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ORIGINAL ARTICLE

Identification of potential vectors of *Dirofilaria immitis* and *Brugia pahangi* (Spirurida: Filariidae): First observation of infective third-stage larva of *B. pahangi* in *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Information on the mosquito species that transmit canine filariosis is scanty. Hence, an experimental study was conducted to identify the potential vectors responsible for the transmission of *D. immitis* Leidy and *B. pahangi* Buckley & Edeson. A total of 367 mosquitoes belonging to six species containing both laboratory and field strains (i.e. *Aedes togoi* Theobald, *Aedes aegypti* Linnaeus, *Aedes albopictus* Skuse, *Culex quinquefasciatus* Say, *Culex vishnui* Theobald and *Anopheles dirus* Peyton & Harrison) were used in this study. All mosquitoes were artificially fed on either *D. immitis* or *B. pahangi* microfilariae (mfs) infected blood by using the Hemotek™ membrane feeding system. Out of 367 mosquitoes, 228 (64.9%) were fully engorged. After feeding on *D. immitis* (20%) and *B. pahangi* (33%) mfs positive blood, the mortality rates for *Cx. quinquefasciatus* were found to be slightly lower than that of other species of mosquitoes. On the other hand, majority of *An. dirus* were found to be incapable to withstand the infection of mfs as the mortality rates were relatively high (*D. immitis* = 71.4%; *B. pahangi* = 100.0%). *Brugia pahangi* was detected in *Ae. togoi* and *Cx. quinquefasciatus* with infection rates of 50% and 25%, respectively. *Aedes togoi* was the only species infected with *D. immitis* with an infection rate of 69%. Our results showed that *Ae. togoi* was an excellent experimental vector for both *D. immitis* and *B. pahangi*. This study also documented the observation of *B. pahangi*, for the first time in the head region of *Cx. quinquefasciatus* under a laboratory setting.

KEYWORDS

Brugia pahangi; *Dirofilaria immitis*; filaria; mosquitoes; vector

1.0 Introduction

Mosquito-borne filarioid nematodes such as *Dirofilaria immitis* and *Brugia pahangi* cause diseases to mammals, especially in domestic dogs and cats [1,2]. *Dirofilaria immitis* has a wide geographical distribution whereas *B. pahangi* is endemic in Southeast Asia (i.e. Malaysia, Thailand and Indonesia) [3,4]. Both parasites principally infect canine host, but the increasing reports of human filariosis highlight their potential as emerging zoonosis globally [2,5]. Transmission of filarioid parasites depends on the availability of microfilaraemic hosts, vectors, and favorable temperatures for the growth of the infectious stages in mosquitoes [6]. Mosquitoes of the genera *Aedes, Culex*, and *Anopheles* are the main vectors of filarioid parasites and they transmit L3 infective larvae of these parasites to potential hosts through their bites [7].

The climate and weather in Malaysia provide a conducive environment for all these species of mosquitoes and consequently, their proliferation can increase the risk of transmission [8]. In Malaysia, *Mansonia indiana* Edwards was identified as the vector of *D. immitis* for the first time in 1937 [9]. Subsequently, *Mansonia dives* Schiner, *Mansonia bonneae* Edwards, *Mansonia annulata* Leicester, *Mansonia uniformis* Theobald, *Anopheles campestris* Reid, *Anopheles lesteri* Baisas, *Anopheles nigerrimus* Giles were also identified as the vectors in Malaysia [10–12]. On the other hand, infection of *B. pahangi* was observed in mosquitoes of five genera, *Mansonia, Aedes, Anopheles, Armigeres* and *Culex*. Notably, *Armigeres obturbans* Walker was reported as an efficient vector in transmitting *B. pahangi* in various studies [13–15].

