J Trop Med Parasitol. 2009;32:82-6.

RESEARCH NOTE

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Refractoriness of *Culex sitiens* to Experimental Infection with Nocturnal Subperiodic *Brugia malayi*

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Abstract

A survey of 4 areas in the tsunami-affected area of Phang Nga Province, Thailand, found *Culex sitiens* to be the predominant species in freshwater sites that had changed into brackish-water. To determine the susceptibility of *Cx. sitiens* to *Brugia malayi*, 400 female mosquitoes were fed on a *B. malayi*-infected cat and were dissected at 14 days' post-feeding. *Cx. sitiens* was found to be completely refractory to experimental infection with nocturnal subperiodic *B. malayi*. Thus, despite the presence of relatively high larval and biting densities of this species, it appears to play no role in *B. malayi* transmission in this area of southern Thailand.

Keywords: *Culex sitiens*; *Brugia malayi;* refractory, nocturnal subperiodic

Southern Thailand has experienced an increase in the prevalence of Brugian filariasis in the tsunami-affected area, which is 954 kilometers in length [1]. Areas ~2-3 kilometers from the coastline were devastated by waves, resulting in several sites being covered with brackish water and some originally freshwater sites being changed to brackish-water sites. A year after the disaster, larvae of *Cx. sitiens* have been observed in every water site at moderate (40-50 larvae/dip) to high densities (>100 larvae/dip) (250-300 ml container). Mosquito landing rates were observed to be 42 mosquitoes per man per 10 minutes at 19:00 h.

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Few studies have been published on the susceptibility of *Culex* spp to *B. malayi* [2], but *Cx. halifaxii* and *Cx. pipiens pallens* are refractory to this parasite [3,4]. Human filariasis is still a public-health problem in southern Thailand. Ninety percent of filariasis cases are caused by *Wuchereria bancrofti*, and most of the remainder by *B. malayi*, a zoonotic infection endemic in Narathiwat, Nakhon Si Thammarat, Surat Thani and Krabi provinces, in southern Thailand. Nocturnal subperiodic types of *B. malayi* are reservoirs and commonly infect domestic cats and wild monkeys [5]. The Annual Report of the Bureau of Vector-Borne Diseases, Department of Disease Control, Ministry of Public Health, Thailand, 2003, indicated that the microfilarial (mf) prevalence rate had been reduced from 11.16% in 1992 to

< 0.43% in 2004. The highest mf rates were 17.29, 16.67, and 5.91 cases per 100,000 population in Narathiwat Province (for Brugian filariasis), Mae Hong Son (Bancroftian filariasis) and Tak (Bancroftian filariasis), respectively. In addition, 1.97% of domestic cats are infected with *B. malayi* in Surat Thani and Nakhon Si Thammarat [6]. This study investigated the ability of *Cx. sitiens* to transmit *B. malayi*.

Laboratory experiments on the colonization and susceptibility of mosquito vectors to *B. malayi* were performed on field-caught insects. For the susceptibility studies, adult *Cx. sitiens* females, aged 4-6 days, were fed directly on *B. malayi*-infected cat blood meal. The protocols for blood-feeding of mosquitoes on infected cats, and for human landing collections, were approved by the Ethics Committee, Faculty of Tropical Medicine, Mahidol University, Bangkok.

Wild-caught adult *Cx. sitiens* females were collected by human landing catch, and species were confirmed using taxonomic keys for the identification of *Culex* mosquitoes [7-9]. Mosquitoes were reared individually to obtain single colonies using a modified procedure [10]. Female mosquitoes were released into a 30×30×30 cm cage as starting colonies and were given a blood meal from a golden hamster. The full engorged females were transferred into paper cups (~15 individuals per cup) containing cotton wool soaked with 10% sugar solution as a food source. At about 3-4 days each mosquito was transferred into a plastic cup containing 15 ml of water from the field study area, for oviposition. Egg rafts were separated individually into plastic cups to observe hatchability. On the following day, the size and hatchability of the eggs were scored from 20 egg rafts. The number of eggs per raft, the duration of different larvae instars, pupae and adults, were counted and recorded every day. Larvae were reared in a plastic tray containing 1,000 ml of field study water. A solution of powdered fish meal in water (35% w/v) was provided as larval food; aliquots of 0.5, 1.0, 1.5, and 2.0 ml were added to each of the plastic trays containing each of the 4 developmental instar stages, respectively. The numbers of male and female mosquitoes were counted. Temperature and relative humidity were recorded.

Ae. togoi stock colony (Taiwan strain) was maintained in the insectary at 28°C and 70-80% relative humidity. As these mosquitoes are highly susceptible to *B. malayi* [11,12], they were used as a positive control for filarial infection.

A cat infected with *B. malayi* was maintained in the animal house, Faculty of Tropical Medicine, Mahidol University, and used as a source of microfilariae. Microfilarial density (range 5-25 mf per µl) was determined from multiple 20 µl blood samples obtained from the marginal ear vein. Nocturnal subperiodic *B. malayi* were used in this study. An infected cat was used as the source of naturally infected parasites, and a 24-hour periodicity study on the frequency and density of circulating mf had already been performed [13].

A total of 400 mosquitoes were fed on the *B. malayi*-infected cat, 200 per species and 50 females per cup, and 4 separate feedings were performed. The 50 adult female mosquitoes per paper cup were starved for 12-24 hours prior to blood feeding, which was carried out for 2-3 hours in the afternoon or evening in a dark room. The cat was anesthetized with Nembutal (0.5 ml per kilogram body weight). Before feeding, microfilarial density was determined from counting of Giemsa-stained thick blood film. To minimize variability as much as possible, both species of mosquitoes were fed at the same time. The fully engorged mosquitoes were transferred into plastic cups and maintained with 10% sugar soaked in cotton wool pad (changed daily). All female mosquitoes were successfully blood fed.

