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RESEARCH ARTICLE

Serological survey of canine vector-borne diseases in two animal shelters in central Peninsular Malaysia

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ABSTRACT

Canine vector-borne diseases (CVBDs) are increasingly becoming a cause for global concern because of their high morbidity and mortality rates in dogs. However, information on their occurrence in Malaysia is still scanty. In this study, a total of 103 dog blood samples were collected from two animal shelters in central Peninsular Malaysia and tested for the antibodies against *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi*, and the antigen of *Dirofilaria immitis*. Of the 103 tested dogs, 44.7% (46) were found to be seropositive for *Ehrlichia* spp., 30.1% (31) for *Anaplasma* spp. and 13.6% (14) for *D. immitis*. Co-infections of *Anaplasma* spp. + *Ehrlichia* spp. (18.5%, 19) were most prevalent, followed by *Anaplasma* spp. + *D. immitis* (1.9%; two) and *D. immitis* + *Ehrlichia* spp. (1.0%; one). Furthermore, three dogs (2.9%) were also found to have triple infection, testing seropositive for *Ehrlichia* spp., *Anaplasma* spp. and *D. immitis*. The dogs which were found to be seropositive with at least one pathogen were 66.7% (32/51) at shelter A, and 55.8% (29/52) at shelter B. Serological evidence showed that the exposure of major vector-borne diseases in dogs in shelters was relatively high in the surveyed areas. Routine detection and control of vector-borne diseases are of paramount importance for reducing the risk of CVBDs transmission in dogs and humans.

Keywords: *Dirofilariasis*, Tick-borne diseases, Infectious diseases, Malaysia.

INTRODUCTION

Canine vector borne diseases (CVBDs) are increasingly causing public health concern. Their zoonotic potential poses a threat to human health, particularly in the tropics and sub-tropics as these climatic conditions are conducive for the growth and proliferation of vectors and other reservoir hosts, thus shortening their generation interval (Madder & Pascucci., 2012; Watanabe *et al*., 2012). The realm of the vectors, and emergence and spread of CVBDs are the consequences of climate changes, deforestation, habitat changes, and increased relocation of dogs, urbanisation and globalisation (Zell, 2004; Harrus & Banneth, 2005; Bowman *et al*., 2009). The increased proximity of human and animal populations due to all the above factors may increase the risk exposure of animals and humans toward these zoonotic pathogens (Otranto *et al*., 2009; Tziporyet *al*., 2010; Chomel, 2011).

Domestic dogs may act as reservoir hosts for several species of zoonotic pathogens such as *Hepatozoon canis*, *Ehrlichia canis*, *Anaplasma platys*, *Anaplasma phagocytophilum, Bartonella henselae*, *Borrelia burgdorferi*, *Dirofilaria repens* and *Dirofilaria immitis* (Otranto *et al*., 2009).

The clinical signs of these diseases are overlapping. *Hepatozoon canis* may induce severe clinical manifestations such as lethargy, fever, anorexia, weight loss, lymphadenomegaly, and anemia associated with a high parasite load (Otranto *et al*., 2009). On the other hand, canine anaplasmosis caused by *A. phagocytophilum* can cause mild to severe acute illness such as anorexia, hyperthermia, vomiting, diarrhea, and neurological signs which is similar to *H. canis* infection (Egenvall *et al*., 1997). Besides, *A. platys* targets platelets cell and leads to canine infectious cyclic thrombocytopenia. In addition, canine ehrlichiosis can range from a mild infection to a severe illness leading to death (Harrus & Banneth, 2005). The diagnosis of canine ehrlichiosis is challenging because of its different phases and multiple clinical-pathological manifestations (Neer *et al*., 2002; Harrus & Waner 2011). On the contrary, *D. immitis* the most pathogenic filarid, causes cardiopulmonary dirofilariasis and it is potentially fatal to canids (or canines) (McCall *et al*., 2008; Rani *et al*., 2010). Besides, *D. immitis* also infects humans but the infection is incidental and not associated with severe clinical sign. However, the increased cases of human dirofilariasis have been documented in region with high prevalence of canine

dirofilarial infections. Thus, the detection of heartworm disease and chemoprophylaxis in dogs are important steps for reducing the transmission rate (Lee *et al.*, 2010).

