Notes on the Blood Sources of Vector Mosquitoes Collected at a Remote Village of Northwestern Thailand (Diptera: Culicidae)

Usavadee Thavara[1]
Chitti Chansang[1]
Apiwat Tawatsin[1]
Prakong Phan-Urai[1]
Hitoshi Sasaki [2]

1 Division of Medical Entomology, National Institute of Health, Department of Medical Sciences.

2 Department of Dairy Science (Applied Entomology), Hokkaido College of Arts and Sciences, Japan.

Published in Journal of Rakuno Gakuen University Vol. 16(1): 1-7, 1991.

บทคัดย่อ

ในประเทศไทยมีรายงานการศึกษาเกี่ยวกับพฤติกรรมการกินเลือดของยุงพาหะ เฉพาะในยุงพาหะโรคไข้เลือดออก และไข้มาลาเรีย ขณะที่ยุงพาหะนำเชื้อโรคอื่น เช่น ไข้สมองอักเสบและโรคเท้าช้างไม่ค่อยมีผู้ศึกษา เนื่องจากมีปัญหาเกี่ยวกับความยุ่งยากของวิธีการและพื้นที่ศึกษามีความเสี่ยงต่อการติดเชื้อสูง เพื่อแก้ปัญหาการขาดข้อมูลที่จะนำไปใช้ในการวางแผนควบคุมโรค คณะผู้วิจัยจึงได้ศึกษาพฤติกรรมการกัดของยุงพาหะ แหล่งเลือด และช่วงเวลาที่กัดในพื้นที่เสี่ยงต่อการติดเชื้อโรคเท้าช้าง และไข้สมองอักเสบ โดยวิธี animal baited traps และ bush trap และตรวจเลือดโดยวิธี ELISA ผลการศึกษาพบว่า ในบรรดายุง 1,054 ตัว ที่จับได้ เป็นยุงพาหะ 178 ตัว (16.9%) จำแนกเป็นยุงพาหะโรคเท้าช้าง 1 ชนิด คือ *Aedes niveus* และยุงพาหะโรคไข้สมองอักเสบ 3 ชนิด คือ *Culex tritaeniorhynchus, Cx. gelidus* และ *Cx. fuscocephala* ช่วงเวลาที่จับยุงได้มากที่สุดคือ ช่วง 18.00-21.00 น. พบยุงพาหะที่กินเลือดแล้ว 127 ตัว (71%) ผลการตรวจเลือดพบว่ายุงที่กินเลือดเกือบทั้งหมดมีเลือดวัวอยู่ด้วย และยุง 43 ตัวมีเลือดสัตว์หลายชนิดรวมกัน รวมทั้งเลือดคน แสดงว่ามีการกินเลือดหลายครั้ง (multiple feeding) และสัตว์ที่ดึงดูดยุงได้มากที่สุดคือวัว ข้อมูลนี้แสดงให้เห็นว่า การที่คนมีโอกาสรับเชื้อโรคจากสัตว์เกิดจากพฤติกรรมของยุงที่กินเลือดหลายครั้ง โดยกัดสัตว์แล้วมากัดคน เกิดเป็นวงจรการแพร่เชื้อ การแก้ปัญหาโดยอาศัยข้อมูลเกี่ยวกับชีววิทยาของยุงพาหะช่วยให้เกิดประสิทธิภาพสูงสุดในการควบคุมโรค

Abstract

Studies of blood-feeding behavior of mosquito vectors of Dengue Haemorrhagic Fever (DHF) and malaria have been continuously carried out in Thailand, whereas those of encephalitis and filariasis are scanty because of difficulty methodology and high risk of infection. To get more information for disease control, we then studied on blood sources, biting time and behavior of mosquito vectors in an endemic area of encephalitis and filariasis. Animal-baited

traps and bush trap were employed for mosquito collections and ELISA technique was used for blood source determination. It was found that 178 (16.9%) out of 1,054 collected mosquitoes were identified to filariasis vector (*Aedes niveus*) and Japanese encephalitis vectors (*Culex tritaeniorhynchus, Cx. gelidus* and *Cx. fuscocephala*). Most of the mosquito vectors were collected between 1800 h and 2100 h, and 127 (71%) were engorged-mosquitoes. The ELISA results showed that cow blood was determined from almost all engorged-mosquitoes and multiple blood sources, including human blood were identified from 43 individual mosquitoes. This means cow was the most attractive host for vector mosquitoes in this study and multiple feedings also occurred. The study reveals that multiple feeding behavior of mosquito vectors could play an important role of pathogen transmission between animals/insects and human beings. Biological and ecological information is then helpful for effective control of vector-borne diseases.

