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# Simian Malaria Parasites: Special Emphasis on *Plasmodium knowlesi* and Their *Anopheles* Vectors in Southeast Asia

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Additional information is available at the end of the chapter

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## 1. Introduction

Simian malaria parasites were first reported in Malayan monkeys by Daniels in 1908 [1]. It had been assumed for a long time that transmission of simian malaria to humans would not be possible. However, an accidental infection of scientists in Atlanta, USA by mosquito bites in the laboratory proved that a simian malaria species— *Plasmodium cynomolgi* can be transmitted to humans [2, 3]. In 1965 the first natural infection in human was reported in an American surveyor who was infected in the jungles of Pahang, Malaysia [4]. Fortunately he returned to USA and was detected first as *Plasmodium falciparum* and later revised to *Plasmodium malariae* due to the band form of the parasite. Further examination proved that it was actually *Plasmodium knowlesi* [4].

*Plasmodium knowlesi* was first found in *Macaca fascicularis* monkeys that were brought to India from Singapore. Drs Knowles and Das Gupta knew that they were dealing with a new malaria parasite but did not provide a binomial nomenclature. It was Sinton and Muligan who formally named the new species as *P. knowlesi* [5] after Dr. Knowles. Studies that were carried out before the first human case was reported unveiled many new simian malaria parasites but no human cases. After the first human case was reported in 1965, blood samples were collected from about 1000 people from surrounding villages in West Malaysia where the case of *P. knowlesi* was found but none were positive for simian malaria [6]. However, a presumptive case was reported from Johore, a southern state in peninsular Malaysia [7].

Mosquito surveys carried out in the area where the first case occurred did not reveal any sporozoite infections in the mosquitoes. However, studies in the coastal areas of Selangor in peninsular Malaysia found *Anopheles hackeri* to be a vector of *P. knowlesi* [8] and this mosquito

was attracted only to non-human primates and would not come to bite humans. Thus, at that time it was concluded that simian malaria parasites would not easily affect humans and if it did human malaria cases would occur at very low levels [9]. In 2004 a large focus of knowlesi malaria among humans in Sarawak, Malaysian Borneo was reported [10]. This significant finding stimulated many scientists who were interested in the field of simian malaria in humans and their vectors and hosts. Southeast Asia has now become a focal point for the distribution of *P. knowlesi* in humans. This chapter will describe the simian malaria parasites in non-human primates, the bionomics of vectors involved in transmission, human cases of knowlesi malaria and the challenges in relation to elimination of malaria.

## 2. Simian malaria parasites and their hosts

In Southeast Asia, there are 13 species of *Plasmodium* affecting non-human primates [11]. Of these *Plasmodium coatneyi*, *P. cynomolgi*, *P. fieldi*, *P. fragile*, *P. inui*, *P. knowlesi* and *P. simiovale* are known to occur in macaques and leaf monkeys [12]. However, of the seven species, *P. fragile* has been reported in both India and Sri Lanka while *P. simiovale* is restricted only to Sri Lanka [12]. *Plasmodium eylesi*, *P. jefferyi*, *P. youngi* and *P. hylobati* are found in gibbons while *P. pitheci* and *P. silvaticum* are found in orangutans in Borneo. These malaria parasites are found throughout mainland Southeast Asia and associated islands within the Wallace's line [13].

Information is currently available on the non-human primate malaria especially in Malaysia. Thus, so far five species of simian malaria parasites in non-human primates (macaques) have been reported from Malaysia [12, 14]. The simian malaria parasite *P. cynomolgi* is a species that had been experimentally transmitted to humans [3, 15]. *Plasmodium cynomolgi* in monkeys has many of the characteristics seen during infection of humans with *P. vivax* [16]. It was always believed that monkey malaria was specific for monkeys and human malaria was specific for humans. However, in 1960 accidental infections in the laboratory of simian malaria to humans by mosquito bites led to investigative studies to be carried out in Malaysia and this resulted in the description of many new simian malaria parasites [17-20].

Simian malaria parasites have been detected in three main species of non-human primates. They are *Macaca fascicularis*, *Macaca nemestrina* and *Presbytis melalophos* [19, 20]. In the 1960's studies on malaria parasites of *M. nemestrina* revealed that this non-human primate can harbour the following simian malaria species: *P. cynomolgi*, *P. inui*, *P. knowlesi* and *P. fieldi* [19]. Of these *P. fieldi* was a new species found in this macaque [17]. Currently, *P. fieldi* has been found as mixed infection in longtailed macaques but less frequently compared to the other simian malaria parasites [14]. Only 4% of the macaques had *P. fieldi* mono-infection in a study in Sarawak, Malaysian Borneo [14]. In Malaysian Borneo the predominant species found in the longtailed macaques was *P. inui* (82%) followed by *P. knowlesi* (78%), *P. coatneyi* (66%) and *P. cynomolgi* (56%) [14]. However, in Singapore *P. knowlesi* was the predominant species among long-tailed macaques (68.2%), followed by *P. cynomolgi* (66.6%), *P. fieldi* (16.7%), *P. coatneyi* (3%) and *P. inui* (1.5%) [21]. In Selangor, out of the 107 samples of macaque blood tested for malaria, 64.5% were positive for *Plasmodium* of which 23.3% were positive for *P. knowlesi* [22].

