THE POTENTIAL FOR *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) TO BE A COMPETENT VECTOR FOR CANINE HEARTWORM, *DIROFILARIA IMMITIS* (LEIDY)

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Abstract. The susceptibility of *Aedes albopictus* (Skuse) to canine heartworm, *Dirofilaria immitis* (Leidy), was determined and compared between Thai (TH) and US (US) strains of this mosquito species to assess the likelihood of its potential to be a competent vector for *D. immitis*. There were 6 individual experiments with 1,053 mosquitoes in this study. *Ae. albopictus* were allowed to feed on *D. immitis* infected dogs with different levels of microfilaremia, which were 663 ± 79 (mean ± SE), 1,410 ± 93, 2,463 ± 208, 3,490 ± 211, 5,000 ± 257 and 7,480 ± 551 microfilariae (mf)/ml of blood. Infection rates with the TH strain infective stage larvae (L3) of *D. immitis* were 5, 40, 13 and 14% after taking a blood meal with 663, 2,463, 3,490 and 5,000 mf/ml, respectively, as determined on day 14 post-blood feeding (PBF). Infection rates with the US strain with L3 of *D. immitis* were 8, 20, 25, 18 and 35% after taking a blood meal with 663, 2,463, 3,490, 5,000 and 7,480 mf/ml, respectively, as determined on day 14 PBF. The vector efficiency index (VEI) is defined as the average number of L3 multiplied by 100 and divided by the average number of ingested mf. VEI of TH strain were 3.2, 8.5, 20 and 2.4 after taking a blood meal with 663, 2,463, 3,490 and 5,000 mf/ml, respectively, as determined on day 14 PBF. VEI of US strain were 4.2, 13.5, 58.3 and 5.7 after taking a blood meal with 663, 2,463, 3,490 and 5,000 mf/ml, respectively, as determined on day 14 PBF. This study indicates that both TH and US strains of *Ae. albopictus* were competent vectors for *D. immitis*, however, field studies need to be carried out to determine the possible role of *Ae. albopictus* in the transmission cycle of *D. immitis* in field conditions.

INTRODUCTION

Filariasis, a disease caused by the filarial nematode, is important in tropical countries, including Thailand. *Brugia malayi* and *Wulchereria bancrofti* are filarial nematodes that cause filariasis or elephantiasis in humans. Important filarial nematodes in dogs are *Dirofilaria immitis* and *Brugia pahangi*, the adults of which are found in the heart and lymph nodes of an infected dog, respectively. *D. immitis* causes hematological and serum chemistry changes (Niwetpathomwat *et al*, 2006) and serious illness in dogs, since adults of this nematode reside in the right ventricle and sometimes in the pulmonary artery. *D. immitis* also infects other mammals, including humans. This nematode, however, cannot complete its life cycle in humans but may cause pulmonary nodules and granulomas and subcutaneous nodules in infected humans (Levinson *et al*, 1979; Tsung and Liu, 2003; Oshiro *et al*, 2004).

The mosquito is a biological vector for this nematode, which facilitates the development of microfilaria to an infective larval stage. Infective larvae are transmitted during the feeding process of the infected mosquito. Different mosquito species have different abilities to be vectors of *D. immitis* because of their anatomy. A study by Tiawsirisup *et al* (2005) found that Thai strains of *Aedes aegypti* and *Culex quinquefasciatus* may serve as biological vectors for *D. immitis* in laboratory conditions. However, there have been no studies of the vector competency of *Ae. albopictus* in Thailand.

Ae. albopictus (Skuse), the Asian tiger mosquito, is a flood water mosquito, considered a competent vector for many pathogens in both field and laboratory conditions. Previous studies have shown its potency as a biological vector of

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various arboviruses, such as chikungunya virus, dengue virus and West Nile virus (Tiawsirisup *et al*, 2004, 2005). This mosquito is widely distributed in America, Africa, Europe, Australia, and Asia, including Thailand (Chareonviriyaphap *et al*, 2003). It was first introduced into Europe in 1979 and the United States in 1985 (Sprenger and Wuithiranyagool, 1986). Used tire shipments between countries were considered the main pathway that brought *Ae. albopictus* from Asian countries to others (Reiter and Sprenger, 1987).

