CULEX QUINQUEFASCIATUS IN PHITSANULOK AS A POSSIBLE VECTOR OF NOCTURNALLY PERIODIC *WUCHERERIA BANCROFTI* TRANSMISSION IN MYANMAR IMMIGRANTS

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Abstract. The present study was undertaken in order to study whether *Culex quinquefasciatus* collected in Phitsanulok Province can be an insect host for the development of *Wuchereria bancrofti* larvae. *W. bancrofti* infected blood from Myanmar workers in Mae Sot, Tak Province was fed to mosquitoes by using the artificial membrane feeding. An infection of *W. bancrofti* was found with the highest density of L_3 in the mosquito thorax on the 14th day after feeding. The infection rate also correlated to the density of microfilaria found in the donor' s blood. Our results showed that *Cx. quinquefasciatus* in Phitsanulok is a possible vector of nocturnally periodic *W. bancrofti*.

INTRODUCTION

Bancroftian filariasis is a disease caused by infection with *Wuchereria bancrofti* and remains a public health problem in Thailand, especially in the western part near the Thai-Myanmar border, such as Tak and Ranong provinces (Division of Filariasis, 1995). The important vectors of this disease are: *Aedes harinasuti*, *Aedes demostes*, *Aedes anandalei*, *Aedes imitator* and *Mansonia diver* (Harinasuta *et al*, 1971; Sucharit and Harinasuta,1975; Gould *et al*, 1982; Suvannadabba, 1993). Recently, *Cx. quinquefasciatus* was reported to be a highly efficient insect host for the larval development of nocturnally periodic *W. bancrofti* (Sucharit and Harinasuta, 1995; Jitpakdi *et al*, 1998).

Culex quinquefasciatus is widely distributed in tropical and subtropical areas and closely associated with human habitation because of its anthropophilic, endophilic blood feeding habits and breeding areas (Forattini *et al*, 1993a,b,c,d). An environmental survey indicated that inadequate disposal of human waste and waste water provide important *Cx. quinquefasciatus* breeding sites that are always found in a big city such as Phitsanulok.

Because of low income in Myanmar and rapid extension of industry in Thailand, a growing number of Myanmar people have illegally migrated to work in Thailand, especially in the urban areas. Phitsanulok is a large city located in the lower northern part of Thailand near Mae Sot, which is an endemic area for *W. bancrofti* (Swaddiwudhipong *et al*, 1996). Like other big cities, rapid extension of urbanization and industrialization in Phitsanulok has enhanced the immigration of Myanmar workers. The presence of waste water, *Cx quinquefasciatus* mosquitoes and Myanmar workers all play an important role in the spread of *W. bancrofti* infections; the migration of infected individuals to places with mosquitoes capable of transmission is a potential danger in non-endemic areas. However, there are many other important factors, such as the presence of suitable vectors, susceptible human population and an environment influenced by the dynamics of lymphatic filariasis transmission (Vanamail *et al*, 1990). This study was undertaken in order to study whether *Cx. quinquefasciatus* mosquitoes collected in Phitsanulok can be a vector for *W. bancrofti* larvae development and onward transmission.

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MATERIALS AND METHODS

Mosquitoes

Colonies of *Cx. quinquefasciatus* established from larva and pupa were collected from breeding sites around Phitsanulok city. Mosquitoes were maintained in insectariums with a temperature of 27±1˚C and a humidity of 80±10%, which was relative to the physical conditions in the natural habitat. Larva and pupae were maintained in plastic containers with water. Pupae were collected every day and transferred to the net cages. Adult mosquitoes were provided with 10% sugar solution. The experiment was started with the 5th generation of *Cx. quinquefasciatus* in our insectariums.

Source of microfilaria

A blood survey was undertaken in selected communities in Mae Sot. Two hundred participating volunteers were screened for the microfilaria of nocturnally periodic *W. bancrofti*. Prior the screening, the purpose of the survey was explained to the community leadership and members. The positive patients were offered appropriate treatment. Blood samples were collected between 20 00 and 23 00 hours by finger-prick. Three positive volunteers participated in the study. Five ml of blood samples were taken from the vein of these three positive cases. Four ml of the blood sample was used to feed mosquitoes by artificial membrane feeding method and one ml of the blood sample was used to examine the density of microfilaria.

Infection of vectors

The experimental mosquitoes were 4-10 day-old females. They were starved of sugar meals for 24 hours before exposure to infected blood meals by artificial feeding methods for 15-30 minutes between 22 00 and 24 00 hours, coinciding with the peak of female *Cx. quinquefasciatus* biting activity (Pipitgool *et al*, 1998). Engorged mosquitoes were transferred to plastic boxes and maintained in insectariums until examination.