Dogs can be infected with several species of zoonotic pathogens concurrently, depending on the presence and abundancy of the vectors around the area (Nicholson *et al*., 2010). In Malaysia, the main vectors transmitting CVBDs among dogs include mosquitoes and the brown dog tick *Rhipicephalus sanguineus* (Vythilingam *et al*., 2005; Low *et al*., 2018).

Sheltered dogs have higher chances of being infected with CVBDs as these dogs were unlikely to receive any treatment (Lau *et al*., 2017). However, the prevalence of CVB pathogens are variable from one another which might be due to the different management protocol in these studied animal shelters. A study conducted in 2016 reported that the seroprevalences of *E. canis* and *A. phagocytophilum* were 39.5% and 9.3%, respectively by using serological method (Koh *et al*., 2015). A recent publication reported that the overall prevalences for *E. canis, A. phagocytophilum* and *D. immitis* in three shelters were 55.6%, 16.7% and 10.0%, respectively (Lau *et al*., 2017). In addition, this study compared the different management protocol of three dog shelters against the prevalence of CVB pathogens and the study suggested that occasional treatment of the dogs might not be effective when compared to scheduled preventive measures (Lau *et al*., 2017). In the present study, we attempted to understand the relationship between the prevalence of CVB pathogens with different management protocols at two different dog shelters in the state of Selangor, Peninsular Malaysia, and establish their co-infection status using SNAP® 4Dx® Plus (IDEXX Laboratories, Westbrook, ME), which can detect the presence of *D. immitis* antigen, and *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B. burgdorferi* antibodies.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Medical Ethics Committee of Department Veterinary Services Malaysia (Reference number: JPV: BPI/500-4/1/2 (18)). Written informed consents were received from the owners of the animal shelters.

Sample Collection

The study was conducted on physically healthy dogs from two different animal shelters (i.e., A and B) located at the state of Selangor, central of Peninsular Malaysia. This study was conducted from the year 2016 to 2017. A total of 103 dog (Shelter A: 51; Shelter B, 52) blood samples (3 ml) were collected by veterinarian in EDTA tubes and stored at 2-4° prior to analysis from both female and male of different breeds and ages. Based on the physical appearance of the dogs, all dogs were healthy, however no further examination was conducted. The various environmental conditions and exposure status of CVB pathogens observed between Shelter A and Shelter B.

Shelter A is surrounded with forest and river and it is located at rural area which is only accessible via laterite road and muddy road. Due to shortage of funding and lack of volunteers, the shelter does not practice routine preventive measures (i.e., de-tick, shower and preventive medicine for heartworm disease). The shelter is divided into two types of confinement system whereby some of the dogs were confined in smaller groups whereas approximately 200 dogs were left to roam freely in a big compound (approximately 6 metres × 5 metres). Dogs confined in smaller groups were puppies, sick dogs and dogs that could not socialise well with other dogs. Some of these dogs were severely infested with ticks and fleas. During the blood collection, ticks samples were also collected and screened for various pathogens (Low *et al*., 2017).

On the other hand, dogs in Shelter B were better maintained, confined in different groups in the kennels with concrete floor. All dogs were allowed to run freely during the fixed schedules in the compound of the animal shelter surrounded by vegetation. Dog kennels were cleaned thrice daily. All dogs were given a bath weekly, and using anti tick shampoo as routine preventive measures to prevent infestation of ticks and fleas. However, heartworm prevention was not administered due to the high cost of the medications. None of the dogs were found infected with ticks and fleas during blood collection. All the dogs in both Shelter A and Shelter B were fed twice a day. All the dogs in the shelter were physically fit and healthy.