Its original habitat is tropical forest. Larvae and pupae are found in natural containers, including bamboo stumps, coconut shells, tree holes and rock holes, however, they are also found in artificial containers. It primarily feeds on humans however it also feeds on animals, including cattle, swine, dogs, cats, rats, and chickens (Ponlawat and Harrington, 2005). Mixed blood meals may be detected from this mosquito; since it is a multiple-host feeder, it may serve as a bridge vector that carries some pathogens from infected animals to humans.

This study investigated whether *Ae. albopictus* in Thailand may be a competent vector for *D. immitis*, and whether there is any difference in vector competency between the Thai (TH) and US (US) strains of *Ae. albopictus*.

MATERIALS AND METHODS

Mosquito specimens

TH and US strains of *Aedes albopictus* raised for more than 10 generations, were used for this study. The TH strain of *Ae. albopictus* was kindly provided by Dr Padet Siriyasatien, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Thailand and the US strain of *Ae. albopictus* was kindly provided by Dr Wayne A Rowley, Department of Entomolgy, Iowa State University, USA. All mosquitoes were maintained in controlled environmental conditions (28 ± 2ºC and 80 ± 10% RH) at the Division of Parasitology, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Ae. albopictus was selected for this study because this mosquito is widely distributed in Thailand. The TH and US strains were selected for this study to examine whether there were any variations in vector competency between the mosquito strains.

Experimental animals

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

Microfilaria counting

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

Blood fed mosquitoes. Mosquitoes were randomly selected after a blood meal. Mosquitoes were individually dissected and the blood meal was removed from the midgut, mixed with distilled water and smeared on a glass slide. The slide was fixed in absolute methanol, stained with 10% Giemsa. The stained slide was then examined and microfilariae counted under a light microscope.

Vector competence of *Ae. albopictus* for *Dirofilaria immitis*

Three- to 5-day-old mosquitoes were used in this study. The mosquitoes were deprived of sucrose for 24 - 48 hours before feeding on the infected dog. The dog was sedated with 2 mg/kg body weight of Xylazine HCl and anesthetized with 10 mg/kg body weight of pentobarbital sodium. The dog was then placed on a mosquito cage where the mosquitoes were allowed to feed for 30 minutes. A group of 50 blood-fed mosquitoes were transferred into plastic cups and maintained in the mosquito laboratory. Mosquitoes were randomly selected from mosquito cups each tested 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 competentcy of *Ae. albopictus* for *D. immitis* in this study was evaluated. The infection rate, defined as the number of blood-fed mosquitoes that had infective stage or third stage larvae (L3) in their body multiplied by 100 and divided by the number of tested mosquitoes. Vector efficiency index (VEI) is defined as the average number of L3 developed in the mosquito multiplied by 100 and divided by the average number of ingested microfilariae.

Infection rates per strain were compared for each microfilaria level and between each tested day. Infection rates were also compared between strains on each tested day at the same microfilaria level. Pairwise Fisher's exact test was used for comparison. Observed differences were considered significant at $p < 0.05$.

RESULTS

There were 6 experiments with different microfilaria (mf) levels in this study. TH and US strains of *Aedes albopictus* were allowed to feed on *Dirofilaria immitis* infected dogs at 663 ± 79, 1,410 ± 93, 2,463 ± 208, 3,490 ± 211, 5,000 ± 257 and 7,480 ± 551 mf/ml of blood. Blood-fed mosquitoes were dissected and examined for third stage larvae (L3) on days 7-18 post-blood feeding (PBF) and defined as being infected mosquitoes if they had L3. The infection rates and vector efficiency index (VEI) are shown in Tables 1 and 2, respectively. Infection rates for the US strain were determined for days 7 and 14 PBF and they were 0 and 35%, respectively ($p < 0.001$). The other evaluations were performed on day 14 PBF and thereafter.