Experimental studies on different mosquito species in Southeast Asia have been conducted to identify the potential vectors of *D. immitis* and *Brugia* spp [16–18]. However, most experimental studies were on *Brugia malayi* Brug and information regarding *in vitro* trial of potential vectors of *B. pahangi* is scarce. Indeed, an experimental study on different mosquito species or different populations is important for incriminating additional vectors responsible for transmission.

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Accordingly, this study was conducted to identify the potential vectors of *D. immitis* and *B. pahangi* through an *in vitro* trial using field and laboratory strains of various mosquito species.

2.0 Methods

2.1 Ethics statement

This study was approved by the Medical Ethics Committee of Department Veterinary Services Malaysia [JPV: BPI/500-4/1/2 (18)] and the Institutional Biosafety and Biosecurity Committee, Universiti Malaya [UMIBBC/NOI/R/TNC/TIDREC-014/13,062,019]. Written informed consents were obtained from the owners of the animal shelters.

2.2 Microfilariae inoculums

Microfilariaemic blood samples (*D. immitis* and *B. pahangi*) were collected from infected dogs in an animal shelter located in Semenyih, Kuala Lumpur, Malaysia. Dog blood samples were collected randomly from the animal shelters and screened for filarial parasites and other pathogens by using serological test and microscopic Giemsa stain technique prior to selecting dogs for the present study [19–21]. After examination, a one-time blood collection was performed on two dogs; one was positive with single infection of *B. pahangi* and another one with single infection of *D. immitis*. Ten milliliters of blood samples were collected in Citrate Phosphate Dextrose Adenine (CDPA) anticoagulated blood tubes by a trained veterinarian and stored at 4°C until further processing. No medications were given to these animals prior to blood collections. The presence, viability, and number of mfs in all samples obtained were confirmed by microscopic technique. Approximately, 100 µl of blood was covered with a cover slide, and mfs were counted by microscopic examination under 40x and 100x magnifications. Steps were repeated thrice, and an average was taken to determine the intensity of the infection. Further species identification of filarioid parasites was performed using a molecular technique [21]. In addition, a dog blood sample that was negative with all the detection techniques was taken and used as a control (control blood). Subsequently, blood was immediately fed to the mosquitoes by using the Hemotek™ system.

2.3 Mosquitoes

2.3.1 Field strains

Host-seeking mosquitoes were collected from urban and sub-urban areas in Klang Valley by using a human-landing catch technique. Briefly, human landing catch was conducted by sitting on the chairs with legs exposed. Collectors caught the landing mosquitoes with a transparent glass tube. *Aedes aegypti* and *Ae. albopictus* were collected from urban areas (GPS coordinate: 3.1162287831250723, 101.65313602754688), whereas *Cx. vishnui* was collected from a sub-urban area (GPS coordinate: 3.5623536415921504; 101.09889656993488). The age of the adult mosquitoes collected from field was unknown. Mosquitoes were transferred into plastic cups with moist cotton wool and transported to the laboratory for oral inoculation to the filarial infected blood.

2.3.2 Laboratory strains

Laboratory strains of *Ae. aegypti, Ae. albopictus, Ae. togoi, Cx. quinquefasciatus* originated from Institute for Medical Research Malaysia, and *An. dirus* originated from Malaria Research and Reference Reagent Resource Center (MR4/BEI resources) were reared and maintained in an insectarium under standard conditions (i.e. 26 ± 2°C) and a relative humidity (RH) of 80 ± 10% under a L12:D12 h light: dark cycle. In addition, 10% of sugar solution was provided daily to the mosquitoes as food source. Female mosquitoes aged 5–7 days were chosen for the inoculation experiments.

