**Plasmodium cynomolgi** in humans: current knowledge and future directions of an emerging zoonotic malaria parasite

Loick P. Kojom Foko1 · Amit Kumar1 · Joseph Hawadak1 · Vineeta Singh1

Received: 15 September 2022 / Accepted: 1 November 2022 / Published online: 19 November 2022 © The Author(s), under exclusive licence to Springer-Verlag GmbH Germany 2022

**Abstract**

*Plasmodium cynomolgi* (*Pcy*), a simian malaria parasite, is a recent perfect example of emerging zoonotic transfer in human. This review summarizes the current knowledge on the epidemiology of natural *Pcy* infections in humans, mosquitoes and monkeys, along with its biological, clinical and drug sensitivity patterns. Knowledge gaps and further studies on *Pcy* in humans are also discussed. This parasite currently seems to be geographically limited in South-East Asia (SEA) with a global prevalence in human ranging from 0 to 1.4%. The *Pcy* infections were reported in local SEA populations and European travelers, and range from asymptomatic carriage to mild/moderate attacks with no evidence of pathognomonic clinical and laboratory patterns but with *Pcy* strain-shaped clinical differences. Geographical distribution and competence of suitable mosquito vectors and non-primate hosts, globalization, climate change, and increased intrusion of humans into the habitat of monkeys are key determinants to emergence of *Pcy* parasites in humans, along with its expansion outside SEA. Sensitization/information campaigns coupled with training and assessment sessions of microscopists and clinicians on *Pcy* are greatly needed to improve data on the epidemiology and management of human *Pcy* infection. There is a need for development of sensitive and specific molecular tools for individual diagnosis and epidemiological studies. The development of safe and efficient anti-hypnozoite drugs is the main therapeutic challenge for controlling human relapsing malaria parasites. Experience gained from *P. knowlesi* malaria, development of integrated measures and strategies—ideally with components related to human, monkeys, mosquito vectors, and environment—could be very helpful to prevent emergence of *Pcy* malaria in humans through disruption of transmission chain from monkeys to humans and ultimately contain its expansion in SEA and potential outbreaks in a context of malaria elimination.

**Keywords** Malaria · *Plasmodium cynomolgi* · Emerging zoonosis · Biology · Epidemiology · Clinical patterns · Drug sensitivity · Future directions

**Abbreviations**

| 18S rRNA | Small subunit ribosomal RNA gene |
| --- | --- |
| ACT | Artemisinin-based combination therapy |
| AMA-1 | Antigen membrane antigen 1 |
| CQ | Chloroquine |
| CSP | Circumsporozoite protein |
| CVC | Caveola—vesicles complexes |
| Cyt-b | Cytochrome b |
| COX-I | Partial cytochrome c oxidase sub-unit 1 gene |
| DBP | Duffy binding protein |
| EBL/EBP | Erythrocyte binding ligand/protein |
| G6PD-d | Glucose-6-phosphate dehydrogenase deficiency |
| msp | Merozoite surface protein |
| NBP/RBP | Normocyte/Reticulocyte binding protein |
| mtDNA | Complete mitochondrial genome |
| Pbra | *Plasmodium brasiliense* |
| Pct | *Plasmodium coatneyi* |
| Pcy | *Plasmodium cynomolgi* |
| Pf | *Plasmodium falciparum* |
| Pfd | *Plasmodium falciparum* |
| Pin | *Plasmodium inui* |
| Pk | *Plasmodium knowlesi* |
| Pm | *Plasmodium malariae* |
| Po | *Plasmodium ovale* |
| Pv | *Plasmodium vivax* |
| PfRh | *P. falciparum* Reticulocyte-binding homologue |

1 Parasite and Host Biology Group, ICMR-National Institute of Malaria Research, Dwarka, Sector 8, New Delhi 110077, India

Vineeta Singh
vineetas_2000@yahoo.com
Introduction

Plasmodium falciparum (Pf) and Plasmodium vivax (Pv) are the main species involved in human malaria burden worldwide [1]. Though Pf is the most geographically distributed species in the world, Pf is the main malaria species responsible for a large fraction of global morbidity and mortality cases [1, 2]. African populations especially children and pregnant women are most vulnerable to malaria contributing to over 90% of the total global burden [1]. Pf is the predominant species in Sub-Saharan Africa (sSA) and Pv is found to be highly prevalent in Latin America and South East Asia (SEA) where it can outcompete Pf as seen in some regions of India [3].

The Pf and Pv burden has been greatly decreased over the last decades due to the implementation and/or scaling-up of control measures such as long-lasting insecticide-treated nets, preventive treatment with sulfadoxine-pyrimethamine (SP), and artemisinin-based combination therapy (ACT) used as first-line treatment for uncomplicated malaria [1]. However, the World Health Organization (WHO) has noted a slowdown of malaria control since 2015 by mitigated reduction in malaria burden for these recent years. The emergence and spread of ACT-resistant Pf isolates is now well established in The Greater Mekong subregion in SEA, the likely reason for this slowdown. ACT resistance has also been recently reported in two African countries (Uganda and Rwanda) [4–6]. Other factors related to mosquito vectors (e.g., insecticide resistance), populations (e.g., self-medication, misuse of malaria preventive tools), and parasites particularly non-Pf/Pv malaria have also greatly impacted the effectiveness of malaria control measures [1].

The epidemiology of non-Pf/Pv species is largely understudied especially in sSA [7, 8], with few reports highlighting the presence of P. ovale spp (Po) and P. malariae (Pm) in a Tanzanian area where concomitant decline in Pf transmission was observed, thereby suggesting a possible shift from Pf to non-Pf malaria in this area [9]. Also, other non-Pf/Pv species such as P. cynomolgi (Pcy) could be a future public health problem as the case with P. knowlesi (Pk) parasites. Indeed, Pk is currently well established in humans especially in SEA countries such as Malaysia where it can elicit severe clinical attacks and deaths [10]. Thus, such emerging malaria species such as Pcy could jeopardize malaria elimination objectives if not addressed timely.

Data on the epidemiology and clinical outcomes of Pcy in humans are currently available from different studies and case reports, but very few reviews are currently available [11, 12]. In the present review, we summarized the current knowledge on the epidemiology of natural infections of humans with Pcy parasites and its biological, clinical and drug sensitivity patterns. Current knowledge gaps and further studies on this emerging zoonotic parasite and related simian malaria parasites are also discussed.

Addendum search strategy

We used PubMed, Google scholar, Google, Scopus, Wiley Online library, the World Malaria Report, The WHO regional websites, and ClinicalTrials.gov to search for publications on Plasmodium cynomolgi, that were published between January 1900 and August 2022, and written either in English or French. The archives of local scientific associations/journals and websites of national malaria programmes were also consulted. We used the search terms “malaria”, “Plasmodium cynomolgi” and “simian malaria”. Additionally, we reviewed relevant articles cited in references of identified literature and included them as primary sources. Publications were considered of interest if they addressed any aspect of Pcy parasites including its biology, prevalence, clinical presentation and course, and treatment aspects. Principal investigators were contacted to request a full length paper and/or more details on studies. The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

Origin and diversity

The first description of Pcy was done in 1907 by M. Mayer who isolated it from a Macaca fascicularis long-tailed macaque, originally designated M. irus, in Java [13]. The strain was then extensively re-described and laboratory-established by H.W Mulligan ~ 30 years later [14]. In the late 1950s, a second Pcy strain was established by Prof. P.C.C. Garnham who named it Pcy bastianelli and is currently called “B” strain [15]. The Pcy strain described by Mulligan was analyzed by research laboratories across the world and variably termed between 1959 and 1963. This strain was finally called M or Mulligan strain in honor of H.W Mulligan [16, 17]. Between 1965 and 1971, other new Pcy strains (e.g., Berok, Cambodian, RO, PT-I, Gombak, Ceylonensis, Smithsonian) was isolated from monkeys and mosquitoes and their developmental phases (i.e., sporogony, erythrocytic
and exoerythrocytic steps) in both mosquito vectors and macaques (Fig. 1) [18–25]. Later, investigations have been conducted to address several aspects of \( P_{cy} \) parasites such as evaluation of candidate anti-relapse drugs, vaccine development, epidemiology in monkeys/mosquitoes/humans, and genetic diversity studies (Fig. 2).