After taking a blood meal with 663 ± 79 mf/ml, infection rates in the TH strain were 5, 8, and 10% and infection rates in the US strain were 8, 10, and 13% on days 14, 15, and 16 PBF, respectively. The ranges of VEI for these mosquitoes were 3.2-6.5 and 4.2-12.5 for TH and US strains, respectively. There were no significant differences in infection rates between the two strains tested.

After taking the blood meal with 1,410 ± 93 mf/ml, infection rates with the TH strain were 3, 13, and 7% and infection rates with the US strain were 10, 3, and 23% on days 16, 17, and 18 PBF, respectively. The ranges of VEI for these mosquitoes were 6.7-13.3 and 2.5-41.7 for the TH and US strains, respectively. There were no significant differences in infection rates between the two strains tested.

After taking a blood meal with 2,463 ± 208 mf/ml, infection rates with the TH strain were 40 and 33% and infection rates with the US strain were 20 and 12% on days 14 and 15 PBF, respectively. Ranges for VEI of these mosquitoes were 8.5-13.4 and 10.8-13.5 for the TH and US strains, respectively. There was a significant difference between the infection rates for the TH and US strains on day 15 PBF at this mf level ($p=0.0391$).

After taking a blood meal with 3,490 ± 211 mf/ml, infection rates for the TH strain were 13 and 41% and infection rates of the US strain were 25 and 28% on days 14 and 15 PBF, respectively. The ranges of VEI for these mosquitoes were 20-46.7 and 45.8-58.3 for the TH and US strains, respectively. There were no significant differences in the infection rates between the strains tested at this mf level.

After taking a blood meal with 5,000 ± 257 mf/ml, infection rates for the TH strain were 14, 14, and 0% and infection rates for the US strain were 18, 16, and 25% on days 14, 15, and 16 PBF, respectively. The ranges of VEI for these mosquitoes were 0-3.2 and 2.9-6.7 for the TH and US strains, respectively. There was a significant difference between the infection rates for the TH and US strains on day 16 PBF at this mf level ($p=0.001$).

Infection rate comparisons within strain for tested days at each mf level showed significant differences in infection rates between days 14 and 15 PBF ($p=0.0227$) and between days 15 and 16 PBF ($p=0.0219$) for the TH strain after taking a blood meal with 3,490 ± 211 mf/ml and significant differences in infection rates between days 15 and 16 PBF ($p=0.0159$) and days 14 and 16 PBF ($p=0.0159$) with the TH strain after taking a blood meal with 5,000 ± 257 mf/ml.

Infection rate comparisons within the TH strain for mf levels on day 14 PBF showed significant differences in the infection rates in mosquitoes that took a blood meal with 663 and

Table 1
Infection rates in Thai (TH) and US (US) strains of *Aedes albopictus* with *Dirofilaria immitis* infective stage larvae determined on days 7-18 post-blood feed (PBF).

Blood meal microfilariae per ml (Mean ± SE)	Mosquito strain	Day (PBF)	No. mosquitoes tested	Infection rate (95% confidence interval)
663 ± 79	TH	14	40	5 (1, 17)
	US	14	40	8 (3, 20)
	TH	15	40	8 (3, 20)
	US	15	40	10 (4, 23)
	TH	16	40	10 (4, 23)
	US	16	30	13 (5, 30)
1,410 ± 93	TH	16	30	3 (1, 17)
	US	16	30	10 (3, 26)
	TH	17	30	13 (5, 30)
	US	17	30	3 (1, 17)
	TH	18	30	7 (2, 21)
	US	18	30	23 (12, 41)
2,463 ± 208	TH	14	20	40 (22, 61)
	US	14	20	20 (8, 42)
	TH	15	52	33 (22, 46)
	US	15	34	12 (5, 27)
3,490 ± 211	TH	14	30	13 (5, 30)
	US	14	20	25 (11, 47)
	TH	15	32	41 (26, 58)
	US	15	43	28 (17, 43)
	TH	16	32	13 (5, 18)
5,000 ± 257	TH	14	50	14 (7, 26)
	US	14	50	18 (10, 31)
	TH	15	50	14 (7, 26)
	US	15	50	16 (8, 29)
	TH	16	40	0
	US	16	40	25 (14, 40)
7,480 ± 551	US	7	40	0
	US	14	40	35 (22, 50)