*Plasmodium coatneyi* was successfully established when sporozoites from *An. hacker* collected from Rantau Panjang Selangor, were inoculated into an uninfected rhesus monkey. The monkey exhibited infection after a prepatent period of 14 days. The young trophozoites were not easily distinguishable from those of *P. falciparum* and demonstrated a tertian cycle thus leading to a new species [23]. This is the first instance of finding a new species of malaria in the vector before it was known from the primate host. Subsequently *P. coatneyi* was also isolated from *M. fascicularis* from the same area and also from the Philippines [24].

The pig-tailed macaque – *Macaca nemestrina* occurs in various sub-species from easternmost India and Bangladesh, through Myanmar and Thailand, Malaysia, Sumatra and Kalimantan [19]. This animal is trained to harvest coconuts from tall trees and is kept as a pet by their owners. They coexist with long-tailed macaques-*M. fascicularis* but are ecologically less diverse in their choice of habitats [19]. They are also less commonly seen compared to *M. fascicularis*. The parasites found in the pig-tailed macaques were *P. cynomolgi*, *P. inui*, *P. knowlesi*, *P. fieldi* and *Hepatocystis* [19].

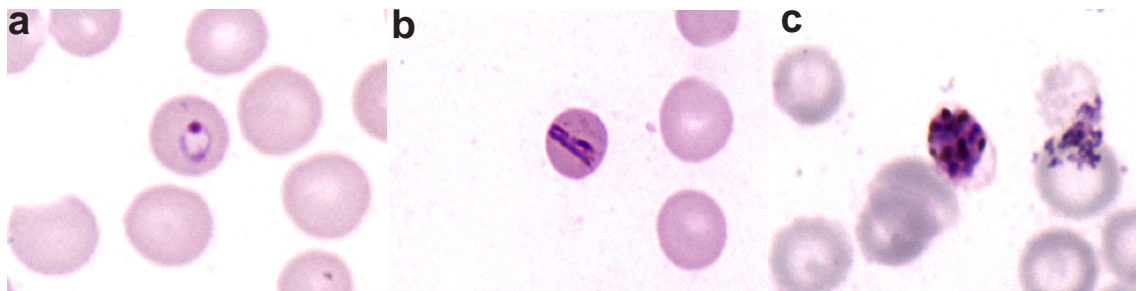
### 3. History of natural infection of *P. knowlesi* in human host

Scientists have always been curious as to the possibility of humans being infected with non-human primate malaria. This interest was intensified when two scientists working in the Memphis laboratory were infected with *P. cynomolgi*. They were conducting infection studies in the laboratory and they were dissecting a large number of mosquitoes heavily infected with malaria parasites two weeks prior to coming down with the illness [2]. Following these infections, scientists decided to survey areas in peninsular Malaysia and search for natural transmission of simian malaria in humans. There were also attempts by scientists to probe into the natural transmission of monkey malaria to humans in the northernmost state of peninsular Malaysia [25]. In the first survey they did not come across any human cases but described new species of monkey malaria parasites in macaques [6].

In 1965, an American surveyor working in Bukit Kertau in Pahang, Malaysia came down with malaria. Fortunately he returned to USA where he was diagnosed as *P. knowlesi* [4]. This was the first natural infection reported in humans. The surveyor was apparently working in the forested area at night. American scientists along with the scientists from the Institute for Medical Research carried out extensive surveys in that area where the surveyor was infected. Blood from 1117 persons from 17 villages were examined for malaria parasites by microscopy using Giemsa stained slides. Blood was also inoculated into rhesus monkeys to determine if there were natural infections of simian malaria in humans. Of these only 28 had malaria infection, 11 were *P. falciparum*, 13 *P. vivax* and four were not identifiable. None of the rhesus monkeys developed malaria parasites [6]. Thus it was concluded that simian malaria would not easily infect humans. In 1970's a presumptive case of *P. knowlesi* was reported from Johore, peninsular Malaysia [7].

#### 4. Cases of knowlesi malaria in Southeast Asia

In 2004, a large focus of human knowlesi malaria cases were reported from Sarawak, Malaysian Borneo [10]. In that study it was found that 58% of the patients, admitted at the Sarawak hospital, were found to be infected with knowlesi malaria using molecular tools. These were misidentified by microscopy as *P. malariae*. Early trophozoites of *P. knowlesi* in the erythrocyte resemble that of *P. falciparum* such as double chromatin dots, multiple-infected erythrocytes and appliqué forms [26]. Besides the late and mature trophozoites, schizonts and gametocytes of *P. knowlesi* in human infections were generally indistinguishable from those of *P. malariae*. Moreover, 'band form' trophozoites, which are a characteristic feature for *P. malariae* parasites [27, 28] were observed in more than half of the blood films examined by Lee *et al* [26]. 'Sinton and Mulligan's stippling' in erythrocytes infected with *P. knowlesi* was noted previously in infections in rhesus monkeys [27] and humans [7]. However, in present knowlesi cases only faint stippling was evident in some of the infected erythrocytes with mature trophozoite and schizont stages [10, 26]. Thus, human infections with *P. knowlesi* have been mistaken for *P. falciparum* malaria when the infecting parasites were predominantly at the early trophozoite or ring form developmental stage and as *P. malariae* when in the late trophozoite or band form. Figure 1 shows the different stages of development of *P. knowlesi*.