Studies have assessed the genetic diversity of \( P_{cy} \) parasites by analyzing the polymorphism within genes and comparing sequences to different \( P_{cy} \) strains and other *Plasmodium* species. The main objective of such studies was to determine the role of evolutionary drivers within \( P_{cy} \) species. On analyzing 18S sequences, Wong and coworkers found a clear pattern of geographical structuration of \( P_{cy} \) populations from two Malaysian regions (Sabah and Peninsular Malaysia) [26]. In Thailand, one study reported high nucleotide diversity in Duffy binding protein genes (DBP) of \( P_{cy} \) (\( P_{cy}DBP1 \)), \( P_{v} \) (\( P_{v}DBP \)), and \( P_{k} \) (\( P_{k}DBPa, P_{k}DBPp, P_{k}DBP\)) populations, with occurrence of positive selection in domain V of \( P_{cy}DBP1 \), and domains I, II and III of \( P_{v}DBP \) [27]. Similarly, high nucleotide and haplotype diversity but occurrence of purifying selection were found on analysis of \( P_{cy}DBP2 \) domain II from \( P_{cy} \) isolates collected from wild macaques in Peninsular Malaysia [28]. Pacheco and colleagues have reported higher genetic diversity in merozoite surface protein 8 and 10 genes of \( P_{cy} \) and *Plasmodium inui* (\( P_{i} \)) compared to that of \( P_{f} \), \( P_{k} \) and \( P_{v} \) isolates. Also, some studies found the role of purifying selection on both \( msp8 \) and \( msp10 \) from \( P_{cy} \) parasites from different geographical origins (Cambodian, Gombak, Ceylonensis, B, Mulligan, PT-I, and RO) [29].

### Epidemiology of natural *P. cynomolgi* infections in monkeys, mosquito vectors and humans

#### Monkeys

Monkeys of the genera *Macaca* and *Presbytis* are natural hosts of \( P_{cy} \) parasites. In addition to *Macaca* monkeys, other genera (i.e., *Aotus* and *Saimiri*) are largely used for experimental studies aiming at addressing different aspects of \( P_{cy} \) infections like parasite biology, drug sensitivity and pathophysiological aspects [30, 31]. Given the difficulties in continuous culture for \( P_{v} \), simian models using \( P_{cy} \) are considered to be a good proxy for \( P_{v} \) biology and pathophysiology for genetic similarities between these two malaria species [32–34]. In natural conditions, \( P_{cy} \) has been reported as mono-infections and/or mixed infections with other simian malaria parasites such as *P. inui* (\( P_{i} \)), *P. coatneyi* (\( P_{c} \)) and *P. fieldi* (\( P_{f} \)) (Table 1 and Supplementary material 1). A recent systematic review reported an average \( P_{cy} \) prevalence of 33.05% in macaques from Malaysia [35]. In Philippines, the prevalence of \( P_{cy} \) was 23.2% in captive and wild *M. fascicularis* long-tailed monkeys [36]. Fungfuang and colleagues reported a similar \( P_{cy} \) prevalence rate (20%) in Thailand among three macaque species (*M. fascicularis*, *M. leonina*, *M. arctoides*) [37]. Great Apes such as orangutans are not natural hosts of \( P_{cy} \) parasites [38, 39].

*Fig. 1* Brief timeline on discovery and description of *P. cynomolgi* strains. *Only mainly studied *P. cynomolgi* strains are depicted*
Mosquitoes

In mosquito vectors, data on infection with \( P_{cy} \) are consistent with those seen in monkeys, with several mosquito species (e.g., \( A. \) dirus, \( A. \) barbirostris) collected from Malaysia and Vietnam where \( P_{cy} \) was found in mono-infections and more frequently in mixed infections with \( P_{v} \), \( P_{in} \), \( P_{k} \), \( P_{fld} \) and \( P_{ct} \) (Table 2 and Supplementary material 1). A brief presentation of distribution and biting behavior of \( Anopheles \) species associated with \( P_{cy} \) transmission to humans both in laboratory and natural conditions is presented in Table 3 [40–45].

Humans

Several reports on accidental and experimental human \( P_{cy} \) infections are a proof of possible detection of natural human infections with this simian malaria parasite, an emerging zoonotic malaria infection [46–50]. The first case of natural human infections was reported in 2014 from a 39-year
### Table 1  
Studies on natural infections of monkeys with *Pcy* parasites using molecular tools

| Monkey species | Country | Total number of samples | Target parasite gene | *Pcy* prevalence rate (n) | Other *Plasmodium* species (n) and association with *Pcy* |
|----------------|---------|-------------------------|----------------------|---------------------------|----------------------------------------------------------|
| *M. fascicularis, M. nemestrina* | Malaysia | Blood (n = 82 and n = 26) | 18S rRNA | 63.4% (n = 52) and 34.6% (n = 9) | In *M. fascicularis*: Dual infections [*Pcy* + Pin, n = 2; *Pcy* + Pk, n = 2], Triple infections [*Pcy* + Pin + Pk, n = 4; *Pcy* + Pct + Pk, n = 3; *Pcy* + Pin + Pin, n = 1], quadruple infections [*Pcy* + Pin + Pk, n = 38], Quintuple infections [*Pcy* + Pct + Pin + Pk, n = 1] |
| *M. fascicularis, M. nemestrina, Pongo pygmaeus* | Malaysia | Blood (n = 15, n = 26 and n = 38) | Cyt-b | 6.7% (n = 1) and 11.5% (n = 3) and 0% (n = 0) | – |
| *M. fascicularis* | Malaysia | Blood (n = 70) | 18S rRNA | 25.7% (n = 18) | Dual infections [*Pcy* + Pin, n = 3; *Pcy* + Pct, n = 1; *Pcy* + Pk, n = 1], Triple infections [*Pcy* + Pin + Pk, n = 4; *Pcy* + Pct + Pin, n = 3; *Pcy* + Pct + Pk, n = 1], quadruple infections [*Pcy* + Pct + Pin + Pk, n = 3] |
| *M. fascicularis* | Laos, Singapore, Cambodia, Philippines, Indonesia | Blood (n = 44, n = 40, n = 54, n = 68 and n = 70) | 18S rRNA | 63.6% (n = 28), 65% (n = 26), 50% (n = 27), 5.9% (n = 4) and 87.1% (n = 61) | Laos: -  
Singapore: Dual infections [*Pcy* + Pin, n = 1; *Pcy* + Pct, n = 1], Triple infections [*Pcy* + Pin + Pk, n = 1]  
Cambodia: Dual infections [*Pcy* + Pin + Pfld, n = 1]  
Philippines: Dual infections [*Pcy* + Pct, n = 1]  
Indonesia: Dual infections [*Pcy* + Pin, n = 2; *Pcy* + Pfld, n = 1], Triple infections [*Pcy* + Pin + Pfld, n = 1] |
| *M. radiata* | India | Blood, spleen and liver | Cyt-b, *msp*142, 18S rRNA | – | One *Pcy* case was identified in blood samples using the three molecular markers |
| Monkey species | Country | Total number of samples | Target parasite gene | Pcy prevalence rate (n) | Other Plasmodium species (n) and association with Pcy |
|----------------|---------|-------------------------|----------------------|------------------------|-------------------------------------------------------|
| *M. fascicularis*<sup>c,d</sup> | Malaysia | Blood (n = 43) | mtDNA, ClpM         | 11.5% (n = 13) using mtDNA, 4.5% (n = 4) using ClpM | –                                                   |
| *M. fascicularis*<sup>3,e</sup> | Philippines | Blood (n = 95) | 18S rRNA | 24.2% (n = 23) | Triple infections [Pcy + Pin + Pfld, n = 7]; Quadruple infections [Pcy + Pct + Pin + Pfld, n = 5; Pcy + Pin + Pfld + Pk, n = 3]; Quintuple infections [Pcy + Pct + Pin + Pfld +Pk, n = 8] |
| *M. fascicularis, M. nemestrina*<sup>d</sup> | Malaysia | Blood (n = 98 and n = 5) | 18S rRNA | 41.8% (n = 41) and 20% (n = 1) | In *M. fascicularis*; Dual infections [Pcy + Pin, n = 13; Pcy + Pin, n = 2]. Triple infections [Pcy + Pin + Pk, n = 6; Pcy + Pfld + Pin, n = 1] and quadruple infections [Pcy + Pin + Pfld + Pk, n = 1] In *M. nemestrina*; Triple infections [Pcy + Pct + Pin, n = 1] |
| *M. fascicularis, M. nemestrina, M. arctoides*<sup>e</sup> | Thailand | Blood (n = 93) | 18S rRNA | 8.6% (n = 8) | Dual infections [Pcy + Pin, n = 1; Pcy + Pct, n = 1]. Triple infections [Pcy + Pfld + Pin, n = 2] |
| *M. fascicularis* | Malaysia | Blood (n = 176) | 18S rRNA | 65.9% (n = 116) | Dual infections [Pcy + Pct, n = 14; Pcy + Pfld, n = 1; Pcy + Pk, n = 14]. Triple infections [Pcy + Pct + Pk, n = 22], quadruple infections [Pcy + Pfld + Pin + Pk, n = 2]. Quintuple infections [Pcy + Pct + Pin + Pfld + Pk, n = 2] Not specified |
| *M. fascicularis, M. nemestrina* | Thailand | Blood (n = 1015) | COX-1 | 16.95% (n = 172) | In *M. fascicularis*; Dual infections [Pcy + Pin, n = 1; Pcy + Pct, n = 1]. Triple infections [Pcy + Pct + Pk, n = 1; Pcy + Pct + Pin, n = 3], quadruple infections [Pcy + Pct + Pin + Pk, n = 4] |
| *M. fascicularis, M. nemestrina* | Malaysia | Blood (n = 48 and n = 25) | 18S rRNA | 18.75% (n = 9) and 12% (n = 3) | In *M. nemestrina*; Dual infections [Pcy + Pin, n = 1], Triple infections [Pcy + Pct + Pin, n = 1] |
old woman in the Hulu Terengganu area, East coast of Peninsular Malaysia [51]. Malaria parasites were microscopically misidentified as Pm/Pk mixed infections at a hospital and then as Pv at a reference medical institute in Malaysia. Molecular data coupled with sequencing and phylogenetic analysis confirmed this infection case with Pcy even though initially Pv was identified using nested polymerase chain reaction (PCR) protocol only [51]. Several other reports of natural human infections with Pcy parasites in Malaysia and other countries (Thailand, Cambodia) have also been published [52–58]. Hartmeyer and colleagues reported natural PCY infections in European traveler (37 years old Danish woman) returning from Thailand and Malaysia [53]. Geographical distribution of naturally acquired Pcy infections in humans are presented in Fig. 3 and supplementary material 2. The Pcy infections have been reported in adults as mono-infections and/or mixed infections with Pf, Pv and Pk from macaques inhabited forest areas restricted currently to Malaysia, Thailand and Cambodia (Supplementary material 2).

