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Review Paper

Plasmodium knowlesi in humans: a review on the role of its vectors in Malaysia

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Abstract. *Plasmodium knowlesi* in humans is life threatening, is on the increase and has been reported from most states in Malaysia. *Anopheles latens* and *Anopheles cracens* have been incriminated as vectors. Malaria is now a zoonoses and is occurring in malaria free areas of Malaysia. It is also a threat to eco-tourism. The importance of the vectors and possible control measures is reviewed here.

INTRODUCTION

In 1901 Daniels discovered malaria parasites in the long-tailed macaque (also known as the crab-eating macaque) (Daniels, 1908). In 1931 Knowles and Das Gupta discovered a new parasite in a long-tailed monkey that was sent to Calcutta from Singapore. Although they knew that it was a new parasite it was only named as *Plasmodium knowlesi* by Stinton & Mulligan (1932). Studies carried out by Knowles and Das Gupta showed that humans were infected when inoculated with the blood from the infectious monkey (Knowles & Das Gupta, 1932). This parasite was then used as a therapeutic agent against neurosyphilis (Ciuca *et al.*, 1937). However, simian malaria gained prominence only when in May 1960, Don Eyles, a scientist was infected with *Plasmodium cynomolgi* by mosquito bites in the laboratory (Coatney,1968). They then realized that simian malaria could be a zoonoses.

This triggered the scientists from the National Institutes of Heatlh of USA to carry out studies in peninsular Malaysia on humans, non human primates and their vectors as the infected monkeys had come from peninsular Malaysia (Sandosham, 1967). They carried out studies along with their colleagues from the Institute for Medical Research. The main objectives of the investigations were to determine (1) the distribution, prevalence and species of malaria parasites in monkeys and apes, (2) the vectors of monkey malaria in nature and also to determine if they attacked humans, (3) the susceptibility of Malayan mosquitoes to monkey malaria, and (4) whether monkey malaria infection was transmissible to humans in Malaysia (Sandosham, 1967).

In 1965, the first case of *P. knowlesi* was reported from Pahang, peninsular Malaysia (Chin *et al.*,1965) and a second suspected case from Johore, (Fong *et al.*, 1971). After the report of the first case, further studies were carried out to determine the vectors and to study the parasites in humans and non human primates. No human cases of *P. knowlesi* were detected during those studies although 1100 blood samples from humans were inoculated into rhesus monkeys (Warren *et al.*, 1970). However, vectors of simian malaria were determined and also many new simian malaria parasites were described (Wharton & Eyles, 1961; Eyles *et al*., 1962a, 1962b, 1962c; Wharton *et al*., 1962; Eyles, 1963). Thus, in the late 1960's, scientists were of the opinion that simian malaria would not easily cross the animal host barrier to infect humans.

After four decades a large focus of *P. knowlesi* infection in Kapit, Sarawak Malaysian Borneo was reported using molecular tools (Singh *et al*., 2004). This stimulated renewed interest in the scientific community of *P. knowlesi* occurring in humans. Since then many other cases of *P. knowlesi* malaria have been reported from many countries in Southeast Asia (Jongwutiwes *et al*., 2004; Zhu *et al*., 2006; Cox Singh *et al*., 2008; Ng *et al*., 2008; Luchavez *et al*., 2008; Vythilingam *et al*., 2008; Van den Eede *et al*., 2009). Recently there was another report of *knowlesi* malaria from Kalimantan – Indonesia Borneo (Berens-Riha, 2009).

Although *P. knowlesi* has now been extensively studied in these countries very little work has been carried out on the vectors. This review attempts to collate all the information on field studies that have been carried out on the vectors of simian malaria. It also seeks to define the role played by the various vectors and where possible reasons for changes in their habitats.

Early studies on vectors of simian malaria

Prior to 1960's, no natural vectors were known for any species of simian malaria although decades ago simian malaria had been detected in non human primates. Most of the studies were carried out in peninsular Malaysia in the 1960's and the interest was first in finding the vector for *P. cynomolgi* since this was the parasite that was first transmitted to humans in the laboratory accidentally by mosquitoes.

