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Chapter 15
Simian Malaria Parasites: Special Emphasis on Plasmodium knowlesi and Their Anopheles Vectors in Southeast Asia

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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 \textit{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 \textit{Plasmodium} affecting non-human primates [11]. Of
these \textit{Plasmodium coatneyi}, \textit{P. cynomolgi}, \textit{P. fieldi}, \textit{P. fragile}, \textit{P. inui}, \textit{P. knowlesi} and
\textit{P. simiovale} are known to occur in macaques and leaf monkeys [12]. However, of the seven species,
\textit{P. fragile} has been reported in both India and Sri Lanka while \textit{P. simiovale} is restricted only to Sri
Lanka [12]. \textit{Plasmodium eylesi}, \textit{P. jefferyi}, \textit{P. youngi} and \textit{P. hylobati} are found in gibbons while
\textit{P. pitheci} and \textit{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 \textit{P. cynomolgi} is a species that
had been experimentally transmitted to humans [3, 15]. \textit{Plasmodium cynomolgi} in monkeys has
many of the characteristics seen during infection of humans with \textit{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 \textit{Macaca fascicularis}, \textit{Macaca nemestrina} and \textit{Presbytis melalophos} [19, 20]. In the 1960's
studies on malaria parasites of \textit{M. nemestrina} revealed that this non-human primate can
harbour the following simian malaria species: \textit{P. cynomolgi}, \textit{P. inui}, \textit{P. knowlesi} and
\textit{P. fieldi} [19] Of these \textit{P. fieldi} was a new species found in this macaque [17]. Currently,
\textit{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 \textit{P.fieldi} mono-infection in a study
in Sarawak, Malaysian Borneo [14]. In Malaysian Borneo the predominant species found in
the longtailed macaques was \textit{P. inui} (82%) followed by \textit{P. knowlesi} (78%), \textit{P. coatneyi} (66%)
and \textit{P. cynomolgi} (56%) [14]. However, in Singapore \textit{P. knowlesi} was the predominant species among
long-tailed macaques (68.2%), followed by \textit{P. cynomolgi} (66.6%), \textit{P. fieldi} (16.7 %),
\textit{P. coatneyi} (3%) and \textit{P. inui} (1.5%) [21]. In Selangor, out of the 107 samples of macaque blood tested for
malaria, 64.5% were positive for \textit{Plasmodium} of which 23.3 % were positive for \textit{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].

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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.
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.

<table>
<thead>
<tr>
<th>Complex Vector species</th>
<th>Species of Plasmodium</th>
<th>Vertebrate hosts</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucosphyrus</td>
<td>An. leucosphyrus Donitz (hv)</td>
<td>Pf, Pv, Pm</td>
<td>Human Indonesia, Sumatra</td>
</tr>
<tr>
<td>An. latens</td>
<td>Sallum &amp; Peyton (hv, sv, fv)</td>
<td>Pf, Pv, Pm</td>
<td>P. inui [78]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. knowlesi, P. inui, P. coatneyi, P. fieldi [79]</td>
</tr>
<tr>
<td>An. introlatus</td>
<td>Colless (sv)</td>
<td>P. cynomolgi, P. fieldi [78]</td>
<td>M. fascicularis [352], M. nemestrina [333]</td>
</tr>
<tr>
<td>An. balabacensis</td>
<td>Colless Information in inadequate</td>
<td>P. knowlesi [45]; possibly P. coatneyi &amp; P. inui [73]</td>
<td>Brunei, Indonesia, East Malaysia, Philippines [56]</td>
</tr>
<tr>
<td>An. baisasi</td>
<td></td>
<td></td>
<td>Luzon, Philippines</td>
</tr>
<tr>
<td>An. dirus</td>
<td>Peyton &amp; Harrison (hv, sv, fv)</td>
<td>P. knowlesi [76-77]</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M. fascicularis [35], Cambodia, China, Vietnam, Laos, Thailand</td>
</tr>
<tr>
<td>An. baimaii</td>
<td>Sallum &amp; Peyton (hv, fv)</td>
<td>Pf, Pv, Pm</td>
<td>Human Bangladesh, India, Thailand, Myanmar, China</td>
</tr>
<tr>
<td>An. mirans</td>
<td>Sallum &amp; Peyton (sv)</td>
<td>P. cynomolgi, P. inui</td>
<td>India, Sri Lanka</td>
</tr>
<tr>
<td>An. hackeri</td>
<td>Edwards (sv)</td>
<td>P. cynomolgi, P. inui, P. fieldi, P. coatneyi, P. knowlesi [52]</td>
<td>East and West Malaysia, Philippines, Thailand</td>
</tr>
<tr>
<td>An. pujutensis</td>
<td>Colless (sv)?</td>
<td>Probable vector of simian malaria parasites [52]</td>
<td>Indonesia, East and West Malaysia, Thailand</td>
</tr>
</tbody>
</table>

1 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 puddles.
Like other members of the Leucosphyrus complex, larval habitats of *An. latens* 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].

<table>
<thead>
<tr>
<th>Species</th>
<th>Light intensity</th>
<th>Turbidity</th>
<th>Water movement</th>
<th>Natural water collections</th>
<th>Artificial water collections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. latens</em></td>
<td>Heliophobic</td>
<td>Clear, turbid, fresh water</td>
<td>Still or stagnant</td>
<td>Small pools, seepage streams, pools</td>
<td>Wheel ruts, hoof prints</td>
</tr>
<tr>
<td><em>An. balabacensis</em></td>
<td>Typical heliophobic</td>
<td>Fresh water</td>
<td>Still or stagnant</td>
<td>Pools, dips</td>
<td>Wheel ruts, hoof prints</td>
</tr>
<tr>
<td><em>An. dirus</em></td>
<td>Heliophobic</td>
<td>Clear, turbid, fresh water</td>
<td>Still or stagnant</td>
<td>Small streams, pools, wells, dips</td>
<td>Borrow pits, wheel ruts, hoof prints</td>
</tr>
<tr>
<td><em>An. hackeri</em></td>
<td>Heliophobic</td>
<td>Clean non saline water, but found in water containing up to 4% sea-water</td>
<td>Still or stagnant</td>
<td>In split bamboo and cavities at the leaf base of nipa palm</td>
<td>In Thailand, in elephant footprints [56]</td>
</tr>
</tbody>
</table>

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 [72, 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. Leucos‐ 

Species Peak biting time Host preference or MBT:HBT Survivorship Sporozoite rate/EIR

An. latens 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] An. balabacensis 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] An. dirus 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 Simian Malaria Parasites: Special Emphasis on Plasmodium knowlesi and Their Anopheles Vectors in Southeast Asia http://dx.doi.org/10.5772/54491 495 Sensitive Document Notice This document can only be viewed within: - Adobe Acrobat - Secure web viewer here
Plasmodium positive bites occur before 21:00 h (Vietnam) [91]. 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].

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].

An. hackeri. 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].

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 protein.
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-spore 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 where 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 [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.
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.

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 \textit{P. knowlesi} thus, thwarting efforts to eliminate malaria.

The vectors of \textit{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 anthropophilic, exophagic and exophilic 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 \textit{P. knowlesi} in the most intensively endemic parts of Southeast Asia. Innovative interventions are needed to control simio-anthropophagic 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 \textit{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 \textit{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].

Simian Malaria Parasites: Special Emphasis on \textit{Plasmodium knowlesi} and Their \textit{Anopheles} Vectors in Southeast Asia

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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 Anopheles mosquitoes - New insights into malaria vectors500 Sensitive Document Notice

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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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