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Research Note

Low prevalence of *Dirofilaria immitis* in dogs in Johor Bahru, Malaysia as a reflection of vector availability?

Ng, K.L.[1], Lee, E.L.[2] and Sani, R.A.[1*]
[1]Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia
[2]Global Pets Clinic, Johor Bahru
[*]Corresponding author e-mail: rehana@vet.upm.edu.my
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Abstract. This study was conducted to investigate the low prevalence of *Dirofilaria immitis* in dogs in Johor Bahru as reported by veterinary practitioners, using wet blood mount, Knott's Concentration Test and two heartworm antigen test kits (IDEXX Canine SNAP® 4Dx and RapiGEN®). This study also compared the two test kits used and determined the microfilaria species. Blood were collected from 100 owned dogs and 50 stray dogs in Johor Bahru via cephalic venipuncture. A thick blood smear was done and examined for samples that were positive for microfilaria species identification. The overall prevalence of *D. immitis* in dogs in Johor Bahru was 1.33% (2/150) and the microfilaria identified was *D. immitis*. The prevalence of heartworm in owned and stray dogs in this study was 1% and 2% respectively. With only one false negative result from RapiGEN® test kit, comparing the sensitivity between the two test kits could not be achieved. The low prevalence of *D. immitis* found in this study confirmed anecdotal evidence that prevalence of dirofilariasis is indeed low in Johor Bahru. Additionally, we speculate that dirofilariasis in dogs might be considered as an indicator of vector availability.

Heartworm disease is of considerable economic importance affecting canine population around the globe. It is caused by the parasitic worm, *Dirofilaria immitis*, in arteries of the lungs of affected animals and occasionally in the right side of the heart (American Heartworm Society, 2007). Mosquitoes are the intermediate host for *D. immitis*. Currently more than 70 species of mosquitoes have been recorded to transmit *D. immitis*. The type of mosquito present depends on locality and different species have different feeding habits (Kittleson, 1998). In Malaysia, the common species are *Armigeres* sp., *Culex* sp. and *Aedes* sp. (Vythilingam *et al.*, 2005).

Johor Bahru city is located at the southernmost tip of peninsular Malaysia, neighbouring Singapore and about 250km south from the densely populated Klang valley which includes the city of Kuala Lumpur. Most studies on heartworm in dogs were done in the Klang valley. Limited information is available in the literature on the occurrence and prevalence of heartworm disease in Johor Bahru. Most dog owners in Johor Bahru do not put their dogs on heartworm prevention programme (personal communication, Dr Tan Check Nam, Global Pets Clinic, Johor Bahru). This practice which differed from that in Klang valley initiated our interest to investigate the anecdotal

evidence from veterinary practitioners in Johor Bahru that dirofilariasis is low or even absent locally. The study was conducted at the end of October till mid- November 2010. The efficacy of two test kits widely used for the diagnosis of dirofilariasis was compared and any microfilaria detected was identified.

A total of 150 dogs were sampled; 100 owned dogs from four veterinary clinics and 50 stray dogs from one animal shelter in Johor Bahru. The criteria for selection were dogs more than seven months old and not on any heartworm prevention programme. Approximately 2ml of blood was collected from each dog via cephalic venipuncture. The blood samples were transferred into EDTA (ethylene-diamine-tetra-acetic acid) tubes and refrigerated at 2-4°C prior to analysis.

Each blood sample was tested for canine heartworm antigen using SNAP® 4Dx test kit and RapiGEN® Canine Heartworm Antigen Test. Each blood sample was also examined for microfilaria using wet blood smear and Knott's Concentration Test (KCT). For samples that were microfilaria positive, a thick blood smear was done to identify the microfilaria species.

Among the samples taken from 100 owned dogs, a microfilaria was detected from one sample using the KCT. The microfilaria was identified as belonging to *D. immitis*. However, all 100 samples tested for the presence of heartworm antigen were negative for both canine antigen test kits. Thus the prevalence of dirofilariasis in owned dogs based on microfilaria detection in Johor Bahru was found to be 1%.

From the 50 stray dogs, several microfilariae were detected in one sample using the KCT. The microfilariae was identified as belonging to *D. immitis*. The same sample was also the only one out of the 50 samples which was positive by the canine antigen test kit (Canine SNAP® 4Dx). Therefore the prevalence of dirofilariasis in stray dogs in Johor Bahru was shown to be 2%.

This is the first record of the prevalence of heartworm in Johor Bahru. The prevalence of heartworm in owned and stray dogs in this study was found to be 1% and 2% respectively The overall prevalence of *D. immitis* in dogs in Johor Bahru was hence only 1.33% (2/150). Yap & Ong (2008) conducted a serological study on heartworm antigenemia in the southern states of peninsular Malaysia (Negeri Sembilan, Melaka and Johor) and recorded two positive cases out of 129 samples. However, none of the positive cases was obtained from dogs in Johor.

