VECTOR COMPETENCE OF *AEDES AEGYPTI* (L.) AND *CULEX QUINQUEFASCIATUS* (SAY) FOR *DIROFILARIA IMMITIS* (LEIDY)

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Abstract. This study was performed to examine the vector competence of *Aedes aegypti* and *Culex quinquefasciatus* for *Dirofilaria immitis*. Eleven individual experiments were conducted in this study. Nonthaburi and Udon Thani strains of *Ae. aegypti* were allowed to feed on infected dogs that had 5,750 and 4,600 microfilariae (mf) per ml of blood, respectively. Three groups of Bangkok-strain *Cx. quinquefasciatus* were allowed to feed on dogs that had 4,800, 5,200, and 5,850 mf per ml of blood. Six groups of Liverpool-strain *Ae. aegypti* were allowed to feed on dogs with 1,650, 1,950, 3,350, 9,000, 9,250, and 11,550 mf per ml of blood. Three to 4% of Nonthaburi-strain, and 0-6% of Udon Thani-strain *Ae. aegypti* became infected and had infective-stage larvae (L3) of *D. immitis* in their probosces. Zero to 1 and 7% of Bangkok-strain *Cx. quinquefasciatus* had L3 in their probosces after taking blood meals with 4,800 and 5,850 mf per ml of blood, respectively. The percent-infected Liverpool-strain *Ae. aegypti* with L3 in their probosces were 3-12, 0-12, 10, 16, 7-19, and 0-21 after taking blood meals with 1,650, 1,950, 3,350, 9,000, 9,250, and 11,550 mf per ml of blood, respectively, when tested at different post-blood-feeding days. This study showed both *Ae. aegypti* and *Cx. quinquefasciatus* from Thailand can become vectors for *D. immitis*; however, Liverpool-strain *Ae. aegypti* are more likely to be competent vectors for *D. immitis* than *Ae. aegypti* and *Cx. quinquefasciatus* from Thailand. The percent infection rates of *Ae. aegypti* and *Cx. quinquefasciatus* with *D. immitis* in the field in Thailand need to be investigated, to confirm the role of these mosquitoes in the life cycle of *D. immitis* in nature.

INTRODUCTION

Dirofilaria immitis (Leidy), a filarial nematode in the family Filariidae, can be found in domestic canines, felines and other wildlife species (Gortazar *et al*, 1998; Nakagaki *et al,* 2000; Kim and Huh 2005; Liu *et al,* 2005). Human pulmonary dirofilariasis is a zoonotic disease caused by *D. immitis,* which is usually transmitted from dogs to humans by mosquito bite. The nematode enters the subcutaneous tissues, travels to the right ventricle, dies and then embolizes the pulmonary vessels, causing a small pulmonary infarction, which subsequently appears as a nodule. Pulmonary nodules, pulmonary granulomas, or subcutaneous nodules, are lesions presenting in infected humans (Rena *et al,* 2002; Tsung and Liu 2003; Mumtaz *et al,* 2004). Microfilariae released from female *D. immitis* that reside in the right ventricle of an infected dog will develop into infective-stage larvae in the mosquito. An infective-stage larva in the proboscis of an infected mosquito will move into the small capillaries of the host during the mosquito's feeding process. Differences in the distribution of *D. immitis* in each area depend upon the variety of mosquito species and sources of infected blood meals. Each mosquito species may play a different role in the life cycle of *D. immitis* in nature.

Aedes aegypti (L.) and *Culex quinquefasciatus* (Say) mosquitoes are widely distributed in rural and urban areas of Thailand. *Ae. aegypti*, or the "yellow fever mosquito" usually bites during the daytime, indoors or in sheltered areas near houses. *Ae. aegypti* is a major vector for dengue virus and yellow fever. *Cx. quinquefasciatus* is often found inside houses and the females are night feeders. *Cx. quinquefasciatus* bites humans vigorously, particularly just after sunset; it also feeds on birds; it plays an important role in the cycle of Japanese encephalitis virus and other viruses in nature.

This study was performed to assess the capacity of the Thailand strains of *Ae. aegypti* and *Cx. quinquefasciatus* to serve as vectors for *D. immitis*.