The studies were carried out mainly in the State of Selangor and Pahang, in peninsular Malaysia. (Wharton *et al*., 1964). In Selangor the studies were carried out in the mangrove swamp forest (Rantau Panjang, Delik and Sungai Burong), lowland swamp forests (Bukit Mandul and Pacific Tin) and inland hill forest of Ulu Lui and Ulu Gombak. In Pahang, work was carried out in lowland swamp forest of Tanah Puteh in Pekan district.

In the coastal mangrove area of Rantau Panjang, *Anopheles hackeri* which belongs to the *leucosphyrus* group was the predominant species. Mosquitoes obtained were more in monkey bait traps (MBT) than human bait traps (HBT). In Delik *Anopheles sundaicus* (= *Anopheles epiroticus Linton* and Harbach, 2005) was the predominant species and was found more in human bait than in monkey bait traps. While in Sg Burong the predominant species were *Anopheles campestris*, *Anopheles sinensis*, and *An. epiroticus* and found more in HBT than MBT. The only species that was found positive for sporozoites was *An. hackeri*. The sporozoites were inoculated into rhesus monkeys and it was found that *An. hackeri* was able to transmit five species of monkey malaria namely *P. knowlesi*, *P. cynomolgi*, *Plasmodium inui*, *Plasmodium coatneyi* and *Plasmodium fieldi*. (Warren & Wharton, 1963). *Anopheles hackeri* is found mainly in peninsular Malaysia. In Thailand it is mainly found in the southern peninsular region (Rattanarithikul *et al*., 2006).

In the Lowland swamp forest (Bukit Mandul and Pacific Tin) the predominant species attracted to human and monkeys was *Anopheles letifer*. However, *Anopheles pujutensis* was collected from monkey bait traps in very small numbers but none were infected with sporozoites. The only species positive were *An. letifer*, *Anopheles donaldi*, *Anopheles roperi* and *Anopheles. umbrosus*. The sporozoites from these mosquito species were inoculated into rhesus monkeys, long tailed macaques and different birds but the parasites failed to develop. They suspected it to be mouse deer malaria- *Plasmodium traugli* (Wharton *et al*., 1964). At Tanah Puteh very few *Anopheles* were caught by HBT and MBT. *Anopheles nitidus* (=*indiensis*) was the predominant (5) species obtained in MBT on canopy. More *Mansonia* mosquitoes were obtained in this area (Wharton *et al*., 1964).

In the forested regions - inland hills of Ulu Lui *Anopheles maculatus* was the predominant species found in human and monkey bait traps. *Anopheles maculatus* is the predominant vector of human malaria. Only four *Anopheles leucosphyrus* (= *Anopheles latens* Sallum and Peyton, 2005) was found in the monkey baited trap. However, in the forested area of Ulu Gombak, *An. leucosphyrus* (*An. latens)* was the predominant species both in the human baited and monkey baited traps. This was closely followed by *Anopheles balabacensis introlatus* (=*Anopheles introlatus* Sallum *et al.*, 2005). These species of mosquitoes preferred to bite monkeys at canopy compared to ground level (Wharton *et al.*, 1964).

Although *An. maculatus* was found positive for sporozoites it did not cause infection in rhesus monkeys, unlike *An. latens* which caused *P. inui* infection (Wharton *et al.*, 1962) and *An. introlatus* which caused *P. cynomolgi* (Eyles *et al.*, 1963) and *P. fieldi* infection in rhesus monkeys (Wharton *et al.*, 1964). In the monsoon rain forest of northern Perlis which is at the northern tip of peninsular Malaysia the early workers were successful in incriminating *Anopheles balabacensis balabacensis* (= *An. cracens* Sallum and Peyton, 2005) as vector for both *P. inui* and *P. cynomolgi* (Cheong *et al.*, 1965).