Role of other zoonotic species in human malaria: Plasmodium brasilianum, P. simium, P. inui and others

There is a growing body of evidence on less restricted host tropism of simian malaria parasites as exemplified by the ability of Pcy to naturally infect other hosts such as humans, even though the human infections seem to be limited in few SEA countries. In this context, it is likely to see human infections with simian malaria species other than Pcy. Analyzing the 18S rRNA and circumsporozoite genes of Plasmodium species, one study reported natural human infections with P. brasilianum (Pbra), an Alouatta monkeys’ parasite, in individuals from the Venezuelan Amazon, South America [59]. Likewise, Pbra was also reported to cause human infection in some coastal areas in Brazil [60]. In Malaysia, Pin, Pin-like, Pct, and P. simiovale parasites were found to infect humans based on analysis of the 18S rRNA and COXI genes [58, 61]. In Brazil, one study reported an outbreak of human malaria infections caused by P. simium [62].

Biological, clinical, and drug sensitivity patterns of P. cynomolgi infections

Biological and clinical patterns

Biological, morphological and clinical characteristics of Pcy are quite similar to non-Pf species with incubation and pre-patent periods roughly shorter than that of Pf (Table 4). The erythrocytic cycle of Pcy parasites lasts for about 48 h,
Table 2  Publications on molecular detection of Pcy parasites in wild Anopheles mosquitoes

| Country   | Anopheles species | Target parasite gene | N   | Total number of Pcy reported | Type of mixed infections                      |
|-----------|-------------------|----------------------|-----|-------------------------------|-----------------------------------------------|
| Vietnam   | A. dirus          | 18S rRNA             | 79  | 11 (6 mono-infections, 5 mixed infections) | Dual: Pcy + Pv (n = 3)                          |
|           |                   |                      |     |                               | Triple: Pcy + Pv + Pk (n = 1)                  |
| Malaysia  | A. balabacensis   | 18S rRNA             | 23  | 8 (4 mono-infections, 4 mixed infections) | Dual: Pcy + Pin (n = 2), Pcy + Pk              |
|           |                   |                      |     |                               | (n = 1), Pcy + Pfd (n = 1)                     |
| Vietnam   | A. dirus, A. maculatus, A. minimus, A. aconitus | 18S rRNA | 49  | 9 (6 mono-infections, 3 mixed infections) | Triple: Pcy + Pv + Pin (n = 3)                |
| Malaysia  | A. balabacensis   | 18S rRNA             | 38  | 24 (5 mono-infections, 19 mixed infections) | Dual: Pcy + Pin (n = 9), Pcy + Pk             |
|           |                   |                      |     |                               | (n = 2)                                        |
|           |                   |                      |     |                               | Triple: Pcy + Pct + Pin (n = 2), Pcy + Pin + Pk (n = 4) |
|           |                   |                      |     |                               | Quadruple: Pcy + Pct + Pin + Pk (n = 2)       |
| Malaysia  | A. barbirostris (s.l.) | 18S rRNA, COXI, ITS2 | 16  | 2 (2 mixed infections)         | Dual: Pcy + Pk (n = 1)                        |
|           |                   |                      |     |                               | Triple: Pcy + Pfd + Pin (n = 1)               |
| Malaysia  | A. laten, A. roperi | 18S rRNA             | 11  | 3 (3 mixed infections)         | Triple: Pcy + Pfd + Pin (n = 1), Pcy + Pct + Pk (n = 1) |
|           |                   |                      |     |                               | Quadruple: Pcy + Pfd + Pin + Pk (n = 1)       |

The references used for this table as listed as supplementary file 2

Pcy: Plasmodium cynomolgi, Pin: Plasmodium inui, Pct: Plasmodium coatneyi, Pfd: Plasmodium fieldi, Pk: Plasmodium knowlesi
18S rRNA: Small subunit ribosomal RNA gene, COXI: Mitochondrial cytochrome c oxidase subunit 1, ITS2: Internal transcribed spacer 2
N: Total number of Plasmodium-infected mosquitoes

*Mosquito species infected with Pcy was not clearly specified

and at the end of this cycle each mature schizont produces 14–20 merozoites in the infected RBC. Once released in bloodstream, the newly produced merozoite can infect fresh RBCs to either multiply asexually or sexually. There is no evidence of rosetting and sequestration of Pcy parasites in the bloodstream as seen in Pf infections. In contrast, the simian species Pcy is quite closer to its Pv sister species on several attributes like preferential invasion of reticulocytes (young erythrocytes), early formation of infectious gametocytes, production of particular structures inside erythrocytes called caveola—vesicles complexes (CVCs), tertian periodicity and relapses of infections due to dormant stages (hypnozoites) (Table 4 and Supplementary material 3). Even within Pcy parasites, these biological characteristics are strain-dependent (e.g., the extent of avidity for reticulocytes was demonstrated to be higher for Cambodian and Berok compared to B and Gombak strains) [63].

Clinical and laboratory findings—Pcy strains/species

Data on the clinical spectrum of Pcy infections are limited to findings from experimental studies in non-immune individuals and fewer from field studies in both non-immune and immune indigenous individuals (Fig. 4). In 1961, an accidental infection case of a 31-year old student with Pcy was reported from New York University, School of Medicine, USA. The student presented chills, tertian-pattern high fever, severe headache and slight alterations in some biochemical (serum bilirubin, lactate dehydrogenase) and hematological parameters (hemoglobin, hematocrit and red blood cell count) [50]. Few years later, accidental infections with the B strain were reported from France in two individuals with high fever, headache, nausea and various aches [49].

