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The prevalence and trends of canine heartworm (*Dirofilaria immitis*) in Kuala Lumpur, Malaysia (1970–2018)

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ABSTRACT

Malaysia is considered a hyperendemic area for canine heartworm (*Dirofilaria immitis*) due to its favorable climate for the completion of the parasite life cycle. This study provides an updated prevalence data on *D. immitis* in owned dogs from Kuala Lumpur, Malaysia and compares the trends of *D. immitis* in Malaysia. In the period between December 2017 and June 2018, 3.85% (5/130) dog blood samples tested positive for the presence of *D. immitis* antigen. A majority of the tested dogs (122/130) were not on rigorous heartworm prevention. After collating and analyzing information from 10 historical studies (1970–2017), we identified a significant decline in prevalence of *D. immitis* antigen in Malaysia, after the year 2000. Historically, the prevalence of *D. immits* antigen in owned dogs was significantly lower than the prevalence seen in stray dogs in Malaysia. This study demonstrates that *D. immitis* remains active in Kuala Lumpur, implying that accurate compliance of heartworm prevention is essential in Malaysia.

1. Background

Canine heartworm disease is caused by a pathogenic mosquito-borne filaroid parasitic nematode, *Dirofilaria immitis* (Brown et al., 2012; Genchi et al., 2014; Hoch and Strickland, 2008; Lu et al., 2017; McCall et al., 2008; Vieira et al., 2014). Clinical manifestation of canine heartworm disease includes cough, dyspnea, weight loss, exercise intolerance, weakness, hemoptysis, cyanosis and congestive heart failure (Hoch and Strickland, 2008). The adult worms of *D. immitis* lodges primarily in the pulmonary artery of dogs and other carnivores, resulting in chronic, progressive cardiopulmonary insufficiencies and is potentially fatal for an infected animal (Bowman and Drake, 2017). The number of worms, host immune response, duration of infection and host exercise levels determines the severity of the cardiopulmonary pathology (Bowman and Drake, 2017; McCall et al., 2008).

Kuala Lumpur is the national capital of Malaysia and is amongst the fastest growing metropolitan regions of South-East Asia. Kuala Lumpur is located on the central west coast of Peninsular Malaysia, covering an area of 328 km^2 with an estimated population of 1.73 million (https://www.dosm.gov.my/; Department of Statistics, Malaysia). Malaysia is located in the equatorial region with high temperature (mean 26.5 °C, ranging from 22 to 33 °C) and humidity (80% to 90%), with copious rainfall. These climate conditions are ideal for vector-borne disease transmission, and are commonly seen throughout South-East Asia (Lau et al., 2017; Lu et al., 2017; Mohd-Zaki et al., 2014). By contrast to other vector borne diseases in South-East Asia, *D. immitis* infections have a greater clinical significance in companion animals, with prevalence rates approaching 100% in locations where prophylaxis is not practiced (Irwin and Jefferies, 2004). Taking into consideration the clinical significance and prevalence of *D. immitis*, and the cost and benefits of heartworm prevention medications, it is advantageous for companion animals in these high risk areas to be on regular heartworm preventatives (Bowman and Drake, 2017).

The aim of this study was to assess the prevalence of *D. immitis* in owned dogs visiting veterinary practices in Kuala Lumpur, Malaysia. The current prevalence was assessed using a rapid *D. immitis* antigen test from December 2017 to June 2018. The current *D. immitis* prevalence was then compared to the historical prevalence data of Malaysia to elucidate the trend of *D. immitis* prevalence in the region.

2. Materials and methods

2.1. Literature search

The following electronic databases were searched on April 2018: Medline via OVID, Web of Science and PubMed. The search criteria terms used were: (dog OR canine) AND (*Dirofilaria immitis* OR dirofilariasis OR dirofilaria OR heartworm) AND (Malaysia OR Kuala

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Lumpur) AND (prevalence). No limits were set in any of the electronic database searches, and each database was searched from the earliest year available to the present: OVID (1946–2018), Web of Science (1900–2018) and PubMed (1966–2018, selectively to 1800). Furthermore, the reference lists of articles, reviews and reports obtained were searched for any citations of published articles apparently relevant to the topic.

