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POTENTIAL MOSQUITO (DIPTERA: CULICIDAE) VECTORS OF *DIROFILARIA IMMITIS* (FILARIIDAE: ONCHOCERCIDAE) IN TWO URBAN AREAS OF KUALA LUMPUR AND ITS PREVALENCE IN STRAY DOGS

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Abstract A study was conducted in the urban areas of Kuala Lumpur using CDC light trap, dog-baited trap and human landing collection to determine the vectors. Eleven species of mosquitoes were obtained, of which only *Aedes albopictus*, *Armigeres subalbatus* and *Culex quinquefasciatus* were in significant numbers. The predominant species was *Ar. subalbatus* in both the human landing and dog bait collection. Only this species was positive for *D. immitis*. Experimental studies also showed *Ar. subalbatus* and *Ae. aegypti* to be susceptible while *Cx. quinquefasciatus* was refractory. Due to the propensity for biting humans and dogs, *Ar. subalbatus* can be considered as the primary vector for *D. immitis* in Kuala Lumpur.
Key Words Dirofilariasis, *Armigeres subalbatus*, *Ae. aegypti*

INTRODUCTION

Dirofilaria immitis (Leidy) commonly known as dog heartworm is a nematode parasite that infects the right ventricle and pulmonary artery of dogs and other carnivores. It has a wide geographical distribution and various mosquito species of *Culex*, *Aedes*, *Anopheles* and *Mansonia* have been incriminated as vectors (Ludlam et al., 1970) but their susceptibility varies from country to country as shown in various studies (Ahid et al., 2000, Brito et al., 1999, Loftin et al., 1995; Theis et al., 2000). In Malaysia, Hodgkin (1937) was probably the first to report the occurrence of larval *D. immitis* in mosquitoes (*Mansonia indiana* Edwards from Sabak Bernam) in the then Federated Malay States. Wharton (1960, 1962) encountered this parasite in *Ma. dives* (Schiner), *Ma. bonneae* Edwards, *Ma. annulata* Leicester and *Ma. uniformis* (Theobald) from various parts of the country. From the Northern states of Perak and Kedah, *D. immitis* was found in *Ma. indiana*, *Anopheles campestris* Reid, *An. lesteri* Baisas and Hu and *An. nigerrimus* Giles (Reid et al., 1962). In a study on the natural infection of mosquitoes with *D. immitis* Basio and Cheong (1977) found infective L3 larvae in *Aedes albopictus* (Skuse), *Aedes* spp., *Armigeres malayi* (Theobald) and *Armigeres* spp.

Besides reporting *dirofilariasis* from dogs in various parts of the country (Ramachandran et al., 1964; Yap et al., 1976; Kan et al., 1977; Faridah and Lee, 1981), *D. immitis* was found in wild black panther, *Panthera pardus* from Pahang (Strauss and Sivanandam, 1966), and in a Malaysian clouded leopard, *Neofelis nebulosa* (Zahedi et al., 1986). *D. immitis* although considered mainly being a dog parasite has in recent years emerged as a zoonotic infection where human cases of dirofilariasis have been reported (Kochar, 1984; Villanueva and Rodriquez, 1993, Kondo and Fujita, 1991; Lee et al., 2000). In Malaysia *D. immitis* has been reported in human by Ambu et al. (1997, 2002).

In view of the importance of this filarial parasite, this study was carried out to determine local vector host associations. The knowledge of local vector host association will provide useful information for incriminating mosquito species most likely to be the primary vector at the given site. This paper reports the prevalence of *D. immitis* in stray dogs in Kuala Lumpur, natural infection in mosquitoes and laboratory experiments carried out on potential vector mosquitoes.