After the first human case of *P. knowlesi* was reported, studies were conducted in the village and forest of Bukit Kertau area where the surveyor was suspected to have been infected with *P. knowlesi* (Warren *et al.*, 1970). *Anopheles introlatus* and *An. latens* were obtained only from the forest and not from the village. In the forest it was also found that both the *leucosphyrus* group of mosquitoes were attracted more to human bare leg catches compared to monkey and human baited traps (Warren *et al.*, 1970). One mosquito from each of the species was positive for sporozoites but non produced infection when inoculated into rhesus monkeys. Table 1 shows the studies and locations where vectors of simian malaria in Malaysia were conducted.

Vectors of *Plasmodium knowlesi* in Kapit District, Sarawak

The first report of *Plasmodium knowlesi* in Sarawak, Malaysian Borneo was reported in 2004 (Singh *et al.*, 2004). In 2005 vector studies were carried out in three different ecological sites – forest, farm and village in Kapit District. It was established that *An. latens* was the vector of *P. knowlesi* (Vythilingam *et al.*, 2006). *Anopheles latens* was attracted to both humans and macaques. In the forest and farm *An. latens* comes to bite humans as early as 18:00 hours but the peak biting time is between 19:00 and 20:00 hours in the forest while in the farm it is between 01.00 to 02:00 hours (Tan *et al.*, 2008).

Besides *An. latens* the other species belonging to the *leucosphyrus* group that were obtained were *Anopheles macarthuri* and *An. pujutensis*. Both these species were obtained from monkey baited traps in the forest but none were positive for oocyst or sporozoites. *Anopheles latens* was the only vector in our study site in Kapit. The role of *An. pujutensis* seems to be elusive. Small numbers were found in our study and perhaps in the deep jungle it may be playing the role of keeping the parasite circulating among the long tailed macaques. Only few *An. pujutensis* may have followed the macaques to the forest fringes.

With the current molecular technology, it was easier to detect the species of sporozoites present. All mosquitoes were dissected and the sporozoites were subjected to DNA extraction and PCR followed by sequencing (Tan *et al.*, 2008). *An. latens* was also found to be a vector of *P. coatneyi*, *P. inui*, *P. fieldi* and *P. cynomolgi* in Kapit Sarawak (Tan, 2008 MSc thesis UNIMAS).

Plasmodium knowlesi has now been detected from various parts of Sarawak and the infection is life threatening (Cox Singh *et al.*, 2008), however, the vector/s of the parasite in other parts of Sarawak remains unknown. It is also interesting to note that 70% of the malaria cases reported from humans admitted to the hospital in Kapit, Sarawak was *P. knowlesi* (Daneshvar *et al.*,