2.4 Infection of mosquitoes with microfilariae

Mosquitoes were starved overnight before the inoculation experiments. Three study groups were included: *D. immitis*-inoculated group, *B. pahangi*-inoculated group and negative control. Number of mosquitoes were recorded and separated into several cups according to species and placed inside a glove box approximately 127 cm × 254 cm × 95 cm in dimensions. Laboratory and field strains of mosquitoes were allowed to feed through Hemotek™ system (Discovery Workshop, UK) which had a surface area of 9.62cm^2 with blood containing single infection of *D. immitis* or *B. pahangi* mfs and negative control blood (negative control: *Ae. togoi* laboratory strain only) in an Arthropod Containment Level 2 (ACL2) laboratory located at Department of Parasitology, Universiti Malaya. Firstly, rubber rings were used to keep five aluminum holders in place after coated with parafilm. Two milliliters of blood were injected into the ports, which were then sealed with plastic stoppers. The temperature of the blood meal was maintained at 37°C using an electric heating element in the Hemotek™ system throughout the blood feeding process. The blood was fed to the mosquitoes by placing the cups (each carrying different species of mosquitoes) underneath the feeder, with the cup's nylon netting in contact with the feeder's membrane. The microfilaraemic counts of *D. immitis* and *B. pahangi* in the blood samples were 1000mfs/ml and 9000mfs/ml, respectively. All mosquitoes were sorted after inoculation. The cups containing mosquitoes were placed in a − 20°C freezer for 30 seconds to induce

a cold anesthesia. The cups which contained anaesthetised mosquitoes were then placed inside an ice box. Each mosquito that had been fed with either *D. immitis* or *B. pahangi* infected blood was inspected under the Olympus SZ51 stereomicroscope to examine if it had a full meal; and to determine the proportion of engorged females with blood bloated abdomens. Fully engorged female mosquitoes were transferred to a new cup according to species. Mosquitoes that did not take a full blood meal or did not take any meal were discarded.

2.5 Maintenance of the mosquitoes after inoculation

Mosquitoes were kept for 14 days [22] in a double containment (inner containment: sterile polystyrene cup, outer containment: small cage of 24 cm × 18.5 cm × 13 cm dimensions). The mosquitoes were maintained in ACL2 at 26°C with relative humidity of 85% and provided with 10% sugar solution. All mosquitoes were dissected individually after 14 days.

2.6 Assessment of mosquito mortality and detection of filarioid larvae in mosquitoes

Mortality of mosquitoes was recorded daily for 14 days, and dead mosquitoes were dissected to examine the presence of filarioid parasites. Mosquitoes were anaesthetized briefly by placing them in a freezer at −20°C for 30 sec. The mosquitoes were immobilized by removing their legs and wings on a chill table. Subsequently, they were kept at 4°C and dissected individually in a drop of buffered saline on a slide before microscopic examination. The head, thorax and abdomen of the mosquitoes were separated. Each body part was transferred to separate drops of buffered saline and macerated to liberate larvae of the filarioid parasites. Clean entomological forceps and needles were used for each dissection. All mosquitoes were dissected under a dissecting microscope. The number and location of larvae, and the stage of development were recorded.

2.7 Data analysis

Cumulative mortality rates were calculated based on the total number of mosquitoes that died naturally on 1-, 7- and 14-day post infection (dpi) divided with the total number of blood-fed mosquitoes. In addition, mortality rates were calculated by dividing the numbers of mosquitoes surviving for 14 days after the infective blood meal by the number of mosquitoes that took complete blood meals [4].

The accumulative numbers of dead mosquitoes were plotted against time, and the correlation coefficient were calculated and compared by using IBM SPSS V21 [23]. Infection rate (IR) was calculated based on the equation below [4].

$$\text{Infectionrate(IR)} = \frac{\text{Numberofbloodfedmosquitoeswith L3inbody}}{\text{Survivingmosquitoesatthe endofincubationperiod}} \text{x}100\%$$

3.0 Results

3.1 Feeding rates

The feeding rates were calculated as shown in Table 1. Three groups of mosquitoes had 100% feeding rate [i.e; *An. dirus* laboratory strain (14/14), *Ae. albopictus* field strain (13/13) feeding on *D. immitis* microfilaraemic blood and *Ae. aegypti* field strain (3/3) feeding on *B. pahangi* microfilaraemic blood]. On the other hand, there were lower feedings rates noted in *Cx. quinquefasciatus* laboratory strain [*D. immitis* = 5/15 (33.3%) and *B. pahangi* = 5/15 (31.6%)] and *Cx. vishnui* field strain [*D. immitis* = 10/24 (41.7%) and *B. pahangi* = 11/26 (42.3%)].