MATERIALS AND METHODS

Mosquitoes

The Nonthaburi and Udon Thani strains of *Aedes aegypti* (L.), and the Bangkok strain of *Culex quinquefasciatus* (Say), < 10^{th} generation were used in this study. These mosquitoes were originally collected from Nonthaburi (central Thailand), Udon

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Thani (northeastern Thailand), and Bangkok (central Thailand), in 2004. The Liverpool strain of *Ae. aegypti* were originally colonized by the Department of Parasitology, University of Georgia, USA. These mosquitoes were maintained in the Mosquito Laboratory, Department of Pathology, Chulalongkorn University, Bangkok, Thailand. All mosquitoes were maintained in controlled environmental conditions (28 ± 2 ºC and 80 ± 10% RH).

Aedes aegypti and *Culex quinquefasciatus* were selected for this study because they are widely distributed in Thailand, including Bangkok. Different Thailand and Liverpool strains were selected to assess variations in vector competency between mosquito strains.

Experimental animals

Two mixed-breed, local dogs naturally infected with *D. immitis* were used in this study as sources of infected blood meals for the mosquitoes. The dogs were kept in the laboratory animal facilities, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Microfilaria counting

Infected dog blood: one ml of blood was collected from the cephalic vein during the mosquito-feeding period. A three-line smear made from 20 μl of blood on a glass slide, was allowed to air dry, hemolized in distilled water, fixed in absolute methanol, and stained with 10% Giemsa. The stained slide was examined, and microfilariae counted under a light microscope.

Blood-fed mosquitoes: blood-fed mosquitoes were randomly selected after blood feeding. The mosquitoes were dissected individually and the blood meal removed from the midgut, mixed with 0.85% NaCl, and smeared onto a glass slide, which was allowed to air dry, hemolized in distilled water, fixed in absolute methanol, and stained with 10% Giemsa. The stained slide was examined and microfilariae counted under a light microscope.

Vector competence of *Dirofilaria immitis*

Three- to 5-day-old mosquitoes were used in this study. The mosquitoes were deprived of sucrose for 24-48 hours before blood-feeding on the infected dog. The dog was sedated with 2 mg/kg body weight Xylazine HCl and anesthetized with 10 mg/kg body weight of pentobarbital sodium. The dog then was placed on a mosquito cage, where the mosquitoes were allowed to feed for 30 minutes. Groups of 50 blood-fed mosquitoes were transferred into plastic cups and maintained in the mosquito laboratory.

Mosquitoes were randomly selected from the mosquito cups each test day. The wings and legs were removed and the mosquitoes were dissected. Each mosquito organ was examined for *D. immitis* larvae under a light microscope.

Data analysis

The vector competence of *Ae. aegypti* and *Cx. quinquefasciatus* for *D. immitis* in this study is presented in descriptive format. Percent infected mosquitoes with L3 in proboscis at day 14 PBF were compared for each microfilaria level in blood meal by species, using pairwise Fisher's exact test. Observed differences were considered significant at $p < 0.05$ and approaching significance at $0.05 \quad p < 0.1$. No statistical comparison was conducted between mosquito species, since they did not feed on the same infected dog at the same time.

RESULTS

There were 11 individual experiments in this study. Nonthaburi and Udon Thani strains of *Aedes aegypti* were allowed to feed on *Dirofilaria immitis*-infected dogs that had 5,750 and 4,600 microfilariae (mf) per ml of blood, respectively. The average mf levels in the mosquito midguts after blood meal were 10 and 4 mf per mosquito, respectively. Three groups of Bangkok-strain *Culex quinquefasciatus* were allowed to feed on *D. immitis*-infected dogs that had 4,800, 5,200, and 5,850 mf per ml of blood. The average mf level in the mosquito midguts after blood meal were 5, 10, and 12 mf per mosquito, respectively. Six groups of Liverpool-strain *Ae. aegypti* were allowed to feed on *D. immitis* infected dogs with 1,650, 1,950, 3,350, 9,000, 9,250, and 11,550 mf per ml of blood. The average mf levels in the Liverpool-strain *Ae. aegypti* midguts after blood meal were 1, 4, 6, and 15 mf per mosquito after blood meal from infected dogs with 1,950, 9,250, 9,000, and 11,550 mf per ml of blood, respectively.

