ENTOMOLOGICAL SURVEYS OF MALARIA IN KHAMMOUANE PROVINCE, LAO PDR, IN 1999 AND 2000

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Abstract. Anopheline mosquitos and their relation to malaria transmission were studied 3 times: in July and August, 1999; in December, 1999; and in August and September, 2000. The studies took place in the malaria endemic villages of Khammouane Province, southeast of Lao PDR. A total of 28 species were collected using human and animal bait. Human bait attracted predominantly *Anopheles dirus* and *An. minimus*, which were identified as vectors by the detection of sporozoites by dissection, PCR, and enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *P. vivax*. The vectorial capacity of *An. dirus* was 0.009-0.428, while that of *An. minimus* was 0.048-0.186. The inoculation rate of *An. dirus* was 0.052-0.137 (Boualapha; August, 2000). *An. nivipes* and its sister species, *An. philippinensis*, were principally zoophilic, although a considerable number of the females were also attracted to human bait in the villages of the paddy field areas. *An. philippinensis* infected with oocysts of *P. vivax* was detected in a specimen collected by animal bait. These two species were considered as vectors in Khammouane Province. Four species, *An. notanandai*, *An. sawadwongporni*, *An. willmori*, and *An. hodgkini*, had not been recored before in Lao PDR. Information is provided on host preference and the nocturnal biting activities of common species and the incidence of malaria in the study areas.

INTRODUCTION

Lao PDR is a landlocked country, surrounded by Vietnam, China, Thailand, Myanmar and Cambodia. It has a population of nearly 4.2 million; 80% of the people live in rural areas. Malaria is the most important health problem and is associated with high morbidity and mortality in rural areas (Pholsena, 1992). According to data collected from all provinces by the Epidemiology Department of the Center of Malariology, Parasitology and Entomology (CMPE), about 300,000 confirmed and suspected malaria cases are reported every year (Phetsouvanh, 2000). However, the exact prevalence of malaria in Lao PDR, is not known because cases are passively recorded: they comprise only patients admitted to hospital. The country's endemic malaria has only recently become clear (Kobayashi *et al*, 1997, 1998, 2000; Toma *et al*, 1999, 2001), due to the renovation of the facilities for malaria diagnosis and the training of microscopists that was supported by the Japan International Cooperation Agency (JICA) and the Primary Health Care (PHC) Project, in cooperation with the Center of Malariology, Parasitology and Entomology (CMPE), Vientiane. The active case detection carried out on four occasions during the period December 1995 to August 1996 by Kobayashi *et al* (1998) showed that 5.3-10.5% of the inhabitants of two villages, Namdik and Nakham, located in mountainous forest in Khammouane Province, were infected with malaria throughout the year.

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Pholsena (1992) studied the mosquitos of Lao PDR: 37 anopheline species were found, of which; *Anopheles minimus*, *An. maculatus*, and *An. dirus* were malaria vectors (Pholsena, 1992; Meek, 1995). Kobayashi *et al* (2000) suspected that *An. nivipes* was a malaria vector in the endemic areas of Khammouane Province, as it accounted for more than 65% of all mosquitos collected using human bait. Recently, Vythilingam *et al* (2001) reported *Anopheles* mosquitos, collected indoors and outdoors, in eight provinces. However, little is known about the vectors or the vectorial transmission capacity and behavior of anopheline mosquitos in the provinces of Lao PDR. In accordance with the government policy on primary health care (1999), the National Malaria Control Program (NMCP) is given top priority in Lao PDR. One of the program's strategies for vector control is the widespread use of insecticide (pyrethroid) impregnated bed-nets (IBM) throughout the country.

The present study aimed to identify the vector mosquitos of malaria. Three mosquito surveys were carried out, the first from July to August 1999 (rainy season) and the second in December 1999 (dry season) at two topographically different areas of Khammouane Province, and the third from August to September 2000 (rainy season) in Koutboun and Thapachon. Mass screening of the blood of village people was carried out in 1999.

MATERIALS AND METHODS

Study area

Khammouaen Province, in the center of the country, is about 350 km southeast of Vientiane, the capital of Lao PDR (Fig 1). The climate of the province is rainy (May to October) and dry (November to April) (Fig 2). Two areas, around Thakhek City and Boualapha district, were selected for entomological survey because of their different topography. The study area in Thakhek City is a plain surrounded by paddy fields and crossed by numberous streams. The area covered Nongceng village (45 km from Thakhek City, the center of the province) in Nhommarath district, Thamlay (25 km from Thakhek) and Bounghouana (South) village (20 km from Thakhek) in Xebangfay district, and Xiengvenh village (10 km from Thakhek) in Thakhek district. Another study area, Boualapha district, is located in a mountainous forest fringe near the border of Vietnam. Three of the district's villages, Napoung, Koutboun, and Thapachon, are situated along Xebangfay river, which runs through a shallow limestone valley at some 50-180 m above sea level. Houses are usually made of wood and bamboo and stand 1-2 meters above the ground in order to avoid flooding during the rainy season. There is no electricity, or piped water; there are no toilets; access is difficult, especially during the rainy season. Domestic animals, such as cows, buffalos, dogs, and chickens, are common in the villages; they stay in their owners' houses at night. The number of households (population) in these villages is shown in Table 1. Annual rainfall and mean temperature data were obtained from the Provincial Meteorology Center in Thakhek City (Fig 2). During a period of the study, maximum and minimum temperatures and humidity were recorded daily.

Active case detection

Active case detection (ACD) was carried out among the inhabitants of the villages that were included in the entomological survey. Giemsa-stained blood films were used to detect malaria parasites. Well-trained microscopists at the CMPE identified *Plasmodium* species by microscopy.

