MALARIA TRANSMISSION BY *ANOPHELES DIRUS* IN ATTAPEU PROVINCE, LAO PDR

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Abstract. A study was carried out in four malaria-endemic villages in Attapeu Province, in the southern region of Lao PDR. All-night human landing collections were carried out in May, August, and October 2002, to determine malaria vectors. At the same time, mass blood surveys were also carried out in the same villages. *Anopheles dirus* was the predominant species in three of the study villages. Sporozoites were found only in *An. drius* from Phou Hom. However, in Beng Phoukham, *An. dirus* was positive for oocysts. The distribution of malaria cases was highest in Phou Hom and this correlated well with the vectorial capacity of *An. dirus*. The risk for infection from *An. dirus* was also high, at 0.99.

INTRODUCTION

Malaria is a serious public health problem in Lao PDR, which is a landlocked country. Most malaria cases are reported from the provinces south of Vientiane, the capital of Lao PDR. Very little is known about the malaria vectors in Lao PDR, as mosquito dissections have not been carried out for about 2 decades (Watson, 1999). However, of late, vector studies have been carried out in Khammouane Province (Toma *et al,* 2002, Kobayashi *et al,* 1997; 2000) and in Sekong (Vythilingam *et al,* 2003).

Khammouane Province is in the center of the country and is about 350 km southeast of Vientiane. In this province, studies have shown that *An. dirus* was the vector for malaria, although it was not the predominant species (Toma *et al,* 2002). In Sekong, a province 700 km south of Vientiane, *An. dirus* was found to be a vector in the forested region that had been cleared for planting hill paddy (Vythilingam *et al,* 2003).

Although *An. dirus* is known to be a vector in Myanmar, Thailand, China, and Indochina (Peyton and Harrison, 1979), it is *An. minimus* that is synonymous with malaria in Lao PDR. Recent studies by Walton *et al* (1999) have shown that *An. dirus* is a species complex and plays an important role in malaria transmission in the Mekong Region. Since *An. dirus* has been found in Sekong, which is a province in the south, we wanted to determine how far south *An. dirus* was present.

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Attapeu Province is the southern-most province of Lao PDR, and it has common borders with Vietnam and Cambodia. There are five districts in Attapeu. Little is known about the vectors in this area as no previous studies have been carried out. However, it is known to be endemic for malaria. In order to sustain a good control program, it is important to determine the vectors and their seasonal variations in different ecological zones. In this paper, we report the patterns of biting activity and vectorial capacity of *An. dirus* in relation to malaria transmission.

MATERIALS AND METHODS

Study area

Attapeu Province is situated on the southern tip of Lao PDR, and has common borders with Cambodia to the south, Vietnam to the east, Champassack to the west, and Sekong to the north. The town is built in a large valley surrounded by mountains. Four villages from three districts were selected for the study. In Phou Vong district, which is mountainous and has a common border with Cambodia, we selected Phou Hom village. In Sammakkixay district, we selected Mixay and Beng Phoukham villages, which are adjacent to each other. This is a forested area and has a mixed ethnic population. The fourth village is Pier Geo in

Sanaxy district, which borders Vietnam.

The houses in all villages are built on stilts and have thatched roofs. The walls are made of bamboo and many of the houses are devoid of proper walls. In Pier Geo, some of the walls of the houses are made of wire mesh and have large leaves inserted into them. Animals found in these areas are cattle, buffalo, dogs, and pigs. However, in Pier Geo, there are not many animals compared with the other three villages. The rainy season runs from May to October, and the dry season from November to April. Our surveys were carried out at the beginning, in the middle, and at the end of the rainy season.

Mosquito collection

All-night human landing collections were performed in May/June, August, and October 2002. In each village, two houses were selected and two nights of collections were carried out from 1800 to 0600 hr with two collectors stationed indoors and two outdoors, simultaneously. These were fixed stations for collection throughout the study period. One team worked from 1800 to 2400 hr and the other from 2400 to 0600 hr. All mosquitos landing on human bait were caught using small tubes, which were subsequently plugged with cotton wool and labeled according to time and site.

The next morning, mosquitos were brought to the field laboratory and were identified morphologically using the keys (Reid, 1968; Harrison and Klein, 1975; Rattanarithikul and Green, 1987). The *Anopheles* mosquitos were dissected to extract the ovaries for parity determination and the guts were examined for oocysts. The heads and thoraxes were pooled according to species and time and stored in individual tubes with silica gel.