The literature search produced ten relevant references for which full text has been available and retrieved. Inclusion criteria for retained references included primary data on *D. immitis* prevalence in dogs, regardless of the method used. For each retained reference, information on the year of study, study area, sample size, number of positive samples, prevalence of *D. immitis*, confidence intervals and the methodology of the study was collated.

2.2. Study population

The study population comprised of 130 dogs that were presented to 3 veterinary clinics (Clinic 1, Clinic 2, Clinic 3) across Kuala Lumpur from December 2017 to June 2018 (Supplementray data). The dog selection for heartworm testing was at the discretion of the registered veterinarian and inclusion criteria of animals were: (1) privately owned dogs regardless of breed, (2) presented to the veterinary clinic for a routine health examination, (3) over 1 year of age, and (4) not living outside the area of interest of the study. Note that full history of the dogs travel was not available. The heartworm test was done on the discretion of the veterinary practitioner as part of routine health assessment. With each sample obtained, information on the age, sex and heartworm prevention status was acquired. The prevention rigor was determined by the veterinary practitioner based on the history of the dog. Each heartworm antigen test was photographed and rechecked by the author to ensure consistency and accuracy.

2.3. Screening for Dirofilaria immitis antigen

Blood samples were screened for *D. immitis* antigen using the Rapid Test Kit Heartworm Ag2.0 (Anigen, BioNote Inc., Seoul, South Korea). As part of routine health check, blood samples (~3 ml) were collected into the kit's provided EDTA blood collection tube by registered veterinarians. The presence of heartworm antigen was tested according to the manufacturer's instructions.

Overall sensitivity and specificity of the Rapid Test Kit Heartworm Ag2.0 for *D. immitis* antigen were reported as 94.4% (68/72) and 100% (102/102), respectively, based on 102 post-mortem negative dogs and 72 post-mortem positive dogs (Lim, 2003). All post-mortem negative dogs returned negative test results; the test returned positive result in 100% of dog, if at least 2 male or female *D. immitis* worms were present (Lim, 2003).

2.4. Statistical analysis

The information collected for all 130 dogs were tabulated on a spreadsheet, where the information on the sex, age, prevention status of the dogs and date of collection were analyzed into proportions. Descriptive statistics were determined using QuickCalcs (https://www.graphpad.com/quickcalcs/), where the prevalence and 95% confidence intervals were calculated by modified Wald method. Proportion comparison was calculated using Chi-square (χ^2) test with degrees of freedom set to 1. Statistical significance is considered for p-value < .05.

3. Results

Dogs presented to three veterinary practices in Kuala Lumpur, Malaysia were screened for *D. immits* antigen. Of the 130 dogs screened, 47% (n = 61) were males and 53% (n = 69) were females, ranging from 1 to 17 years old (median = 6 years old, mean = 6.13 years old) (Fig. 1). The age for two dogs in this study was unknown. For eight dogs (6%, 8/130), the *D. immitis* prevention was considered rigorous based on the veterinary practitioner assessment, thirty-three dogs (25%, 33/130) were inconsistent with the *D. immitis* prevention and a majority (69%, 89/130) of dogs had no heartworm prevention.

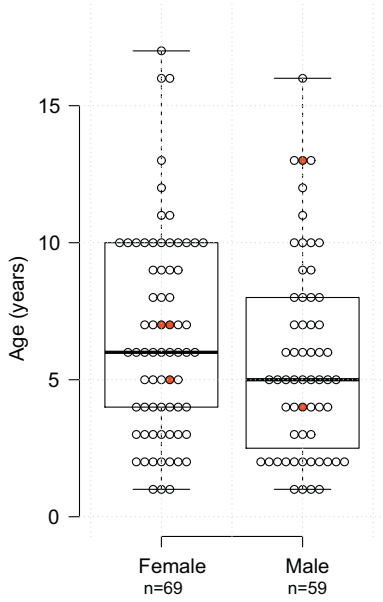

Fig. 1. Age distribution of owned dogs infected and uninfected with *Dirofilaria immitis* in Kuala Lumpur, Malaysia. Box plot age distribution of dogs tested with those that tested positive for *D. immitis* antigen indicated in red.

