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BLOOD PROTOZOA FINDINGS IN PET DOGS SCREENED IN IPOH, MALAYSIA

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ABSTRACT. A total of 103 blood samples from pet dogs around Ipoh were screened for common blood protozoa. A total of 14 samples were found positive for *Ehrlichia canis* and one sample was found positive for microfilaria of *Dirofilaria immitis*. Both these diseases are transmitted by vectors; ticks (*Rhipicephalus sanguineus*) and mosquitoes respectively. In the hot and wet tropical environment where vectors are abundant, pet care, hygiene and regular screening will help veterinarians detect these infections early to facilitate treatment.

Keywords: vector borne blood parasites, pet dogs, *E. canis*

INTRODUCTION

Humans and dogs share a long history of a close relationship. Dogs have known to become human companions in many instances such as hunting, working and pets. According to recent genetic analyses, they have been under human domestication for something in the order of

100,000 years—longer by several orders of magnitude than any other domestic species. The term domesticated dogs refer to *Canis lupus familiaris* that belong to Canidae family of the mammalian order Carnivora (Kjemtrup *et al.*, 2000). However, dogs are competent reservoirs as host to several zoonotic agents and their increased close relationship with humans in developing countries as pets and companions pose new concerns for public health (Otranto, Dantas-Torres and Breitschwerdt, 2009). As in previous years, the Veterinary Research Institute (VRI) has an initiative to monitor diseases of domesticated or pet dogs in Malaysia. According to Erwanas *et al.* (2014), the clinical signs of parasitic infection are variable and occasionally some infected animals are asymptomatic. As asymptomatic dogs can still transmit disease, it is very important to monitor the type of pathogen and the number of animals that carry it.

According to the VRI annual report, there are several types of blood parasites commonly found in Malaysian pet and

stray dogs, which are *Babesia gibsoni*, microfilaria (*Dirofilaria immitis*), *Ehrlichia canis*, *Babesia canis*, and *Hepatozoon canis*. Other than microscopic detection of parasites in peripheral blood smears, diagnosis can be by serological tests, flow cytometry and polymerase chain reaction (PCR) (Nalumba *et al.*, 2011). The other commonly found blood protozoan in Malaysia, as indicated in VRI annual report is microfilaria. *Dirofilaria immitis* is commonly found in the pulmonary arteries and right ventricle of dogs and other canids, and cause canine heartworm disease. In the past ten years, the geographic range of canine heartworm infection has been markedly increased (Simón *et al.*, 2009). Diagnosis of microfilaria are usually by microscopic examination and PCR. Next is *Ehrlichia canis*, which is a Gram-negative, highly pleomorphic bacterium, which is enveloped with a rippled thin outer membrane (Rikihisa *et al.*, 1985, 1997). *H. canis* infection is frequently diagnosed by microscopic detection of intracellular gamonts in stained blood smears, an indirect fluorescent antibody test (IFAT) for Anti-*Hepatozoon canis* antibodies, enzyme-linked immunosorbent assay (ELISA) and PCR (Baneth, 2011). Another blood parasite that is taken in consideration is Leishmania. Leishmania is a protozoan parasite belonging to the order of trypanosomatid that causes leishmaniasis. This disease is considered to be one of the “neglected tropical diseases” due to its increasing number of infection as well its relation with poverty, famine,

war and immunosuppression. PCR is the best technique to identify Leishmania because it enhances sensitivity and aids the identification of the infecting species and it obviates the need for parasite culture (Uezato 1998).