**Figure 1.** Giemsa stained thin blood film of *P. knowlesi* as seen with 100 x objective. a). trophozoite b) band form of trophozoite, c) schizont

After the publication in 2004 [10], more cases were reported in Malaysia [29-32] and also from other countries in Southeast Asia with the exception of Lao PDR. To date cases have been reported from Thailand [33-35], Philippines [36], Vietnam [37], Indonesia [38], Cambodia [39], Myanmar [40] and Singapore [41]. Malaysia has reported the highest number of cases in the region. *Plasmodium knowlesi* is now considered as the fifth malaria parasite affecting humans [42] and is detected by molecular methods. However, some still believe that it is a simian malaria since human to human transmission has not been proven [13].

A study has shown that *M. fascicularis* experimentally infected with *P. knowlesi* erythrocytic parasites from humans developed pre patent infection on day seven and demonstrated diurnal sub-periodic pattern [43]. It is the only primate malaria with a 24-hour erythrocytic cycle [44] while *P. falciparum* has a 48 hour cycle and *P. malariae* a 72 hour cycle.

Knowlesi malaria has shown to be life threatening and mortality has been reported [29, 31]. From December 2007 to November 2009 six (27%) out of 22 patients with severe knowlesi malaria died in Sabah [31]. Cases of knowlesi malaria are also occurring in areas where human malaria cases have been reduced or in malaria free areas [45]. People can contract malaria either outside their houses in rural settings, in farms where they work or in the forest while hunting or working.

## 5. Knowlesi malaria associated with travellers to Southeast Asia

Naturally acquired cases of *P. knowlesi* have been reported from travellers visiting this region. A New Zealand pilot working in Sabah and Sarawak north of Bintulu Malaysian Borneo was diagnosed as *P. knowlesi* in New Zealand when he fell ill. The sequence of the parasite had a 100% homology to the Vietnam strain [46]. A lady born in the Philippines and residing in USA for more than 25 years came down with knowlesi malaria after visiting Palawan in the Philippines where she stayed in a log cabin close to the forest edge. She fell ill and on her return to USA was diagnosed as *P. knowlesi* [47]. A Finish traveller spent about 5 days in the jungle on the north-western coast of peninsular Malaysia and fell ill after he returned to Finland. He was diagnosed with *P. knowlesi* parasitaemia by PCR and sequencing showed 100% homology with *P. knowlesi* sequence from Malaysian Borneo and a *Macaca mullata* from Colombia [48]. A Swede who travelled to the Bario Highlands in Malaysian Borneo came down ill on his return to Sweden and was diagnosed as suffering from knowlesi malaria [49]. A Spanish traveller who spent six months travelling around Southeast Asia – in forested areas was diagnosed as knowlesi malaria when he returned to Spain [50]. A French tourist returning from Thailand was diagnosed as *P. knowlesi* [51]. This shows that the knowlesi malaria is currently a serious public health problem and not just single occasional episodes.