Serologic Test

A serological diagnostic test was performed by using a commercial lateral flow assay, (SNAP 4Dx test kits IDEXX Laboratories, Westbrook, ME) in accordance with the manufacturer's protocol. This kit can detect antibodies against *A. phagocytophilum*/*A. platys*, *E. canis*/*E. ewingii*, *B. burgdorferi* and antigen of *D. immitis*. The seropositive values of tick-borne pathogens (*Anaplasma* spp., *Ehrlichia* spp., *B. burgdorferi*) reported in the present study were interpreted as current infection with/or previous exposure to the pathogens. The test results collected were compiled accordingly and all seroprevalence of the pathogens were calculated and expressed in percentage. The 95% confidence interval of all estimates was calculated.

RESULTS

Exposure to at least one vector-borne pathogen was documented in 63 (61.2%) dogs (Table 1). Out of 103 dogs blood samples, antibodies of *Ehrlichia* spp. and *Anaplasma* spp. were detected in 46/103 (44.7%) and 31/103 (30.1%) of dogs, respectively. On the other hand, antigen of *D. immitis* was detected in 14/103 (13.6%) of dogs. However, antibodies of *B. burgdorferi* were not detected in this study. A total of 19 (18.5%) dogs were tested seropositive for *Anaplasma* spp. + *Ehrlichia* spp., two (1.9%) dogs seropositive for *Anaplasma* spp. + *D. immitis* and one (1.0%) dog seropositive with *D. immitis* + *Ehrlichia* spp. Interestingly, three (2.9%) dogs were tested seropositive with triple infections (*Anaplasma* spp. + *Ehrlichia* spp. + *D. immitis*).

In shelter A, 26 out of 52 dogs (51%), which represent half of the population were seropositive for *Ehrlichia* spp. and 22 dogs (43.1%) were seropositive for *Anaplasma* spp. Four dogs (7.9%) were infected with *D. immitis*. Mixed infection of *Ehrlichia* spp., *Anaplasma* spp. and *D. immitis* (double infection and triple infection) was observed in 15 dogs (29.4%).

On the other hand, in shelter B, out of 53 dogs, 20 (38.5%) dogs and nine (17.3%) dogs were seropositive for *Ehrlichia* spp. and *Anaplasma* spp., respectively. However, the infection of *D. immitis* was higher in shelter B, in which nine dogs were infected (19.23%). Eleven dogs (21.2%) were observed to have mixed infection of CVBDs (double exposure and triple exposure) in this shelter. None of the dogs were found seropositive with *B. burgdorferi* for both shelters.

Table 1. Exposure status of *D. immitis*, *Anaplasma* spp., *B. burgdorferi* and *Ehrlichia* spp. in 103 dogs in Selangor, Malaysia

Pathogens	Shelter A (N=51)			Shelter B (N=52)			Total (N=103)		
	N	%	CI (95%)	n	%	CI (95%)	n	%	CI (95%)
Overall									
D. immitis	4	7.84	2.2 – 18.9	10	19.23	9.0 – 32.0	**14**	**13.60**	**7.6 – 21.8**
Ehrlichia spp.	26	50.98	36.6 – 65.2	20	38.46	24.8 – 52.1	**46**	**44.66**	**34.9 – 54.8**
Anaplasma spp.	22	43.14	29.3 – 57.8	9	17.31	8.1 – 29.8	**31**	**30.10**	**21.4 – 39.9**
Single infection									
D. immitis	1	1.96	0.0 – 10.4	7	13.46	5.6 – 25.8	**8**	**7.77**	**3.4 – 14.7**
Ehrlichia spp.	10	19.61	9.8 – 33.1	12	23.08	12.5 – 36.8	**23**	**22.3**	**14.7 – 31.6**
Anaplasma spp.	8	15.69	7.0 – 28.6	0	0.00	–	**7**	**6.80**	**2.8 – 13.5**
Double infection									
Anaplasma spp. + *Ehrlichia* spp.	12	23.53	12.8 – 37.5	7	13.46	5.6 – 25.8	**19**	**18.45**	**11.5 – 27.3**
Anaplasma spp. + *D.immitis*	1	1.96	0.0 – 10.4	1	1.92	0.0 – 10.3	**2**	**1.94**	**0.2 – 6.8**
D. immitis + *Ehrlichia* spp.	1	1.96	0.0 – 10.4	0	0.00	–	**1**	**0.97**	**0.0 – 5.3**
Triple infection									
Anaplasma spp. + *Ehrlichia* spp. + *D. immitis*	1	1.96	0.0 – 10.4	2	3.85	0.5 – 13.2	**3**	**2.91**	**0.6 – 8.3**