Keywords

Blood sources, mosquitoes, Thailand

Introduction

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

The authors investigated the blood suckling behaviour of 4 species of the vector mosquitoes by means of the animal baited traps at a remote Karean village in Tak province, northwestern Thailand (Figure 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).

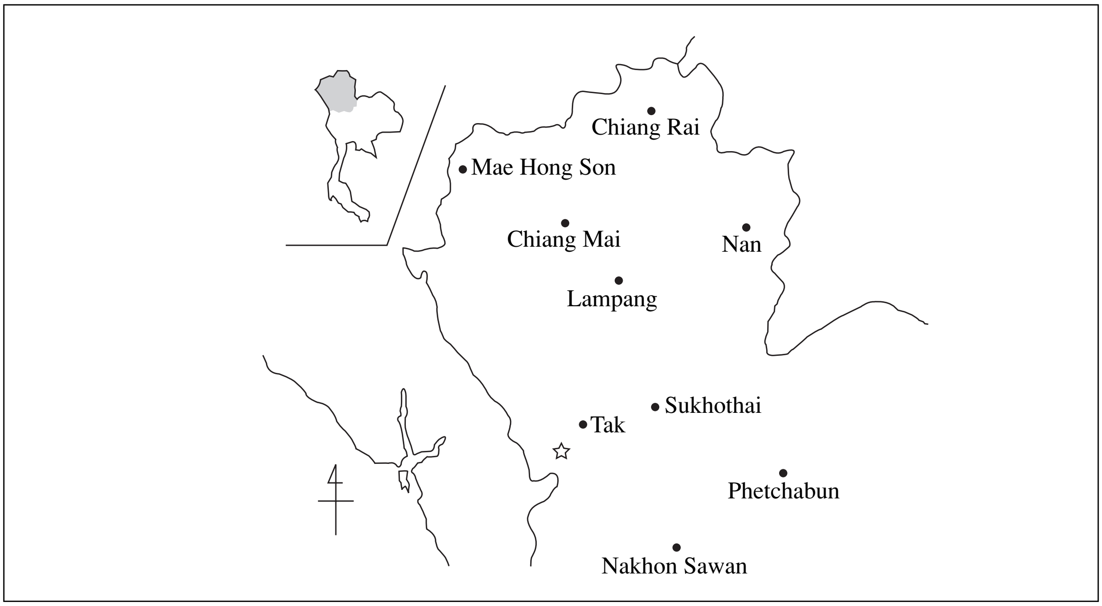

Figure 1   Map of Thailand ☆ shows the investigated place.

Materials and methods

1. Collection of the mosquitoes

3 m x 4 m x 2 m of Nylon mosquito-net was used as a trap, each with different bait species (Figure 2).

Figure 2   Chicken baited mosquito-net trap.

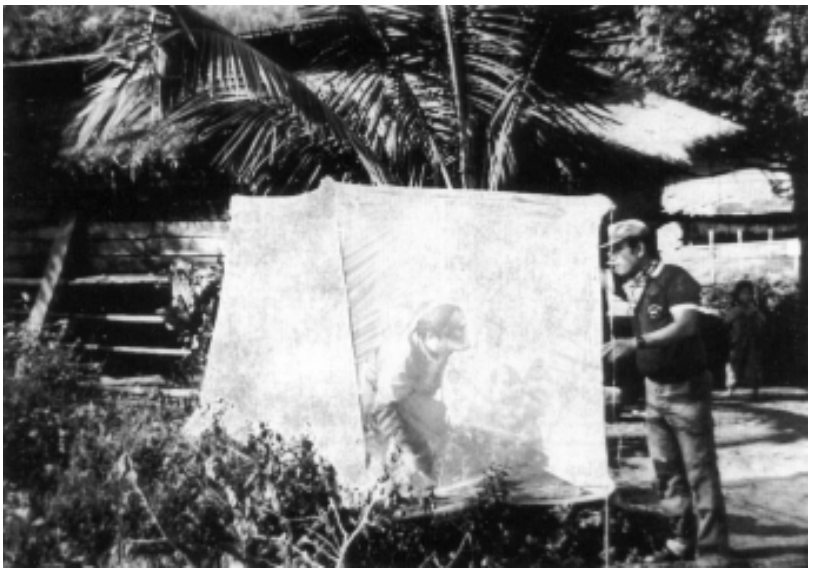

Figure 3 Modified bush trap.

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 1800 h to 900 h the following morning, and the lured mosquitoes were collected 3 times daily at 2100, 0600 and 0900 h from 7 to 11 December, 1989.

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

2. Blood source determination

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

The filter papers were dried with diphosphorous pentoxide in a desiccator and carried back to the laboratory. The smeared part of the filter papers were cut off and put into 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 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-phenylenediamine 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 using a Millititer HA plate were performed at room temperature and those using a plastic microplate were done at 37 °C.