Enzyme-linked immunosorbent assays (ELISA)

In the laboratory the heads and thoraxes were prepared for ELISA. The procedure followed that of Burkot *et al* (1984) and Wirtz *et al* (1987). A sample was considered positive if it gave a visually-detectable green color with an OD value at least twice the mean OD of 8 negative control wells on that plate. All positive samples were re-examined.

Determination of probability of daily survival, life expectancy in days, and vectorial capacity

Daily survival was compared using the methods of Davidson (1954). Life expectancy was determined using the formula described by Garrett-Jones and Grab (1964). Vectorial capacity was calculated using the formula of Garret-Jones and Shidrawi (1969).

Mass blood smears

Mass blood smears were carried out during the same months when mosquito collections were done. All people in the study villages were informed by the respective villages heads to be present on the appointed day at respective mobile field stations to have their blood screened for malaria. Thick and thin blood smears from finger-prick blood were collected from all those who came to the field station. Samples were stained with Giemsa and examined under a microscope. The species were identified and density determined based on white blood cell count. All positive cases were treated with chloroquine and Fansidar (sulfadoxine/pyrimethamine) according to the Lao Ministry of Health guidelines on the management of malaria. This project was approved by the Ethics Committee of the Ministry of Health, Malaysia.

Statistical analysis

Analysis was performed using Statistica Stat Soft and Epi-Info^{Tm6}. The confidence interval for sporozoite and parous rate was calculated using Fleiss quadratic 95% CI (Fleiss, 1981). Chi-square analysis was carried out to test for significance between indoor and outdoor biting.

RESULTS

Species composition

A total of 21 A*nopheles* species was caught in the study villages, of which the predominant species in three of the villages was *An. dirus*. Besides *An. dirus*, *An. maculatus* ss, *An. minimus*, *An. sawadwongporni/notanandi* were also obtained in considerable numbers (Table 1).

Species biting rate by village

The results, summarized in Table 2, show the biting rates for the six most common species and compares them by village. There were differences in both species composition and abundance in the different villages. *An. dirus* was the predominant species in the three villages but the biting rates differed. The highest biting rate was 11.4 bites/man/night in Phou Hom.

Endo and exophagy

Estimate of the degree of endophagy and

Table 1
List of *Anopheles* mosquitos in the four study villages, from May to October 2002.

Anopheles species	Phou Hom Number (%)	Pier Geo Number (%)	Beng Phokham Number (%)	Mixay Number (%)
aconitus	9 (1.2)	1 (0.5)	9 (1.4)	7 (1.1)
barbirostris	14 (1.9)	1 (0.5)	2 (0.3)	0
dirus	535 (71)	8 (4.2)	229 (35.8)	174 (27)
donaldi	2 (0.3)	0	4 (0.6)	1 (0.2)
dravadicus	0	1 (0.5)	4 (0.6)	2 (0.3)
hyrcanus	1 (0.1)	1 (0.5)	6 (0.9)	7 (1.1)
karwari	1 (0.1)	12 (6.3)	0	2 (0.3)
kochi	4 (0.5)	0	8 (1.3)	12 (1.9)
maculatus ss	6 (0.8)	2 (1.0)	48 (7.5)	169 (26.1)
minimus	119 (15.8)	4 (2.1)	51 (8.0)	39 (6.0)
nivipes	13 (1.7)	46 (24)	18 (2.8)	19 (2.9)
notanandai	2 (0.3)	9 (4.7)	26 (4.1)	24 (3.7)
pallidus	0	1 (0.5)	0	0
pampanai	0	0	3 (0.5)	1 (0.2)
philippinensis	13 (1.7)	30 (15.6)	33 (5.2)	24 (3.7)
pseudowilmori	0	1 (0.5)	0	0
sawadwongporni	5 (0.7)	11 (5.7)	23 (4.0)	43 (6.7)
sawadwongporni/notanandai	8 (1.1)	57 (29.7)	152 (23.8)	109 (16.9)
splendidus	0	0	21 (3.3)	13 (2.0)
tessalatus	5 (0.7)	1 (0.5)	1 (0.2)	0
varuna	3 (0.4)	5 (2.6)	1 (0.2)	0

Table 2
Human biting rate (bites/man/night) by village and species.

