COLLECTION OF ANOPHELINE MOSQUITOS IN THREE VILLAGES ENDEMIC FOR MALARIA IN KHAMMOUANE, LAO PDR

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Abstract. Anopheline mosquito collections were made during the months of May and June (dry season) and August (rainy season), 1996 at villages, Nakham, Namdik and Hinboon Neua in Khammouane Province. Larval collections were also made in 3 slow running streams around Nakham villages in May 1996. Altogether, 3,549 adult females consisting 19 *Anopheles* species were collected by 3 nights of human and 6 animal bait traps and 3 resting collections. Among them, *Anopheles vagus* (19.75%), *An. philippinensis* (15.02%), *An. nivipes* (11.55%), *An. pallidus* (11.27%), *An. peditaeniatus* (8.34%), *An. kochi* (8.00%) and *An. barbirostris* (7.27%) were predominant species. The immatures of *An. barbirostris, An. minimus, An. maculatus* sensu lato, and *An. culicifacies* were found in the streams of Nakham. It is noteworthy that *An. minimus, An. dirus* and *An. maculatus* sensu lato, which are well known malaria vectors in Thailand, were recorded for the first time in this area.

INTRODUCTION

The malaria network for Khammouane Province has been supported from 1995 by the Japan International Cooperation Agency (JICA), Primary Health Care (PHC) project with cooperation of the Institute of Malariology, Parasitology and Entomology (IMPE) in Vientiane, Lao PDR. According to the parasitological survey carried out from October 1995 to August 1996, in Nakham and Namdik villages located in the limestone forest shallow valley, malaria parasites were detected in about 7% of the villagers and many cases have been recorded in Hinboon district hospital, Mahaxay district hospital and the provincial hospital in Khammouane, Lao PDR. However, study of the malaria vector mosquitos in this region has not been carried out until the present time. In the present study, mosquito collections were made in three villages, Nakham, Namdik and Hinboon Neua where the malaria parasitological survey was done by us.

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MATERIALS AND METHODS

Khammouane Province (Fig 1) is located at about 350 km south-east from Vientiane. The following 3 villages were chosen for mosquito collection. Nakham village, Mahaxay district situated in a limestone mountain valley, 30 km east of Thakhek city, the capital of Khammouane province. The valley is 50 to 100 m above sea level. Namdik village in Hinboon district is located near the limestone mountain forest, about 30 km northwest of Thakhek city. Around these villages there are many streams in the forest and rice fields and the number of streams increased greatly in the rainy season. Hinboon Neua village is located along the Mekong river and is enclosed by river and rice fields. Much of the original forest in the valley has been cleared by logging operation of for agriculture. The climate is rainy from June to October, dry from November to May. There is no electricity in the villages. The houses are made of wood and bamboo, being constructed at 1 to 2 meters above ground to avoid flood in the rainy season.

According to our investigation, the populations of Nakham, Namdik and Hinboon Neua are 402,

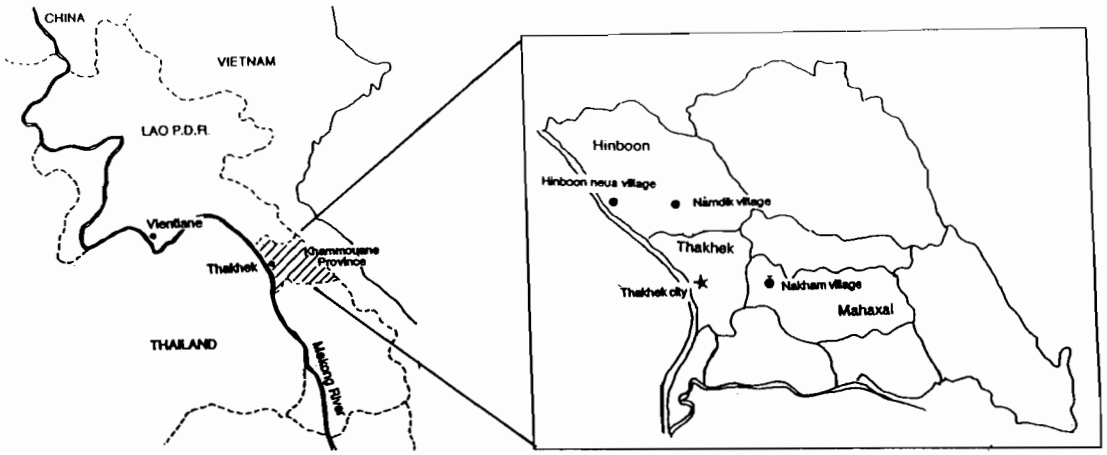

Fig 1–Three villages where mosquito collection were carried out in Khammouane Province, Lao PDR. The one village, Hinboon Neua is located along Mekong River and a few cases of malaria were detected. While other two villages, Nakham and Namdik located in forest were malaria positive, being about 5-8% of the villagers.