Based on the one false negative result demonstrated by the RapiGEN® Canine Heartworm antigen test kit, this lateral immunochromatographic test appeared to be less sensitive than Canine SNAP® 4Dx in heartworm antigen detection. The false negative result by RapiGEN® Canine Heartworm antigen test in this study may be due to the presence of immature female worms. However, it is probably not accurate to state that RapiGEN® Canine Heartworm antigen test appears to be less sensitive than Canine SNAP® 4Dx in heartworm antigen detection based on one false negative result.

Prior to the present study, no other formal studies were conducted specifically to determine the prevalence of dirofilariasis in Johor Bahru. The detection of heartworm in this study showed that dirofilariasis is present in Johor Bahru. However, the prevalence rate was much lower compared to previous studies reported in Klang Valley. Mullin (1970) conducted a study of *D. immitis* in Kuala Lumpur and Petaling Jaya and recorded a 30.4% prevalence rate while Retnasabapathy & Khoo (1976) recorded the prevalence in Malaysia to be 25.8%; Dhaliwal & Sani (1993) recorded a prevalence of 42% in Kuala Lumpur; Toh (2002) recorded a prevalence rate of 31.7% in Selangor and Yap (2006) recorded in Kuala Lumpur the prevalence rate of 33.34%.

The low prevalence observed among owned dogs in Johor Bahru was probably due to a number of reasons. Firstly, it could be due to that most of the owned dogs were pet dogs and not guard dogs and so they were kept indoors most of the time. As such, they were less likely to be bitten by infected mosquitoes.

With regards to the multiplicity of canine types, there are no inherent variations in the susceptibility of canine breeds to heartworms. Haddock (1987) reported that one major factor that appears to make a difference in the susceptibility of various canine populations to heartworm infection is the type of housing provided for the animals. He contended that yard dogs are at a greater risk of exposure to mosquitoes as opposed to house dogs. In the present study, stray dogs had a slightly higher prevalence compared to owned dogs as they were more exposed to infected mosquitoes than pet dogs which were more likely to be kept indoors. This is in agreement with the previous studies done (Mullin, 1970; Retnasabapathy & Khoo, 1976; Dhaliwal & Sani (1993); Toh, 2002; Yap, 2006) where the prevalence of *D. immitis* in stray dogs from 1970 to 2006 ranged from 25.8% to 42% based on parasitological detection methods (KCT and necropsy) compared to studies on owned dogs where the prevalence was 10% (Dhaliwal & Sani, 1993) based on parasitology and 1.5% based on serology (Yap & Ong, 2008).

The low prevalence of *D. immitis* in Johor Bahru was mostly likely related to lower mosquito abundance as compared to Selangor. According to the latest figures released by the Ministry of Health, Malaysia in a press statement in October 2010, there was higher incidence of dengue fever cases in Selangor (16,179 cases) which was almost four times the number of cases recorded in Johor at 4355 cases. As dengue cases are directly related to vector numbers, the *Aedes* mosquitoes, this analogy is suggestive of the lower abundance of mosquitoes in Johor as compared to Selangor.

Kartman (1953) and McGeevy *et al.* (1974) reported that the survival of the mosquito following its infection by *D. immitis* varies from species to species as well as it does within the same species. This means that a particular species that serves as an effective vector in one locale may be a totally ineffective vector at a different location. They believed the reason for these variations appears to be genetic in nature. In Malaysia, few studies have been done to identify the principal vector of *D. immitis*. In a study by Vythilingam *et al.* (2005) in two urban areas in Kuala Lumpur, *Armigeres subalbatus* was incriminated as a potential vector of *D. immitis*. It was further strengthened that *D. immitis* was able to develop to infective stage in the head, thorax and abdomen of this mosquito. This finding was also in agreement with Cheong *et al.* (1981) who incriminated *Armigeres subalbatus* as an important vector of *D. immitis* in urban Kuala Lumpur.

A lack of water or an over abundance can have marked consequences for the survival of mosquito populations (Carpenter, 1955). The role of vegetation in the life support of mosquitoes is often intertwined with human actions. Proximity of mosquitoes to humans and their pets was probably enhanced by landscaping new housing projects with wide ornamental plants such as bamboos and banyan trees which supports mosquito larvae (Miyahara *et al.*, 1976). Many additional human activities within the environment can have an impact on vector populations. Some examples are mosquito abatement programmes which act to reduce the insect numbers, presence of trash disposal facilities, junk yards and construction sites that provide potential breeding sites for mosquitoes by producing numerous catchments for water (Sears *et al.*, 1980).

The higher mosquito population of mosquito in Klang Valley could be largely due to the rapid socio-economic development in Klang Valley as compared to Johor Bahru. We would expect more construction sites with stagnant water bodies and clogged up drains contributing to the abundance of mosquitoes in Selangor. Merely for comparison, through personal communication with Dr. Shane Ryan, President of the Singapore Veterinary Association, the incidence of heartworm is low in Singapore due to their mosquito control measures as well as widespread use of prophylaxis. This study confirmed anecdotal evidence that the prevalence of *D. immitis* is indeed low in Johor Bahru. It is tempting to suggest that the prevalence of dirofilariasis in a locality may serve as an indicator of mosquitoe abundance in that area.

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