Village	Person nights	*An. dirus*	*An. maculatus* ss	*An. minimus*	*An. notanandi*	*An. sawadwongporni*	*An. sawad/nota*
Phou Hom	48	11.4	0.1	2.5	0.04	0.1	0.2
Pier Geo	48	0.2	0.04	0.08	0.2	0.2	1.2
Mixay	48	3.6	3.5	0.8	0.5	0.9	2.3
Beng Phoukham	48	4.8	1.0	1.1	0.5	0.5	3.2

exophagy were obtained when relative proportions of *An. dirus* attempting to bite indoors and outdoors were compared. *An dirus* was more endophagic with an in/out ratio of 1.6. The ratio of *An. dirus* caught indoors/outdoors did not differ significantly (χ^2 =1.166, p > 0.05) (Table 3).

Changes in landing rate over time

Fig 1 shows the average bites/man/night of *An. dirus* in the different villages. Peak biting was observed in August in all the villages. This coincided with heavy rainfall the previous month.

Fig 2 shows the biting cycle of *An. dirus*. The percentages of human landings per hour were plotted separately for indoor and outdoor collections. *An. dirus* starts to bite as early as 1900 hr but the peak is around 2200 hr, and it continues to bite until 0600 hr.

Parous rate, probability of survival, life expectancy and vectorial capacity

The parous rate of *An. dirus* was higher in May and October, compared with August. The probability of survival was similar in all three villages, but the vectorial capacity was highest in Phou Hom in August and October (Table 4). Life

Table 3
Biting rate of common anopheline mosquitos and in/out ratio in study areas.

Species	Total number of mosquitos		Percentage of total		Ratio
	In	Out	In	Out	In/Out
An. dirus	581	367	61.3	38.7	1.6
An. maculatus ss	65	160	28.9	71.1	0.41
An. minimus	114	96	54.3	45.7	1.2
An. sawadwongporni/notanandi	209	276	43.1	56.9	0.76

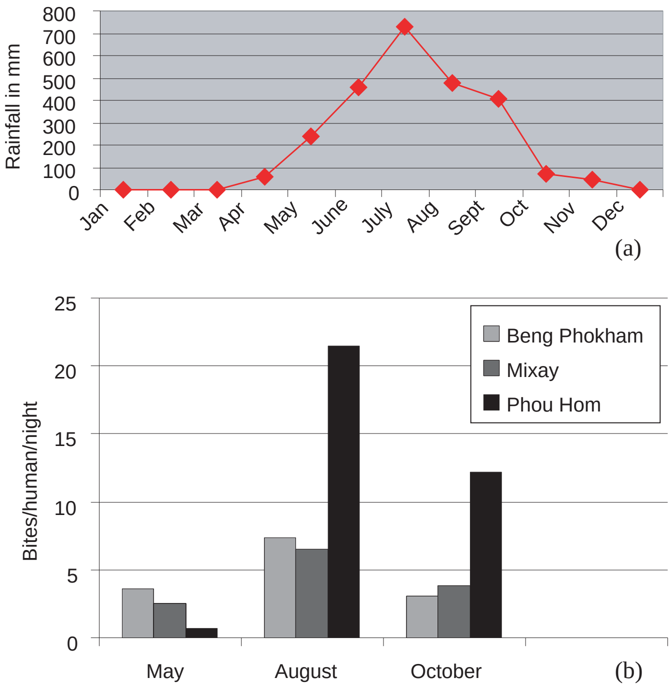

Fig 1–Rainfal and bites/human/night of *An. dirus* in Attapeu.

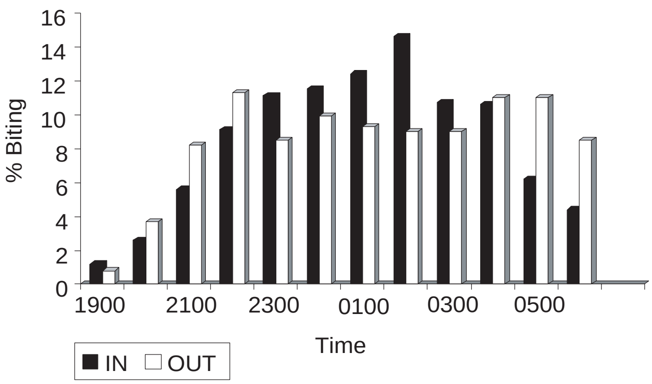

Fig 2–Indoor/outdoor biting cycle on *An. dirus.*

expectancy was also highest (10.6) in October.

