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Notes on the blood sources of vector mosquitoes collected at a remote village of north-western Thailand (Diptera : Culicidae)

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Introduction

The mosquitoes have been well known to get their blood-meals from some domestic and wild animals together from human[1),2),3)], but the knowledge on the blood source animals of the vector mosquitoes in Thailand are still incomplete, although many ecological and epidemiological studies on the vector mosquito species, with respect to such diseases as filaliasis, dengue, malaria and Japanese Encephalitis, were carried out in Thailand[4),5)].

The authors investigated the blood sucking behaviour of the 4 species of the vector mosquitoes by means of the animals baited mosquito-net traps at a remote Karean village in Tak province, north-western Thailand (Fig. 1) in the winter season of 1989 and determined the blood-meals of the captured mosquitoes by the use of the enzyme-linked immunosorbent assay (ELISA).

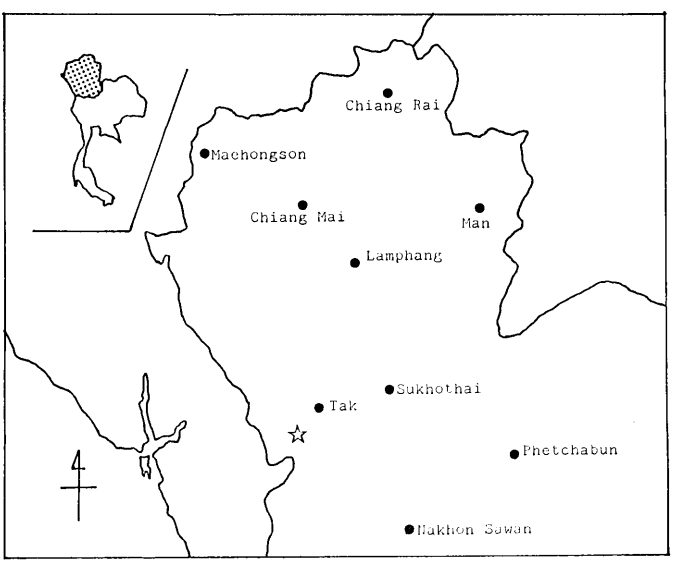

Fig. 1. Map of Thailand ☆ shows the investigated place.

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Methods

1. Collection of the mosquitoes

3 m × 4 m × 2 m of Nylon mosquito-net was used as a trap, each with a different

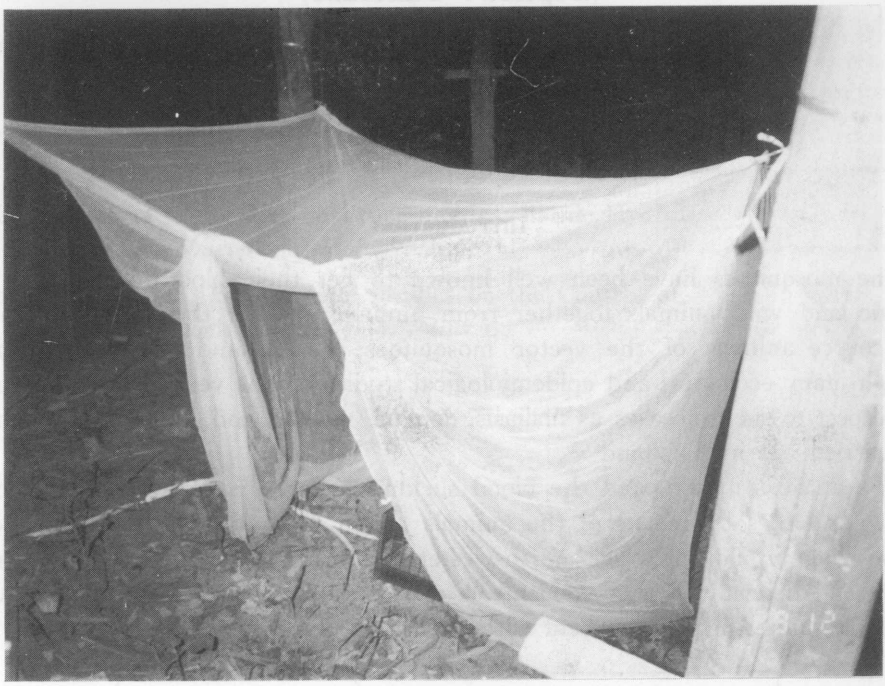

Fig. 2. Chicken baited mosquito-net trap.

