literature.

NWH: new world monkeys; C: Chimpanzees; B: Bonobos; G: Gorillas; M: Macaca

DRC: Democratic Republic of the Congo