Table 1: Studies on vectors of simian malaria in Malaysia

Terrain	Locality	Geographical coordinates	Vector Species	Species of *Plasmodium*	Author & Year
Coastal Mangrove Forest	Rantau Panjang, Selangor	3º 4' 0" N, 101º 25' 0" E	*An. hackeri*	*P. knowlesi*, *P. cynomolgi*	Wharton & Eyles, 1961
Lowland swamp forest	Pacific Tin, Selangor	3º 22' 47" N, 101º 42"E	*An. pujutensis*	Nil	Cheong *et al.*, 1965
	Jabor Valley	3º 9' 5" N, 103º 31' 7" E	*An. lecuosphyrus (=latens)*	Nil	Cheong *et al.*, 1965
Inland Hill Forest	Ulu Lui, Selangor	3º 11' 5" N, 101º 67" E	*An. leucosphyrus (=latens)*	*P. inui*	Wharton *et al.*, 1964
	Ulu Gombak, Selangor	3º 18" N, 101º 47" E	*An. balabacensis introlatus (=introlatus)*	*P. cynomolgi*, *P. fieldi*	Wharton *et al.*, 1964
	Ulu Bendol, Negri Sembilan	2º 7' 3' 3" N, 102º 1' 3" E	*An. leucosphyrus (=latens)*	Nil	Wharton *et al.*, 1964
Monsoon Rain Forest	20th Mile Padang Besar Rd, Perlis	6º 6' 7" N, 100º 31' 7" E	*An. b. balabacensis (=An. balabacensis)*	*P. cynomolgi*, *P. inui*	Cheong *et al.*, 1965
Island Type	Pulau Aur	02º 35' N, 104º 10' E	*An. hackeri*	Nil	Cheong *et al.*, 1965
Deep forest	Bukit Kertau, Pahang	3º .4' 5" N, 102º 6' 7" E	*An. balabacensis introlatus (=introlatus)*	Gland & gut infection (could be of primate origin)	Warren *et al.*, 1970
			An. leucosphyrus (=latens)		
			An. pujutensis	Nil	
Oil palm plantation	Penjom, Pulau Kuching, Kuala Lipis	4º .13' 33' N, 102º 16' 7" E	*An. cracens*	Nil	Vythilingam unpublished document
Village	Kg. Serunai Mela, Kuala Lipis	4º .7' 0" N, 102º 11' 9" E	*An. cracens*	*P. knowlesi*	Vythilingam *et al.*, 2008
	Sg. Ular, Kuala Lipis	4º 15' 6" N, 102º 4' 9" E	*An. cracens*	Nil	Vythilingam unpublished document
	Sg. Damak, Kuala Lipis	4º 16" N, 102º 2' 7" E	*An. cracens*	Nil	Vythilingam unpublished document
Fruit orchard	Kebun Sg Ular	4º 15' 7" N, 102º 4' 8" E	*An. carcens*	*P. knowlesi*	Vythilingam *et al.*, 2008
Secondary jungle	Kuala Lipis – secondary jungle behind Sek. Meng. Teknik	4º 18' 3" N, 102º 12' E	*An. cracens*	Nil	Vythilingam unpublished document

Village	Kg. Dura, Terengganu	5º 4' N, 102º 56' E	*An. cracens*	Nil	In Sallum *et al.* (2007)
	Kg. Tapah, Terengganu	5º 6' N, 102º 55' E	*An. cracens*	Nil	In Sallum *et al.* (2007)
	Kg Jenagor	5º 20' N, 103º 8" E	*An. cracens*	Nil	1999 Unpublished report
	Kg Basong	5º 5' N, 103º 1' E	*An. carcens*	Nil	2003 Unpublished report
Farm	Sg Sut, Kapit, Sarawak	1º 54' N, 112º 51 E	*An. latens*	*P. knowlesi*	Unpublished report
Forest	Forest 4.5 Km East of Kapit Town Centre, Sarawak	2º 0' N, 112º 55' E	*An. latens*	*P. knowlesi*, *P. inui*, *P. coatneyi*, *P. fieldi*	Tan 2008
Farm	Ulu Sg Yong, Kapit, Sarawak	02º 15' N, 112º 58' E	*An. latens*	*P. knowlesi*, *P. inui*	Tan 2008
Farm	Rugarding, Ranau, Sabah	06º 02' N, 116º 40' E	*An. balabacensis*	*P. knowlesi*	Vythilingam unpublished document

2009). With such large numbers of cases being reported, are appropriate control measures being instituted? Therefore, there is a need for more detailed studies on the vectors in the state of Sarawak before control measures can be planned.

Cases of *P. knowlesi* in peninsular Malaysia

Now cases of *P. knowlesi* have been reported throughout peninsular Malaysia with the exception of the northern most state of Perlis. The cases are on the increase year after year. All these cases have been detected by PCR. Most of these cases are occurring in malaria free areas (Vythilingam *et al.*, 2008). Some have mixed infection of *P. knowlesi* and other human malaria parasites. Death due to *knowlesi malaria* has also been reported. Molecular tools have helped in the detection of simian malaria in humans. Studies have also shown that blood from infected humans (Pk by PCR) when inoculated into long-tailed macaques in the laboratory, developed parasitemia after a pre-patent period of 7 days and a 24 hour periodic cycle was observed in the macaques (Anderios *et al.*, 2010).