The percent-infected mosquitoes with infective-stage larvae (L3) in their proboscis after taking blood meals from *D. immitis* infected dogs, and tested at different days post-bloodfeeding are shown in Tables 1 and 2. Three to 4% of Nonthaburi-strain *Ae. aegypti* and 0-6% Udon Thani-strain *Ae. aegypti* became infected, and had L3 of *D. immitis* in their probosces. No Bangkok-strain *Cx. quinquefasciatus* had L3 in their probosces after taking blood meal with 5,200 mf per ml of blood. However, 0-1 and 7% Bangkok-strain *Cx. quinquefasciatus* had L3 in their probosces after taking blood meals with 4,800 and 5,850 mf per ml of blood, respectively.

The percent infected Liverpool-strain *Ae. aegypti* mosquitoes with L3 in their probosces were 3-12, 0-12, 10, 16, 7-19, and 0-21 post-bloodmeal with 1,650, 1,950, 3,350, 9,000, 9,250, and 11,550 mf per ml of blood, respectively, tested on different days post-bloodfeeding.

The percent-infected mosquitoes with L3 in the proboscis at day14 PBF were compared between each microfilaria level in blood meal, within species. There were statistical differences between the *Cx. quinquefasciatus* that took blood meals with 4,800 and 5,850 mf per ml of blood ($p = 0.0066$), and between *Ae. aegypti* that took blood meals with 1,650 and 11,550 mf per ml of blood ($p = 0.0386$)

DISCUSSION

Aedes aegypti and *Culex quinquefasciatus* mosquitoes can be found worldwide, particularly in Thailand. They are potential vectors for many pathogens; however, the vector competence of these mosquitoes for any pathogen may vary, depending upon the strain of mosquito.

The vector competence of mosquitoes for pathogens can be determined in many ways. This study indicates the vector competence of *Ae. aegypti* and *Cx. quinquefasciatus* for *Dirofilaria immitis* as a percent of blood-fed mosquitoes with third-stage (L3), or infective-stage larvae, of *D. immitis* in their probosces. Vector competence, however, can also be determined as a vector efficiency index (VEI), which is the number of L3 multiplied by 100 and divided by the number of ingested microfilariae (mf).

The percent infection rate for Rio de Janeiro laboratory strain *Ae. aegypti* after ingestion of blood containing 3,000, 5,000, and 7,000 mf/ml were 55.3, 66.7, and 100%, respectively, and the VEI ranged from 1.6-9.3 (Serrao *et al,* 2001). Our study, however, found that percent infection rates for Liverpool-strain *Ae. aegypti* at day14 post-bloodfeeding (DPBF) were 9, 12, 10, 16, 19, and 21% after ingestion of blood containing 1,650, 1,950, 3,350, 9,000, 9,250, and 11,550 mf/ml, respectively; the percent infection rate for Thailand-strain *Ae. aegypti* at 14 DPBF was only 4% after ingestion of blood containing 4,600 and 5,750 mf/ml, respectively.

Table 1
Percent-infected Nonthaburi- and Udonthani-strain *Aedes aegypti* and Bangkok-strain *Culex quinquefasciatus* mosquitoes with infective-stage larvae (L3) in their probosces post-bloodmeal from *Dirofilaria immitis*-infected dogs, tested on different days post-bloodfeeding.

Exp	Mosquito species	Microfilaria level in blood meal (mf/ml)	Day (PBF)	Percent-infected mosquitoes with L3 in the proboscis* (infected/tested)
1	*Ae. aegypti* (Nonthaburi strain, F2)	5,750	7	4 (3/70)
			10	3 (2/60)
			14	4 (7/175)[a]
2	*Ae. aegypti* (Udon Thani strain, F4)	4,600	7	0 (0/13)
			10	6 (4/70)
			14	4 (6/163)[a]
			21	2 (4/183)
3	*Cx. quinquefasciatus* (Bangkok strain, F7)	5,200	14	0 (0/14)[b,c]
4	*Cx. quinquefasciatus* (Bangkok strain, F8)	4,800	7	0 (0/12)
			10	0 (0/17)
			14	1 (1/163)[b]
5	*Cx. quinquefasciatus* (Bangkok strain, F8)	5,850	14	7 (6/83)[c]

* Percent-infected mosquitoes with L3 in the proboscis at day14 PBF were compared for each microfilaria level in the blood meal, within species. Percent-infected mosquitoes not sharing the same superscript are significantly different ($p < 0.05$).