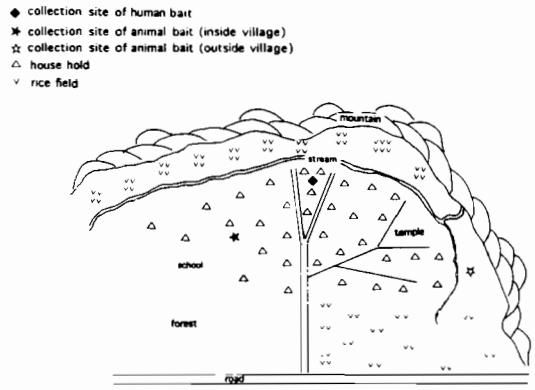

Fig 2–Collection site of mosquito in Nakham village, Mahaxay.

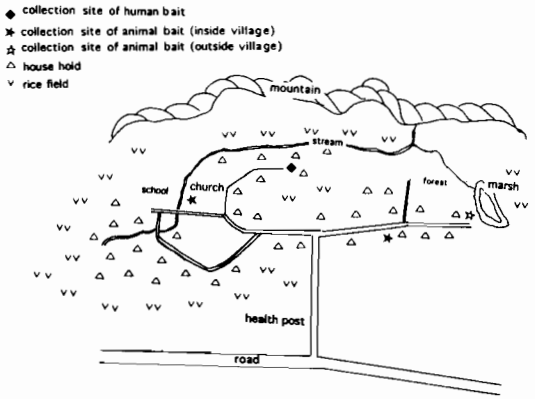

Fig 3–Collection site of mosquito in Namdik village, Hinboon.

449 and 439 respectively. The people make their living by farming of rice fields, hunting animals in the forest and fishing in the pond and river. Cattle, buffalos and pigs are the commonest large domestic animals in the villages where about 150 cattle and 160 buffalos were gazing on the hillside in the daytime and staying around their owners' houses in the night.

Mosquitos were collected in Nakham, Namdik and Hinboon Neua villages by the following conventional methods on 16-18 May, 11-13 June and 19-22 August 1996.

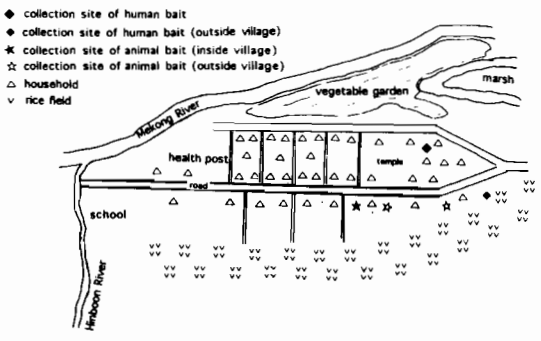

Fig 4–Collection site of mosquito in Hinboon Neua village, Hinboon.

Animal bait collection

Large net (4 × 4 × 2 m) was put up around the village and a cow or buffalo was used as bait animal restrained inside it throughout the night from 7 pm to 5 am. All mosquitos in the net were collected 3 times 21.00, 23.00 and 05.00 hours by sucking tube. The collections were made for 4 nights in each the 3 villages.

Human bait collection

Two collectors, sitting outdoor from 19.00 - 05.00 hours collected female mosquitos biting their legs by sucking tube. The collections were made 4 nights in each village.

Resting collection

Mosquitos resting on the wall inside the house were collected by two persons using sucking tubes from 05.00 to 06.00 hours in the 3 villages.

Immature collections

Collections of immatures (3rd and 4th instar larvae and pupa) were done in 3 slow running streams (cave stream, stream 1 and 2) around Nakham village on 16-19 May (Fig 5). The water of the stream was derived from the cave located about 200 m east of Nakham village. The water in the stream was slightly polluted by animal and human excretions and the water volume was apparently reduced, but according to the village people, the stream never dried up throughout the year. Stream 1 is a forest clean water stream, about 1 km from the villages and is the source of water supply for the villagers. The volume of the water was small but never dried up even in the dry season. Stream 2, located 8 km east of Nakham village and flowing in the foot of the mountain, had a considerable amount of water but dried up in the dry season. The water was unpolluted. Using dipper and pipette, collections were made along about 100 m of the streams for 30 minutes by 3 collectors. The immatures were reared in the laboratory to the adults for identification. The female *Anopheles* mosquitos were identified using the key prepared by Malaria Center 2, Division of Communicable Disease Control, Ministry of Health, Thailand, with reference to the following literature; Buttiker *et al* (1959), Colless (1956), Harrison (1980), Peyton and Scanlon (1966), Reid (1953, 1968).