3.2 Survivability rates of mosquitoes

Mortality and survivability rates of mosquitoes are summarized in Table 1. At 1dpi, *B. pahangi*-inoculated group (0.8%) had a higher overall mortality rate (for both laboratory and field strains) compared to *D. immitis*-inoculated group (0.2%). No mortality was observed in all laboratory strains in *D. immitis*-inoculated group. However, mortality was observed in all field strains in *D. immitis*-inoculated group (*Ae. albopictus* = 7.69%; *Cx. vishnui* = 10%). On the other hand, mortality was observed in all laboratory strains (*Ae. albopictus* = 11.8%; *Ae aegypti* = 8.3%; *Cx. quinquefasciatus* = 1.7%; *Ae. togoi* = 3.3%) in *B. pahangi*-inoculated group except *An. dirus*. Besides, except *Ae. albopictus* (19%) which had the highest mortality rate among all other groups, no mortality was observed in field strains of *Cx. vishnui* and *Ae. aegypti* in *B. pahangi*-inoculated group.

On 7 dpi, all mosquito species in both microfilaraemic-inoculated groups had mortality rates ranging from 10 to 46.2%. *Aedes albopictus* (6/13; 46.2%) laboratory strain in *D. immitis*-inoculated group and *Ae. aegypti* (5/12; 41.7%) laboratory strain in *B. pahangi*-inoculated group had the highest cumulative mortality rates.

Of all mosquito species, laboratory strain of *Cx. quinquefasciatus* which was blood-fed on *D. immitis* and *B. pahangi* mfs had the lowest mortality rates of 20% (1/5) and 33.3% (2/6), respectively. On the other hand, *An. dirus* was shown to be incapable to

Table 1. Feeding and mortality rates of mosquitoes during infection trials with *D. immitis* and *B. pahangi*.

	No. of mosquitoes						
				Mortality rates (%)			
Species of mosquito	Total mosquitoes	No. of engorged mosquitoes with blood meal N (%)	Average no. of mortality daily (range)	1 dpi N (%)	7 dpi N (%)	14 dpi N (%)	No. of surviving more than 14 days (N)
D. immitis							
Laboratory strain							
Ae. aegypti	13	10 (76.7)	0.35 (0–1)	0	1 (10.0)	5 (50.0)	5
Cx. quinquefasciatus	15	5 (33.3)	0.07 (0–1)	0	1 (20.0)	1 (20.0)	4
Ae. togoi	29	24(82.6)	0.71 (0–4)	0	5 (20.8)	10 (41.7)	14
An. dirus	14	14(100)	1.00 (0–2)	0	3 (21.4)	10 (71.4)	4
Field strain							
Ae. albopictus	13	13(100)	0.79 (0–3)	1 (0.08)	6 (46.2)	11 (84.6)	2
Cx. vishnui	24	10(41.7)	0.36 (0–1)	1 (10.0)	1 (10.0)	5 (50.0)	5
B. pahangi							
Laboratory strain							
Ae. albopictus	18	17(94.4)	0.43 (0–1)	2 (11.8)	4 (23.5)	6 (35.3)	11
Ae. aegypti	14	12(81.0)	0.57 (0–1)	1 (8.3)	5 (41.7)	8 (66.7)	4
Cx. quinquefasciatus	19	6(31.6)	0.14 (0–1)	1 (1.7)	1 (1.7)	2 (33.3)	4
Ae. togoi	36	30(83.3)	1.29 (0–4)	1 (3.3)	8 (26.6)	18 (60.0)	12
An. dirus	19	12(63.2)	0.57 (0–5)	0	4 (33.3)	12 (100.0)	0
Field strain							
Ae. albopictus	22	21(95.5)	1.0 (0–7)	4 (19.0)	6 (28.6)	14 (66.6)	7
Cx. vishnui	26	11(42.3)	0.64 (0–3)	0	4 (36.4)	9 (81.8)	2
Ae. aegypti	3	3(100)	0.07 (0–1)	0	1 (33.3)	1 (33.3)	2
Negative control							
Laboratory strain							
Ae. togoi	50	40(80)	0.2 (0–2)	0	3(7.5)	8(20.0)	32