Vectors of *P. knowlesi* in peninsular Malaysia

Studies on the vectors of *P. knowlesi* were carried out in the district of Kuala Lipis, in the State of Pahang since most number of cases was reported from this district. Preliminary studies were carried out in many villages before selecting 2 sites for detailed longitudinal studies.

The vector of *P. knowlesi* in Kuala Lipis in Pahang was *An. cracens* (Vythilingam *et al.*, 2008). Besides *P. knowlesi* it was also found to be the vector of *P. cynomolgi*. *Anopheles cracens* were early biters coming to bite human as early as 19.00 hours and the peak biting time was 19:00 to 21:00 hours.

The human: macaque biting ratio was 2:1. This showed that *An. cracens* prefers to bite humans more than monkeys. It was found to be the only vector and the sporozoite rate was low. However the prevalence of malaria parasites among the macaques in Pahang was very high (97.3%) (Vythilingam *et al.*, 2008). Therefore there is a probability that another vector may be responsible in maintaining the cycle among the macaques in the deep jungle. Our study was carried out in the forest fringe and in nearby villages.

The other species of mosquito belonging to the *leucosphyrus* group was *An. pujutensis*. This was found in small numbers and only in the monkey baited traps. None were positive. However, it may be possible that *An. pujutensis* may be playing the role of keeping the parasites in circulation among the monkeys in the deep forest. Besides, studies similar to the Kuala Lipis study have not been carried out in the other States of peninsular Malaysia.

In nature *An. balabacensis* has been incriminated as a vector of *P. knowlesi* in Rugarding Ranau District, Sabah (Vythilingam unpublished document). In Vietnam *Anopheles dirus* which is also a member of the *An. leucosphyrus* group of mosquitoes has been incriminated as a vector of *P. knowlesi* (Nakazawa *et al.*, 2009). *Anopheles dirus* is also present in many countries in Southeast Asia.

Laboratory infection of vectors

Laboratory studies on susceptibility of vector mosquitoes to simian malaria parasites have been painstakingly conducted by Collins *et al.* (1966, 1967, 1971, 2001). They carried out susceptibility studies on *Anopheles freeborni*, *Anopheles quadrimaculatus*, *An. balabacensis*, *An. maculatus* and *Anopheles stephensi* to the H strain (1st natural infection) of *P. knowlesi*. Of these only *An. quadrimaculatus* failed to become infected. However, *An. balabacensis* was the most efficient vector having over more than 1000 sporozoites and it also caused infection in monkeys after a prepatent period of 7-8 days. The *An. maculatus* was obtained from Malaysia and only 3 out of 57 mosquitoes developed sporozoites but of low intensity (Collins *et al.*, 1967). *Anopheles maculatus* was also susceptible to *P. inui* in the laboratory (Collins *et al.*, 1966). *Anopheles dirus* and *An. maculatus* were also susceptible to *P. coatneyi* (Collins *et al.*, 2001).

The author fed *An. maculatus* on a laboratory infected macaque (in the Institute for Medical Research, Malaysia) but only one mosquito was found positive with only one oocyst in the midgut. Thus, its chances to be a vector for simian malaria is slim.

Human cases of *knowlesi* malaria due to the changing role of vectors and environment?

In the 1960's after the first case of *knowlesi* malaria in humans and extensive vector studies in the country it was stated that *knowelsi* malaria may not be a serious problem since the vectors were found only in the deep jungle. Thus the scientists then felt that humans would not be easily infected by the bite of these mosquitoes (Chin *et al.*, 1968).