Table 2
Percent-infected Liverpool-strain *Aedes aegypti* mosquitoes with infective-stage larvae (L3) in the proboscis post-bloodmeal from *Dirofilaria immitis* infected dogs, tested on different days post-bloodfeeding.

Exp	Mosquito species	Microfilaria level in blood meal (mf/ml)	Day (PBF)	Percent infected mosquitoes with L3 in the proboscis* (infected/tested)
6	*Ae. aegypti* (Liverpool strain)	1,650	14	9 (10/115)[a]
			21	12 (10/90)
			28	3 (2/60)
			34	8 (1/13)
7	*Ae. aegypti* (Liverpool strain)	1,950	14	12 (6/50)[a,b]
			21	0 (0/20)
8	*Ae. aegypti* (Liverpool strain)	3,350	14	10 (3/30)[a,b]
			28	10 (2/20)
9	*Ae. aegypti* (Liverpool strain)	9,000	14	16 (8/50)[a,b]
10	*Ae. aegypti* (Liverpool strain)	9,250	14	19 (13/70)[a,b]
			28	7 (4/59)
11	*Ae. aegypti* (Liverpool strain)	11,550	7	0 (0/33)
			14	21 (10/48)[b]

* Percent-infected mosquitoes with L3 in the proboscis at day14 PBF were compared for each microfilaria level in the blood meal. Percent-infected mosquitoes not sharing the same superscript are significantly different ($p < 0.05$).

The study by Ahid and Lourenco-De-Oliveira (1999) showed 54.5% of collected mosquitoes from Brazil were *Cx. quinquefasciatus,* and it was the only species collected every month of the year. The percent infection rate for this mosquito with L3 of *D. immitis* was 0.1%. The field study in Taiwan by Lai *et al* (2001) showed 4.28% of *Cx. quinquefasciatus* infected with *D. immitis,* with an infection intensity of 2.89 larvae per mosquito. The percent infection rate for Taiwan-strain *Cx. quinquefasciatus* was only 1.1% after ingestion of blood containing 7,500 mf/ml with a VEI of 3.47.

The study by Lowrie (1991) showed the VEIs of the Haiti and Covington strains of *Cx. quinquefasciatus,* after feeding on approximately 5,500 mf/ml of blood with *D. immitis*, were 1.2 and 0.3%, respectively; the VEIs increased to 1.6 and 0.5%, respectively, when the mf levels in the blood increased to about 20,000 mf/ml.

The vector competence of *Cx. quinquefasciatus* from five localities in Brazil to *D. immitis* was evaluated by Ahid *et al* (2000). The mosquitoes were fed on an infected dog with 6, 000 mf/ml of blood; the range of ingested mf was 4.8-24.6 mf/mosquito, and the mean number of infective larvae detected in the heads and probosces was 1-1.5 at 15 DPBF. The survival rates for all *Cx. quinquefasciatus* were 50-75%. The survival rate of *Ae. aegypti* assayed simultaneously for comparison was only 24.7%, while the VEI was much higher than *Cx. quinquefasciatus*. In this study, the percent infections of Thailand-strain *Cx. quinquefasciatus* were only 1 and 7% at 14 DPBF after ingestion of blood containing 4,800 and 5, 850 mf/ml, respectively.

The variations in vector competence between mosquito species and strains may be due to mosquito immunity and defense mechanisms. The poor vector efficiency or competence of *Cx. quinquefasciatus* is probably due to the formation of long, needle-like oxyhemoglobin crystals in the blood meal, which prevents the migration of microfilariae to the Malpighian tubules (Lowrie, 1991). *Ae. aegypti* females showed melanization of microfilariae in the Malpighian tubules on the midgut wall and in the hemocoel. A

cellular melanization response was observed in the hemocoel, whereas, a humoral melanization response was observed in the Malpighian tubules (Mahmood, 2000).

This study showed both *Ae. aegypti* and *Cx. quinquefasciatus* from Thailand can become vectors for *D. immitis*. However, Liverpool-strain *Ae. aegypti* are more likely to be competent vectors for *D. immitis* than *Ae. aegypti* or *Cx. quinquefasciatus* from Thailand. Percent infection rates of *Ae. aegypti* and *Cx. quinquefasciatus* with *D. immitis* in the field in Thailand need to be investigated, to confirm the role of these mosquitoes in the life cycle of *D. immitis* in nature.

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