## 6. Bionomics of simian malaria vectors and trapping techniques

### 6.1. Distribution

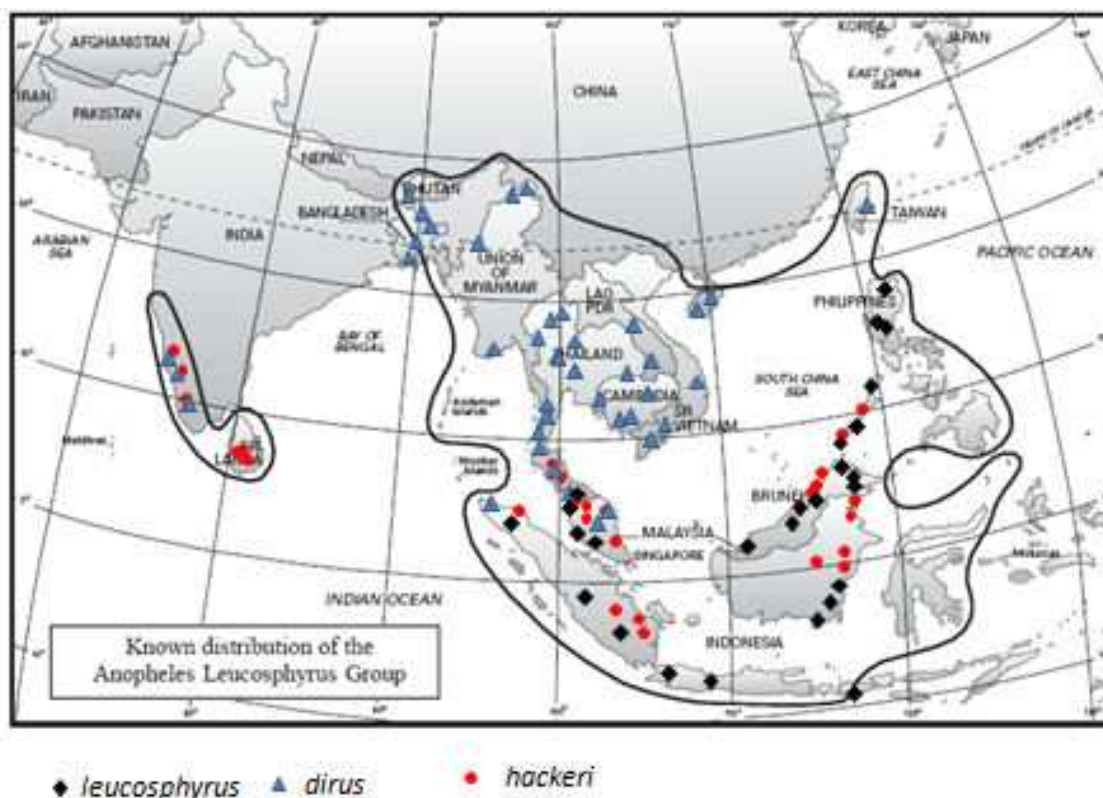
The distribution of *P. knowlesi* in the natural monkey hosts and transmission to humans are restricted to mosquito vectors of the *Anopheles* Leucosphyrus Group confined to Southeast Asia [52]. It is currently recognized that under natural forest conditions, most if not all members of the Leucosphyrus Group apparently feed primarily on monkeys in the canopy, transmitting various plasmodia [53]. In Harbach's review [54], the Leucosphyrus Group in the Neomyzomyia Series contains 20 named species [55, 56], one unnamed species (aff. *takasagoensis*) and two geographical forms (Con Son form from island off South Vietnam and Negros form from Negros island in Philippines) [55] divided between the Hackeri, Leucosphyrus and Riparis Subgroups. According to Manguin *et al.* [57] and Sallum *et al.* [56], the Leucosphyrus Subgroup consists of the Dirus and Leucosphyrus complexes, which includes seven and five sibling species, respectively. Species belonging to the Leucosphyrus complex are also important

vectors of human malaria and lymphatic filariasis and are distributed in the South and Southeast Asia regions. The current vectorial status and geographical distribution of the Leucosphyrus Group are listed in Table 1 and Figure 2.

Complex	Vector species	Species of <i>Plasmodium</i>	Vertebrate hosts	Distribution
Leucosphyrus	<i>An. leucosphyrus</i> Donitz (hv) <sup>1</sup>	<i>Pf, Pv, Pm</i> <sup>2</sup>	Human	Indonesia, Sumatra
	<i>An. latens</i> Sallum & Peyton (hv, sv, fv)	<i>Pf, Pv, Pm</i> <i>P. inui</i> [78] <i>P. knowlesi, P. inui, P. coatneyi, P. fieldi</i> [79]	Human <i>M. fascicularis,</i> <i>M. nemestrina</i> <i>P. melalophos</i> [19-22]	Indonesia, East Malaysia, West Malaysia, Thailand Sarawak: East Malaysia: Sarawak[45,75] West Malaysia [56]
	<i>An. introlatus</i> Colless (sv)	<i>P. cynomolgi, P. fieldi</i> [78]	<i>M. fascicularis,</i> <i>M. nemestrina</i> <i>P. melalophos</i> [19-22]	Indonesia West Malaysia, Thailand [56]
	<i>An. balabacensis</i> Colless (hv, sv, fv)	<i>P. knowlesi</i> [45]; possibly <i>P. coatneyi</i> & <i>P. inui</i> [73]	<i>M. fascicularis</i> [73]	Brunei, Indonesia, East Malaysia, Philippines [56]
	<i>An. baisasi</i> Colless	Information inadequate		Luzon, Philippines
Dirus	<i>An. dirus</i> Peyton & Harrison (hv, sv fv)	<i>P. knowlesi</i> [76-77]	Human <i>M. fascicularis,</i> [35]	Cambodia, China, Vietnam, Laos, Thailand
	<i>An. cracens</i> Sallum & Peyton (hv, sv, fv)	Probable vector of human malaria [56] <i>P. knowlesi</i> [30,72] <i>P. cynomolgi, P. inui</i> [80]	<i>M. fascicularis</i> [30]	Indonesia, West Malaysia, Thailand
	<i>An. baimaii</i> Sallum & Peyton (hv, fv)	<i>Pf, Pv Pm</i>	Human	Bangladesh, India, Thailand, Myanmar, China
Hackeri	<i>An. mirans</i> Sallum & Peyton (sv)	<i>P. cynomolgi, P. inui</i> [56] <i>P. inui shortii; P. fragile</i> [56]		India, Sri Lanka
	<i>An. hackeri</i> Edwards (sv)	<i>P. cynomolgi, P. inui, P. fieldi, P. coatneyi, P. knowlesi</i> [52]	<i>M. fascicularis</i>	East and West Malaysia, Philippines, Thailand
	<i>An. pujutensis</i> Colless (sv)?	Probable vector of simian malaria parasites [52]		Indonesia, East and West Malaysia, Thailand