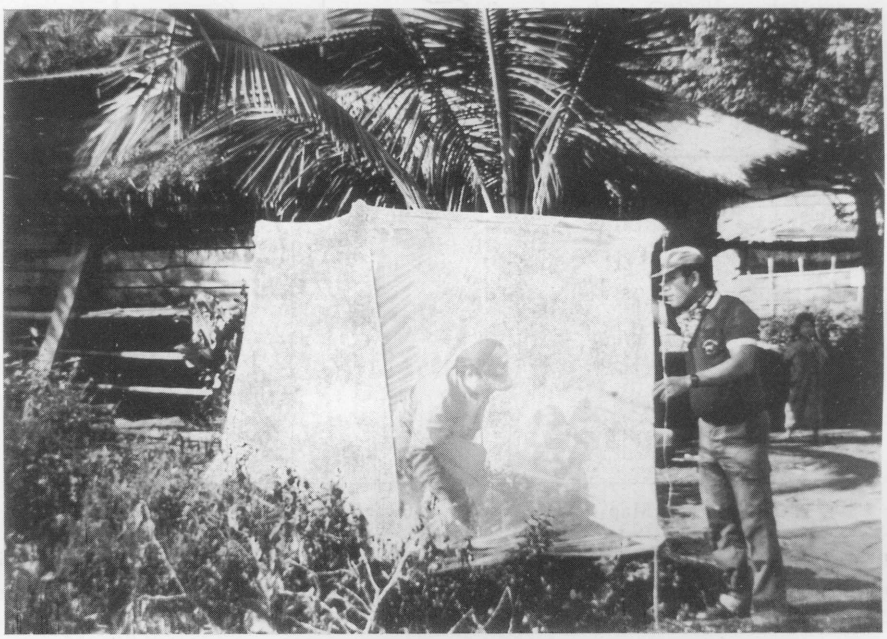

Fig. 3. Modified bush trap.

bait species (Fig. 2). Four animal species were used as bait, namely human, cow, pig and chicken. The traps were situated at a distance from each other to eliminate any interfering or influencing effect.

The traps were set in the evening from 18 : 00 to next 9 : 00 the following morning, and the lured mosquitoes were collected 3 times daily at 21 : 00, 6 : 00 and 9 : 00 from Dec. 7th to 11th of 1989.

Modified bush traps (Fig. 3) were also used to collect the resting mosquitoes.

2. Blood source determination

The procedures of the blood-meal determination were performed in accordane with Sasaki[6], with the following modifications: The captured mosquitose were identified into species and counted their number and pressed onto the filter paper (Whatman No. 1) individually in order to get their blood-meals.

The filter papers were dried with diphosphoros pentaoxide in a desiccator and carried back to the laboratory.

The smeared part of the filter papers were cut off and put in small tubes individually and stored in a freezer until the assay.

Blood smeared filter papers were dipped in 400 μl of physiological saline (pH =7.0) for 2 hr, then disassembled with an insect pin to extract smears (blood-meal) completely. The solutions were then centrifuged at 5,000 rpm for 5 min.

Forty μl of extract were put into each well of a Millititer HA plate (Millipore Co.) or a flat bottomed plastic microplate individually and incubated for 2 hr. The plate was then washed three times with washing buffer (0.1% of Tween 20 dissolved in phosphate buffered saline (PBS pH=7.2)). Then 80 μl of coating buffer (4% bovine serum dissolved in PBS when anti-chicken serum were used and 4% chicken serum dissolved in PBS when anti-mammalian sera were used) were added to each well and incubated for 1 hr. The plate was then washed three times with washing buffer again. Forty μl of antiserum were then added to each well and reacted for 2 hr. The plate was washed three times with washing buffer and 40 μl of the conjugate (commercial horse radish peroxidase conjugated to the IgG fraction of goat anti-rabbit serum) were added to each well. Two hr after the addition of the conjugate, the plate was rewashed 6 times with washing buffer and then 80 μl of substrate solution (40 mg of *o*-phenylene diamine dissolved in 100 ml of citric acid-Na_2HPO_4 buffer solution (pH=5.0) and 20 μl of H_2O_2) were added to each well. The plate was then incubated in a dark box. The reaction was stopped 15 min later by the addition of 40 μl of 4N sulfuric acid. All procedures of assay used a Millititer HA plate were performed at room temperature and those used a plastic microplate were did at 37°C.

The results were visually assessed and the yellow colour produced in the wells of tested extracts was compared with the colour of the positive and negative controlled wells.

Results and Discussion

The weather of during the collecting period was consistently fine and the temperature and humidity data of the investigated area are shown in Table 1. The

range of temperature during the investigation was from 12 to 32°C.

Table 1. Temperature and humidity data at Tak, Thailand

date	temperature		at 21 : 00		at 6 : 00	
	max.	min.	humid.	temp.	humid.	temp.
07/Dec.	27.0	15.0	7.30	19.5		
08/	28.0	14.0	74.0	17.4	85.6	14.8
09/	30.0	14.0	73.5	18.3	90.5	15.6
10/	32.0	12.0	80.6	15.6	91.8	14.9
11/					90.2	13.8
Average	29.3	13.8	75.3	17.7	89.5	14.8

A total of 1,054 individuals of mosquitoes, including 176 individual vector mosquitoes, belonging to 2 genera and 4 specie, were collected. From this study, it is determined that the trap used a cow as bait was the most effective and that, 18 : 00 to 21 : 00 is, generally, the most effective time to collect mosquitoes (Table 2, 3). Specificially concerning the 4 vector species however, the most effective time of collect was found to be night time. This disagreement may due to the fact that the most dominant species among the 4 vector species active during the night time.