DISCUSSION

Of 103 dogs examined, 46 (44.7%) and 31 (30.1%) dogs were tested seropositive for *Ehrlichia* spp. and *Anaplasma* spp., respectively. The results obtained from the present study were comparable with previous studies conducted in Malaysia. A recent study reported higher seroprevalence of *Ehrlichia* spp. (55.6%), and lower seroprevalences of *Anaplasma* spp. (16.7%) and *D. immitis* (10.0%) as compared to our study (*Anaplasma* spp. = 30.10%; *D. immitis* = 13.6%) (Lau *et al*., 2017). In another study, the seroprevalences of *Ehrlichia* spp. (39.5%) and *Anaplasma* spp. (9.3%) were relatively lower (Koh *et al*., 2015).

Molecular identification of these pathogens was performed on the blood samples in shelter A in a recent study (Low *et al*., 2018). DNAs of *A. platys* (8/51=15.67%) and *E. canis* (31/51=60.78%) were detected, confirming that *A. platys* and *E. canis* were the causative agents for the tick-borne diseases in this study area.

While there was a moderate agreement (62.50%) between the molecular assay and SNAP 4Dx results for *E. canis*, the results for *A. platys* were less satisfactory (36.40%). These results were expected because antibody test cannot distinguish current infection or previous exposure to the pathogens. In contrast, molecular detection can provide high sensitivity and specificity results for current infection of the pathogens. Thus, it is suggested that the combined use of PCR and serological assays could heighten the detection of infection with or exposure to CVBDs effectively. Additionally, eights dogs and five dogs were detected positive with the DNA of *Hepatozoon canis* (15.7%) and *Babesia vogeli* (9.8%) respectively, in shelter A (Prakash *et al*., 2018a, 2018b).

Borrelia burgdorferi is a tick-borne spirochete that causes Lyme diseases in many animals including dogs, horses, cattle and human (Joppert *et al*., 2001; Vorou *et al*., 2007). It can cause severe arthritis or, rarely, glomerulonephritis in dogs, although some dogs can be asymptomatic (Herrin *et al*., 2018). *Borrelia burgdorferi* in dog was not found in the present and previous studies in Malaysia (Koh *et al*., 2015). Nevertheless, the presence of antibodies against this bacterium was found in human blood donors and patients in Malaysia (Tay *et al*., 2002).

An earlier study conducted in southern Peninsular Malaysia reported a very low seroprevalence of *D. immitis* (1.33%) (Ng *et al*., 2012), possibly because of majority of the samples were pet dogs. Thus, exposures of these pet dogs to the infected mosquitoes were lower as compared to the shelter dogs from our study. Likewise, similar results were also reported in owned dogs. In 1993, 10% of owned dogs were detected positive with *D. immitis* based on microscopic analysis (Dhaliwal & Sani, 1993.). By contrast, prevalence of *D. immitis* in shelter dogs ranged from 26 to 42%, based on Knott's Concentration Test (KCT) and necropsy technique (Mulim, 1970; Retnasabapathy & Khoo, 1976; Toh, 2002).