Mosquito collection

Mosquito collections in the 1st survey were carried out in Nongceng, Thamlay, and Bounghouana (South) on July 17-28, in Xiengvenh on August 19-20, and in Napoung, Koutboun, and Thapachon on August 2-13, 1999. In the 2nd survey, the collections were in Thamlay on 5-10 December and in Napoung, Koutboun and Thapachon on December 15-31, 1999. The 3rd survey was carried out in Koutboun and Thapachon on August 20 to September 15, 2000. Mosquitos were collected by 2 methods:

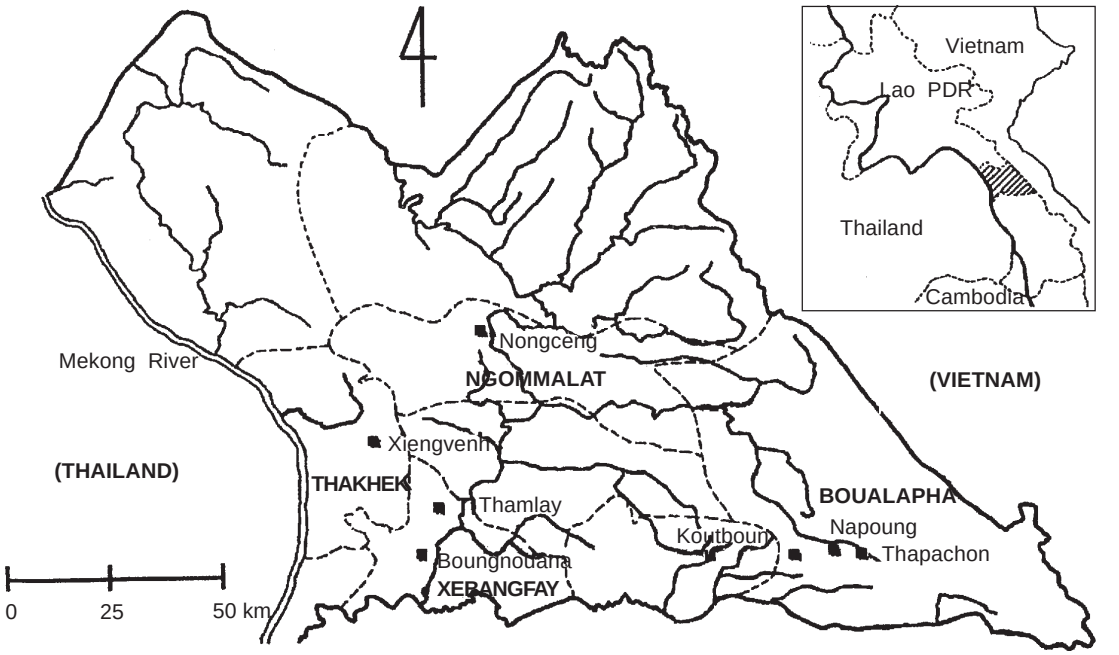

Fig 1–Study areas in Khammouane Province, Lao PDR.

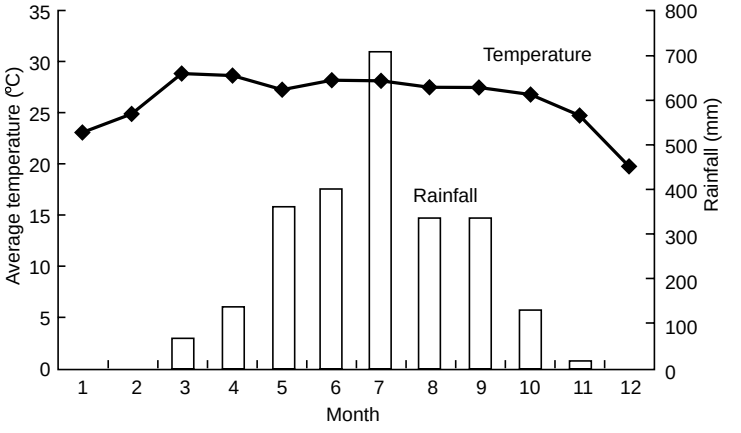

Fig 2–Monthly average temperature and rainfall (1999) in Thakhek City, Khammouane Province.

animal bait and human bait. For the animal bait collections, large double nets (outer net 4 x 4 x 2 m) were set up in the villages and a cow or buffalo was tethered inside the inner net; the skirt of the outer net was opened from 18.00 to 24.00 hours to facilitate the entry of mosquitos. Between one and four nets were set up each night. All the mosquitos that were trapped inside the outer net were collected with a sucking tube at 21.00 and 24.00 hours. Human bait collections were made by 8 collectors (4 pairs) simultaneously with the animal bait collection. The collectors sat outdoors, exposed their legs, and caught all the mosquitos that landed on them with a sucking tube from 18.00 to 24.00 hours. All mosquitos collected were brought to a field station and transferred to cages (20 x 20 x 30 cm) with a cotton ball soaked in a sugar solution. An additional mosquito collection was made, during the early morning from 5 August to 16 September, 2000, by two instructors who followed the method described by Uza *et al* (2002): 92 bednets in Thapachon households and 75 bednets in Koutboun were sampled. The mosquitos species under the stereomicroscope using keys (Harrison and Scanlon, 1975;

Table 1
Prevalence of *Plasmodium* infection in villages of Khammouane Province, 1999.

District village	Date examined	Total population	No. of households	No. of families	No. of people examined	Examined rate (%)	Malaria species					Malaria positive rate (%)
							Pf	*Pv*	*Pm*	*Pf* and *Pv*	*Pf* and *Pm*	
Ngoumalat												
Nongceng	Jul-Aug (1st survey)	293	60	62	191	65.2	32	8	0	1	0	21.4
Xebangfay												
Thamlay	Jul-Aug (1st survey)	435	83	90	338	77.7	45	3	0	0	0	14.2
	Dec (2nd survey)	435	83	90	326	74.9	31	0	0	0	0	9.5
Bounghouana (South)	Jul-Aug (1st survey)	554	111	119	128	23.1	9	0	0	0	0	7.0
Thakhek												
Xiengvenh	Jul-Aug (1st survey)	370	70	77	244	65.9	32	0	0	0	0	13.1
Boualapha												
Napoung	Jul-Aug (1st survey)	426	75	80	289	62.6	60	3	1	0	0	22.1
	Dec (2nd survey)	426	75	80	264	57.1	63	8	0	1	0	27.3
Koutboun	Jul-Aug (1st survey)	255	40	48	171	67.1	44	3	0	0	0	27.5
	Dec (2nd survey)	255	40	48	163	63.9	76	7	0	5	0	54.0
Thapachon	Jul-Aug (1st survey)	267	41	47	103	38.6	27	6	0	0	0	32.0
	Dec (2nd survey)	267	41	50	127	47.6	29	0	0	0	0	22.8