The clinical signs/symptoms of Pcy infections are similar to those of other malaria species with slight differences between the Pcy strains. One experimental study outlined similar major symptoms (high fever, cephalgia, anorexia, myalgia and nausea) for the Pcy M and B strains, but differences in duration, frequency of fever and spleen enlargement [16]. Longer duration and higher frequency of the tertian fever, higher frequency of splenomegaly were seen in Pcy M—infected volunteers as compared to their Pcy B—infected counterparts. Also, chills and vomiting were specifically seen in individuals experimentally infected with the strain M [16]. The sister species Pcy and Pv exhibit similar clinical course which is mild to moderate for Pcy (B strain), and moderately severe for Pv [47]. Several other Pcy-related signs/symptoms like diffuse abdominal pain, thrombocytopenia, generalized malaise, reduction in adrenal response, anemia-accompanying reticulocytosis, leucopenia, increase in erythrocyte sedimentation, low back pain, hypoalbuminemia, and hypergammaglobulinemia have also been reported [47, 48]. Some of the above mentioned signs/symptoms (i.e.,
### Table 3  Distribution and bionomics of *Anopheles* species infected with *Pcy* parasites in natural conditions

| Anopheles species | Geographical distribution (countries)** | Biting behavior | Laboratory susceptibility to *Pcy* infections |
|-------------------|----------------------------------------|----------------|---------------------------------------------|
| *A. dirus*        | Asia–Pacific (Myanmar, Thailand, Malaysia) | Zoophilic and anthropophilic, Both endophagic and exophagic | – |
| *A. maculatus*    | Asia–Pacific (Myanmar, Singapore) | Zoophilic and anthropophilic, Both endophagic and exophagic | High |
| *A. minimus*      | Asia–Pacific (India, Myanmar, Thailand, Malaysia, Vietnam) | Zoophilic and anthropophilic, Both endophagic and exophagic | – |
| *A. aconitus*     | Asia–Pacific (Australia, Bangladesh, Bhutan, Cambodia, India, Indonesia, Laos, Myanmar, Sri Lanka) | Zoophilic and anthropophilic, Both endophagic and exophagic | Low |
| *A. balabacensis* | Asia–Pacific (Indonesia, Malaysia, Philippines) | Zoophilic and anthropophilic, Strongly exophagic | High |
| *A. barbirostris* (s.l.) | Asia–Pacific (Myanmar, India, Indonesia, Philippines, Thailand) | Zoophilic and anthropophilic | Low to high |
| *A. latens*       | Asia–Pacific (Indonesia, Malaysia, Thailand) | Zoophilic and anthropophilic, Both endophagic and exophagic | – |
| *A. roperi*       | Asia–Pacific (Cambodia, India, Indonesia, Malaysia, Thailand) | Zoophilic and anthropophilic, Both endophagic and exophagic | Low |
| *A. freeborni*    | North America (Canada, Mexico, USA) | Zoophilic and anthropophilic, Both endophagic and exophagic | High |
| *A. atroparvus*   | Europe and Middle East (France, Germany, Iran, Italy, Portugal, Spain) | Zoophilic and anthropophilic, Both endophagic and exophagic | High |
| *A. stephensi*    | Asia–Pacific (Bangladesh, Myanmar, India, Iraq, Iran), Horn of Africa (Ethiopia, Djibouti) | Zoophilic and anthropophilic, Both endophagic and exophagic | Moderate to high |
| *A. elegans*      | Asia–Pacific (India, Solomon Islands, Sri Lanka, Thailand) | Zoophilic and anthropophilic, Both endophagic and exophagic | High |
| *A. kochi*        | Asia–Pacific (Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Singapore, Thailand, Vietnam) | Zoophilic and anthropophilic, Both endophagic and exophagic | Moderate to high |
| *A. letifer*      | Asia–Pacific (Cambodia, Indonesia, Malaysia, Singapore, Thailand, Vietnam) | Zoophilic and anthropophilic, Strongly exophagic | Moderate |
| *A. lesteri*      | Asia–Pacific (China, Japan, Korea, Philippines) | Zoophilic and anthropophilic, Both endophagic and exophagic | Moderate |
| *A. sandacus*     | Asia–Pacific (India, Indonesia, Malaysia, Thailand, Vietnam) | Zoophilic and anthropophilic, Both endophagic and exophagic | High |
| *A. vagus*        | Asia–Pacific (Afghanistan, Bangladesh, Bhutan, Cambodia, Guam, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, People’s Republic of China, Philippines, Singapore, Sri Lanka, Thailand, Vietnam) | Zoophilic and anthropophilic, Both endophagic and exophagic | Low to moderate |
| *A. quadrimaculatus* | North America (Belize, Canada, Costa Rica, Cuba, Dominican Republic, Mexico, Panama, Puerto Rico, USA) | Zoophilic and anthropophilic, Both endophagic and exophagic | Moderate to high |
| *A. philippinensis* | Asia–Pacific (Bangladesh, Bhutan, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, People’s Republic of China, Philippines, Thailand, Vietnam) | Zoophilic and anthropophilic, Both endophagic and exophagic | Moderate |

Underlined term outlines the main biting behaviour of the mosquito species

*Field studies found *Pcy* parasites in salivary glands of these mosquito species

*These mosquito species have shown moderate-to-high susceptibility to *Pcy* infections and successful transmission and infection to monkey with viable sporozoites[13, 19–22]*

Several *Pcy* strains were used for experimental infections of *Anopheles* mosquitoes: Berok, Cambodian, Gombak, Solomon. Some examples of countries where this mosquito species/complex are given in brackets.
anemia, thrombocytopenia, and anemia-accompanying reticulocytosis) have also been reported in macaques experimentally infected with Pc y B strain sporozoites [30, 32].

Regarding field investigations mild-to-moderate fever, chills and rigors, cough and cold were reported in a Danish woman visiting the Terengganu state, Malaysia [51]. Another study reported fever, chills, rigors, headache and myalgia in a man living in the Kelantan state, Malaysia (Fig. 4) [55].

Drug sensitivity patterns and hypnozoites

The shared biological peculiarity for Pv, Po and Pcy parasites to provoke malaria relapses is due to reactivation of dormant development stages ("hypnozoites") in several weeks to years after the first malaria attack [64]. Current antimalarial drugs (e.g., chloroquine, sulfadoxine-pyrimethamine, atovaquone-proguanil, and ACTs) are effective against blood stages of all human malarias, but unable to kill hypnozoites to prevent relapses. Two 8-aminoquinolones, primaquine (PQ) and tafenoquine (TQ), have high hypnozoiticidal activity, but their therapeutic response against Pv blood stages is slower than that of ACTs and chloroquine (CQ), and this jeopardizes their usage as monotherapy [65, 66].

In practice, the concomitant elimination of blood stages and exoerythrocytic stages, including hypnozoites, known as radical cure, is obtained by utilization of a blood stage-killing drug (e.g., CQ or Atovaquone-proguanil) and an anti-relapse drug [67]. Radical cure with PQ is WHO-endorsed and is adopted in national guidelines of several Pv-endemic countries [68]. However, the routine administration of PQ and TQ is challenging in these areas due to compliance, economic, ethical and safety issues [69, 70]. These hypnozoiticidal drugs can elicit severe hemolytic anemia in patients.
| Characteristics                                      | *P. falciparum* | *P. vivax*   | *P. malariae* | *P. ovale* | *P. knowlesi* | *P. cynomolgi* |
|-----------------------------------------------------|-----------------|--------------|---------------|-------------|---------------|----------------|
| Sporogonic cycle duration (days)                    | ~8–21           | ~8–15        | ~14–17        | ~12–14      | ~9–15         | ~7.5–28        |
| Pre-erythrocytic cycle (days)                       | ~5–10           | ~6–12        | ~14–16        | ~9          | ~5–9          | ~8–10          |
| Incubation period from the infecting mosquito bite (days) | ~9–14           | ~12–17       | ~18–40        | ~9–14       | ~9–12         | ~15–37         |
| Pre-patent period (days)                             | ~6–25<sup>c</sup> | ~11–13<sup>b</sup> | ~15–59        | ~10–20      | ~5–12         | ~7–16<sup>d</sup> |
| Erythrocytic cycle (hours)                          | 48 (Tertian)    | 48 (Tertian) | 72 (Quartan)  | 50 (Tertian) | 24 (Quotidian)| 48 (Tertian)   |
| Number of merozoites released by erythrocyte infected| ~8–20<sup>e</sup> | ~14–20<sup>e</sup> | ~6–14         | ~8–20       | ~16           | ~14–20<sup>f</sup> |
| Early development of mature gametocytes<sup>**</sup>  | No              | Yes          | Yes           | Yes         | Yes           | Yes            |
| Dormant stages (Hypnozoites)                        | No              | Yes          | No            | Yes         | No            | Yes            |
| Relapse latency<sup>†</sup>                          | -               | Short to Long| No            | No          | No            | Short          |
| Rosetting                                           | Yes             | Yes          | No            | No          | Yes<sup>f</sup> | No             |
| Sequestration in human bloodstream                   | Yes<sup>f</sup> | Yes<sup>f</sup> | No            | No          | Yes<sup>f</sup> | No             |
| Preferred erythrocytes for invasion                 | All             | Reticulocytes| Older normocytes| Reticulocytes| All          | Reticulocytes  |
| Some examples of ligands for human erythrocyte invasion<sup>**</sup> | EBL (EBA-175, EBA-140, EBA-1, & EBA-181); PfMSP1; PfRh (PfRh1, PfRh2, PfRh3, PfRh4, PfRh5) | DBP (PvDBP1, PvDBP2)<sup>h</sup>; RBP (PvRBP1a, 1b, 2a, 2b, 2c); PfMSP1; PvTRAg (PvTRAg38, PvTRAg35.2) | RBP (PmRBP1a, 1b, 2a, 2b and 3) | RBP | DBP (PkDBP-α); NBP (NBPXa, NBPXb) | DBP (PcyDBP-1 and Pcy-DBP-2); RBP (PcyRBPl, 1a, 1b, 2a, 2b, 2c, 2d, 2e, 2f, 3) |
| Stained structures in the infected erythrocytes on microscopic examination | None | Schüffner’s dots | Maurer’s clefts | Schüffner’s dots | Sinton-Mulligan’s clefts | Schüffner’s dots |
| Ring stage chromatin                                 | Single to Double | Single      | Single        | Single     | Single to Double | Single         |
| Severe malaria in humans                             | Yes             | Yes         | Yes<sup>i</sup> | Yes<sup>i</sup> | Yes         | No             |
Table 4 continued