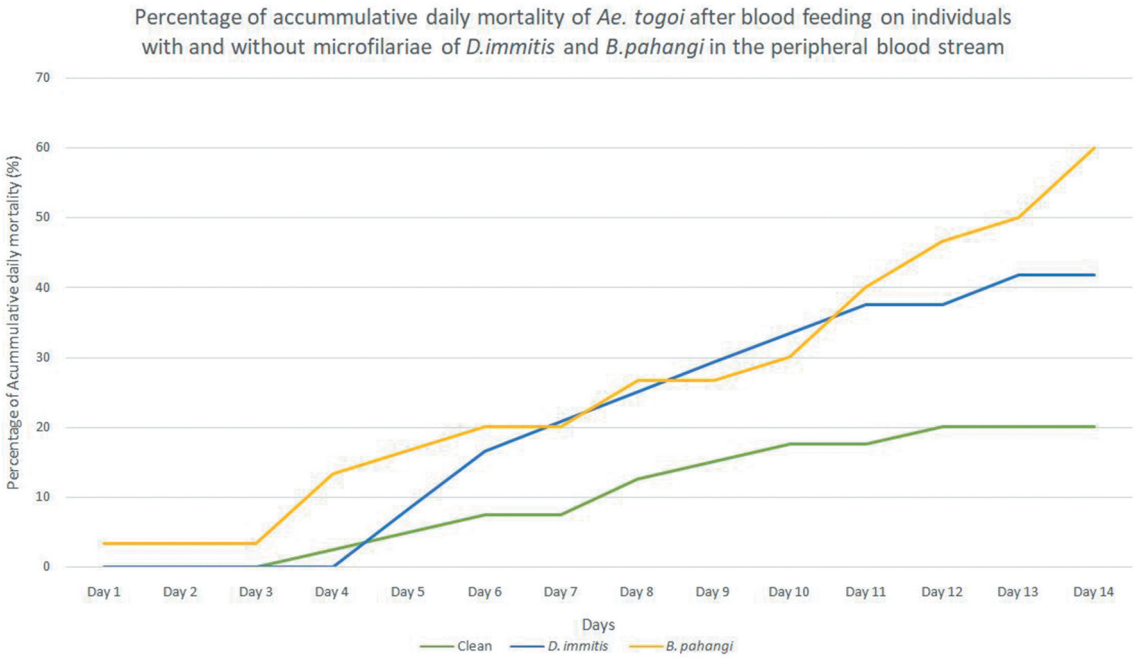

Figure 1. Percentage of accumulative daily mortality of *Ae. togoi* after blood feeding on individuals with and without microfilariae of *D. immitis* and *B. pahangi* in the peripheral blood stream.

Table 2. Infective rates and parasite load of *Ae. togoi* and *Cx. quinquefasciatus* after feeding on blood containing *D. immitis* (microfilarial density = 1000mf/100 µl) and *B. pahangi* microfilariae (microfilarial density = 9000 mf/100 µl), with all mosquitoes dissected 14 days after feeding.