MATERIALS AND METHODS

Study Sites. Two sites in Kuala Lumpur were selected for the study. Federal Hill is a residential area situated in the heart of Kuala Lumpur. It is a hilly forested area with individual bungalow houses. Most people living in this area are from higher income group and are of multi ethnic origin. Dogs are also present in most houses of the non Muslims. Air Panas is situated in the east of Kuala Lumpur and is a Chinese New Village. Most people in this area are from the middle to lower income groups. Dogs are present in most houses. There are lots of trees and vegetation in the area. The city hall dog pound is also situated in this area.

Mosquito Collections and Processing. Three methods of mosquito collections were carried out and in each site trapping was carried out once a month for three months from June to August 2003. In total, 18 CDC battery-operated light traps augmented with 150 g of dry ice were operated at fixed sites. Landing catches on human bait were conducted outdoors near human habitation by two teams of three men. The men using flash-lights actively collected mosquitoes landing on them with 50 x 19 mm tubes. A dog-baited net trap was also set up in both sites. A medium size mosquito net measuring 180 cm in length, 130 cm in width and 150 cm in height was suspended from four poles so that there was a gap of about 20 cm between the ground and the bottom of the net. A dog was securely fastened in the centre of the net and as it was tame, it posed no problems. All mosquitoes in the dog-baited trap were collected at the end of 2200 hours. All procedures commenced at 1800 hours and terminated at 2200 hours.

All mosquitoes caught were identified, recorded and pooled according to species and collection type. The mass dissection technique described by Cheong (1985) was used. Briefly this consists of a glass funnel approximately 16 cm in diameter and 1.5 cm at the narrow end and across this narrow end was placed a wire gauze of approximately 70 mesh and a scissor clamp was fixed to an extension rubber tubing. Cotton gauze of approximately 55-60 mesh was placed above the wire gauze. The pooled mosquitoes were placed in a petri dish to which normal saline was added and a flat bottom tube was used to grind the mosquitoes. The contents were then poured into the funnel and left for one hour, after which the clamp was gently opened to allow the sediment containing infective larvae to be collected in a petri dish. This was then examined under a dissecting microscope. Mosquitoes that were blood-fed from the dog-baited trap were identified and maintained in paper cups for 3 days before dissection.

Identification of larvae. All larvae were transferred to Bless fluid to kill the worm and straighten it. It was then transferred to a ringed slide containing filarial mounting medium. A cover slip was placed over this and was sealed. All worms were identified using the description in Yen et al. (1982).

Field and Laboratory Infection of Mosquitoes. Wild caught *Ar. subalbatus* (Coquillett) and *Ae. albopictus* (since we did not have a laboratory colony) were introduced into an infected dog-baited trap. From previous experience, we know that wild caught mosquitoes do not easily feed when exposed to bait. It is for this reason that the mosquitoes were introduced into the dog- baited trap so that they could take their time to feed. The blood-fed mosquitoes were maintained for 15 days before dissection. Three species of laboratory bred mosquitoes namely *Ae. aegypti* (Linnaeus), *Ae. togoi* (Theobald) and *Cx. quniquefasciatus* Say were allowed to feed on an infected dog. Briefly, 150 mosquitoes of each species in different cages were allowed to feed on the anaesthetized dog infected with dirofilaria (5.5 microfilariae per µl of blood at time of feeding). The different species of mosquitoes were fed on the same dog, two cages at a time. The fed mosquitoes were maintained in the insectary at a temperature of about 26° C with relative humidity of 85% and were provided with sugar solution. Mosquitoes were dissected individually starting from day 6 to day 18.

Examination of Dog Blood. Two ml of blood was obtained from 104 stray dogs at random from the City hall dog pound at Air Panas. All samples were examined by wet mount method for the determination of dirofilaria. In the wet mount method, about 200 µl of blood was placed on a slide and a cover slip was placed over it. It was then examined under 100X magnification. All positive samples and 10% of the negative samples were subjected to further examination whereby 60 µl of blood was spread on to a clean slide and dried overnight in an incubator. The slides were then dehaemoglobinized and fixed in methanol for 30 seconds and stained with Giemsa stain. The slides were then examined under a compound scope.