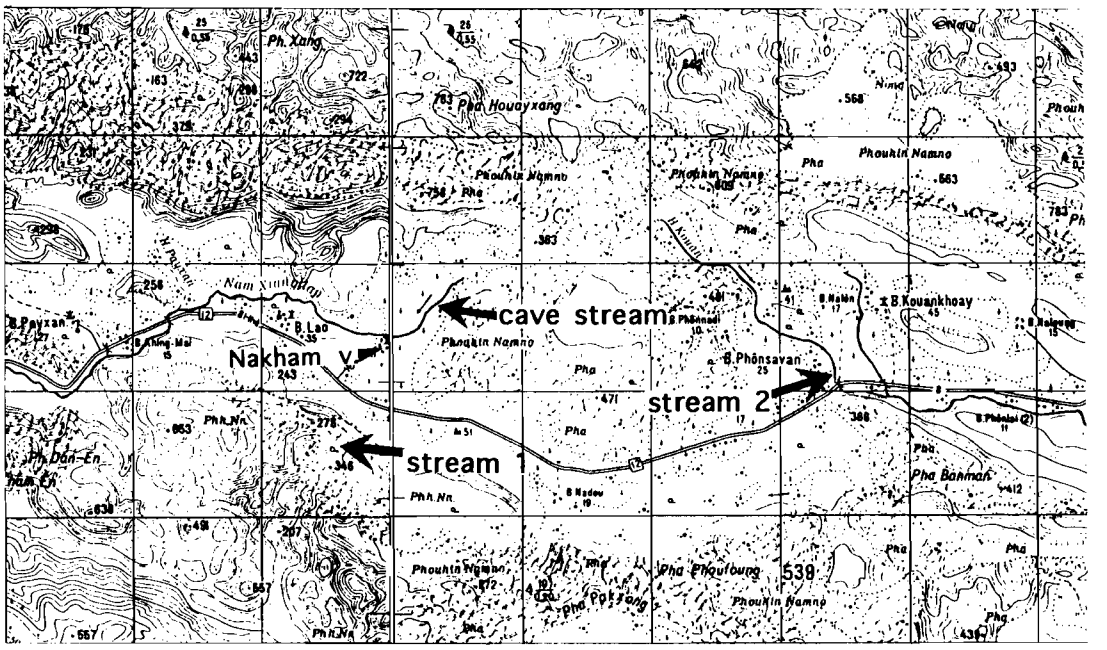

Fig 5–Map showing 3 stream for immature collection around Nakham village.

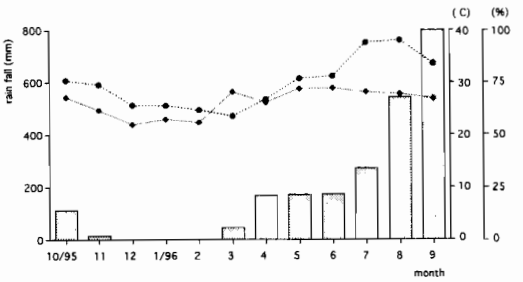

Fig 6–Rain fall, temperature and humidity in Thakhek city, Khammouane Province from October, 1995 to September, 1996.

rain fall ···◆··· temperature --●-- humidity

Table 1

Collection of the immatures of Anopheline mosquitos in 3 streams around Nakham village, Mahaxay district, 16-19 May, 1996.

Species collected	Cave stream	Stream 1	Stream 2
An. minimus	0	2	11
An. maculatus	5	3	1
An. culicifacies	1	0	0
An. barbirostris	5	0	13
Total	11	5	25