After 14 days, the mosquitoes were lightly anesthetized with ether. The mosquito bodies were separated into head, thorax, and abdomen, using a dissecting needle, and examined for the presence of larvae. Larvae were picked up with a dissecting needle and transferred individually to a Bless fluid drop to fix the larvae on a glass cavity block. The numbers of larvae in all body parts were counted and then transferred into a micro-

Table 1 Mortality and infective rates of *Cx. sitiens* and *Ae. togoi* after 14 days feeding on *B. malayi*-infected cat.

Exp	Mosquito	No. of fed mosquitoes	No. of dead mosquitoes (%)	No. of dissected mosquitoes	No. of infected mosquitoes (%)	Average no. of 3rd stage larvae per infected mosquito
1	*Cx. sitiens*	200	12 (6.0)	188	0	-
	Ae. togoi	200	143 (71.5)	57	14 (24.6)	5.2
2	*Cx. sitiens*	200	13 (6.5)	187	0	-
	Ae. togoi	200	178 (89.0)	22	9 (40.9)	5.9

tube containing 70% alcohol to confirm species.

PCR was employed using *HhaIR* and *HhaIF* primers to confirm the presence of *B. malayi* mf in cat and human blood [13,14]. An amplicon of 320 bp is indicative of *B. malayi* mf.

The results from the two experiments, each employing 200 mosquitoes, for the susceptibility of *Cx. sitiens* and *Ae. togoi* to *B. malayi* infection, showed that *Cx. sitiens* was not susceptible, whereas *Ae. togoi*, the control mosquito, had infective rates of 24.6 and 40.9% (Table1). PCR assay also confirmed that the infective larvae in *Ae. togoi* were *B. malayi* (data not shown). Following infection, the percentages of dead mosquitoes in the two experiments were 6.0 and 6.5 for *Cx. Sitiens,* and 71.5 and 89.0 for *Ae. togoi*.

Although *Cx. sitiens* has been implicated on a few occasions as a vector of *W. bancrofti*, there has not been any other reported instance of *Cx. sitiens* serving as a vector of *B. malayi* anywhere in the world, except for the report of Iyengar [15] of *Cx. sitiens* naturally infected with *B. malayi* in Thailand. Bangs *et al* in 1995 [2] provided the first conclusive evidence that *Cx. tarsalis* and *Cx. erythrothorax* could be infected with *B. malayi*. *Cx. (Lutzia) halifaxii* and *Cx. pipiens pallens* are refractory [3,4].

In southern Thailand, *Mansonia uniformis* and *Ma. bonneae* are the primary natural vectors of subperiodic *B. malayi* [16]. Chiang *et al* in 1989 [17] compared 5 strains of *Ma. uniformis* in Malaysia for susceptibility to subperiodic *B. malayi*, and found susceptibility to infection ranged from 62 to 100%, with no significant differences between 5 mosquito strains. The susceptibility rate is directly related to the microfilarial density of the cat at the time of feeding. Sarataphan *et al* [18] tested *Ma. indiana* collected from a non-endemic area for human lymphatic filariasis for their susceptibility to infection with nocturnally subperiodic *B. malayi* using a naturally infected cat, and showed susceptibility ranging from 30 to 70%, indicating that *Ma. indiana* collected from a non-endemic area can transmit nocturnally subperiodic *B. malayi*. Lek-Uthai and Tomoen, in 2005 [16], found the highest numbers of 3rd-stage filarial larvae in 10-day-old *Ma. uniformis* from 41.9% of dissected mosquitoes, with 40.3 and 17.8% in 5-day and 15-day old mosquitoes, respectively. Chiang *et al*, in 1991 [11], studied the susceptibility of *Cx. tritaeniorhynchus, Cx. gelidus,* and *Cx. vishnui* to *B. malayi* in Malaysia, and found that the control mosquitoes, *Ma. uniformis* and *Ae. togoi*, were highly susceptible to subperiodic *B. malayi,* with infection rates of 86.4-100% and 80-89.2%, respectively. Arrested development of mf in the abdominal cavity and thoracic muscle has not been observed. However, in this study, the cibarial denticles on the cibarial crest of *Cx. sitiens* were not inspected, which may damage imbibed mf, contributing to refractoriness. In *Ae. togoi*, the 3rd-stage larvae in the mosquitoes were detected following

dissection at 14 days' post-feeding. Mf had not developed in *Cx. sitiens* head, thorax and abdomen, and 3rd-stage larva were not found. Further studies to examine the impact of mf infection density on different mosquito species in relation to mosquito mortality should be conducted.

Acknowledgements

We gratefully acknowledge the help and support provided by Mr Supit Yodmek, Office of Disease Control, Ministry of Public Health, Thailand, and the staff of the Department of Medical Entomology, Faculty of Tropical Medicine, Department of Parasitology and Entomology, Faculty of Public Health and Faculty of Graduate Studies, Mahidol University, Bangkok, Thailand. We also thank Prof Dr Prapon Walairat, Faculty of Science, Mahidol University, and Prof Dr Alister Craig, Liverpool School of Tropical Medicine, Liverpool, UK, for their kind reading and very helpful comments. This study was partially supported by the China Medical Board, Faculty of Public Health, Mahidol University, Bangkok, Thailand.

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