This project was approved by the Animal Use Committee of the Ministry of Health, Malaysia.

RESULTS

A total of 11 species of mosquitoes were obtained by the various trapping techniques. However, only 3 species were predominant in both sites as shown in Fig. 1. They were *Ae. albopictus, Ar. subalbatus* and *Cx. quinquefasciatus*. Of these *Ar. subalbatus* and *Ae. albopictus* were more attracted to human bait while *Cx. quinquefasciatus* was attracted to light trap. Mass dissection of field collected mosquitoes showed the presence of L3 *D. immitis* in *Ar. subalbatus* from Federal Hill and Air Panas.

Table 1 shows the results of the field collected mosquitoes that were introduced into an infected dog-baited trap. The survival rate of *Ar. subalbatus* on day 14 was 57.6% (19/33) and for *Ae. albopictus* was 40% (8/20). Only one *Ar. subalbatus* was positive with L3 worm of *D. immitis* in head, thorax and abdomen, thus the percentage infective was 5.26%. For *Ae. albopictus*, one was positive and L3 larvae were found in the malphigian tubules.

Table 1. Infection rates of mosquitoes collected and introduced to a dog baited trap (*Dirofilaria* infected dog).

Species	Days after feeding	No. dissected	No. positive	% positive	Worm load	Larval stage	Location in mosquitoes
Ae. albopictus	14	8	1	12.5	3	III	Malphigian tubules
Ar. subalbatus	14	19	1	5.26	11	III	Head, thorax abdomen

Table 2. Total number of mosquitoes exposed to infected dog, percentage fed and dissected.

Species	No. exposed	No. blood fed (%)	Total dissected (%)	Total died (%)
Ae. aegypti	150	135 (90)	75 (55.6)	60 (44.4)
Ae. togoi	150	130 (86.7)	38 (29.2)	92 (70.8)
Cx. quinquefasciatus	150	50 (33.3)	21 (42)	29 (58)

Table 3. Susceptibility status of laboratory infected mosquitoes

Species	Days after feeding	No. dissected	No. positive	Worm load	Larval stage	Location in mosquitoes	Comments
Ae. aegypti	6	10	7	23	I	Malphigian tubules	55.8% infected
	7	12	6	42	I	Malphigian tubules	
	13	8	3	16	Late	Malphigian tubules	1.4% infected
	14	8	6	36	II	Malphigian tubules	
	15	10	3	13	Late	Malphigian tubules	1 mosquito had
	16	5	4	23	II	Malphigian tubules	stage III worm in

	17	13	7	31	Late	Malphigian tubules	head, thorax and
	18	9	3	20	II	Malphigian tubules	abdomen
					III	Head, thorax,	
					III	abdomen	
					III		
Ae. togoi	6	5	3	19	I	Malphigian tubules	
	7	5	1	1	I	Malphigian tubules	55.2% infected
	10	12	9	33	II	Malphigian tubules	
	13	5	2	3	Late	Malphigian tubules	
	14	8	5	41	II	Malphigian tubules	
	16	3	1	4	III	Malphigian tubules	
					III		
Cx. quinquefasciatus	6	5	0	0	-	-	
	7	6	2	3	I	Malphigian tubules	7.14% infected
	10	3	0	0	0	-	
	13	3	0	0	0	-	
	17	4	0	0	0	-	

In the laboratory study although 150 mosquitoes were exposed to the infected dog, a large percentage of the mosquitoes died before dissection as shown in Table 2. The infected rate in *Ae. aegypti*, *Ae. togoi* and *Cx. quinquefasciatu*s was 55.8, 55. 2 and 7.14 respectively (Table 3). Only one *Ae. aegypti* had stage III larvae in head, thorax and abdomen, thus the infective rate was 1.4. In most instances the stage III larvae were found in the malphigian tubules. Of the 104 dogs examined for *D. immitis* only 4 were positive. Thus the prevalence rate was 3.85.