Rate of infected mosquitos

In August, one *An. dirus* specimen from Phou Hom was positive for oocysts out of 294 examined, as shown in Table 5. Thus, the infection rate was 0.34. In Beng Phoukham, two *An. dirus* were positive for oocysts out of the 108 examined, and thus the infection rate was 1.85. One midgut had more than 250 oocysts. Another heavy infection was observed in October in Phou Hom, where the midgut had 112 oocysts.

Infection rate and risk

Table 6 shows the sporozoite rate, entomological inoculation rate, and risk, in Phou Hom village. Only mosquitos from Phou Hom were positive for sporozoites. In August and October, the inoculation rate was similar and the risk of infection from *An, dirus* was high, at 0.99.

Calculated and actual gametocyte rates

The calculated and actual gametocyte rates are shown in Table 7. The formula postulated by Macdonald (1975) was used. Since the sporozoite rates are known, it was possible to calculate the expected gametocyte rate. Actual gametocyte prevalence was obtained from the thick blood films. There was no significant difference between observed and expected values.

Slide positivity rate of malaria

Fig 3 shows the slide positivity rate (SPR) of malaria in the study villages.

Table 4
Parous rate, probability of survival, life expectancy and vectorial capacity of *An. dirus* in the study villages.

Months	Villages	% Parous (95 % CI)	Probability of survival	Life expectancy in days	Vectorial capacity
May/June	Phou Hom	60 (27.4-86.3)	0.84	5.7	0.58
	Pier Geo	Nil			
	Mixay	77.5 (61.1-88.6)	0.92	12	4.8
	Beng Phoukham	70.2 (56.4-81.2)	0.89	8.6	2.7
August	Phou Hom	59.8 (54.2-65.1)	0.84	5.7	5.02
	Pier Geo	16.7 (0.9-63.5)	0.54	1.6	2.2
	Mixay	59.5 (47.8-70.2)	0.84	5.7	1.54
	Beng Phoukham	60.9 (51.1-69.9)	0.84	5.7	1.7
October	Phou Hom	76.4 (69.7-82.1)	0.91	10.6	13.8
	Pier Geo	100 (30.0-100)	1		
	Mixay	70 (50.4-84.6)	0.88	7.8	2.1
	Beng Phoukham	71.4 (56.5-83.0)	0.89	8.6	2.2

Table 5
Rate of infected *An. dirus* (with oocyst) in study villages in August and October 2002.

Villages	August			October		
	No. dissected	No. with oocyst	% Infected (95 % CI)	No. dissected	No. with oocyst	% Infected (95% CI)
Phou Hom	294	1 (3 oocyst)	0.34 (0.18-1.88)	159	4 (one with 112 oocyst)	2.52 (0.81-6.72)
Pier Geo	7	Nil		3	Nil	
Mixay	90	Nil		30	Nil	
Beng Phoukham	108	2 (one with 5 oocyst, one with > 200 oocyst)	1.85 (0.32-7.19)	49	Nil	

Table 6
Man-biting rate of *An. dirus*, sporozoite rate, entomological inoculation, estimated mean inoculation per month and risk of receiving *P. falciparum* in Phou Hom village.

Month	Man-biting rate (Ma)	Sporozoite rate (S%) (95 % CI)	Entomological inoculation rate (EIR)	Estimated EIR	Risk[a]
August 02	21.5	1.45 (0.54-3.56)	0.31	9.61	0.99
October 02	12.19	2.56 (0.95-6.21)	0.32	9.92	0.99

[a]Krafsur (1977) $1\text{-}e^{-\text{ inoculation/month}}$, e is the base of natural logarithms.

In Phou Hom, the SPR was highest in May and October (41.3, 41.7), the beginning and end of the rainy season. In Pier Geo, it was 35.4% in May, and 22.6 in August and October. However, in Beng Phoukham and Mixay, the SPR was highest in August (38.9; 37.3). However, there were no significant differences between the parasite rates.

Table 7
Comparison of number of gametocyte carriers predicted from entomological data with actual number found in Phou Hom.