<sup>1</sup> hv,sv and fv indicate human malarial, simian malarial and human lymphatic filarial vectors; sv? Vectorial status awaiting confirmation

**Table 1.** Simian malaria parasites of Southeast Asia: their Leucosphyrus Group natural vectors, hosts and geographical distribution (modified from Sallum et al [56])



**Figure 2.** Known limit of the distribution of the *An. leucosphyrus* Group (*An. hackeri* Subgroup, *An. dirus* complex and *An. leucosphyrus* complex) of mosquitoes in South and Southeast Asia adopted from Sallum et al 2005. Only the distribution of those species mentioned in Table 1 are shown

As a member of the *Leucosphyrus* complex, *An. latens* is widely distributed in Borneo (Kalimantan, Sarawak, Sabah) together with *An. balabacensis* in the forested areas of eastern Borneo (Figure 2). *Anopheles latens* and *An. introlatus* are sympatric with members of the closely related *Dirus* complex in the Malay Peninsula, including southern Thailand [58, 59] (Figure 2).

The *Dirus* complex is well known because its species are widespread in forest and forest foothills throughout the Oriental Region from southwestern India eastwards and from 30° north parallel to the Malaysian peninsula [60-62] (Figure 2), whereas the *Leucosphyrus* complex has been investigated to a much lesser degree in Malaysia Borneo and Kalimantan Borneo. *Anopheles cracens* (*Dirus* complex) was the predominant mosquito species in a recent study and was never reported previously from Pahang, Malaysia [30]. Earlier reports indicate that *An. cracens* was found in Perlis (Northern most state of Peninsular, Malaysia) and in Terengganu (east Coast State of Peninsular Malaysia [56]. Its geographic distribution within peninsular Malaysia is unknown [63].

## 6.2. Larval biology

Table 2 shows a summary of *Anopheles* larval habitat characteristics adapted from Sinka et al [64]. As forest-dwelling species, the immature stages share an affinity for humid, shaded environments where they make use of transient or temporary larval habitats such as pools and pud-



dles. Like other members of the *Leucosphyrus* complex, larval habitats of *An. lateans* and *An. balabacensis* are mostly shaded temporary pools and natural containers of clear or turbid water on the ground in forest areas (Table 2). Larvae of *An. latens* are usually found in clear seepage pools in forest swamps in peninsular Malaysia [65] and in pools beside a forest stream and in swampy patches in hilly areas [66]. Habitats occupied by *An. latens* in Thailand include stump ground holes, sand pools, stream margins, seepage-springs, wheel tracks and elephant foot prints [53, 59]. Typical breeding places of *An. balabacensis* are small pools in clay soil containing fairly clean seepage or rainwater, still or slow moving, and under some shade, with the upper altitudinal limit of 4000 ft in Borneo (1220 meters) [66]. Other adventitious and rare breeding sites include swamp edges or in rock pools, bamboo stumps, split bamboos, tins and other artificial containers [66] and wells in Sandakan, Sabah (unpublished report by Dr David Muir, WHO consultant). In inland forest *An. hackeri* was found breeding in split bamboo while in the coastal area it was found breeding in the cavities of leaf bases of nipah palm [8].

Species	Light intensity	Turbidity	Water movement	Small natural water collections	Small man-made water collections
<i>An. latens</i> & <i>leucosphyrus</i>	Heliophobic	Clear, turbid, fresh water Muddy pool (W Malaysia [56])	Still or stagnant	Small streams, seepage streams, pools	Wheel ruts, hoof prints
<i>An. balabacensis</i>	Typical heliophobic	Fresh water	Still or stagnant	Pools; dips in the ground	Wheel ruts, hoof prints
<i>An. dirus</i>	Heliophobic	Clear, turbid, fresh water	Still or stagnant	Small streams, pools, wells, dips in the ground	Borrow pits, wheel ruts, hoof prints
<i>An. hackeri</i>	Heliophobic	Clean non saline water, but found in water containing up to 4% sea-water	Still or stagnant	In split bamboo and cavities at the leaf base of nipa palm	In Thailand, in elephant footprints [56]

**Table 2.** Larval habitat characteristics of monkey malaria vectors (adapted from Sinka et al [64]) including individual studies reported in the literature.

### 6.3. Biological characteristics

The important biological characteristics of the known vectors of simian malaria are shown in Table 3 which has been modified from Meek [67]. Of the known vectors, *An. hackeri* is known to bite only monkeys and rarely comes to bite humans [8]. Although *An. latens* is a vector of human malaria in East Malaysia [68-70], the current studies have shown that the species is more attracted to monkeys compared to humans [71], whilst *An. cracens* is attracted to both monkeys and humans [72]. In Palawan Island, Philippines, *An. balabacensis* was more attracted

to a monkey bait trap compared to carabao (water buffalo) and human bait traps [73, 74]. It was also found positive for oocysts and sporozoites but could not be confirmed if it was of monkey origin [73]. However, infection studies carried out by the same authors proved that *An. balabacensis* was the vector of simian malaria in Palawan [73]. So far only the *An. Leucosphyrus* Group (*An. latens*, *An. cracens*, *An. balabacensis*, *An. hackeri* and *An. dirus*) of mosquitoes have been found positive for simian malaria parasites in nature [30, 45, 71, 72, 75-80]. However, *An. dirus* is also a main vector of both human malaria, with sporozoite rates as high as 14% in Myanmar and as low as 2.5% in Lao PDR [61, 81], and *Wuchereria bancrofti* [82].