Dissecting of mosquitoes

To determine the distribution of infective larvae in mosquitoes, the mosquitoes were individually dissected at day 14 after exposure to an infective blood meal that corresponded to the time needed for microfilariae of *W. bancrofti* to develop into third-stage larvae. For dissection, mosquitoes were put on a slide and separated into mouth part, head, thorax, and abdomen. Each part was placed in a drop of 0.85% normal saline and examined for third-stage larvae under a dissection microscope at ×40 magnification. The number of third-stage larvae found in each part was recorded.

Assessment of the results

The susceptibility of *Cx quinquefasciatus* to nocturnally periodic *W. bancrofti* was assessed by the equation proposed by Ramachandran (1970).

$$\text{Infectivity rate} = \frac{\text{No. of mosquitoes containing } L_3 \text{ after 14 days}}{\text{No. of mosquitoes surviving for 14 days}}$$

$$\text{Intensity of infection} = \frac{\text{No. of } L_3}{\text{No. of mosquito with } L_3}$$

RESULTS

The infective rate of *Cx. quinquefasciatus* with nocturnally periodic *W.bancrofti*.

The results of dissection of all infected mosquitoes on day 14 showed that *Cx. quinquefasciatus* collected from Phitsanulok city contained L3. The infective rates were 34.38%, 10.09% and 50.79% in case 1, case 2 and case 3, respectively. More infective rates were found in the mosquitoes that were fed with blood meals that contained a high density of microfilaria. The infective rates of *Cx. quinquefasciatus* are shown in Table 1.

The distribution of larvae of *W. bancrofti* in different parts of the body of *Cx. quinquefasciatus*.

On day 14 after infection, dissection of the mosquitoes revealed that the majority of larvae had already molted to L3 and migrated from thorax to other parts of the body of mosquitoes. From the examination, 25.00%, 0.63%, 52.50% and 21.88% of L3 were found in the mouth part, head, thorax and abdomen of dissected mosquitoes, respectively. There were more L3 in the thorax than the other parts. Further analysis was carried out to determine whether there was a difference in distribution of L3 in the body of vectors based on L3 density. The results showed that the distribution patterns of L3 in low and high intensity groups were similar. The distribution of larvae of *W. bancrofti* in different parts of the body of *Cx. quinquefasciatus* are shown in Table 2.

The intensity of infection

The intensity of infection of *Cx. quinquefasciatus* with nocturnally periodic *W. bancrofti* was 3.36 ± 1.15, 1.64 ± 0.92 and 3.28 ± 0.97 in case 1, case 2 and case 3, respectively as shown in Table 1.

DISCUSSION

This study was undertaken to investigate whether *Cx. quinquefasciatus* collected in Phitsanulok could support the development of *W. bancrofti* larvae and, therefore, possible transmission. It was shown that *Cx. quinquefasciatus* mosquitoes of Phitsanulok origin were susceptible to nocturnally periodic *W. bancrofti.* The results obtained in this study were similar to other reports that discovered *Cx. quinquefasciatus* was susceptible to nocturnally periodic *W. bancrofti* (Sucharit and Harinasuta, 1975; Jitpakdi *et al*, 1998). Interestingly, more infective rates were found in mosquitoes that were fed with blood meals that contained a high density of microfilaria. This finding suggests that the possibility of transmission depends on the density of microfilaria in the infected host. This report also suggests that the migration of infected persons from Myanmar and the existence of effective vectors may lead to the transmission of this disease. Therefore, in non-endemic areas like Phitsanulok

Table 1
Infective rates and parasite loads in *Cx. quinquefasciatus* after artificial feeding on the nocturnally periodic *W. bancrofti* infected blood on day 14 after feeding.

No. of cages	Mf density per mm^3	No. dissected	No. infected (%)	Average no. of larvae per infected mosquitoes
Case I	15 ± 1.12	32	11 (34.38)	3.36 ± 1.15
Case II	1 ± 0.53	109	11 (10.09)	1.64 ± 0.97
Case III	25 ± 1.19	63	32 (50.79)	3.28 ± 0.92

Table 2
Distribution of infective larvae of nocturnally periodic *W. bancrofti* in mouth part, head, thorax and abdomen of *Cx. quinquefasciatus* on day 14 after feeding.

No. of cages	No. of infected larva found in (%)				
	Mouth part	Head	Thorax	Abdomen	Total
Case I	8 (21.62)	0 (0.00)	16 (43.24)	13 (35.14)	37 (100)
Case II	4 (22.22)	1 (5.56)	9 (50.00)	4 (22.22)	18 (100)
Case III	28 (26.67)	0 (0.00)	59 (56.19)	18 (17.14)	105 (100)
Total (%)	25.00	0.63	52.50	21.88	100

where the vector exists, the arrival of infected persons such as Myanmar immigrants can initiate disease transmission.

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