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

Results and Discussion

The weather 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 temperatures during the investigation was from 12 to 32 °C.

Table 1 Temperature and humidity data at Tak, Thailand

Date	Temperature		at 2100 h		at 0600 h	
	max.	min.	humid.	Temp.	humid.	Temp.
07/ Dec	27.0	15.0	73.0	19.5		
08/ Dec	28.0	14.0	74.0	17.4	85.6	14.8
09/ Dec	30.0	14.0	73.5	18.3	90.5	15.6
10/ Dec	32.0	12.0	80.6	15.6	91.8	14.9
11/ Dec					90.2	13.8
Average	29.3	13.8	75.3	17.7	89.5	14.8

A total of 1,052 individuals of mosquitoes, including 178 individual of Filariasis and Encephalitis vectors, belonging to 2 genera and 4 species, were collected. From this study, it is determined that the trap using a cow as bait was the most effective and that, 1800 h to 2100 h is, generally, the most effective time to collect mosquitoes (Table 2, 3). This result may be due to the fact that the most dominant species among the 4 vector species was active during the night time.

Table 2 Number of mosquitoes collected at Tak, Thailand (classified by blood sources)

	human	swine	chicken	cow	bush	Total
Aedes niveus	1	0	0	5	1	7
Culex fuscocephala	1	0	0	135	1	137
Cx. gelidus	1	0	0	3	0	4
Cx. tritaeniorhynchus	0	1	0	27	0	28
Others	12	9	3	844	8	876
Total	15	10	3	1014	10	1052

Table 3 Number of mosquitoes collected at Tak, Thailand (classified by collected time)

	0600-0900	1800-2100	2100-0600	Total
Aedes niveus	0	5	1	6
Culex fuscocephala	40	55	41	136
Cx. gelidus	0	2	2	4
Cx. tritaeniorthnchus	7	10	11	28
Others	125	543	200	868
Total	172	615	255	1042 *

* except for 10 mosquitoes from bush trap.

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 using a cow as bait. No mosquitoes collected from human and pig-baited traps had any blood-meals in their alimentary canals.

Table 4 Engorged number of mosquitoes collected at Tak, Thailand (classified by blood sources)

	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 (classified by collected time)

	0600-0900	1800-2100	2100-0600	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 *

* except for 2 mosquitoes from bush trap.

The engorged rates of the 4 vector species collected by the animal-baited trap varied from 50% to 88.9% (Table 6). In *Cx. fuscocephala* the highest engorgted rate was obtained from the specimens collected at 0600 h (lured from 2100 h to 0600 h) (Table 5). This species is well-known to be active during the night time, as mention above, and this concurs with the results obtained in the investigation.

Table 6 The blood sources of 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 (62) human + cow (7) cow + swine (14) human + cow + swine (9) unidentified (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)

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, cow, swine or chicken. There were 3 individuals of *Cx. fuscocephala* that could not be identified the blood-meal by anti-human, cow, swine and 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 for 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 were both human and cow blood though the mixture in some included swine blood.

From the 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 knowledge of host preference and biting behaviour. The lack of it, will affect our ability to control the vector-borne diseases.

Acknowledgements

This work was supported in part by Japan International Cooperation Agency (JICA).

References

1. Burkot, T. R., W. G. Goodman and G. R. DeFoliart (1981): Identification of mosquito blood meals by enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg., 30(6): 1336-1341.
2. Burkot, T. R. and G. R. DeFoliart (1982): Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a shouthern Wisconsin forest endemic for La Crosse encephalitis virus. *ibid*, 31(2): 376-381.
3. Edrissian, Gh. H. and A. Hafizi (1982): Application of enzyme-linked immunosorbent assay (ELISA) to identification of *Anopheles* mosquito bloodmeals. Trans. Royal Soc. Trop. Med. Hyg., 76(1): 54-56.
4. Harbach, R. E., J. B. Gingrich and L. W. Pang (1987): Some entomological observations on malaria transmission in a remote village in northwestern Thailand. J. Amer. Mosq. Control Assoc., 3(2): 296-301.
5. Nutsathapana, S., P. Sawasdiwongphorn, U. Chitrarop and J. R. Cullen (1986): A mark-release-recapture demonstration of host-preference heterogeneity in *Anopheles minimus* Theobald (Diptera: Culicidae) in a Thai village. Bull. Ent. Res., 76(2): 313-320.
6. Sasaki, H. (1988): Morphological and immunological studies on the blood sources of black flies in Hokkaido, Japan (Diptera : Simuliidae). J. Rakuno Gakuen Univ., 13(1): 29-82.