Month	Calculated gametocyte rate x #	No. expected	No. found
May 02	-	-	2
August 02	0.0058	1.98	1[a]
October 02	0.0033	1.11	10

$x = s(-\log_e p)/a\ (p^n - s)$; where s = sporozoite rate; p= probability of survival, n=12 length of sporogonic cycle; a=0.33 daily rate of blood feeding on man.
[a]difference between observed and expected not significant, at 0.05% level of χ^2 with Yates correction.

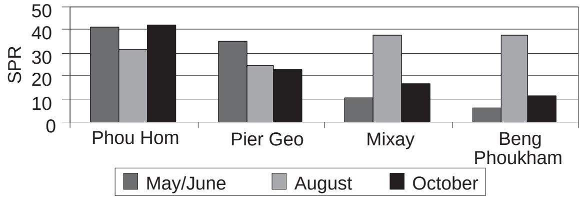

Fig 3–Slide positivity rate of malaria.

DISCUSSION

This brief study indicated that *An. dirus* was the most important, and sole, vector in the study area. It was found biting man and was also positive for *P. falciparum*. *An. dirus* has adapted very well to the habits of man. In the early part of the night, from 1900 hr until 2200 hr, and in the early hours of the morning, from 0500 hr to 0600 hr, it bites more outdoors than indoors. The rest of the time indoor biting was higher than outdoor. It has been observed that most people, at least 80%, are in bed by 2200 hr and they arise before 0600 hr. It was found that 12.5% and 10% of *An. dirus* biting outdoors between 1900-2000 hr and 2000-2100 hr, respectively, were infective. Indoors it was found that 20% biting between 2200-2300 hr and 5.6% between 2300-2400 hr, and 7.7% between 0400-0500 hr, were infective. Thus, although treated mosquito nets were available, it is still possible for people to become infected. However, since *An. dirus* comes indoors to bite man, the use of insecticide-treated nets would help reduce vector density. The biting pattern seen here is very similar to what has been described in Sekong (Vythilingam *et al*, 2003).

The biting rate of *An. dirus* was highest in August, which coincided with the heaviest rainfall during the previous month. Large numbers of breeding sites would have been available. The parous rate was lower in August, compared with October. The parous rate, the vectorial capacity and life expectancy in days of *An. dirus* correlate very well with the increase in cases in October. It looks like *An. dirus* peaks during the period of heavy rains and then tapers off during the onset of the dry season. However, the survival rate and vectorial capacity was at its height in October, along with an increase in the prevalence of gametocytes. It is known that *An. dirus* breeds in small, fresh, temporary pools and its numbers and proportion infective have usually been observed to peak some time during the wet season (Scanlon and Sandinand, 1965; Wilkinson *et al*, 1978; Rosenberg and Maheswary, 1982). In this study, and in the previous study in Sekong (Vythilingam *et al*, 2003), it was found that the proportion infective was higher at the end of the rainy season.

It has also been reported that *An. dirus* is not only very resistant to control within its habitat but that it is an extraordinarily efficient vector, so long-lived and anthrophilic that only a small population is necessary to maintain high malaria endemicity (Rosenberg *et al*, 1990). Although *An. minimus* has always been synonymous with malaria in Lao PDR, we feel very strongly that *An. dirus* is the main vector, at least in the southern region. In earlier years, it was reported to be more of a forest species, but now *An. dirus* seems to have adapted well to man's changing environment. In Myanmar, it has been found breeding in wells (Oo *et al*, 2002; Htay-Aung *et al*, 1999). Unlike the previous study in Sekong (Vythilingam *et al*, 2003), where *An. dirus* was the main vector in the farm which was a clearing in the forest, this study indicated that *An. dirus* was also found in scrub areas with low vegetation. Also, unusually high numbers of oocysts have been found in the *An. dirus* in this region.

Further extensive studies in other provinces of Lao PDR should be carried out to determine the vectors of malaria. At present, it is believed that *An. dirus* plays a role only in the south, while in the north, other vectors are involved. Thus the bionomics of *An. dirus* should be studied in great depth as it seems to be the important vector, and control programs can be better managed if the habits of the vector are known.

ACKNOWLEDGEMENTS

This project was supported by a WHO WPRO Inter-country Small Research Grant (MVP/MAA/01/01). We wish to thank Dr Eva Christophel of WHO and Dr Lye Munn Sann, Director of IMR, for their support. The technical assistance of SW Tan is gratefully acknowledged. We also like to thank the staff of CMPE and the Malaria Station of Attapeu for their assistance in the field.

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