The information on total population was obtained from village leaders.
Pf: Plasmodium falciparum, Pv: Plasmodium vivax, Pm: Plasmodium malariae

Rattanarithikul, 1982; Rattanarithikul and Green, 1986; Peyton, 1989). *An. minimus* was identified by its pattern of pale and dark wing spots. Most of the species were *An. minimus* A; the proportion of *An. minimus* C was very low. In this study *An. minimus* includes both A and C. *Anopheles dirus* in this paper is *An. dirus* Peyton and Harrison, 1979 (*An. dirus* A) (Miyagi and Toma, 2000). Damaged specimens of *An. nivipes* and *An. philippinensis* were regarded *An. nivipes* or *An. philippinensis* (Tables 2-5). The average number of anopheline mosquitos was calculated for each village.

Host preference and nocturnal feeding activities

The anthropophilous index (AI) was calculated from the number of mosquitos collected by human and animal bait collections in the 1st and 2nd surveys. The nocturnal feeding activities of common anopheline mosquitos collected in the 1st and 2nd surveys, 1999, were examined by comparing the number collected hourly from 18.00 to 21.00 and 21.00 to 24.00 hours. In the 3rd survey, *An. dirus* and *An. minimus* attracted by human bait were collected hourly from 18.00 to 06.00 hours from August 20 to September 15, 2000, in Thapachon.

Detection of parity, *Plasmodium* oocyst, and sporozoites

The mosquitos were anesthetized by ethylether and dissected under aseptic conditions: head with anterior thorax (HT) and abdomen with posterior thorax (AB) were seperated, as described by Tsuzuki *et al* (2001). Parity was determined by the degree of development of the ovaries, which were examined microscopically (x 200) according to the method of Detinova (1962). The parous rate for each species was calculated. The salivary glands of parous females were examined microscopically for sporozoites (x 400); the midguts were examined for oocysts (x 200). The HT samples with sporozoites and the AB samples with oocysts were put into separate tubes with 15 ml of 99.5% ethanol for species identification. Only mosquitos with oocysts or sporozoites were used for the identification of *Plasmodium* parasites by PCR, as described by Tsuzuki *et al* (2001). Dry specimens that could not be dissected in the field and brought back to Japan were dissected (HT and AB) and examined for *P. falciparum* and *P. vivax* sporozoites by ELISA using the method developed by Wirtz *et al* (1985, 1987). Vectorial capacity was calculated by the formula given by Bruce-Chwatt (1985). The inoculation rate is defined as the number of infective mosquitos biting per man-night.

RESULTS

Active case detection carried out in different seasons and different topographical areas in Khammouane Province in 1999, revealed that the rainy-season infection rate of *P. falciparum* was 21.4% in Nongceng, 14.2% in Thamlay, 7.0% in Bounghouana, and 13.1% in Xiengvenh (Table 1). The rates in the remote area, Boualapha district, were apparently higher than those in the villages around Thakhek: 22.1% in Napoung, 27.5% in Koutboun, and 32.0% in Thapachon. *Plasmodium malariae* was detected only in Napoung; *P. vivax* was found in Nongceng, Thamlay, Napoung, Koutboun, and Thapachon; *P. falciparum* was the dominant species in all the villages surveyed. Even in the cool dry season, the malaria infection rates among the inhabitants were almost the same as those of the rainy season.

In the 1st survey (rainy season, July to August), 4,691 mosquitos of the subgenus *Cellia* (12 species) and 809 of the subgenus *Anopheles* (7 species) were captured in 34 animal-nights; 1,017 *Cellia* (12 species) and 124 *Anopheles* (5 speices) were collected in 160 man-nights (Tables 2, 3). The number of anopheline mosquitos per animal-night ranged from 3.4 in Napoung to 608.5 in Thamlay. *An. nivipes* was the dominant species in all the villages, accounting for about 70% of the specimens, except in Koutboun (35.6%) and Thapachon (37.1%). The lowest collection rate was one anopheline mosquito per man-night (Bounghouana); the highest rate was 18.6 (Nongceng). In Nongceng, Thamlay, Boungouana, Xiengvenh, and Koutboun, *An. nivipes* was

Table 2
Number (%) of *Anopheles* collected by animal bait collection in villages of Khammouane Province from July to August (1st survey, rainy season), 1999.