Table 2. Number of mosquitoes collected at Tak, Thailand

	human	swine	chicken	cow	buse	Total
Aedes niveus	1	0	0	5	1	7
Culex fuscocephala	1	0	0	138	1	140
Cx. gelidus	1	0	0	3	0	4
Cx. tritaeniorhynchus	0	1	0	26	0	27
Others	12	9	3	844	8	876
Total	15	10	3	1016	10	1054

Table 3. Number of mosquitoes collected at Tak, Thailand

	6 : 00-9 : 00	18 : 00-21 : 00	21 : 00-6 : 00	Total
Aedes niveus	0	5	1	6
Culex fuscocephala	40	57	42	139
Cx. gelidus	0	2	2	4
Cx. tritaeniorhynchus	7	9	11	27
Others	125	543	200	868
Total	172	616	256	1044

Among the 4 vector species, *Culex fuscocephala* was the most dominant species (140 indiv., 78.7%) collected in this study. Of the other 3 species, a smaller number of individuals was collected.

A total of 127 individuals out of 178 vector mosquitoes had blood-meals and most engorged individuals were collected by the trap used a cow as bait. No mosquitoes collected from the human- and pig-baited traps had any blood-meals in their alimentary canals.

The engorged rates of the 4 vector species collcted by the cow-baited trap

Table 4. Engorged number of mosquitoes collected at Tak, Thailand

	human	swine	cow	bush	Total
Aedes niveus	0/1	—	5/5	1/1	6/7
Culex fuscocephala	0/1	—	94/138	1/1	95/140
Cx. gelidus	0/1	—	2/3	—	2/4
Cx. tritaeniorhynchus	—	0/1	24/26	—	24/27
Total	0/3	0/1	125/172	2/2	127/178

Table 5. Engorged number of mosquitoes collected at Tak, Thailand

	6:00-9:00	18:00-21:00	21:00-6:00	Total
Aedes niveus	—	4/5	1/1	5/6
Culex fuscocephala	27/40	32/57	35/42	94/139
Cx. gelidus	—	1/2	1/2	2/4
Cx. tritaeniorhynchus	6/7	7/9	11/11	24/27
Total	33/47	44/73	48/56	125/176

Table 6. The blood sources os mosquitoes collected at Tak, Thailand

	No. of collected	engorged (%)	blood sources
Aedes niveus	7	6 (85.7)	cow (2)
			human+cow (2)
			human+cow+swine (1)
			chicken (1)
Culex fuscocephala	140	95 (67.9)	cow (58)
			swine (2)
			human+cow (7)
			cow+swine (4)
			human+cow+swine (4)
			inidentified (3)
Cx. gelidus	4	2 (50.0)	cow (1)
			cow+swine (1)
Cx. tritaeniorhynchus	27	24 (88.9)	human (1)
			cow (14)
			cow+swine (8)
			human+cow+swine (1)

varied from 50% to 88.9% (Table 4). In *Cx. fuscocephala*, the highest engorged rate was obtained from the specimens collected at 6 : 00 (lured during from 21 : 00 to 6 : 00) (Table 5). This species is well known to be active during the night time, as mentioned above, and this concurs with the results obtained in this investigation.

In one hundred and twenty four out of 127 individuals of the 4 vector species, the origins of their blood-meals were determined to be human, bovine, swine or chicken. The multiple feeding was observed in 43 individuals of the 4 species (Table 6).

Almost all individuals of 4 vector species examined had cow blood in their alimentary canals, except of one *Cx. tritaeniorhynchus* individual and one *A. niveus* individual. These were found to contain only human and chicken blood, respectively.

In many of the individual mosquitoes, there was both human and cow blood through the mixture in some included swine blood.

From these results obtained in this investigation, the role of domestic animals in the reproduction of the 4 vector species was clarified.

A program for the control of these vector species should be based upon the results of this study.

Acknowledgements

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摘　　要

タイ国西部ターク県メソット市郊外のカレン族の集落で，ヒト，ウシ，ブタ，ニワトリを用いた動物おとり蚊帳トラップ法と，ブッシュトラップ法で，1989年12月，蚊の採集調査を行なった。

その結果，フィラリア症や日本脳炎(JE)の媒介種である *Aedes niveus*, *Culex fuscocephala*, *Cx. gelidus*, *Cx. tritaeniorhynchus* の4種176個体を含む1,044個体を得た。

媒介種4種について，その blood-meal の同定を ELISA で行い，62.5% にあたる110個体について，その吸血源動物を明らかにした。検討した個体の中には，ヒト，ウシ，ブタの血液をその blood-meal に同時に含むものもあり，これら媒介種の繁殖に及ぼす家畜の役割が改めて確認された。