RESULTS AND DISCUSSION

During 19 nights of collecting, 3,549 anopheline female mosquitos were captured. The collections contained 19 species of *Anopheles; Anopheles (Anopheles) argyropus* Swellengrebel, *An. (Ano.) barbirostris* Van der Wulp, *An. (Ano.) barbumbrosus* Strickland and Chowdhury, *An. (Ano.) nigerrimus* Giles, *An. (Ano.) peditaeniatus* Leicester, *An. (Ano.) umbrosus* group, *An. (Cellia) aconitus* Doenitz, *An. (Cel.) annuralis* Van der Wulp, *An. (Cel.) dirus* Peyton and Harrison, *An. (Cel.) jeyporiensis* James, *An. (Cel.) kochi* Doenitz, *An. (Cel.) maculatus* sensu lato, *An. (Cel.) minimus* Theobald, *An. (Cel.) nivipes* Theobald, *An. (Cel.) pallidus* Theobaldo, *An. (Cel.) philippinensis* Ludlow, *An. (Cel.) subpictus* Grassi, *An. (Cel.) tessellatus* Theobald, and *An. (Cel.) vagus* Doenitz. The most dominant species were *An. vagus, An. philippinensis, An. nivipes* and *An. pallidus*, accounting for 57.6% of the specimens. *An. pediateniatus, An. kochi, An. barbirostris* and *An. barbumbrosus* were next in abundance. Most of them were zoophilic and attracted to the bait animals in the rainy season. The other 11 species were collected in low numbers. The numbers of mosquitos collected in May (dry season) by 2 night bait collections were 0 for human and 185 for animal at Namdik, and 0 and 187, respectively, at Nakham; in August (rainy season), the numbers were 6 for human and 946 for animal baits at Namdik, and 9 and 367 respectively at Nakham. In the Hinboon Neua, 1 for human and 758 for animal baits were collected in June, while 10 for human and 1,051 for animal were collected in August. Among a total of 21 females comprising 5 species collected by 12 nights of human bait collections in the 3 villages, 13 were *An. nivipes*, 4 were *An. dirus*, 2 were *An. palidus*, and 1 each of *An. minimus* and *An. philippinensis*. In the (162 nights) animal bait collections from the villages, there were 63 *An. maculatus* sensu lato, 14 *An. dirus* and 26 *An. minimus* which are the principal vectors of malaria in Thailand. Due to many cattle and buffalos staying around human dwellings during in the night, only a small number of the female mosquitos might be attracted to the human bait.

Four species of *Anopheles* were identified from a total of 41 larvae and pupae collected in the 3 streams. These included 18 *An. barbirostris*, 13 *An. minimus*, 9 *An. maculatus* sensu lato., and 1 *An. culicifacies* Giles (Table 1). Rattanarithikul *et al* (1994) found larvae of *An. minimus* A, *An. speudowillmori* (Teobald), one of the *maculatus* complex, and *An. culicifacies* B during a dry season in the Mekong River, North eastern Thailand. In spite of our limited larval survey, considerable numbers of *An. minimus* and *An. maculatus* were collected in the stream margins in hilly regions. The character of the rivers in Khammouane Province changes greatly between rainy and dry seasons. In the rainy season, rivers and streams are flooding and the breeding habitats of the mosquitos are expanded greatly and hence difficult to find the stream breeders of these anopheline larvae. During the late dry season, most of the rivers shrink or dry up and the breeding places are limited. However, once suitable breeding habitats such as stream margins, sand

pools next to streams, seepage pools in springs in hilly regions, were found, *An. minimus, An. maculatus* and *An. culicifacies* may be collected easily. *An. dirus* is known as a primary vector and a forest dwelling mosquito, breeds in small, fresh temporary pools and its numbers have been observed to peak during the rainy season in Thailand (Gingrich *et al*, 1990; Rosenberg *et al*, 1990; Scanlon and Sandhinand, 1965; Wilkinson *et al*, 1978). We did not find the larval habitat of *An. dirus* during this study. However, several females were found in the collected by human and animal baits during the rainy season. *An. minimus* is the other main malaria vector in Thailand. It is mainly found in forest and foothill areas where stream water cleared are running slowly. At a shallow valley in northern Thailand, because of both its relatively high density and high feeding index to human bait, *An. minimus* was the most important malaria vector in the study area (Takagi *et al*, 1995). *An. maculatus* group includes 8 nominal forms of a sibling species complex which are morphologically are genetically distinguishable species in Thailand (Rattanarithikul and Green, 1987). *An. pseudowillmori*, a member of the group, is an important vector at Mae Sot in northwestern Thailand, but other members of the group in the same area have not yet been found to be infected with *Plasmodium* (Green *et al*, 1991). Due to the difficulty of correct identification of the members of this group, the specimens collected in this survey were treated as *An. maculatus* sensu lato. In the present survey, *An. philippinensis* was one of the most abundant zoophilic anophelines. Its sister species, *An. nivipes* was also attracted the bait animals as well as human. *An. philippinensis* has been found infected with human malaria parasites in India (Covell, 1944; Ganguli, 1947). *An. nivipes* was implicated as a vector based on the detection of sporozoite infections using ELISA for *Plasmodium falciparum* and *P. vivax* in the Karen village in northwestern Thailand (Harbach *et al*, 1987).

Although this is a fragmental mosquito study, there is no published information about the distribution of the malaria vectors in Khammouane Province. It is noteworthy that considerable numbers of very important vector species, *An. dirus, An. minimus* and *An. maculatus*, were recorded for the first time in an endemic area of malaria in Khammouane.

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