The references used for this table as listed as supplementary file 1

DBP: Duffy binding protein, EBL/A: Erythrocyte binding ligand/Antigen, MSP1: Merozoite surface protein 1, NBP/RBP: Normocyte/Reticulocyte binding protein, PIRh: *P. falciparum* reticulo-cyte-binding homologue, Pcy: *P. cynomolgi*, Pf: *P. falciparum*, Pv: *P. vivax*, TRAg: Tryptophan-rich antigen

1 Parasite development in the mosquito till production and migration of sporozoites to salivary glands. These values are strongly dependent on several factors such as parasite strains and environment temperature

2 Period spacing the liver development of sporozoites and releasing merozoites into the bloodstream

3 Time period between the sporozoite inoculation and the appearance of symptoms

4 Time between the sporozoite inoculation and first detection of parasites in the bloodstream

5 This time is > 10 days for *Pf*, and ≤ 3 days for others species

6 Some studies occasionally reported shorter incubation period

7 Very long incubation and pre-patent periods were reported for this malarial species

8 Based on studies with several *Pf* strains (e.g., Panama, McLendon, New Guinea, Rhodesian, Thailand; Santee-Cooper)

9 Based on studies with several *Pcy* strains (B, M, Cambodia, Ceylonensis, Berok, Gombak, RO)

10 The usual number of merozoites released is 16 for these species

11 Only demonstrated experimentally

12 Demonstrated both using experimental and field studies

13 Some reports outlined the ability of *Pv* to infect Duffy-negative reticulocytes

14 These species can occasionally elicit severe malaria

15 Duration of the latency is strain-specific. Short relapse latency (3–4 weeks) and long relapse latency (8 to 9 months)

16 These are the main parasite ligands involved in erythrocyte invasion
suffering from glucose-6-phosphate dehydrogenase deficiency (G6PD-d) and testing for G6PD-d should be routinely done before PQ/TQ administration [3]. There is a need for new drugs with high hypnozoiticidal activity and little or no risk of hemolysis in \( P_v \)-infected patients. The discovery of such drugs is hampered by the absence of robust in vitro continuous culture systems of \( P_v \) parasites [71]. Its sister species \( P_{cy} \), which is now reported to cause natural infections in humans, is cultivable in vitro for longer periods [34], and this offers an opportunity to develop safer hypnozoiticidal drugs for control \( P_{cy}, P_o \) spp and \( P_v \) relapses.

Several authors addressed these aspects by evaluating PQ derivatives and repurposing drugs of inflammatory, bacterial, parasitic and viral ailments (rheumatic problems, lymphatic filariasis, human immunodeficiency virus infection, urinary tract infections). Very few molecules such as elubaquine (CRDI 80/53), a PQ analogue, have exhibited interesting anti-relapse potential and little adverse effects on oxidative functions in \( P_v \)-infected individuals from India and Thailand [72–74], while in vitro studies reported potent \( P_{cy} \)- and \( P_v \)-related anti-hypnozoite activity of antiparasitic compounds including KAI407 (non-8-aminoquinoline) and KDU691 (imidazopyrazine) [75, 76]. These molecules are still under investigation and not currently recommended by WHO for managing relapses. In contrast, repurposed drugs like antibiotics (trimethoprim, sulfamethoxazole, demeclocycline, cyclophosphamide, mirincamycin), antiparasitic (ivermectin), antiviral (lopinavir), anti-inflammatory (dexamethasone) and other drugs (guanylhydrazone, tetrahydrofuran derivative) have failed to prevent \( P_{cy} \) relapses in experimentally infected macaques [77–83].

**Knowledge gaps and future directions**

Human infections with \( P_{cy} \) parasites are a growing public health problem which raises many challenges. The first challenge and gap is mainly the diagnosis as this parasite is mostly misdiagnosed given its morphological similarities with \( P_v \) using light microscopy at health facilities and reference microscopy centers in SEA countries, and the lack of awareness of microscopists vis-à-vis to \( P_{cy} \). \( P_{cy} \). The microscopic detection of \( P_{cy} \) can still pose another problem as \( P_{cy} \) infections can be submicroscopic [52, 57]. In this regard, sensitization/information campaigns coupled with training and assessment sessions of microscopists on \( P_{cy} \) are greatly needed.

Molecular methods are also needed to overcome challenges related to microscopic detection of \( P_{cy} \) parasites. The 18S rRNA gene is commonly targeted in nested and quantitative PCR protocols for \textit{Plasmodium} species, but high similarity of the 18S rRNA gene of \( P_{cy}, P_v, P_k, P_m \) can produce
false-positive as seen in Malaysia, Cambodia, and French Guiana [51, 54, 55] [84, 85]. One solution to the problem would be to target at least two nucleic and mitochondrial genes such as cyt-b, COXI and msp142, and further analyses including sequencing, phylogenetic analyses and external cross-checking. These challenges on microscopic and molecular diagnostic explain greatly the underlying difficulties about estimation of real burden of Pcy in humans. Furthermore, clinicians from SEA settings should be sensitized on Pcy malaria and its treatment for better management. As emerging parasites in human, it is possible that clinicians are not knowledgeable about Pcy parasite relapses, and clinicians are to be made aware that prescription of PQ to be associated with a main antimalarial drug treatment [55, 86].

The absence of evidence on natural circulation of Pcy and other simian parasites in humans in Latin America, Western Asia and sSA is likely due to the lack of studies addressing zoonotic transmission of simian Plasmodia in these areas. In today’s era of world globalization due to human migration, surveillance of zoonotic malaria is no doubt crucial for achieving malaria control and elimination objectives. This will also serve better for imported malaria cases in countries where successful malaria elimination has been achieved [8, 87].

The SEA area, especially Thailand, Malaysia, and Cambodia, seems to be the only foci of Pcy infections in humans based on the current data. It is clear that zoonotic infections with Pcy parasites are shaped by a conjunction of factors: driving closer contacts between monkeys (natural vertebrate hosts), mosquito (invertebrate host) and human (accidental vertebrate host). This relationship between monkeys, mosquitoes, and human is crucial for emergence of and spread of Pcy parasites in humans. The expansion of humans and anthropogenic activities such as deforestation, forestry activities, and climate change are probably the leading drivers, thereby increasing chances for simian parasites to enlarge their host spectrum through emergence to local human populations from these countries, as seen for P. knowlesi and other emerging pathogens [88–91]. Such possibility for invading humans is also driven by the presence of competent and anthropophilic mosquito vectors. Several factors related to bionomics, vector capacity and competence of Anopheles species as well as Pcy parasites and monkey hosts are also crucial [18–20]. Evidently, its prevalence and distribution could be likely underestimated as discussed here. It would be worthwhile to address these questions in future research topics i) If the zoonotic transmission of Pcy is advantageous? ii) how Pcy infection dynamics and natural history are influenced in mixed infections with Pf and Pv? and iii) what is the scope of expansion in Pcy human infections in SEA?