However, the situation has now changed. In the 1960's, two-thirds of the country was virgin forest and mosquitoes of the *leucosphyrus* group were only found in the deep jungle with the exception of *An. hackeri* which was found in the mangrove swamp (Wharton *et al.*, 1964). *Anopheles hackeri* however, was not attracted to humans. With environmental degradation and deforestation the *leucosphyrus* group of mosquitoes along with the primates have come to the forest edge and some vector species like *An. cracens* is even found in the villages. This could be one of the reasons why cases of simian malaria which was a rare occurrence in humans in the early days, now seems more predominant. In studies carried out by the author, *Anopheles cracens* was found outside some of the houses where positive cases were detected. Thus, people are being bitten by these vector mosquitoes while outside their houses at dusk. However, it has been shown that the *An. cracens* does not enter houses.

Though most of the cases are occurring in malaria free areas there are still no appropriate control measures in place. With the reduction in the number of malaria cases, people would have lost their immunity due to reduced exposure. This coupled with the presence of vectors and the infected long tailed macaques, is contributing to increase in the number of simian malaria cases among humans over the years (unpublished records). In the early days there was a distinct simian cycle in the nonhuman primates and a human malaria cycle in the villages as shown by Warren *et al.* (1970). However, now we are seeing malaria as a zoonoses.

In Sarawak, *An. latens* is the vector of human malaria (Colless, 1956; Zulueta, 1956; Chang *et al.*, 1995) and now it has been incriminated as the vector of simian malaria (Vythilingam *et al.*, 2006). Thus, it is interesting to note why people are now coming down with *knowlesi* malaria and not before. In a study by Lee *et al.* (2009), it has been shown that archivial blood obtained in 1996 were actually *P. knowlesi* and not *P. malariae*. *Anopheles latens* is known to enter houses and to bite humans (Chang *et al.*, 1995). Thus, to say that currently human to human transmission of *knowlesi* malaria is not occurring is hard to discern. Laboratory studies have shown that monkey-mosquito-human-mosquito-monkey transmission can occur (Chin *et al.*, 1968). Thus unless large scale epidemiological studies on simian malaria in humans, nonhuman primates and its vectors are carried out in Malaysia it will be difficult to institute control measures for the malaria vectors.

Importance of vector studies

It is very important to determine the vectors of *P. knowlesi* that are responsible for the transmission of the parasite to humans. Otherwise people get the impression that humans get malaria by other means such as needle sharing (McCutchan, 2008). It may occur but it is very rare. Thus it is important to determine the vectors in all areas to overcome this kind of perception. Only if the vectors are known can steps to be taken to control and manage the spread of *knowlesi* malaria.

In the early history of our country (then Malaya) it was realised that the ravages of malaria could only be controlled by the destruction of mosquito larvae and their breeding places. The pioneering work of Sir Malcom Watson in Port Swettenham (now Port Klang) is well known (Hodgkin, 1956). During the malaria eradication programme, indoor residual spraying with DDT 2gm/m^2 was effective in controlling the malaria vectors in the coastal plain. However, the malaria of the hilly region still persisted due to different vectors and their behaviour. This problem was later overcome by the introduction of insecticide treated bednets to malarious areas (Vythilingam *et al.*, 1995). The use of insecticide treated bednets has successfully controlled malaria in many countries (Lengeler, 2004).

The question now is can these current methods available be used for the control of malaria which is a zoonoses? If we are to base it on the current information that we have, the answer is no. It is difficult to answer this question at this point of time since we have very scanty information on the vectors. The information available is only from a few locations in two states in Malaysia.

A case of *knowesi* malaria has also been reported from a Finnish traveller who spent some time in the jungle about 80 km south of Ipoh, Perak and was diagnosed as *P. knowlesi* infection when he returned to Finland (Kantele *et al.* 2008). There was also a case of a Swedish visitor who had trekked the jungles of Bario highlands in Sarawak, Malaysian Borneo and developed *knowlesi* malaria (Bronner *et al.*, 2009). With the promotion of eco-tourism it is essential that people are warned about this health hazard and appropriate control measures instituted. We have to understand that humans these days travel the globe and the parasites are also carried along by them from place to place. With cheap air travel available in Southeast Asian countries more visitors can be expected to visit Malaysia. Those who choose to spend time trekking the jungles may be easily infected and the disease may not be detected until long after return.