Species	No[a] (%) mosquitos collected								
	Around Thakhek					Boualapha			
	Nongceng (4)[b]	Thamlay (4)	Bounghouana (4)	Xiengvenh (4)	Total (16)	Napoung (8)	Koutboun (6)	Thapachon (4)	Total (18)
(Cellia)									
aconitus	7.3 (2.4)	0.5 (0.1)	0.5 (0.6)	0.5 (0.3)	2.2 (0.8)	0.1 (3.7)	1.2 (0.9)		0.4 (0.9)
annularis	0.3 (0.1)		1.3 (1.5)	0.3 (0.2)	0.4 (0.2)		0.8 (0.7)		0.3 (0.6)
dirus		1.3 (0.2)			0.3 (0.1)		0.7 (0.5)		0.2 (0.4)
kochi		1.3 (0.2)		0.3 (0.2)	0.4 (0.1)	0.3 (7.4)	7.7 (6.0)	13.0 (44.8)	5.6 (11.0)
maculatus							0.5 (0.4)		0.2 (0.3)
minimus				0.3 (0.2)	0.1 0.0		2.2 (1.7)		0.7 (1.4)
nivipes	216.0 (71.9)	471.5 (77.5)	51.0 (60.9)	113.5 (73.0)	213.0 (74.2)	2.1 (63.0)	45.3 (35.6)	10.8 (37.1)	18.4 (36.6)
nivipes or phili.	9.0 (3.0)	3.5 (0.6)	5.8 (6.9)	0.5 (0.3)	4.7 (1.6)		0.2 (0.1)		0.1 (0.1)
philippinensis	24.0 (8.0)	42.3 (6.9)	2.0 (2.4)	5.5 (3.5)	18.4 (6.4)		5.7 (4.4)	2.0 (6.9)	2.3 (4.6)
sawadwongporni							0.5 (0.4)		0.2 (0.3)
tessellatus		0.3 (0.0)			0.1 0.0		0.8 (0.7)		0.3 (0.6)
vagus	6.3 (2.1)	47.0 (7.7)	13.8 (16.4)	2.5 (1.6)	17.4 (6.1)	0.6 (18.5)	3.7 (2.9)	0.8 (2.6)	1.7 (3.3)
willmori							0.2 (0.1)		0.1 (0.1)
spp	6.3 (2.1)	2.0 (0.3)			2.1 (0.7)				
(Anopheles)									
barbirostris gr	13.8 (4.6)	20.0 (3.3)	6.0 (7.2)	21.0 (13.5)	15.2 (5.3)		1.8 (1.4)	0.8 (2.6)	0.8 (1.5)
hyrcanus gr	11.8 (3.9)	11.0 (1.8)	1.0 (1.2)	10.3 (6.6)	8.5 (3.0)	0.3 (7.4)	56.3 (44.2)	1.8 (6.0)	19.3 (38.2)
spp	5.8 (1.9)	2.8 (0.5)	2.5 (3.0)	1.0 (0.6)	3.0 (1.0)				
Anopheles spp		5.3 (0.9)			1.3 (0.5)				
Total	300.3 (100)	608.5 (100)	83.8 (100)	155.5 (100)	287.0 (100)	3.4 (100)	127.5 (100)	29.0 (100)	50.4 (100)
Total no. of mosquitos collected during all animal-nights	1,201	2,434	335	622	4,592	27	756	116	908

[a]per 1 animal-night, [b]No. of animal-nights.

the dominant species, constituting 22.3-87.5%. In Koutboun, *An. minimus* was second in abundance, accounting for 19.9%. *An. dirus* was the dominant species, accounting for some 50%, in Napoung and Thapachon.

In the 2nd survey (dry season, December), 1,221 mosquitos of the subgenus *Cellia* (16 species) and 78 of the subgenus *Anopheles* (6 species) were captured in 49 animal-nights; 57 *Cellia* (9 species) and 2 *Anopheles* (2 species) were collected in 136 man-nights (Tables 4, 5). The number of anopheline mosquitos per animal-night ranged from 0.67 in Koutboun to 51.7 in Thamlay. In Thamlay, *An. philippinensis* was the dominant species, accounting for 39.5% of the specimens. In Thapachon and Napoung, the dominant species was *An. minimus* (32.4% and 60.6% respectively). The number of anopheline mosquitos collected by human bait was one (female) during 48 man-nights in Thamlay, 34 during 40 man-nights in Napoung, none during 24 man-nights in Koutboun, and 24 during 24 man-nights in Thapachon. The dominant species in Boualapha was *An. miminus*: 64.7% in Napoung and 20.8% in Thapachon. During the 3rd survey (December 8 to 31), maximum and minimum temperatures were recorded outdoor. It was very cool: 14.5-27ºC (daytime) and 10.5-18.0ºC (early morning) in Boualapha.

The anthropophilous indices (AIs) are shown in Tables 6 and 7. From July until August, the AIs of *An. nivipes* and *An. philippinensis* were 0.037 and 0.049 respectively. That of *An. dirus* was 2.526, the highest of all the anopheline species examined. In December the AIs of *An. nivipes*, *An. philippinensis*, and *An. minimus* were 0.003, 0.004, and 0.037 respectively. In the 1st survey of the rainy season, many females were collected from 21.00 to 24.00 hours (Fig 3). In the 2nd dry season, most of the mosquitos were collected from 18.00 to 21.00 hours (Fig 3). About 80% of *An. dirus* were collected from 21.00 to 24.00 hours in the rainy season. The number of *An. dirus* collected by human bait increased from 21.00 hours and peaked at midnight (Fig 4). The number of *An. minimus* was quite contrast throughout. In the early

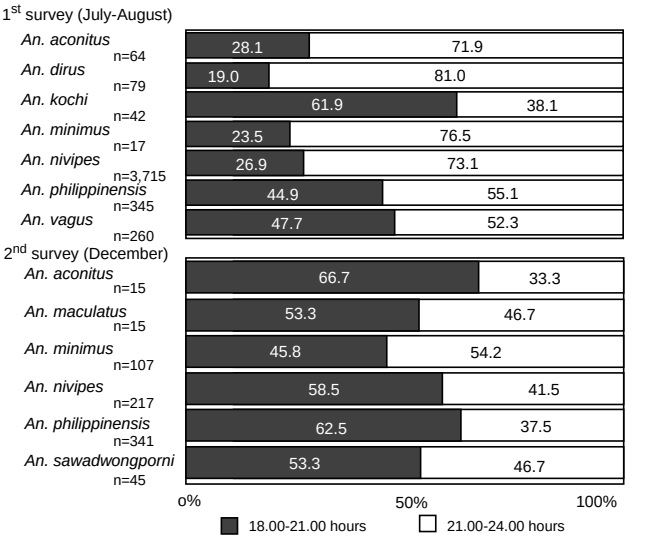

Fig 3–The feeding activities of anopheline females collected by human and animal baits in villages of Khammouane Province, 1999.