Species	Peak biting time	Host preference or MBT:HBT	Survivorship	Sporozoite rate/EIR
<i>An. latens</i>	Sarawak: Around midnight in forested areas and soon after dusk in village settlements [68] Forest: 1900-2000 h; farm: 0100-0200 h [69] Monkey biting rate at 6, 3 m above ground and at ground: 6.8:3.2:1.0. HBR highest at forest fringe (6.74%), within the forest (1.85%) and at long house (0.28%) [71]	Similar host preference 1.0 : 1.3 [71]	Sarawak: parous rate 65.8% (farm), 53.7% (forest), 65.8% (longhouse) [71] VC: 2.86 (farm), 0.60 (forest), 0.85 (longhouse) [71]	Sarawak: 1.18% (pooled from forest fringe, forest & longhouse), 0.7% (farm), 1.4% (forest), all confirmed Pk by PCR; EIR 11.98 (farm), 14.1 (forest) [71]
<i>An. balabacensis</i>	Palawan: In and out; 20.00-03.00 h [73] Sabah: 22:00-02:00 h [85- 86];after midnight [87] Out (76%): 19:00-20:00 h, in(24%): 22:00-23:00 h;[83] Lombok: 19:00-21:00h) [84]	Attracted to humans, monkey & water buffalo; more frequently caught in monkey traps [74]	Sabah: highest in Nov, lowest in July [83]	Kalimantan: 1.3% [88] Palawan: 12.5% sporozoite rate; 29% attracted to monkeys were positive for oocysts [74]
<i>An. dirus</i>	Late or early biting, usually around 22:00 h [60-62]	Highly anthropophilic,	Higher parous rate (76%) & life expectancy during dry season	Human sporozoite rates vary with season and location: from

Species	Peak biting time	Host preference or MBT:HBT	Survivorship	Sporozoite rate/EIR
	9 of 13 <i>Plasmodium</i> positive bites occur before 21:00 h (Vietnam) [91]	exophagic as well as endophagic and exophilic [61-62]	compared to wet season (62.4%) in Lao [81]	7.8% in Assam (India) to 14% in Myanmar [61] and 2.5% in Laos [81] 43% of 72 salivary glands were PCR-positive for Pk CSP and Pk 18s rRNA. Mixed infections of Pk with Pv and Pf were common in Vietnam [77]
<i>An. cracens</i>	Thailand: 1900-2100 h [60] West Malaysia: 2000-2100 h; 74% biting before 2100 h; predominantly exophagic (1.11 bites/man-night) in both forest (1.24 bites/man-night) and fruit orchard (4.15 bites/man-night); 60% biting at ground level to 3 m high before 00:00 h; more biting at canopy level (6 m) compared to earlier collections at the same level [72].	West Malaysia: 1: 2.6 [7.2]	West Malaysia: parous rate 65.7% (fruit orchard), 71.5% (forest) VC: 2.46 (fruit orchard), 1.09 (forest) [72]	West Malaysia: 0.60% (fruit orchard), 2.9% (forest) EIR: 0.08 [72]
<i>An. hackeri</i>	Not known since most bite monkeys and rarely found in human bait traps [78]	Most attracted to monkeys at canopy level in mangrove forest does not come to bite humans [78]	No data available	In coastal area of Rantau Panjang West Malaysia 0.7% [78]

<sup>1</sup>VC - vectorial capacity; EIR - entomological inoculation rate; PCR - polymerase chain reaction; HBR - human biting rate; MBT- Monkey bait trap; HBT- human bait trap; CSP - circumsporozoite

**Table 3.** Biological variations among adults of simian malaria vectors in Southeast Asia (modified from Meek 1995 [67]).

The peak biting times of *An. balabacensis* vary from place to place as shown in Table 3. It seems to bite as early as 19:00 h in recent years compared to being late night biters in the previous decades [83-89]. *An. dirus* s.s. tends to bite between 20:00 and 23:00 h [53, 56, 60] and there is significant biological variability within the Dirus complex, depending on the local circumstances [90]. In Vietnam, sporozoite positive bites from *An. dirus* occur before 21:00 h [91] and co-infections of *P. knowlesi*, *P. falciparum* and *P. vivax* [76, 77] in mosquitoes are indicative of simultaneous transmission. *Plasmodium knowlesi*-sporozoite infective *An. latens* and *An. cracens* were detected from human landing and monkey bait collections in Sarawak and Pahang, Malaysia, respectively [71, 72] suggesting that *P. knowlesi* is being transmitted to both humans and macaques by these two vector species. Generally the parous rates of the Leucosphyrus Group of mosquitoes were relatively high as shown in Table 3. Overall parous rate of *An. latens* was 59% and those caught in the forest was significantly lower than those caught at the farm or long house (where native people of Sarawak live) [71], while for *An. cracens* the parous rate in the forest was higher than in the farm (Table 3), and on average was above 60% [72]. Heterogeneity in biting rates and parous rates indicates that the vectorial capacities are relatively higher in farms or orchards compared to forests (Table 3), and has significant implications for vector control. Understanding the importance of natural heterogeneity in *P. knowlesi* transmission is necessary to elucidate the key variation undermining existing control efforts and to target the vector species for focused interventions [92].