			No. (%) of L3 found		
Mosquito Species	Infection rate % (N)	Average No L3 per infected mosquito (range)	% Head (No.)	% Thorax (No.)	% Abdomen (No.)
D. immitis					
Ae. togoi	69 (9/13)*	5 (1–12)	71.1 (32)	28.9 (13)	0 (0)
B. pahangi					
Ae. togoi	50 (8/16)*	4.75 (1–9)	39.5 (15)	55.3 (21)	5.3 (2)
Cx. quinquefasciatus	25 (1/4) *	1 (1)	100 (1)	0 (0)	0 (0)

* Number of mosquitoes infected/Total mosquitoes dissected after 14 days

withstand the infection of mfs as the mortality rates were relatively high (*D. immitis* = 71.4% (10/14); *B. pahangi* = 100% (12/12)).

Given the availability of high number of *Ae. togoi* to test against all inoculated groups, a graph was plotted to determine the vector competency of *Ae. togoi* toward the infection of both *B. pahangi* and *D. immitis*. The cumulative mortality of *Ae. togoi* infected with *D. immitis* and *B. pahangi*, and *Ae. togoi* fed with negative microfilaraemic blood was plotted against time with correlation coefficient of 0.979, 0.942 and 0.980, respectively for each curve (Figure 1). In addition, the mortality rates for both microfilaraemic-inoculated groups had significant differences (*D. immitis* and *B. pahangi* p = 0.02) compared to control group. However, there was no significant difference (p = 0.901) when compared between both microfilaraemic-inoculated groups.

3.3 Infection rate of mosquitoes

Out of six mosquito species, L3 larvae could only be found in two species of mosquitoes (Table 2). The infection rates for *Ae. togoi* in both microfilaraemic (*D. immitis* and *B. pahangi*) groups and *Cx. quinquefasciatus* in *B. pahangi* group were 69%, 50%, and 25%, respectively. For *D. immitis*-inoculated group, only *Ae. togoi* was found to be infected with L3 larvae at the head (71.1%) and thorax (28.9%) regions of mosquitoes with infection rate of 69%. Figure 2 shows the picture of L3 larva of *B. pahangi* in the head region of *Aedes togoi*. In addition, *Ae. togoi* and *Cx. quinquefasciatus* were also found to be infected with L3 larvae of *B. pahangi* at the head (*Ae. togoi* = 39.5%; *Cx. quinquefasciatus* = 100%), thorax (*Ae. togoi* = 55.3%) and abdomen (*Ae. togoi* = 5.3%) regions of mosquitoes with infection rates of 50% and 25%, respectively. However, only one larva of *B. pahangi* was found in

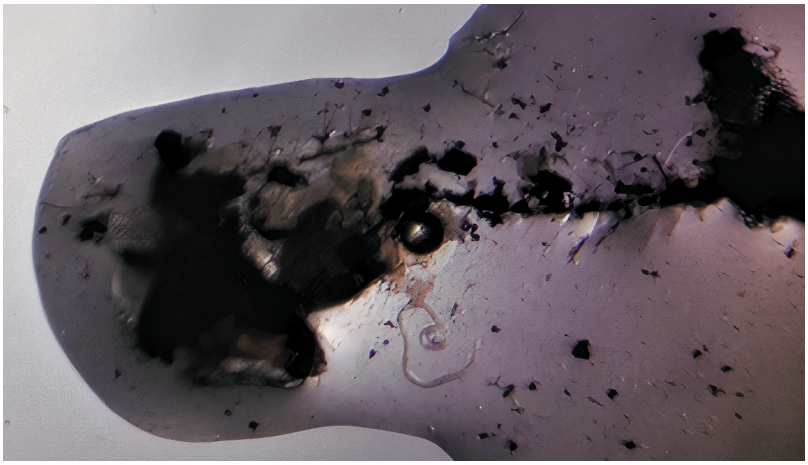

Figure 2. L3 larva of *B. pahangi* at the head region of *Ae. togoi*.

a *Cx. quinquefasciatus*. There was no significance difference (p = 0.296) between *Ae. togoi* infected with *D. immitis* and *B. pahangi*.