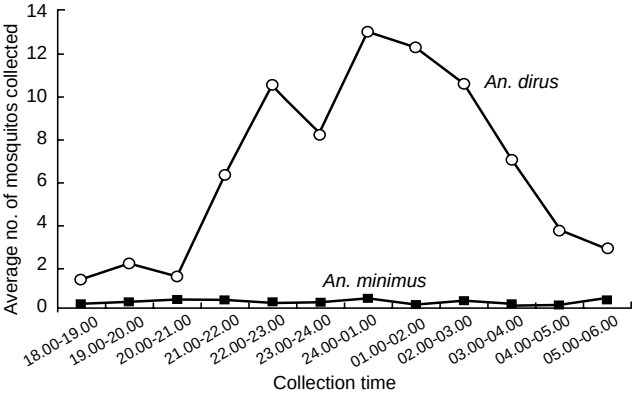

Fig 4–Nocturnal biting activities of *An. dirus* and *An. minimus* females captured by human baits in Thapachon, August to September, 2000.

morning catches in the 92 bednets in Thapachon, 21 mosquitos were collected from 9 nets, including 12 *An. dirus* (5 nets) and 3 *An. philippinensis* (2 nets). In Koutboun, 18 mosquitos were collected from 4 of the 75 nets, including 4 *An. minimus* (single net), 3 *An. hyrcanus* group (2 nets) and one *An. philippinensis* (single net). Most of the bednets that harbored mosquitos were in poor condition: old and with several holes (Table 8).

Table 3
Number (%) of *Anopheles* collected by human bait collection in villages of Khammouane Province from July to August (1st survey, rainy season), 1999.

Species	No[a] (%) mosquitos collected								
	Around Thakhek					Boualapha			
	Nongceng (24)[b]	Thamlay (24)	Bounghouana (24)	Xiengvenh (16)	Total (88)	Napoung (32)	Koutboun (24)	Thapachon (16)	Total (72)
(Cellia)									
aconitus	1.3 (7.2)	0.2 (5.1)		0.3 (1.8)	0.45 (5.2)		0.8 (9.5)		0.3 (5.3)
annularis				0.1 (0.5)	0.01 (0.1)		0.04 (0.5)		0.01 (0.3)
dirus		0.1 (2.5)		0.1 (0.9)	0.05 (0.5)	1.6 (50.0)	0.8 (9.5)	2.1 (52.4)	1.4 (27.5)
kochi	0.04 (0.2)				0.01 (0.1)	0.1 (3.0)	0.2 (2.4)	0.6 (14.3)	0.2 (4.5)
maculatus	0.04 (0.2)	0.04 (1.3)		0.1 (0.5)	0.03 (0.4)		0.2 (1.9)	0.1 (3.2)	0.1 (1.6)
minimus	0.1 (0.4)			0.4 (3.2)	0.10 (1.2)	0.1 (3.0)	1.8 (19.9)		0.6 (12.0)
nivipes	14 (77.6)	2.1 (64.6)	0.9 (87.5)	9.1 (66.8)	6.41 (73.5)	1.1 (34.0)	2.0 (22.3)	0.9 (22.2)	1.3 (25.4)
nivipes or phili.	0.04 (0.2)				0.01 (0.1)				
philippinensis	1.8 (9.4)	0.3 (7.6)	0.04 (4.2)	1.2 (8.8)	0.77 (8.9)	0.1 (2.0)	0.3 (2.8)	0.1 (3.2)	0.1 (2.7)
sawadwongporni				0.1 (0.9)	0.02 (0.3)	0.03 (1.0)	0.3 (2.8)		0.1 (1.9)
tessellatus							0.2 (1.9)		0.1 (1.1)
vagus	0.2 (1.1)	0.08 (2.5)			0.08 (0.9)	0.03 (1.0)	0.1 (0.9)	0.1 (1.6)	0.1 (1.1)
spp	0.1 (0.7)	0.04 (1.3)			0.05 (0.5)	0.03 (1.0)			0.01 (0.3)
(Anopheles)									
barbirostris gr	0.3 (1.6)	0.2 (6.3)	0.04 (4.2)	1.7 (12.4)	0.45 (5.2)	0.1 (3.0)	0.33 (3.8)	0.1 (1.6)	0.2 (3.2)
hyrcanus gr	0.1 (0.7)	0.3 (8.9)	0.04 (4.2)	0.5 (3.7)	0.22 (2.5)	0.1 (2.0)	1.9 (21.3)	0.1 (1.6)	0.7 (12.8)
spp	0.04 (0.2)			0.1 (0.5)	0.02 (0.3)		0.04 (0.5)		0.01 (0.3)
Anopheles spp	0.08 (0.4)				0.02 (0.3)				
Total	18.6 (100)	3.3 (100)	1.00 (100)	13.6 (100)	8.72 (100)	3.1 (100)	8.79 (100)	3.94 (100)	5.2 (100)
Total no. of mosquitos collected during all man-nights	447	79	24	217	767	100	211	63	1,134

[a]per 1 man-night, [b]No. of man-nights.

Table 4
Number (%) of *Anopheles* collected by animal bait collection in villages of Khammouane Province, December (2nd survey, dry season), 1999.