Both sSA and Latin America not only have the highest forest area diversity and non-human primates of the world [92, 93], but also have highly diverse Anopheles species fauna [42, 45]. In Western Asia, the primate diversity is also high with numerous forested areas [93]. Few of Anopheles species such as A. freeborni and A. stephensi are among the major forested areas in North America and Western Asia, respectively, which are also able to produce viable Pcy sporozoites and transmit them to humans in laboratory studies [42, 45, 46, 50]. Also, the risk of Pcy spreading to other countries is also probably due to given reports of A. stephensi in the horn of Africa (e.g., Djibouti, Ethiopia) [94, 95]. Mosquitoes such as A. freeborni and A. stephensi are both anthropophilic and zoophilic, moderately to highly sensitive to Pcy infections, and are able to successfully transmit parasites to monkeys in laboratory conditions [13] (Table 3). Thus, these mosquito species could be vectors of zoonotic malaria parasites such as Pcy in areas where these mosquito species are present. In addition, the nature, distribution and susceptibility of monkey and non-primate hosts (e.g., New World monkeys, apes, macaques) to Pcy infections is also a key determinant modulating the risk and establishment of zoonotic transmission of Pcy to humans. Evidence pointed out directional transmission between humans and African apes as well as humans and New World monkeys for species such as Pv and Pm [96]. Africa and Americas have high diversity of monkeys, but data on efficiency of transmission of Pcy from these monkeys to mosquito vectors and humans in these areas are still lacking. Despite this set of zoonotic transmission-favoring factors in these areas, no report of natural human infections with Pcy has been reported outside SEA till now.

As seen for Pk, control of Pcy malaria could pose a problem in the absence of strategies for controlling the mainly forest-dwelling mosquito vectors and primate reservoir of Pcy parasites [86]. Repellents, odor-based mosquito traps, and insecticide-treated clothes have been proposed, but evidences for their effectiveness are still lacking [97]. Thus, there is need for more studies on Pcy especially on development, evaluation, and implementation of integrated intervention measures (vector control, personal level protection and community engagement, environment control) to curb the chain of transmission of simian parasites from animals to humans [97, 98].

Finally, treatment of Pcy malaria is a significant knowledge gap. No strong evidence for efficacy of current antimalarial drugs is available, even though some studies reported resolution of clinical and laboratory symptoms in Pcy-infected patients treated with Atovaquone + proguanil followed by PQ in European traveler, artemether + lumefantrine followed by PQ in Malaysian patients, and CQ + PQ or artesunate + mefloquine in Thailand patients [53, 55, 57, 99]. With an increasing number of reports on Pcy infections and its therapeutic management, evidence could be generated
Concluding remarks

We reviewed the current knowledge on the epidemiology of natural Pcy infections in humans, mosquitoes and monkeys, along with its biological, clinical and drug sensitivity patterns. A brief snapshot of emergence of other simian malaria parasites was also presented. Also, important knowledge gaps and further studies on Pcy malaria in human were presented and discussed. There are evidences on the ability of Pcy parasites to naturally infect humans, with all natural infection cases reported from either local populations or European travelers returning from SEA countries (Malaysia, Thailand, Cambodia). Clinical presentation of Pcy malaria encompasses mild-to-moderate signs which are mainly represented by fever, chills, and rigor associated with microscopic or submicroscopic parasitemia. Complex interaction involving factors related to human, monkeys (natural vertebrate hosts), mosquitoes, and environment is crucial for emergence of Pcy and other Simian parasites (e.g., P. brasilianum, P. simium, P. inui) in humans from SEA region and outside. Unfortunately, the knowledge of microbiopsists and clinicians on Pcy malaria is still low, thereby jeopardizing its diagnosis, epidemiological study-related findings, and therapeutic management. Also, in the absence of RDTs for simian malaria, molecular methods have been developed to specifically detect Pcy parasites. However, the high level of similarities of target Pcy genes with those of other species is still a big challenge to molecular diagnostics due to chances of false-positive results. From experience gained from Pk malaria control, development of integrated measures and strategies—ideally with components related to human, monkeys, mosquito vectors, and environment—could be very helpful to prevent emergence of Pcy malaria in humans through disruption of transmission chain from monkeys to humans and, ultimately contain its expansion in SEA and potential outbreaks.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s15010-022-01952-2.

Acknowledgements Authors LPKF and JH are supported by The World Academy of Sciences (TWAS), Trieste, Italy, & The Department of Biotechnology (DBT), New Delhi, India. Author AK is ICMR Post-Doctoral Fellow. We are grateful to Dr Wepnje Banda Godlove, PhD (University of Buea, SWR, Cameroon) for English language editing and proofreading. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the funding bodies and institutions with which they are affiliated.

Authors contributions LPKF and VS conceived the paper. LPKF, AK and JH did the literature search. JH and LPKF generated maps. LPKF conceived figures and drafted the first version of the manuscript with the help of AK and JH. AK, JH and VS revised the manuscript for important intellectual content. VS supervised the work at all stages. All authors read and approved the final version of the paper before submission.

Funding The authors did not receive support from any organization for the submitted work.

Data availability All manuscript related data are available in the tables provided and in supplementary material. If any further information is needed, the corresponding author may be contacted for the same.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

References

1. WHO. World malaria report 2021. Geneva; 2021. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021
2. Kojom Foko LP, Narang G, Tamang S, Hawadak J, Jakhan J, Sharma A, et al. The spectrum of clinical biomarkers in severe malaria and new avenues for exploration. Virulence. 2022;13:634–54.
3. Kojom Foko LP, Arya A, Sharma A, Singh V. Epidemiology and clinical outcomes of severe Plasmodium vivax malaria in India. J Infect. 2021;82:231–46. https://doi.org/10.1016/j.jinf.2021.03.028.
4. Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020. https://doi.org/10.1038/s41591-020-1005-2.
5. Balikagala B, Fukuda N, Ikeda M, Kature OT, Tachibana S-I, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. N Engl J Med. 2021;385:1163–71.
6. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, et al. Association of Plasmodium falciparum kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. Lancet Infect Dis. 2021;21:P1120-1128.
7. Kojom Foko L, Kouemo Motse FD, Kamgain Mawabo L, Pande V, Singh V. First evidence of local circulation of Plasmodium ovale curtisi and reliability of a malaria rapid diagnostic test among febrile outpatients in Douala, Cameroon. Infect Genet Evol. 2021;91:104797. https://doi.org/10.1016/j.meegid.2021.104797.
8. Zhou R, Li S, Zhao Y, Yang C, Liu Y, Qian D, et al. Characterization of Plasmodium ovale spp. imported from Africa to Henan Province, China. Sci Rep. 2019;9:2191.
9. Yman V, Wandell G, Mutemi DD, Miglar A, Asghar M, Hammar U, et al. Persistent transmission of Plasmodium malariae and Plasmodium ovale species in an area of declining Plasmodium falciparum transmission in eastern Tanzania. PLoS Negl Trop Dis. 2019;13:634–54.
10. Kotepui M, Kotepui KU, Milanez GD, Masangkay FR. Prevalence of severe Plasmodium knowlesi infection and risk factors related to severe complications compared with non-severe P. knowlesi and severe P. falciparum malaria: a systematic review and meta-analysis. Infect Dis Poverty. 2020;9:106. https://doi.org/10.1186/s40249-020-00727-x.

11. Bykersma A. The new zoonotic malaria: Plasmodium cynomolgi. Trop Med Infect Dis. 2021;6:46.

12. Kotepui M, Masangkay FR, Kotepui KU, Milanez GDJ. Preliminary review on the prevalence, proportion, geographical distribution, and characteristics of naturally acquired Plasmodium cynomolgi infection in mosquitoes, macaques, and humans: a systematic review and meta-analysis. BMC Infect Dis. 2021;21:259.

13. Coatney R, Collins W, Warren M. The primate malarias. US Gov Printing Office. 1971;26:317–33.

14. Mulligan H. Descriptions of two species of monkey Plasmodium isolated from Silenus irus. Archir fur Protistenkunde. 1935;84:285–314.

15. Garnham P. A new sub-species of Plasmodium cynomolgi. Riv Parasitol. 1959;20:273–8.

16. Contacos PG, Elder HA, Coatney AR. Man to man transfer of two strains of Plasmodium cynomolgi by mosquito bite. Am J Trop Med Hyg. 1962;11:186–93.

17. Eyles DE. The species of Simian malaria: taxonomy, morphology, life cycle, and geographical distribution of the monkey species. J Parasitol. 1963;49:866–87.

18. Bennett G, Warren M, Cheong W. Biology of the simian malarias of Southeast Asia. III. Sporogony of the Cambodian strain of Plasmodium cynomolgi. J Parasitol. 1966;52:632–8.

19. Bennett G, Warren M, Cheong W. Biology of the Simian malarias of Southeast Asia. IV. Sporogony of four strains of Plasmodium cynomolgi. J Parasitol. 1966;52:639–46.