A current prevalence of *D. immitis* antigen in dogs was recorded at 3.85% (5/130, 95%CI = 1.42–8.92%). None of the dogs under vigorous prevention tested positive for *D. immitis* antigen. Excluding the dogs with rigorous prevention, the prevalence was 4.10% (5/122, 95%CI = 1.52 to 9.48%). Of the five positive samples, one dog did not receive any heartworm preventatives, whereas four dogs had inconsistent heartworm prevention possibly due to poor client compliance (Table 1). The dogs that tested positive for *D. immitis* antigen had an average age of 7.2 years old (median = 7 years old). No information about presence of microfilaria was available for any of the *D. immitis* antigen positive dogs.

The literature search yielded 10 relevant publications reporting *D. immitis* survey data from Malaysia from 1970 to 2017 (Table 2). Based on the prevalence determined from previous studies and the current study, there was a significant difference in prevalence of *D. immitis* in dogs in Malaysia before and after the year 2000; 25.5% (95%CI = 23.68–27.41%) and 4.0% (95%CI = 2.55–6.08%), respectively (χ^2 = 111.88, DF = 1, p < .0001). The average prevalence of *D. immitis* in owned and stray dogs in Malaysia was 18.4% (95%CI = 16.33–20.60%) and 24.1% (95%CI = 21.91–26.49%), respectively. These results show a significant decrease (χ^2 = 12.58, DF = 1, p = .0004) in prevalence of *D. immitis* in owned dogs compared to stray dogs in Malaysia.

Table 1
Summary of positive *Dirofilaria immitis* antigen test in dogs from Kuala Lumpur, Malaysia.

Sample no	Clinic	Sex	Age (years old)	Prevention status
3	1	M	13	Inconsistent
54	1	F	7	None
89	1	F	5	Inconsistent
102	2	M	4	Inconsistent
116	3	F	7	Inconsistent

Table 2
Dirofilaria immitis surveys in dogs from Malaysia (1970–2018).

Year	Study area	Sample size	Positives	Prevalence (%)	Confidence interval (%)	Methodology	Reference
1970	Kuala Lumpur & Petaling Jaya	101 stray	28	30.4	19.3–37.5	Blood smear	(Mullin, 1970)
1976	Malaysia	764 stray	197	25.8	22.7–29.0	Necropsy	(Dhaliwal, 1988)
1977	Seremban	370 owned	120	32.4	27.7–37.5	Blood smear, counting chamber method, millipore filter method	(Kan et al., 1977)
1981	Kuala Lumpur	430 owned	78	18.1	14.6–22.1	Blood smear, modified Knott's test	(Noor and Chong, 1981)
1981	Kuala Lumpur, Petaling Jaya & Klang	233 owned	28	12	8.1–16.9	Blood smear, modified Knott's test, millipore filter method	(Dhaliwal, 1988)
1993	Kuala Lumpur	200 stray	84	42	35.1–49.2	Necropsy, blood smear, modified Knott's test	(Dhaliwal and Sani, 1993)
2005	Kuala Lumpur	104 stray	4	3.85	1.1–9.6	Wet blood mount method	(Vythilingam et al., 2005)
2012	Johor Bahru	150 i) 100 owned ii) 50 stray	2 i) 1 ii) 1	1.33 i) 1 ii) 2	0.2–4.7	Wet blood mount method, modified Knott's test, IDEXX Canine SNAP 4Dx, RapiGEN test	(Ng et al., 2012)
2015	Klang Valley	30 stray	0	0	0–11.6	IDEXX Canine SNAP 4Dx	(Koh et al., 2016)
2017	Peninsular Malaysia	90 stray	9	10	4.7–18.1	IDEXX Canine SNAP 4Dx	(Lau et al., 2017)
2018	Kuala Lumpur	130 owned	5	3.85	1.42–8.92	Canine Heartworm Antigen Rapid Test Kit	Current study