Species	No[a] (%) mosquitos collected				
	Around Thakhek	Boualapha			
	Thamlay (16)[b]	Napoung (14)	Koutboun (12)	Thapachon (7)	Total (33)
(Cellia)					
aconitus	0.3 (0.5)	1.4 (5.3)		0.9 (5.7)	0.8 (5.3)
annularis	0.1 (0.2)				
culicifacies		0.6 (2.2)		0.1 (1.0)	0.3 (1.9)
jamesii	0.1 (0.1)				
kochi	0.1 (0.1)	0.3 (1.1)		0.3 (1.9)	0.2 (1.3)
maculatus		1.1 (4.4)	0.25 (37.5)	0.7 (4.8)	0.7 (5.1)
minimus	0.8 (1.5)	15.6 (60.6)	0.17 (25.0)	4.9 (32.4)	7.7 (53.9)
nivipes	12.5 (24.2)	0.8 (3.1)		2.7 (18.1)	0.9 (6.3)
nivipes or phili.	11.3 (21.8)	0.2 (0.8)		0.3 (1.9)	0.2 (1.1)
notanandai		0.1 (0.3)			0.0 (0.2)
pallidus				0.4 (2.9)	0.1 (0.6)
pampanai	0.1 (0.2)				
philippinensis	20.4 (39.5)	0.9 (3.6)	0.08 (12.5)	0.9 (5.7)	0.6 (4.2)
sawadwongporni	0.4 (0.8)	3.6 (13.9)	0.08 (12.5)	1.6 (10.5)	1.9 (13.1)
vagus	0.4 (0.8)				
varuna				1.7 (11.4)	0.4 (2.5)
willmori	0.1 (0.1)	0.4 (1.7)			0.2 (1.3)
spp	1.2 (2.3)			0.1 (1.0)	0.0 (0.2)
(Anopheles)					
barbirostris gr	0.4 (0.8)	0.1 (0.6)	0.08 (12.5)	0.1 (1.0)	0.1 (0.8)
hyrcanus gr	3.2 (6.2)	0.5 (1.9)		0.1 (1.0)	0.2 (1.7)
umbrosus gr	0.3 (0.6)	0.1 (0.3)		0.1 (1.0)	0.1 (0.4)
Anopheles spp	0.1 (0.1)				
Total	51.7 (100)	25.7 (100)	0.67 (100)	15.0 (100)	14.3 (100)
Total no. of mosquitos collected during all animal-nights	826	360	8	105	473

[a]per 1 animal-night, [b]No. of animal-nights.

The parous rates of dominant species are shown in Table 9. In the 1st survey, 291 anopheline females (11 species) were dissected. The parous rate of *An. nivipes* was 35.7-56.8%; that of *An. dirus* was 33.3-50%. In the 2nd survey, 420 females (12 species) and 3 species groups of the subgenus *Anopheles* were dissected. The parous rates of *An. minimus* in Thapachon and Napoung were 75.9% and 50.2% respectively. In the 2nd survey, the parous rate was 75.9% in Thapachon and 50.2% in Napoung. In the 3rd survey, the parous rate of *An. dirus* was high in Koutboun (66.6%). Oocysts detected microscopically on the midgut of one of the ten *An. philippinensis* collected by animal bait in Napoung in the 1st survey were identified as *P. vivax* by PCR. By microscopy, oocysts on the midgut (abdomen with posterior portion) of one of the 391 *An. dirus* collected from Thapachon were found to contain mixed infection of *P. falciparum* and *P. vivax* by PCR. No *Plasmodium* parasites were detected by microscopy in the salivary glands (head and posterior thorax) of the same specimen. Sporo-

Table 5
Number (%) of *Anopheles* collected by human bait collection in villages of Khammouane Province, December (2nd survey, dry season), 1999.

Species	No[a] (%) mosquitos collected				
	Around Thakhek	Boualapha			
	Thamlay (48)[b]	Napoung (40)	Koutboun (24)	Thapachon (24)	Total (88)
(Cellia)					
aconitus		0.03 (2.9)			0.01 (1.7)
culicifacies				0.04 (4.2)	0.01 (1.7)
dirus		0.03 (2.9)			0.01 (1.7)
kochi				0.1 (12.5)	0.03 (5.2)
maculatus		0.03 (2.9)		0.1 (12.5)	0.05 (6.9)
minimus		0.6 (64.7)		0.2 (20.8)	0.3 (46.6)
nivipes		0.03 (2.9)		0.04 (4.2)	0.02 (3.4)
nivipes or phili.	0.02 (100)			0.1 (12.5)	0.03 (5.2)
philippinensis				0.2 (16.7)	0.05 (6.9)
sawadwongporni		0.2 (17.6)		0.2 (16.7)	0.1 (17.2)
(Anopheles)					
barbirostris gr		0.05 (5.9)			0.02 (3.4)
Total	0.02 (100)	0.9 (100)	0 (0)	1.00 (100)	0.7 (100)
Total no. of mosquitos collected during all man-nights	1	34	0	24	58

[a]per 1 man-night, [b]No. of man-nights.

zoites were not detected microscopically in any mosquitos. *Plasmodium falciparum* from one of the 63 *An. dirus* from Thapachon and *P. vivax* from two of the 13 *An. dirus* collected from Koutboun in 2000 (3rd survey) were detected by ELISA. Daily survival rates and vectorial capacities are shown in Table 9. The vectorial capacity and inoculation rate of *An. dirus* in Thapachon were 0.428 and 0.137 respectively; those in Koutboun were 0.385 and 0.052 respectively (3rd survey).

DISCUSSION

The infection rates of malaria in the present ACD survey were comparatively high: 7.0-21.4% in the villages around Thakhek; 22.1-54% in the villages of Boualapha district. In both the rainy and the dry seasons, the rates were almost the same, except in Koutboun. In all villages, *P. falciparum* was common; *P. vivax* and *P. malariae* were also found. On the basis of these results, it is clear that malaria is the most important health problem in the villages of the province.

Kobayashi *et al* (1997) recorded 19 *Anopheles* species in the province including *An. vagus*, *An. philippinensis*, and *An. nivipes* as the predominant species; immature *An. minimus*, *An. culicifacies*, *An. barbirostris*, and *An. maculatus* were also found. Kobayashi *et al* (2000) suspected *An. nivipes* to be the most important malaria vector in Nongceng, which is surrounded by the rice fields of the Nhommarath district.

The present entomological surveys, conducted twice in the rainy (June-August) and the dry season (December) in 1999, yielded 28 species: *An. aconitus* Doenitz, *An. annularis*

Table 6
Anthropophilous anopheline females collected by human and animal baits in Khammouane Province, July to August, 1999.