2,463 mf/ml, at 2,463 and 3,490 mf/ml and at 2,463 and 5,000 mf/ml.

Infection rate comparisons in the US strain among the mf levels of on day 14 PBF showed a significant difference in the infection rates between the mosquitoes that took a blood meal with 663 and 7,480 mf/ml.

DISCUSSION

Canine heartworm, *Dirofilaria immitis*, is a common filarial nematode in dogs in Thailand (Choochote *et al*, 1987; Niwetpathomwat *et al*, 2006). The life cycle of this nematode involves infected dogs and a mosquito vector. The risk for

Table 2
Vector efficiency index (VEI) for Thai (TH) and US (US) strains of *Aedes albopictus* for *Dirofilaria immitis* determined on days 14-18 post-blood feed (PBF).

Blood meal microfilariae (mf) per ml (Mean ± SE)	Mosquito strain	No. mf in mosquito midgut (Mean ± SE)	No. L3 in mosquito (Mean ± SE)	Day (PBF)	VEI[a]
663 ± 79	TH	3.1 ± 0.4	0.1 ± 0.1	14	3.2
	US	2.4 ± 0.4	0.1 ± 0.1	14	4.2
	TH	3.1 ± 0.4	0.2 ± 0.1	15	6.5
	US	2.4 ± 0.4	0.2 ± 0.1	15	8.3
	TH	3.1 ± 0.4	0.2 ± 0.1	16	6.5
	US	2.4 ± 0.4	0.3 ± 0.2	16	12.5
1,410 ± 93	TH	1.5 ± 0.2	0.1 ± 0.1	16	6.7
	US	1.2 ± 0.2	0.1 ± 0.1	16	8.3
	TH	1.5 ± 0.2	0.2 ± 0.1	17	13.3
	US	1.2 ± 0.2	0.03 ± 0.03	17	2.5
	TH	1.5 ± 0.2	0.1 ± 0.1	18	6.7
	US	1.2 ± 0.2	0.5 ± 0.2	18	41.7
2,463 ± 208	TH	8.2 ± 1.8	0.7 ± 0.2	14	8.5
	US	3.7 ± 0.7	0.5 ± 0.3	14	13.5
	TH	8.2 ± 1.8	1.1 ± 0.3	15	13.4
	US	3.7 ± 0.7	0.4 ± 0.2	15	10.8
3,490 ± 211	TH	3.0 ± 0.6	0.6 ± 0.4	14	20.0
	US	2.4 ± 0.4	1.4 ± 0.7	14	58.3
	TH	3.0 ± 0.6	1.4 ± 0.5	15	46.7
	US	2.4 ± 0.4	1.1 ± 0.5	15	45.8
	TH	3.0 ± 0.6	0.4 ± 0.2	16	13.3
5,000 ± 257	TH	12.5 ± 1.8	0.3 ± 0.1	14	2.4
	US	10.5 ± 2.2	0.6 ± 0.3	14	5.7
	TH	12.5 ± 1.8	0.4 ± 0.2	15	3.2
	US	10.5 ± 2.2	0.3 ± 0.2	15	2.9
	TH	12.5 ± 1.8	0	16	0
	US	10.5 ± 2.2	0.7 ± 0.2	16	6.7

[a] VEI defined as the average number of L3 developed in the mosquito multiplied by 100, divided by the average number of ingested microfilariae.

infection with *D. immitis* depends on the level of mircrofilaremia in the infected dog and mosquito. To determine a vector in nature, many criteria have to be considered, including the number of mosquitoes in that area, the number of infected dogs, blood feeding preference, frequency of mosquitoes, and the ability of the mosquito to facilitate the development of the infective larval stage (L3).