VRI has been consistent in studying the infections of blood parasites in dogs as shown in studies on incidences of parasites in pet dogs and cats that was conducted from the year 2009 until 2011. From routine analyses, results from the screening shows 1 positive case of *Babesia gibsoni* found each year. Besides that, 5 positive cases of microfilaria *Dirofilaria immitis* were recorded in 2009. One case of *Ehrlichia canis* and *Ancylostoma* sp. was recorded in 2010. Subsequently, *Ascaridia* (2 cases), *Toxocara* sp. (1 case) and *Babesia canis* (1 case) were identified in year 2011. Whereas, for cat samples only 3 parasite species were found which is mite (1 case) in 2009, 8 positive cases of *Toxoplasma gondii* in 2010 and 5 cases of *Ancylostoma* sp. Another study conducted by VRI, on the presence of ectoparasites and endoparasites found in pet and stray dogs showed that there were two positive cases of *Demodex canis*, one positive case of *Rhipicephalus microplus*, seven cases of *Rhipicephalus sanguineus*, four cases of *Ctenocephalides canis* and for endoparasites one positive case whereby an adult *Ancylostoma caninum* worm was found in one dog’s gut contents, ova of *Ancylostoma* sp. in 8 faecal samples, *Toxocara canis* adult worm in one dog, *Gardia* sp. in one dog, and lastly *Ascaris* sp.

ova in 3 dogs (Erwanas *et al.*, 2014). Thus, the aim of this study was to determine the current situation of blood parasites in pet dogs, which have a close relationship with humans. Knowledge on the current disease scenario in pet dogs will enable a more structured control programme for these diseased dogs, thereby preventing transmission to humans.

MATERIALS AND METHOD

Samples

Blood samples from dogs were obtained from government veterinary clinic and one private practice which gave a total of 103 blood samples. The blood samples were taken when these dogs were brought to the clinics for routine examination, vaccination or treatment. The owners' approval was obtained prior to the sampling. The dog's information such as breed type, age and sex were obtained from the clinic registry. An informal interview was conducted with the pet's owner about the condition of their dog and also their feeding and management of their pet dog. The purpose of the interview was to get better information regarding their pet's living environment since habitat of the animal plays an important role in the pathogenesis of parasites. All blood samples were obtained from the cephalic vein using needles (23G) and 3 ml syringes before transfer into 3 ml EDTA tubes.

Parasitology techniques

All blood samples were subjected to microscopic examination, by using two different types of smears, namely the thin blood smear using a drop of EDTA blood and thick smear using buffy coat. This is to differentiate between blood parasites that infect red blood cells and white blood cells. Both types of smears were stained with Giemsa solution. However, only the thin blood smear were fixed with methanol before staining. All sample slides were examined under 1000× magnification with a compound microscope.

Thin Blood Smear

A small drop of blood is placed at one end of the glass slide. Then, a spreader is placed on top of the drop of blood at 45° and waited till the blood extends along the edge of the spreader. The spreader is quickly pushed forward along the slide. This action pulls the blood over the slide, leaving a thin blood smear. The slide is left to air-dry overnight and stained with Giemsa solution. (MAFF, 1981)

Thick Buffy Coat Smear

For thick buffy coat smear, the blood sample was drawn into the capillary tube and sealed at one end. The tube is then centrifuged at 12000 rpm for 5 minutes to gain the buffy coat layer. The capillary tube is cut using a diamond cutter pen right above the buffy coat layer. The buffy

coat layer is smeared on to a clean glass slides to make a thick smear and left to air-dry overnight. The smear is stained with Giemsa solution. (MAFF, 1981)

RESULTS

Results obtained are shown in Table 1.

A total of 103 pet dogs were screened for blood protozoa whereby two isolations of parasites were found from 14 samples. Both isolations were identified as *Ehrlichia canis* (Figure 1) and microfilaria (*Dirofilaria immitis*) (Figure 2). No other parasites were identified. *The E. canis* is characterized by intracytoplasmic morulae inclusions in blood monocytes. Microfilaria of *D. immitis* is identified in blood smears as a multicellular organism about 300-500 μm in length. All animals tested appeared healthy, had good appetites and were active. All results were notified to the authorities for follow-up treatment.

DISCUSSION

Domestic pets are important carriers of zoonotic diseases. As such, regular screening of household pets is advocated. This study shows that vector borne

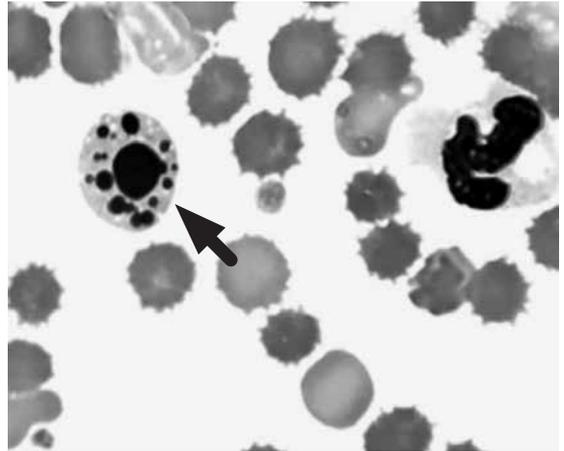


FIGURE 1. *Ehrlichia canis* in white blood cell.