Species	Animal[a]	Human[b]	AI[c]
(Cellia)			
aconitus	1.265	0.375	0.297
annularis	0.353	0.013	0.035
dirus	0.265	0.669	2.526
kochi	3.118	0.113	0.036
maculatus	0.088	0.056	0.638
minimus	0.412	0.331	0.804
nivipes	110.0	4.119	0.037
philippinensis	9.912	0.488	0.049
sawadwongporni	0.088	0.056	0.638
tessellatus	0.176	0.025	0.142
vagus	9.059	0.069	0.008
(Anopheles)			
barbirostris gr	7.147	0.263	0.037

Animal bait and human bait collections were conducted for 34 animal-nights and 160 man-nights.
[a]No. Anophelines per animal-night, [b]No. Anophelines per man-night, [c]Anthropophilous index (AI=Human/Animal).

Table 7
Anthropophilous anopheline females collected by human and animal baits in Khammouane Province, December, 1999.

Species	Animal[a]	Human[b]	AI[c]
(Cellia)			
aconitus	0.592	0.007	0.012
culicifacies	0.184	0.007	0.040
kochi	0.143	0.022	0.154
maculatus	0.490	0.029	0.060
minimus	5.429	0.199	0.037
nivipes	4.694	0.015	0.003
philippinensis	7.082	0.029	0.004
sawadwongporni	1.408	0.074	0.052

Animal bait and human bait collections were conducted for 49 animal-nights and 83 man-nights.
[a]No. Anophelines per animal-night, [b]No. Anophelines per man-night, [c]Anthropophilous index (AI=Human/Animal).

Van der Wulp, *An. culicifacies* Giles, *An. dirus* Peyton and Harrison, *An. jamesii* Theobald, *An. kochi* Doenitz, *An. maculatus* Theobald, *An. minimus* Theobald (A), *An. nivipes* (Theobald), *An. notanandai* Rattanarithikul and Green, *An. pallidus* Theobald, *An. pampanai* Buettiker and Beales, *An. philippinensis* Ludlow, *An. sawadwongporni* Rattanarithikul and Green, *An. tessellates* Theobald, *An. vagus* Doenitz, *An. varuna* Iyengar and *An. willmori* (James) belonging to the subgenus *Cellia*; *An. barbirostris* Van der Wulp, *An. barbumbrosus* Strickland and Chowdhury, *An. campestris* Reid, *An. crawfordi* Reid, *An. hodgkini* Reid, *An. nigerrimus* Giles, *An. nitidus* Harrison, *An. paraliae* Sandosham, *An. peditaeniatus* (Leicester) and *An. sinensis* Wiedemann belonging to the subgenus *Anopheles*. Four species, *An. notanandai*, *An. sawadwongporni*, *An. willmori*, and *An. hodgkini*, had not been recorded before in Lao PDR. Adding these species to those previously recorded (Pholsena, 1992; Kobayashi *et al*, 1997, 2000; Vythilingam *et al*, 2001), it can be shown that the anopheline mosquito fauna of Lao PDR now comprised 45 species.

In the rainy season (July to August), *An. nivipes* was predominant, accounting for 74.2% and 36.6% of the total number of mosquitos caught in Thakhek and the Broualapa district respectively by animal bait collections. Considerable numbers of the *An. hyrcanus* group (38.2%) and *An. kochi* (11.0%) were also collected in Boualapha district. During human bait collections, *An. nivipes* was predominant (73.5% in Thakhek), while *An. dirus* (27.5%), *An. minimus* (12.0%), and *An. nivipes* (25.4%) were predominant in Boualapha.

In the dry season (December), the numbers of mosquitos collected by animal and human baits were small. One noteworthy finding, however, was that *An. minimus* was predominant in the human (46.6%) as well as the animal (53.9%) bait collections in Boualapha. Due to the low temperature in the province, small numbers of *Anopheles* mosquitos were collected in the dry season. The minimum temperature (10.5ºC) was recorded during the field survey in December at Thamlay, near Thakhek City, where only one anopheline mosquito was collected during 48 man-nights. In Koutboung, at 15ºC during the noctural surveys, no mos-

Table 8
Number and species of mosquitos found inside bednets in Boualapha district, Khammouane Province.

Bednet no.	Bednet condition[a]	Mosquito species (No.) collected
Thapachon		
1-1	poor	*Cx. vishnui* subgr (1)
5-2	exellent	*Cx. vishnui* subgr (1)
15-2	exellent	*An. dirus* (1)
16-2	poor	*An. dirus* (4) *An. philippinensis* (1), *Cx. vishnui* subg. (1)
103-3	poor	*An. dirus* (3)
107-1	poor	*An. dirus* (2)
108-1	poor	*Cx. whitmorei* (1)
124-1	poor	*An. philippinensis* (2)
124-4	normal	*An. dirus* (2), *Cx. vishnui* subgr (1), *Cx. whitmorei* (1)
Koutboun		
1	poor	*An. philippinensis* (1), *An. hyrcanus* gr (1), *An. nivipes* (6)
5	poor	*An. minimus* (4), *An. hyrcanus* g (2), *Ae. niveus* gr (1)
4	poor	*Ae. niveus* gr (2)
16	poor	*Cx. gelidus* (1)

In Thapachon and Koutboun villages, 92 and 75 bednets were examined, and mosquitos were collected from 9 and 4 nets, respectively.
[a)] Poor: old with holes; normal: available; excellent: new.

quito was encountered during 24 man-nights. The nocturnal biting activities of anopheline mosquitos in the province had decreased because of the temperature in December. They were inactive when the temperature fell below 15ºC after midnight. The predominant species of *Anopheles* varied according to topography, collection method, and season.

The common species collected by human bait were *An. dirus*, *An. minimus*, *An. nivipes*, and *An. philippinensis*. Of these, *An. dirus* had the highest AI (2.526), followed by 0.804 for *An. minimus* in the rainy season. The AIs of *An. nivipes* and *An. philippinensis* were 0.003-0.037 and 0.004-0.049 respectively in rainy and dry seasons. *Anopheles dirus* was collected at the rate of 0.8-1.6 per man-night in the rainy season (1st), while *An. minimus* was collected at the rate of 0.2-0.6 per man-night in the dry season (2nd survey) in Boualapah. The parous rate of the common species, *An. dirus*, was 33.3-66.7% in rainy seasons; that of *An. minimus* was 50.2-75.9% in the dry season in Boualapha. Oocysts on the midgut of one female *An. dirus* from Thapachon were found by microscopy, and a mixed infection of *P. vivax* and *P. falciparum* was detected by PCR in the same midgut, following preservation in ethanol. Sporozoites were confirmed in one *An. dirus* from Thapachon, and in 2 *An. dirus* from Koutboun; they were identified as *P. vivax* and *P. falciparum* respectively by ELISA.