20. Bennett G, Warren M, Cheong W. Biology of the Simian malarias of Southeast Asia. II. The susceptibility of some Malaysian mosquitoes to infection with five strains of Plasmodium cynomolgi. J Parasitol. 1966;52:625–31.

21. Collins WE, Warren M, Galland GG. Studies on infections with the Berok strain of Plasmodium cynomolgi in monkeys and mosquitoes. J Parasitol. 1999;85:268–72.

22. Collins WE, Warren M, Sullivan JAS, Galland GG, Nace D, Williams A, et al. Studies on two strains of Plasmodium cynomolgi in new world and old world monkeys and mosquitoes. J Parasitol. 2005;91:280–3.

23. Dissanaike A, Nelson P, Garnham P. Two new malaria parasites, Plasmodium cynomolgicyceliensis subsp. Nov. and Plasmodium fragile sp. Nov., from monkeys in Ceylon. Ceylon J Med Sci. 1965;14:1–9.

24. Shorr H, Garnham P. The exoerythrocytic parasites of Plasmodium cynomolgi. Trans R Soc Trop Med Hyg. 1948;41:705–15.

25. Shorr H, Garnham P. The pre-erythrocytic development of Plasmodium cynomolgi and Plasmodium fragilis. Trans R Soc Trop Med Hyg. 1948;41:785–95.

26. Wong ML, Ahmed MA, Sulaiman WYW, Manin BO, Leong CS, Quan FS, et al. Genetic diversity of zoonotic malaria parasites from mosquito vector and vertebrate hosts. Infect Genet Evol. 2019;73:26–32. https://doi.org/10.1016/j.meegid.2019.04.010.

27. Putapornpit C, Kiumobs N, Wongwutiss W. Sequence diversity and positive selection at the Duffy-binding protein genes of Plasmodium knowlesi and P cynomolgi: analysis of the complete coding sequences of Thai isolates. Infect Genet Evol. 2016;44:367–75. https://doi.org/10.1016/j.meegid.2016.07.040.

28. Latif ENM, Shahari S, Amir A, Cheong FW, Lau YL, Abdullah ML, et al. Genetic diversity of Duffy binding protein 2 region II of Plasmodium cynomolgi from wild macaques in Peninsular Malaysia. Trop Biomed. 2022;39:66–72.

29. Pacheco AM, Elango AP, Rahman AA, Fisher D, Collins WE, Barnwell JW, et al. Extended evidence of purifying selection on merozoite surface protein 8 (MSP8) and 10 (MSP10) in Plasmodium spp. Infect Genet Evol. 2012;12:978–86.

30. Joyner CJ, Wood JS, Moreno A, Garcia A, Galinski MR. Severe and complicated cynomolgi malaria in a rhesus macaque resulted in similar histopathological changes as those seen in human malaria. Am J Trop Med Hyg. 2017;97:548–55.

31. Tachibana SI, Kawai S, Katakai Y, Takahashi H, Nakade T, Yasutomi Y, et al. Contrasting infection susceptibility of the Japanese macaques and cynomolgus macaques to closely related malaria parasites, Plasmodium vivax and Plasmodium cynomolgi. Para- sitol Int. 2015;64:274–81. https://doi.org/10.1016/j.parint.2014.10.004.

32. Joyner C, Moreno A, Meyer EVS, Cabrera-Mora M, Kissinger JC, Barnwell JW, et al. Plasmodium cynomolgi infections in rhesus macaques display clinical and parasitological features pertinent to modelling vivax malaria pathology and relapse infections. Malar J. 2016;15:451.

33. Martinelli A, Culleton R. Non-human primate malaria parasites: out of the forest and into the laboratory. Parasitology. 2018;145:41–54.

34. Chua ACY, Ong JHY, Millaret B, Suwanarasuk R, Kosasavee V, Zeeman AM, et al. Robust continuous in vitro culture of the Plasmodium cynomolgi erythrocytic stages. Nat Commun. 2019;10:1–13. https://doi.org/10.1038/s41467-019-11332-4.

35. Sam J, Shamsusah NA, Ali AH, Hod R, Hassan MR, Agustar HK. Prevalence of simian malaria among macaques in Malaysia (2000–2021): a systematic review. PLoS Negl Trop Dis. 2022;16:e0010527. https://doi.org/10.1371/journal.pntd.0010527.

36. Gamalo LE, Dimalibot J, Kadir KA, Singh B, Paller VG. Plasmodium knowlesi and other malaria parasites in long-tailed macaques from the Philippines. Malar J. 2019;18:147. https://doi.org/10.1186/s12936-019-2780-4.

37. Fungfuang W, Udom C, Tongthainan D, Kadir KA, Singh B. Malaria parasites in macaques in Thailand: stump-tailed macaques (Macaca arctoides) are new natural hosts for Plasmodium knowlesi, Plasmodium inui, Plasmodium cootneyi and Plasmodium fieldi. Malar J. 2020;19:350. https://doi.org/10.1186/s12936-020-03424-0.

38. Reid MJ, Ursic R, Cooper D, Nazzari H, Griffiths M, Galdikas BM, et al. Transmission of human and macaque Plasmodium spp. to ex-captive orangutans in Kalimantan, Indonesia. Emerg Infect Dis. 2006;12:1902–8.

39. Singh B, Divis PCS. Orangutans not infected with Plasmodium vivax or P. cynomolgi, Indonesia. Emerg Infect Dis. 2009;15:1657–8.

40. Massey NC, Garrod G, Wiebe A, Henry AJ, Huang Z, Moyes CL, et al. A global bionomic database for the dominant vectors of human malaria. Sci Data. 2016;3:160014.

41. Hay SI, Sinka ME, Okara RM, Kabaria CW, Mbiti PM, Tago CC, et al. Developing global maps of the dominant anophelines vectors of human malaria. PLoS Med. 2010;7:e1000209.

42. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, et al. The dominant Anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010;3:72.

43. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010;3:117.

44. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, et al. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2011;4:89.
45. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonvirapaph T, Coetzee M, et al. A global map of dominant malaria vectors. Parasit Vectors. 2012;5:69.

46. Eyles DE, Coatney GR, Getz ME. Vivax-type malaria parasite of macaques transmissible to man. Science. 1960;131:1812–3.

47. Kuvin SF, Beye HK, Stohlman F, Coatney GR. Clinical and physiological responses in sporozoite-induced B strain Plasmodium cynomolgi and Plasmodium vivax infections in normal volunteers. Trans R Soc Trop Med Hyg. 1962;56:371–8.

48. Kuvin SF, Beye HK, Stohlman F, Contacos PG, Coatney GR. Malaria in Man. Infection by Plasmodium vivax and the B strain of Plasmodium cynomolgi. JAMA. 1963;184:1018–20.

49. Druilhe P, Trape J, Leroy J, Godard C. Gentilini M [Two accidental human infections by Plasmodium cynomolgi bastianellii. A clinical and serological study]. Ann Soc Belg Med Trop. 1969;60:349–54.

50. Most H. Plasmodium cynomolgi malaria: accidental human infection. Am J Trop Med Hyg. 1973;22:157–8.

51. Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. First case of a naturally acquired human infection with Plasmodium cynomolgi. Malar J. 2014;13:68.

52. Grignard L, Shah S, Chua TH, William T, Drakeley CJ, Fornace CM. Natural human infections with Plasmodium cynomolgi and other malaria species in an elimination setting in Sabah, Malaysia. J Infect Dis. 2019;220:1946–9.

53. Hartmeyer GN, Stensvold CR, Fabricius T, Marmolin ES, Hoegh SV, Nielsen HV, et al. Plasmodium cynomolgi as cause of Malaria in tourist to Southeast Asia, 2018. Emerg Infect Dis. 2019;25:1936–9.

54. Imwong M, Madmanee W, Suwannasin K, Kunasol C, Fornace CM. Asymptomatic natural human infections with the simian malaria parasites Plasmodium cynomolgi and Plasmodium knowlesi. J Infect Dis. 2019;219:695–702.

55. Mohd Nor F, Azeana R, Aziz AA, Azimuthul M, Zakaria A, Adura S, et al. P. vivax or P. cynomolgi?: Public health challenges in detection and control measures. Int J Public Health Clin Sci. 2020;6:2289–7577. https://doi.org/10.32827/ijphc.6.6.33.

56. Raja TN, Hu TH, Kadir KA, Mohamad DSA, Rosli N, Wong LL, et al. Naturally acquired human Plasmodium cynomolgi and P. knowlesi infections. Malaysian Borneo. Emerg Infect Dis. 2020;26:1801–9.