Aedes albopictus was selected for this study because it is found in rural and urban areas in Thailand. They are an important vector, similar to *Ae. aegypti*. Both infection rate and vector efficiency index (VEI) were used in this study to

indicate the vector competency of this mosquito in laboratory conditions. We found no L3 in blood-fed mosquitoes examined on day 7 PBF. This is similar to the findings of Nayar and Knight (1999) who found the development of microfilaria (mf) to L3 in the Malpighian tubules of the mosquito happened on days 14-17 PBF.

The potential for mosquitoes to be infected with *D. immitis* is higher when mf levels in the blood meal are higher (Tiawsirisup and Nithiuthai, 2006). The development of *D. immitis* mf to L3 in the mosquito takes place in the mosquito's Malpighian tubules. Large concentrations of microfilariae cause injury to the Malpighian tubules which can cause mosquito mortality, particularly during the first week PBF (Apperson *et al*, 1989; Nayar and Knight, 1999).

The VEIs in the TH strain of *Ae. albopictus* were 3.2, 8.5, 20 and 2.4 and in the US strains of *Ae. albopictus* were 4.2, 13.5, 58.3 and 5.7 after taking blood meals with 633, 2,463, 3,490 and 5,000 mf/ml, respectively, when tested on day 14 PBF. This shows there is a correlation between VEI and microfilaria level in the blood meal except when the microfilaria level in the blood meal is higher than 5,000 mf/ml, which may cause mosquito mortality.

There are many levels of susceptibility of *Ae. albopictus* to the development of *D. immitis* mf to L3. A previously study of *Ae. albopictus* in the United States found the development of mf to L3 could not occur (Apperson *et al*, 1989). The development of *D. immitis* larva in some strains of *Ae. albopictus* was arrested at the end of the first larval stage in the Malpighian tubules, and was an expression of refractoriness, *ie* the infection rate of *Ae. albopictus* with L3 will be less than what it was supposed to be. The susceptibility of *Ae. albopictus* to *D.immitis* infection also has a genetic basis (Nayar and Knight, 1999). This study showed the US strain of *Ae. albopictus* used in this study is a competent vector for *D. immitis*. The US strain in this study was collected from the state of Missouri, USA. The infection rate with L3 was 28% and the VEI was 45.8 after a blood meal with 3,490 ± 211 mf/ml, tested on day 15 PBF.

This study tested the susceptibility of some strains of *Ae. albopictus* from Thailand and the United States to *D. immitis* in laboratory conditions. Field studies need to be performed to assess the role of this mosquito in the transmission of *D. immitis* in nature. The susceptibility of this mosquito in nature may be different due to inbreeding in a restricted environment. Inbreeding can result in large numbers either susceptible or resistant (Nayar and Knight, 1999). *Ae. albopictus* has been found to be a natural vector for *D. immitis* in some countries, such as Japan and Italy (Konishi, 1989; Cancrini *et al*, 2003).

Infection of the mosquito with *D. immitis* can also cause an elevated dissemination rate of some viruses in the mosquito, such as chikungunya virus, because the mf of *D. immitis* cause a hole during penetration through the midgut epithelial layer. When the midgut of the mosquito is punctured immediately after the mosquito ingested a virus, a higher dissemination rate is observed for that mosquito (Zytoon *et al*, 1993a). Transovarial transmission of the virus in the mosquito has also been observed in laboratory conditions (Zytoon *et al,* 1993b). Co-infection with arbovirus and microfilaria of any filarial nematode are more likely to increase the infectivity of the mosquito with the virus.

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