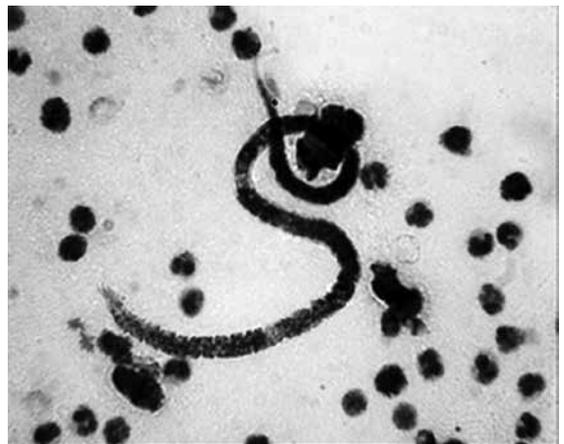


FIGURE 2. Microfilaria (*D.immitis*) in buffy coat smear.

TABLE 1. Blood protozoa identified in pet dogs in 2014

Parasites species	No. of dogs positive / total no. tested (%)	
<i>Ehrlichia canis</i>	14/103	(13.6%)
Microfilaria (<i>Dirofilaria immitis</i>)	1/103	(0.97%)
Total no. of isolations	15/103	(14.6%)

diseases are prevalent among domestic pet dogs. The presence of Ehrlichiosis also known as canine rickettsiosis, canine hemorrhagic fever, canine typhus, tracker dog disease, dog AIDS and tropical canine pancytopenia is a tick-borne disease of dogs usually caused by the organism *Ehrlichia canis* (Ettinger *et al.*, 1995). Traditional diagnostic techniques (hematology, cytology, serology and isolation) are valuable diagnostic tools for Canine Monocytic Ehrlichiosis (CME). However, a definitive diagnosis of *E. canis* infection requires molecular techniques. A common blood parasite found in Malaysia is *Hepatozoan canis* but was not observed in this study. Hepatozoon species are apicomplexan parasites that belong to the family Hepatozoidae and are phylogenetically closely related to the piroplasms and haemosporinids (Barta, 2001; Baneth *et al.*, 2003). However, one case of heartworm microfilaria was seen. Heartworm (*Dirofilaria immitis*) is a parasitic roundworm that is spread from host to host through the bites of mosquitoes. As these pets were kept in households, it is evident that good care in vector control such as giving dogs tick baths and keeping them in a clean environment free from mosquitoes will greatly reduce the infection rates. Canine babesiosis is another vector-borne disease caused by intra-erythrocytic protozoa that induces anaemia, fever, jaundice, splenomegaly, thrombocytopenia and, occasionally, haemoglobinuria (Nalubamba *et al.*, 2011). According to

Nalubamba *et al.*, (2011) and Kjemtrup *et al.* (2000), although there are many strains in canine babesia species, two organisms *B. canis* and *B. gibsoni* are commonly the cause of the disease. Historically, babesia infection in dogs was identified based on morphological appearance in the erythrocyte. All large Babesia were designated *B. canis*, whereas all small Babesia were thought to be *B. gibsoni* (Boozer and Macintire, 2003). However, Babesiosis was not found in the dogs in this study. Regular screening is worthwhile as it will enable the veterinarian to intervene with reliable treatment to save the animal. Other blood protozoans such as *Babesia canis* or trypanosomes were not observed although it has been reported previously from diagnostic cases of the Veterinary Research Institute. This indicates that pet animal management is fairly good with fewer animals getting these infections. In view of the animal ethics and care protocols, it is important to disseminate awareness on proper management of pet animals so that household pets have a good quality of life with their owners.

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