DISCUSSION

It was observed that in our study sites more mosquitoes were attracted to human bait than light trap or dog bait trap. In transmission of dirofilaria, this observation seems paradoxical. However, Pinger (1985) noted that perhaps dogs are not nearly attractive to most species of mosquitoes as are humans. Our findings incriminate *Ar. subalbatus* as a potential vector of *D. immitis*. This was further strengthened where *D. immitis* was able to develop to the infective stage in the head, thorax and abdomen of this mosquito. This finding is in agreement with Cheong et al.(1981) who incriminated *Ar. subalbatus* as an important vector of *D. immitis*.

Although the *Culex* sp. were observed as vectors of dirofilaria in Queensland and Brisbane, Australia (Bemrick and Moorhouse, 1968), there was no evidence in this study that could incriminate the *Culex* mosquitoes as vectors. Only two *Cx. quinquefasciatus* in our laboratory study had stage I larvae in the malpahgian tubules on day 17. However, the vector efficiency of *Cx. quinquefasciatus* differs from country to country. Perhaps based on circumstantial evidence it may have been incriminated as a vector. Studies carried out by Lowrie (1991) proved that the vector efficiency of *Cx. quinquefasciatus* following infection with *D. immitis* was regarded as poor.

A study in Brazil on vector competence of *Cx. quinquefasciatus* (Ahid et al., 2000) observed that 15 days after being fed on infected blood, the infection and infective rates were low in the populations of *Cx. quinquefasciatus*. This observation took into account the vector efficiency, the number of microfilariae ingested and the number of infective larvae. Due to its low susceptibility to *D. immitis* infection, the study considered this species as a secondary vector of the disease. However, in a laboratory study in Singapore Chellappah and Chellappah (1968) found 58% of the *Cx. quinquefasciatus* were positive for *D. immitis* and more than 90% had L3 larvae in the head. However, we feel strongly that *Cx. quinquefasciatus* is refractory to *D. immitis*

infection based on poor feeding on infected dog (33.3%) and previous studies have shown that they prefer avian blood (Vythilingam et al., 1996).

The other species that was predominant in the study but could not be incriminated as a vector of *D. immitis* was *Ae. albopictus* as none of the wild caught were infected. Although Sulaiman and Jeffery (1986) reported the presence of *D. immitis* larva in the proboscis of *Ae. albopictus*, we only found L3 larvae in the malphigian tubules of the mosquitoes exposed to infected dog. Thus the role of *Ae. albopictus* as a vector of dirofilaria remains doubtful.

In our laboratory study we found 55.8 % of *Ae. aegypti* was infected but only 1.4% was infective. Studies by Chellepah and Chellapah (1968) in Singapore and Brito et al. (1999) in Brazil found *Ae. aegypti* susceptible to *D. immitis*, which shows that it has the potential to be a vector.

Among all three methods of mosquito collection, the dog bait trap attracted the least percentage of mosquitoes. Reasons for this are difficult to fathom. However, *Ar. subalbatus* was the predominant species in the dog-baited trap and it was also predominant in the human landing collection. It was the only species found positive in nature. Thus from this study we can hypothesis that *Ar. subalbatus* is the primary vector of *D. immitis* in our study areas.

The first confirmed case of dirofilariasis in Malaysia was in a 29 year old Malay female from Kampung Batu Lima, near Yong Peng in Johore. The worm was identified as *D. repens*, commonly found in domesticated cats (Mak and Thanalingam, 1984). In a survey of *D. immitis* infection among cardiovascular disease patients at the National Heart Institute Kuala Lumpur, 8 out of 299 blood specimens were positive for *D. immitis* (Ambu et al., 2002). These findings showed that we have in our midst an emerging arthropod borne disease which for obvious reasons can no longer be ignored. Further studies should be carried out to determine secondary vectors.

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