4.0 Discussion

A broad range of mosquito species have been experimentally tested as the vectors for *D. immitis* and *B. pahangi*. For *D. immitis*, the vectors responsible for transmission include *Ae. aegypti* [24–26], *Ae. albopictus* [27], *Ae. koreicus* Edwards [28] and *Cx. quinquefasciatus* [25]. In addition, a recent study by 29,also found that *Anopheles maculipennis* Meigen, *Aedes caspius* Pallas, *Aedes vexans* Meigen, *Culex theileri* Theobald and *Culex pipiens* Linnaeus were the potential vectors of *D. immitis* with the detection of DNA in head-thorax pools. On the other hand, *Ae. togoi* [30], *Ma. annulata* and *Ma. dives* were found to be susceptible to *B. pahangi*.

Besides, a study was conducted to investigate the relationship between microfilarial load in human host and uptake and development of *Wuchereria bancrofti* Cobbold mfs by *Cx. quinquefasciatus* [31]. Out of 504 mosquitoes, 20% of the mosquitoes died before reaching 12 dpi and 64% of these dead mosquitoes harbored a parasite of any stage. The mean number of developing larvae per infected dead mosquitoes was significantly higher than the mosquitoes dissected after 12 days. This study is comparable with our study where lower mortality rate was observed in *Ae. togoi* (20%) which served as the negative control in the current study compared to mosquitoes infected with *B. pahangi* (60.0%) and *D. immitis* (41.7%). This could be an indication of higher mortality among mosquitoes with higher parasitic load [31].

Aedes aegypti, Ae. albopictus and *Cx. quinquefasciatus* were reported to be the vectors for *D. immitis* in various countries [32–35]. However, our study could not identify these species as the potential vector because none of the field-collected mosquitoes were found to be positive with *D. immitis* mfs. A previous study reported the development of *D. immitis* mfs in *Cx. vishnui* during the initial observation [36]. Unfortunately, there was no evidence that larvae of mfs could complete their development to the infective stage. This result is comparable with our study whereby none of the *Cx. vishnui* mosquitoes were tested positive for *D. immitis*. The variation of vector found across the world might be due to the differences of vector efficiency index based on rapid parasitic development, proper nursing and low parasitic damage or death across different populations [37]. In addition, a systemic review by 38,showed that different geographical properties, such as latitude, longitude, mean temperature, yearly rainfall, sea level, and humidity could influence the mosquito prevalence rates in field investigations. The parameter 'country' should be interpreted from various factors, including the respective national vector control strategies and the ecological setting [39]. In addition, biological variation of mosquitoes can be influenced by a variety of ecological and geographical factors. Climate change, in combination with altering weather patterns, could promote the growth of *D. immitis* and the development of larvae within the mosquitoes [40]. Collectively, climatic patterns could play important role in vector-borne disease transmission.

Culex quinquefasciatus and *Ae. aegypti* collected from Manila, the Philippines were found to be infected with *D. immitis* mfs. From the experiment, both species had equal capability to allow the development of *D. immitis* within the mosquitoes [41]. 42,collected *Cx. quinquefasciatus* from different habitats and infected them at different times during the year. The study revealed a variation in percentage of infection with *D. immitis* mfs. Furthermore, 15,discovered that *Armigeres subalbatus* Coquillet was positive with *D. immitis* after 14 days of inoculation and it was considered as the primary vector for *D. immitis* in Kuala Lumpur, Malaysia. In addition, *Ar. subalbatus*

was also incriminated as a potential vector for *B. pahangi* in Malaysia [14]. Regrettably, *Ar. subalbatus* was not included in our study as we were unable to obtain this mosquito species from the sampling sites.