Similar studies were also conducted in other countries in Southeast Asia. A recent study in Thailand reported lower seroprevalences of *Anaplasma* spp. (29.4%) and *Ehrlichia* spp. (25.0%) (Piratae *et al*., 2019) while the seroprevalences of *Anaplasma* spp. (47.1%), *Ehrlichia* spp. (22.51%), *D. immitis* (17.8%) reported in Northeast India (Borthakur *et al*., 2015) were higher compared with the results observed in our study. Result variation and inconsistency in different countries can be due to different target population either from owned dogs or sheltered dogs, and different geographical and ecological variations (Jung *et al*., 2012).

Co-infection of pathogens may complicate the interpretation of the clinical manifestations typically associated with single vector-borne disease. This can occur simultaneously or a sequential exposure to several infected vectors, or by a single vector that was infected with multiple pathogens (Kordick *et al*., 1999; Beall *et al*., 2008; Gaunt *et al*., 2010). Overall, the co-infection rates (24.27%) were higher compared to a previous study (Koh *et al*., 2015). The co-infection of several pathogens can lead to severe clinical manifestations. The complicated clinical manifestations which are similar to other diseases may complicate diagnosis and veterinarians often mistakenly linked other diseases to CVBDs (Dantas-Torres, 2008; Beugnet & Marie, 2009; Menn *et al*., 2010). In the current study, the total seroprevalence of Shelter A (66.7%) was higher than Shelter B (55.8%). The seroprevalences of *Anaplasma* spp. and *Ehrlichia* spp. were found to be higher at Shelter A (*Anaplasma* spp.: 43.1%, *Ehrlichia* spp.: 51.0%) than Shelter B (*Anaplasma* spp.: 17.3%, *Ehrlichia* spp.: 38.5%). The most likely explanation is the fact that the dogs in Shelter B

were bathed every week and routine preventive measures were done to prevent infestation of ticks and fleas. This decreases the infestation of ticks which act as vectors in transmitting both pathogens from one dog to another. All dogs in Shelter A were hardly bathed due to the lack of manpower. Indeed, most of the animal shelters in Malaysia were overcrowded with dogs and operated with insufficient or limited funding, inexperienced staffs and expertise in managing the infections and diseases. All these limitations resulted in different management protocols of CVBDs for the animal shelters. Management varies depending on their socioeconomic status, expertise and manpower (Vorou *et al*., 2007; Lau *et al*., 2018). On the other hand, the sero-prevalence of *D. immitis* in Shelter B (19.23%) was higher than Shelter A (7.84%). Unfortunately, mosquito surveillance was not conducted on both sites to determine the vector that may contribute to the infection of *D. immitis*. However, *Aedes albopictus* which has been discriminated as a vector for *D. immitis* (Cancrini *et al*., 2003) could be found in the study sites. Proper mosquito control needs to be done to reduce the risk of infection.

In the nutshell, the current study showed that 61.2% (63/103) of the dogs were seropositive with at least one pathogen. The prevalence was alarmingly high, suggesting that the exposure to CVBDs was ubiquitous, especially among dogs in the animal shelters. Limited veterinary care and financial constraints in animal shelters were other compounding factors. As a result, most of the animal shelters in Malaysia are not able to provide heartworm and tick prevention periodically.

This study provides a better understanding on exposure of CVBDs infecting sheltered dogs in Selangor, Malaysia which may serve as baseline information for future investigations and preventive action in order to protect dogs and control the risk of transmission of CVBDs to human and other animals. Further investigations involving a combined dog and human tick-borne disease surveillance data could enhance both public health and animal health. Surveillance studies on other vector-borne pathogens also need to be conducted to get a better understanding of the current status of CVBD transmission in Malaysia.

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Conflict of interest statement

No potential conflicts of interests with respect to authorship and/ or publication of this article.

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