WHO is gearing up on the strategy for the elimination of malaria. This means the complete interruption of mosquito borne malaria transmission in a defined geographical area (WHO, 2008). Is it possible to eliminate malaria, now that malaria is a zoonoses? There is also a possibility that besides *P. knowlesi*, the other simian malaria parasites like *P. cynomolgi* and *P. inui* can also be transmitted to humans (Coatney, 1968). The current vectors *An. latens* and *An. cracens* can also develop these parasites. This is another area that has to be studied.

It is also interesting to note that mixed infection of *P.knowlesi* and human malarias have been reported in humans (Singh *et al*., 2004; Cox-Singh *et al*., 2008, Vythilingam *et al*., 2008). However, in our limited vector studies we have not found infection of mixed species of humans and simian malarias in the mosquitoes.

The question that remains unanswered is: are the people with mixed infection getting infected by just the bite of a single mosquito or many mosquitoes? In a study carried out in Vietnam in a village in the clearing of the forest, sporozoites were found in the salivary glands of *An. dirus* (Nakazawa *et al*., 2009). They were the first to incriminate mixed infection of *P. falciparum*, *P. vivax* and *P. knowlesi* in that mosquito. Thus, there is now some evidence to prove that a single vector mosquito can harbour human and simian malaria parasites.

The next question that comes to mind is: is anthroponosis occurring, that is human malarias in non-human primates? Past studies have shown that, in New World monkeys 3 species of human malaria can be transmitted from monkeys back to humans by mosquito bites (Contacos, 1970). Therefore, more studies are needed in these areas to establish the pathway of transmission.

Possible vector control measures.

It would be advisable for people camping out in the forest to sleep in long lasting insecticidal hammocks (LLIH). A study in Vietnam showed that use of LLIH by the people when they go to the forest had reduced malaria incidence and prevalence (Tang *et al*., 2009). Those who go to work early in the mornings and return at dusk from farms and plantations are advised to use insecticide treated clothing if possible along with repellents to prevent being bitten by mosquitoes. Studies on insecticide treated uniforms by military personnel showed that permethrin treated uniforms do provide some protection against mosquito bites, thereby reducing malaria transmission (Deparis *et al*., 2004).

Repellents can also be used but these can be expensive and effective for only about 6 hours. DEET MC is a formulation of DEET in which the formulation is gradually released from a capsule that binds the repellent. In a study carried out by N'guessan *et al.* (2008) on DEET MC treated mosquito nets, it was found that the repellent was effective for 6 months. Thus treating anklets, wrist bands and head bands with DEET MC formulation and using these when in the forest may provide protection against mosquito bites. There was a study that showed that DEET impregnated anklets and headbands gave good protection against bites of *Mansonia* mosquitoes in the field (Chiang & Eng, 1991). Therefore the use of DEET impregnated personal protection measures should be advocated for use by individuals who live or work in malarious environment. It would also be useful to field test some of these methods for their efficacy.

CONCLUSION

Four decades ago it was thought that humans became infected after they intruded into the territory of the non human primates and were bitten accidentally by the aboreal *Anopheles* mosquitoes. However with the current knowledge on environmental degradation and its impact on disease transmission there is renewed interest on the subject. It is apparent now that deforestation in many areas has brought the monkeys and the forest dwelling *Anopheles* vectors closer to human habitation. These *Anopheles* mosquitoes and the nonhuman primates now colonize our villages. Public health authorities in Malaysia and other neighbouring countries in Southeast Asia should come up with a collaborative approach to determine the extent of this problem, its impact on the population, the infrastructure to deal with the problem and prevention and control programmes. Malaria is a zoonoses, therefore it needs vigilant surveillance to combat its spread. We can no longer say that we can have 'Anophelism' without malaria. Therefore, it is timely also for countries in the region to strengthen their public health service to combat simian malaria in humans.

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