However, an experimental study using laboratory strain of mosquitoes in Singapore reported that *Cx. quinquefasciatus, Ae. aegypti* and *Ae. albopictus* were susceptible to *D. immitis* except *Ar. subalbatus*. In addition, *Cx. quinquefasciatus, Ae. aegypti* and *Ae. albopictus* were found to harbor more than 90% of L3 larvae in the head region [43]. A study done in 2005 by Vythilingam et al. observed only one *Ae. aegypti* which harbored L3 larvae in the head, thorax and abdomen regions. In addition, only one L1 larva was found in the malpighian tubes of *Cx. quinquefasciatus* [15]. Although 43 observed more than 90% of L3 of *D. immitis* larvae located in the head region of *Cx. quinquefasciatus*, 15,suggested that *Cx. quinquefasciatus* was refractory to *D. immitis* infection based on its poor feeding on infected dog, which is comparable to the feeding rate in our study (33.3%). Furthermore, previous study also showed that *Cx. quinquefasciatus* preferred avian blood compared to mammalian blood [44]. Although these mosquitoes were found to be infected with *D. immitis* under experimental settings in other studies, yet, none of these mosquito species (*Cx quinqiefasciatus, Ae. aegypti* and *Ae. albopictus*) were found to be susceptible to *D. immitis* in our study.

High infection rate of *B. pahangi* ranging from 95–100% in the laboratory strains of *Mansonia crassipes* Wulp, *Ma. annulata* and *Anopheles barbirostris* Wulp were observed in an earlier study [13]. However, *Ae. albopictus* showed no evidence of infection, whereas *Ae. aegypti* showed a small number of infective larvae. The results are comparable with our study in which none of the *Ae. albopictus* and *Ae. aegypti* were found positive. On the other hand, there were few experimental studies performed on other *Brugia* spp. against *Ae. togoi*. These studies found that *Ae. togoi* was a good experimental vector for both *B. malayi* and *Brugia patei* Buckley [16, 45; 17, 18, 46]. In addition, a study in Thailand [47] showed that *Ae. togoi* was a suitable laboratory vector for *B. pahangi*. Likewise, this finding parallels with our observation. Furthermore, *Cx. quinquefasciatus* was known to be resistant to *B. pahangi* in a study conducted in Kenya [48]. However, in our study, a L3 larva of *B. pahangi* was found in the head region of *Cx. quinquefasciatus* suggesting its potential as a vector for *B. pahangi*. It would be worthwhile to repeat these experiments by expanding the number of the mosquitoes in order to get a clearer picture on the infection rate and vector competency for *B. pahangi* in *Cx. quinquefasciatus*.

Anopheles dirus, a well-known vector of *Plasmodium* parasites in Thailand, Cambodia, Laos and Vietnam [49–52] was found to be responsible for filariosis in western Pacific and Southeast Asia [53]. Notably, *An. dirus* from northern Peninsular Malaysia was found to have a certain level of susceptibility toward *B. pahangi* [54]. However, none of the *An. dirus* was susceptible to *D. immitis* and *B. pahangi* in our study and the mortality rate of *An. dirus* was found to be very high (*D. immitis* = 71.4%, *B. pahangi* = 100%). *Anopheles dirus* was previously known as a species complex comprising at least seven members [55]. Different members or species might have different susceptibility toward the infection; however, we could not confirm whether the true *A. dirus* was used in 54.

More than 360 individuals of mosquitoes were used in the present study. Unfortunately, the reduced numbers of blood-fed mosquitoes and mosquitoes that survived at 14 dpi have made the sample size smaller, which is the limitation of the present study. An increased sample size of mosquitoes in future study would be beneficial in understanding the infection rate and vector competency of the tested mosquito species for both *D. immitis* and *B. pahangi*.

5.0 Conclusion

Laboratory strain of *Ae. togoi* remains as a good experimental vector for *D. immitis* and *B. pahangi*. This study showed for the first time that the laboratory strain of *Cx. quinquefasciatus* has the potential to be a vector for *B. pahangi*. However, extensive studies by increasing the sample size of mosquitoes and dissecting them on different days to show the developmental stages of the parasites should be carried out. Natural infection of *B. pahangi* in the field populations of *Cx. quinquefasciatus* should also be investigated.

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