#### 6.4. Laboratory susceptibility studies

In laboratory experiments with *P. knowlesi*, *An. balabacensis* was found to be a successful vector [93]. However, *An. maculatus* only developed few oocysts and sporozoite infection in salivary glands was of low intensity. Laboratory feeding experiments, *An. maculatus* was susceptible to *P. inui* and was able to transmit the parasite to the non-human primate host after a prepatent period of 11 days [94]. In a series of experiments infectivity conducted in the Institute for Medical Research, with the Gombak strain of *P. cynomolgi*, the following mosquitoes were found with salivary gland infections: *An. maculatus*, *An. kochi*, *An. sundaicus* (= *An. epiroticus*), *An. vagus* and *An. introlatus* [16]. However, in field situation it was observed that *An. maculatus* was not attracted to macaques, with only three female mosquitoes entering the monkey bait trap [72]. While *An. kochi* was the second predominant mosquito entering monkey baited trap, none were positive for oocyst or sporozoites [72]. Thus, although species other than the Leucosphyrus Group were able to develop the simian malaria parasites to sporozoites, none were incriminated in nature except the Leucosphyrus Group.

#### 6.5. Trapping techniques

Various trapping methods were tested for the collection of *Anopheles* mosquitoes attracted to non-human primates. Earlier observations indicated that these mosquitoes prefer to feed well above ground level and especially about 6-8 m above ground level. Thus, platforms were built among foliage in the forest or plantations to house the non-human primates for mosquito collections. The following traps that were tested [95] are described hereunder.

## Net Traps

This is similar to the human-bait-net trap introduced by Gater [96]. This method provided the best results when tested [95]. The platforms were constructed among the branches of trees to a height of 6 meters. Special metal cages measuring 90 cm x 90 cm x 90 cm and covered by wire mesh were used to house the monkeys on the platform measuring 300 cm X 200 cm. The meshed cages provided a physical barrier to prevent the monkeys from grabbing the collectors and also to prevent the entry of snakes. It is ideal to have two monkeys sharing a cage to increase vector attraction. A mosquito net measuring 190 cm x 180 cm x 150 cm with an opening of about 40 cm lifted on either ends was used to cover the cages with monkeys on each platform. The traps were operated from 18:00 to 06:00 hours and were searched at regular intervals [71, 72]. A collector, upon entering the net, closed the openings and collected all resting mosquitoes with the use of aspirators. Mosquitoes in the aspirator were then transferred to paper cups and were brought to the laboratory for identification and dissection. Platforms were built at various heights, ground level, 3 and 6 meters above ground. Figure 3 shows two different platforms in operation.



**Figure 3.** Monkey Baited Net Traps at different levels on platform.

The other traps used were Shannon net trap, drum funnel-trap, Lumsden suction trap and light traps. Detailed descriptions can be found in Wharton [95]. Of all the traps tested, it was found that the monkey-baited traps were superior compared to other types of traps. Although it is a difficult task to collect mosquitoes from the platforms at regular intervals, it is no doubt important to study the behaviour of the mosquitoes. Studies by Wharton [95] demonstrated that 83% of the *An. hackeri* were collected in catches made before midnight, compared to only 62% and 65.8% of *An. latens* and *An. cracens* caught before midnight respectively [71, 72]. Thus,

it seems that all night collection is still important despite logistical difficulties, costliness, tediousness and human fatigue.

## 7. Implications for control

Currently insecticide treated bednets (ITN) and indoor residual spraying (IRS) are the two most important tools for the control of malaria vectors. Scaling up ITN, IRS, artemisinin-based combination therapies and intermittent preventive treatment for infants and pregnant women have contributed to the reported reductions in malaria on a global scale [97]. As part of the Global Malaria Action Plan, the RBM Partnership and World Health Organization has recommended “malaria eradication worldwide by reducing the global incidence to zero through progressive malaria elimination in countries” [98]. However, if human malaria could be eliminated, forests in Southeast Asia provide favourable environments for zoonotic transmission of *P. knowlesi* thus, thwarting efforts to eliminate malaria.

The vectors of *P. knowlesi* malaria have been incriminated only from certain districts or locations in Malaysia [71, 72, 75]. Given that the vectors of monkey malaria show anthroponophilic, exophilic and exophagic tendencies, it is obvious that the existing front-line vector control tools (IRS, ITN) will not be sufficient to reduce vector density and break the transmission cycle of *P. knowlesi* in the most intensively endemic parts of Southeast Asia. Innovative interventions are needed to control simio-anthropophilic and acrodendrophilic vectors that do not rest and feed indoors. There are two major problems that need to be addressed before considering malaria elimination. It is known that *P. knowlesi* can be life threatening [99] and mortality due to it is increasing [31, 100]. Thus it is important to determine the vectors throughout the country; study the behavior and ecology of the species of mosquitoes and apply the most effective strategy(ies) for control of these vector. To achieve these outcomes, several key areas for strategic investment relevant for malaria elimination have been proposed [101]. Second, there will always be a problem of human population movement (HPM) and thus people moving into the jungle may introduce the parasite which could give rise to new infections if suitable vectors are present and readily establish local transmission. HPM is common among migrants in the Greater Mekong Subregion [102] and in Southeast Asia [103].