57. Putaporntip C, Kuamsab N, Pattanawong U, Yanmanee S, Srivilairit S, Thanachartwet V, et al. Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) vs primaquine in double blind clinical trial. Curr Sci. 2001;80:561–3.

58. Yap NJ, Hossain H, Nada-raja T, Ngui R, Muslim B, Ho H, et al. Natural human infections with Plasmodium cynomolgi, P. inui, and 4 other simian malaria parasites, Malaysia. Emerg Infect Dis. 2021;27:2187–91.

59. Lalremruata A, Magris M, Vivas-Martínez S, Kocher M, Esen M, Kempaiah P, et al. Natural infection of Plasmodium brasilianum in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. EBioMedicine. 2015;2:1186–92. https://doi.org/10.1016/j.ebiom.2015.07.033.

60. Buery JC, de Alencar FEC, de Duarte AMRC, Loss AC, Vicente CR, Ferreira LM, et al. Atlantic forest malaria: a review of more than 20 years of epidemiological investigation. Microorganisms. 2021;9:132.

61. Liew JWK, Bukhari FDM, Jeyaprakasam NK, Phang WK, Vythilingam I, Lau YL. Natural Plasmodium inui infections in humans and Anopheles crassus mosquito, Malaysia. Emerg Infect Dis. 2021;27:2700–3.

62. Brasil P, Zalis MG, de Pina-Costa A, Siqueira AM, Júnior CB, Silva S, et al. Outbreak of human malaria caused by Plasmodium simium in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. Lancet Glob Health. 2017;5:e1038–46.

63. Warren M, Skinner JC, Guinn E. Biology of the simian malarial of Southeast Asia I Host cell preferences of young trophozoites of four species of Plasmodium. J Parasitol. 1966;52:14–6.

64. White NJ. Determinants of relapse periodicity in Plasmodium vivax malaria. Malar J. 2011;10:297.

65. Putkittayakamsee S, Vanijanonta S, Chantra A, Clemens R, White NJ. Blood stage antimalarial efficacy of primaquine in Plasmodium vivax malaria. J Infect Dis. 1994;169:932–5.

66. Fukuda MM, Krudsood S, Mohamed K, Green JA, Warrask S, Noedl H, et al. A randomized, double-blind, active-control trial to evaluate the efficacy and safety of a three day course of tafenoquine monotherapy for the treatment of Plasmodium vivax malaria. PLoS ONE. 2017;12:e0183736.

67. Chu CS, White NJ. The prevention and treatment of Plasmodium vivax malaria. PLoS Med. 2021;18:e1003561. https://doi.org/10.1371/journal.pmed.1003561.

68. WHO. Guidelines for the treatment of malaria—Third edition [Internet]. Geneva; 2015. https://www.ncbi.nlm.nih.gov/books/NBK294440/pdf/Booksshelf_NBK294440.pdf.

69. Peters AL, Van Noorden CJF. Glucose-6-phosphate dehydrogenase deficiency and malaria: cytochemical detection of heterozygous G6PD deficiency in women. J Histochem Cytochem. 2009;57:1003–11.

70. Lubell Y, White L, Varadan S, Drake T, Yeung S, Cheah PY, et al. Ethics, economics, and the use of primaquine to reduce falciparum malaria transmission in asymptomatic populations. PLoS Med. 2014;11:e1001704.

71. Gunalan K, Rowley EH, Miller LH. A way forward for culturing Plasmodium vivax. Trends in Parasitology. 2020;36:512–9. https://doi.org/10.1016/j.pt.2020.04.002.

72. Dutta G, Puri S, Bhaduri A, Seth M. Radical curative activity of a new 8-aminooquinoline derivative (CDRI 80/53) against Plasmodium cynomolgi B in monkeys. Am J Trop Med Hyg. 1989;41:635–7.

73. Valecha N, Adak T, Bagga A, Asthana O, Srivastava J, Joshi H, et al. Comparative antirelapse efficacy of CDRI compound 80/53 (Bulaquine) vs primaquine in double blind clinical trial. Curr Sci. 2001;80:561–3.

74. Krudsood S, Wilairatana P, Tangpukdee N, Chalermrut K, Krudsood L, et al. Targeting Plasmodium PI(4)K to eliminate malaria. Nature. 2013;504:248–53. https://doi.org/10.1038/nature12782.

75. McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Nagle A, et al. Naturally acquired human Plasmodium vivax malaria in Thailand. Korean J Parasitol. 2009;47:221–9.

76. Lubell Y, White L, Varadan S, Drake T, Yeung S, Cheah PY, et al. Ethics, economics, and the use of primaquine to reduce falciparum malaria transmission in asymptomatic populations. PLoS Med. 2014;11:e1001704.

77. Hobbs CV, Dixit S, Penzak SR, Sahu T, Orr-Gonzalez S, Lambert D, et al. Targeting Plasmodium Pl(4)K to eliminate malaria. Nature. 2013;504:248–53. https://doi.org/10.1038/nature12782.

78. Zeeman AM, Van Amsterdam SM, McNamara CW, Voorberg-van Der Wel A, Klooster EJ, Van Den Berg A, et al. KA1407, a potent non-8-aminooquinoline compound that kills Plasmodium cynomolgi early dormant liver stage parasites in vitro. Antimicrob Agents Chemotherapy. 2014;58:1586–95.

79. Hobbins CB, Dixit S, Penzak SR, Sahu T, Orr-Gonzalez S, Lambert L, et al. Neither the HIV protease inhibitor lopinavir-ritonavir nor the antimalarial trimethoprim-sulfamethoxazole prevent malaria relapse in Plasmodium cynomolgi-infected non-human primates. PLoS ONE. 2014;9:115506.

80. Kumar A, Dutta GP. Antimalarial activity of demeclocycline against Plasmodium cynomolgi bastianellii in rhesus monkeys. Ann Trop Med Parasitol. 1989;83:199–206.

81. Hu Y-M, Nie M. Effects of dexamethasone and cyclophosphamide on development of exo-erythrocytic form of Plasmodium cynomolgi bastianellii in rhesus monkey. Acta Pharmacol Sin. 1992;13:478–80.
80. Fracisco S, Teja-Isavadharm P, Gettyacamin M, Berman J, Li Q, Melendez V, et al. Anti-relapse activity of mirincamycin in the Plasmodium cynomolgi sporozoite-infected Rhesus monkey model. Malar J. 2014;13:409.
81. Vanachayangkul P, Im-erbsin R, Tungtaeng A, Kodchakom C, Roth A, Adams J, et al. Safety, pharmacokinetics, and liver-stage Plasmodium cynomolgi effect of high-dose ivermectin and chloroquine in Rhesus macaques. Antimicrob Agents Chemother. 2020;64:e00741-e820.
82. Corcoran K, Hansukjariya P, Sattabongkot J, Ngampochjana M, Schmidt L. Activities of the tetrahydrofuran derivative, Plasmodium knowlesi in rhesus monkeys. Antimicrob Agents Chemother. 1985;27:146–50.
83. Jeyaprakasam NK, Liew JWK, Low VL, Wan-Sulaiman WY, Hansen M, Potapov P, et al. First detection of Anopheles stephensi Liston, 1901 (Diptera: culicidae) in Ethiopia using molecular and morphological approaches. Acta Trop. 2018;188:180–6. https://doi.org/10.1016/j.actatropica.2018.09.001.
84. Seyfarth M, Khairieh BA, Abdi AA, Bouh SM, Faulde MK. Five years following first detection of Anopheles stephensi (Diptera: Culicidae) in Djibouti, Horn of Africa: populations established—malaria emerging. Parasitol Res. 2019;118:725–32.
85. Sharp PM, Pledgerleith LJ, Hahn BH. Ape origins of human malaria. Annu Rev Microbiol. 2020;74:39–63.
86. Mohammad AH, Naserrudin NA, Syed Abdul Rahim SS, Jelip J, Cahill R, Sazali MF, et al. Narrative review of the control and prevention of knowlesi malaria. Trop Med Infect Dis. 2022;7:178.
87. Babu R, Sridhar S, Sasa T, Rajahram GS, et al. Clinical management of Plasmodium knowlesi infection: a systematic review. Int J Environ Res Public Health. 2022;19:3675.
88. Hansen M, Potapov P, Moore R, Hancher M, Turubanova S, Tyukavina A, et al. High-resolution global maps of 21st-century forest cover image. Science. 2013;342:850–3.
89. Estrada A, Garber PA, Rylands AB, Roos C, Fernandez-Duque E, Di FA, et al. Impending extinction crisis of the world’s primates: Why primates matter. Sci Adv. 2017. https://doi.org/10.1126/sciadv.1600946.