4. Discussion

The current study defines the prevalence of *D. immitis* at 3.85% ($n = 5$, 95%CI = 1.42–8.92%), therefore presenting an active heartworm activity in owned dogs from Kuala Lumpur, Malaysia. Heartworm preventative drugs are expected to have perfect efficacy, as a 100% efficacy during experimental testing is required prior to the approval and registration of the drug (Blagburn et al., 2016; Bowman, 2012). A majority of the dogs tested positive in this study had inconsistent heartworm prevention, indicating that the preventative drugs do not provide adequate protection against heartworm infection when applied irregularly. Application of canine heartworm preventatives with excellent client compliance should abolish any circulating microfilaria, however there is a non-lethal effect on the adult *D. immitis* female worms (Blagburn et al., 2016; Bowman, 2012; Nguyen et al., 2016). The non-lethal effect on adult female worms allows the possibility of microfilaria reappearance before the following monthly dose is administered, particularly if multiple doses are missed due to poor client compliance (McCall, 2005). It is hard to determine if the lack of heartworm preventative efficacy in Kuala Lumpur is due to resistant forms of microfilaria or problems with lack of client compliance resulting in under dosing and reinfections. It is advised, that pet owners in Kuala Lumpur remain vigilant and administer a heartworm preventative that is 100% effective against susceptible heartworm. The current study only determined presence of *D. immitis* antigen. To improve the diagnosis antigen testing should be followed by diagnosis of circulating *D. immitis* microfilaria, such as using the Knott test or PCR testing to confirm *D. immitis* (Geary et al., 2011; Nguyen et al., 2016).

The widespread distribution of *D. immitis* mosquito vectors previously identified in Malaysia may be attributed to the year-round tropical humid climate (Vythilingam et al., 2005). Additionally, a suitable environment for mosquito breeding is established by the increased water source and vegetation as a result of high levels of urbanization and human activities in Kuala Lumpur (Mohammed et al., 2016; Ng et al., 2012). A peaked distribution of mosquito vectors in Kuala Lumpur compared to other states have been identified, hence potentially exacerbating the transmission of *D. immitis* in this area (Mohd-Zaki et al., 2014). With dengue virus also being endemic in Malaysia, mosquito vector control was implemented in the National Dengue Strategic Plan (NDSP) in 2009 by the Ministry of Health to curb dengue cases (Hii et al., 2016). These efforts resulted in a sharp decline in prevalence in dengue and may have indirectly resulted in a decrease in prevalence of *D. immits* in Malaysia (Mohd-Zaki et al., 2014). Supportingly, a significant decline in the averaged prevalence of *D. immitis* in dogs in Malaysia has been observed after the year 2000. As dengue virus and *D. immitis* share mosquitoes as vectors, it may be possible to implement more vigorous *D. immitis* prevention treatment via community awareness in the high risk areas of the climate-based dengue forecast modelling system. As climate conditions are uncontrollable, effective mosquito vector control measures is a sustainable method of limiting the spread of *D. immitis* across the country, to reduce the morbidity and mortality seen attributed to the disease.

Stray dogs pose serious human health, animal health and animal welfare problems, and have a socio-economic and environmental impact in many countries (OIE, 2018). Infected stray dogs act as a reservoir for the survival of *D. immitis* in Malaysia, making it hyper endemic (Lau et al., 2017). The average prevalence of *D. immitis* in stray dogs was significantly higher than the average prevalence of *D. immitis* recorded in owned dogs in Malaysia. This may be attributed to stray dogs living in unhygienic outdoors conditions supporting mosquitoes, lack of preventive treatment, and the activities that presumably increases its vector exposure rates (Erwanas et al., 2014; Koh et al., 2016). Stray animal population control is essential in reducing the population of *D. immitis* animal reservoirs. As suggested in the OIE Terrestrial Animal Health Code, the best methods of reducing the stray dog population is through education and legislation for responsible

ownership, registration and identification of dogs, reproductive control, control of dog movement domestically and internationally, regulation of commercial dog dealers, and euthanasia (OIE, 2018).

In conclusion, the presented data completes the epidemiological picture of *D. immitis* in Kuala Lumpur, Malaysia. Moreover, the control of mosquito vectors, compliant application of heartworm preventatives and control of stray dog populations are possible factors that could reduce the prevalence of *D. immitis* in Kuala Lumpur. This study provides veterinary authorities with current heartworm prevalence to enable establishment of future control programs for *D. immitis* in Kuala Lumpur.

Conflict of interest

There is no conflict of interest that can be declared.

Ethics

The samples were collected as part of routine veterinary procedures. No ethics required.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2019.100272.

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