In Vietnam, forest malaria caused by *An. dirus* was controlled because workers going into the forest used long lasting insecticide hammocks (LLIH) [104]. The use of LLIH can be encouraged in ecotourism areas where people stay overnight in the community managed guest houses or camps in the forest. However, other types of personal protection methods need to be evaluated for forest workers. A study has demonstrated that military personnel who used permethrin treated uniforms were protected against mosquito bites, thereby reducing malaria transmission [105].

The use of repellents as personal protection measures have been advocated for malaria control. However, this needs to be evaluated in forest settings and large scale implementation will be a public health challenge. Among US Military troops, malaria cases have been reported due to non-compliance of personal protective measures and failure of chemoprophylaxis [106].

Currently in Malaysia people are getting infected when they visit plantations or forests for work or recreational activities as some important vectors do not enter houses [72].

## 8. Challenges

There is no reason to doubt the possibility and biological capacity of other simian malaria species to infect humans [13, 107]. *An. latens* can develop all the five species of simian malaria [79] and has a biting preference for both humans and macaques, the possibility of humans being infected with *P. cynomologi* or *P. inui* needs to be addressed. As stated by Baird [108], in areas where macaques and vectors are in close proximity to humans and when malaria occurs other species should also be considered and not just the human malarias and *P. knowlesi*.

Currently only three species of mosquitoes have been incriminated as simian malaria vectors in Malaysia (*An. balabacensis*, *An. cracens* and *An. latens*) [45, 71, 72, 75] and one in Vietnam (*An. dirus*) [76, 77]. However, it is beyond doubt that there would be several more species involved that would feed on both humans and monkeys and establish natural transmission. Before the inception of the malaria eradication program there were many more *Anopheles* species that were vectors [109], but some species were successfully brought down to very low levels due to their endophilic/endophagic behaviours and susceptibility to residual insecticides. Thus the aggressive national control programme has resulted controlling in only three to four important vectors occurring in Malaysia (*An. balabacensis*, *An. flavirostris*, *An. latens*, *An. maculatus*), [110-113].

In Thailand, the main vectors for human malaria are *An. dirus*, *An. minimus* and *An. maculatus*, mosquitoes [114]. Although *An. dirus* mosquitoes which belong to the Leucosphyrus Group and have been identified as potential vectors for *P. knowlesi* in Vietnam [76, 77], its distribution and abundance have significantly decreased in all major malaria-endemic areas of Thailand during the past decade [34]. Human cases of *P. knowlesi* have been reported from Thailand at a low prevalence (0.57% in 2006-2007), however the vector remains unknown [34].

According to Obsomer et al [61] the mean temperature below 20° C seems to limit the northern distribution of the Dirus complex to just beyond the border of India with Nepal and Bhutan. Rainfall is probably the limiting factor to the west with annual rainfall per year under 800 mm. Thus the lack of information on the distribution and occurrence of *P. knowlesi* cases in large non-forested areas of Thailand, southern Vietnam and central India is probably linked with the lack of suitable habitats [61]. The absence of the complex (besides the newly described species aff. *takasagoensis*) in north of Vietnam is puzzling as this area is still forested and members of the complex occur at the same latitude in neighbouring countries. Laos PDR is the only country in the Greater Mekong Subregion that has not reported the occurrence of *P. knowlesi* malaria. This may be due to the fact that so far investigations have not been carried out for *P. knowlesi*.

Thus it is timely to determine all the vectors of simian malaria throughout the Southeast Asian region. Although old records stating the distribution of the various *Anopheles* species are

available, it may not depict the current situation since landscape ecology and vegetation cover have significantly changed over time. The distribution of vectors, in relation to forest areas and human settlements using modern technology such as the GPS, GIS and the behavioral ecology of the vectors, needs to be addressed. These and other key areas identified for specific strategic investment in ecological research [101] should assist to define the target product profiles of completely new control technologies and delivery systems.

## 9. Conclusion

Since many malaria control programmes in Southeast Asia are moving towards elimination of malaria [115], it is important to determine the prevalence of knowlesi malaria in these countries. In the Greater Mekong Subregion including Bangladesh and India *An. dirus* is one of the primary vector of human malaria and thus it is important to determine if other vectors are involved in knowlesi transmission. Among habitats shared by macaques and vector mosquitoes, it is possible for humans who encroach these areas to be infected. Thus, important issues that need to be determined are as follows: Are other simian malaria parasites affecting humans? Is human to human transmission occurring? What are the other vectors transmitting simian malaria to humans (apart from *An. cracens*, *An. latens*, *An. dirus* and *An. balabacensis*) in the region and what roles do they play in host switching? What innovative technologies or biting prevention are appropriate for the control of these vectors? Thus, knowlesi malaria remains a great challenge for the future.

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