The number of *An. dirus* collected per man-night was 5.76 in Thapachon in August 2000 (3rd survey); the parous rate was 48.3%. The vectorial capacity and inoculation rate of *An. dirus* were 0.428 and 0.137 respectively in Thapachon; in Koutboun, these values were 0.385 and 0.052. The mosquito numbers collected in each season, the AIs and the parasite infection rates considered as a whole suggest that malaria transmission is caused by *An. dirus* in the rainy season and by *An. minimus* in the dry season, especially in Boualapha district. *Anopheles dirus* is the most important vector in the countries that border Lao PDR (Meek,

Table 9
Parity, daily survival rate, vectorial capacity, and inoculation rate of females *Anopheles* in Khammouane Province, 1999 and 2000.

Anopheles *(Cellia)*	No. mosquitos /man-night	No. mosquitos examined[a]	Parous rate (%)	Daily survival rate	Vectorial capacity	Inoculation rate
1st survey (Aug, 1999)						
Nongceng						
nivipes	14.5	39	38.5	0.727	0.328	0
philippinensis	1.75	15	40.0	0.737	0.048	0
Thamlay						
nivipes	2.13	28	35.7	0.709	0.033	0
Napoung						
dirus	1.56	27	48.1	0.784	0.114	0
nivipes	1.06	37	56.8	0.828	0.192	0
Koutboun						
dirus	0.83	9	33.3	0.693	0.009	0
minimus	1.75	20	40.0	0.737	0.048	0
nivipes	1.96	22	40.9	0.742	0.061	0
Thapachon						
dirus	2.06	12	50.0	0.794	0.184	0
nivipes	0.88	8	50.0	0.794	0.079	0
2nd survey (Dec, 1999)						
Napoung						
minimus	0.55	203	50.2	0.795	0.050	0
nivipes	0.03	11	54.5	0.817	0.004	0
philippinensis[b]	0	10	40.0	0.737	0.000	0
Thapachon						
minimus	0.21	29	75.9	0.912	0.249	0
nivipes	0.04	11	81.8	0.935	0.088	0
3rd survey (Aug, 2000)						
Thapachon						
dirus[c]	5.76	464	48.3	0.785	0.428	0.137
minimus	0.30	13	69.2	0.885	0.186	0
nivipes	2.21	152	30.3	0.671	0.015	0
philippinensis	1.60	125	39.2	0.732	0.040	0
Koutboun						
dirus[d]	0.80	31	66.6	0.873	0.385	0.052
minimus	1.55	48	47.9	0.783	0.110	0
nivipes	4.40	125	39.2	0.732	0.110	0
philippinensis	0.70	26	26.9	0.646	0.003	0

[a]Mosquitos were dissected microscopically and examined for oocysts and sporozoites. After detection of malaria, identification of the species was carried out by PCR. In 63 of 464 *An. dirus* from Thapachon and 13 of 31 *An. dirus* from Koutboun in the 3rd survey, sporozoites were examined directly by ELISA, without confirmation of oocysts and sporozoites by dissection.

[b]Oocysts from one *An. philippinensis* collected by animal bait were detected and identified as *P. vivax* by PCR.

[c]Oocysts of *P. vivax* and *P. falciparum* from one of 391 *An. dirus* were detected by microscopy and identified by PCR, and sporozoites of *P. falciparum* from one of the other 63 *An. dirus* were detected by ELISA.

[d]Sporozoites from one of 13 *An. dirus* were detected by ELISA.

1995): Thailand (Gingrich *et al*, 1990; Rosenberg *et al*, 1990), Cambodia (Baker *et al*, 1987), and Myanmar (Tun-Lin *et al*, 1995, Htay-Aung *et al*, 1999). *Anopheles minimus* is also an important vector during the dry season in Thailand (Harbach *et al*, 1987; Rattanarithikul *et al*, 1996). In the present survey, the oocysts of *P. vivax* were detected on the midgut of one *An. philippinensis* from Boualapha. *Anopheles philippinensis* and *An. nivipes* are zoophilic, and sporozoites have been detected from these species in Thailand (Rattanrithikul *et al*, 1996), India (Covell, 1944; Ganguli *et al*, 1947), Pakistan (Quraishi *et al*, 1951) and Myanmar (Feegrade, 1926). Kobayashi *et al* (2000) suspected *An. nivipes* to be a vector in the paddy field areas in Nongceng village, Khammouane Province, as it was mostly attracted to human bait. *Anopheles philippinensis* and *An. nivipes* might play a part in malaria transmission in paddy field areas.

Uza *et al* (2002) carried out a questionnaire study of malaria-related knowledge and behavior in the villages featured in the present study. It was clearly demonstrated that even though most (73%) of the villagers kept and used bednets, their knowledge of the mosquito transmission of malaria was low (40%) and the incidence of malaria, at least in Boualapha, was comparatively high. The survey of the use of mosquito nets and an entomological study conducted in Thapachon in 2000 revealed that most of the bed nets used by villagers were in bad condition and were offen holed. Many mosquitos, including the important, *Anopheles dirus* and *An. minimus*, penetrated the nets and were collected by early morning catches. *An. dirus* was strongly anthropophilic and its feeding activities were at their greatest around midnight, when inhabitants had fallen into deep sleep. It seems that *An. dirus* in the high-transmission Boualapha district are late biters, a feature that could be exploited to control malaria or vectors by the correct use of bed nets, which would ideally be impregnated with insecticide. It is also important to develop educational materials that will strengthen the practice of and knowledge about malaria prevention in villages.

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