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Short Communication

ANAPLASMOSIS IN DOGS IN THE WESTERN PROVINCE OF SRI LANKA: SEROPREVALENCE, CLINICAL AND LABORATORY FINDINGS

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SUMMARY: Canine anaplasmosis is a zoonotic disease caused by *Anaplasma phagocytophilum* and *Anaplasma platys* transmitted by ticks. In Sri Lanka, only a few studies are available as this disease is often neglected (underdiagnosed) in clinical practice due to the non-specific clinical signs. This clinical communication describes seroprevalence, clinical and laboratory findings of 266 dogs seropositive for *Anaplasma spp*. identified from 450 anaemic dogs presented from Gampaha, Colombo and Kalutara areas of Western Province, Sri Lanka. Accordingly, the seroprevalence of *Anaplasma spp.* among this population of anaemic dogs was 59% (266/450). The majority of the Anaplasma seropositive dogs had non-specific clinical signs with low packed cell volume and thrombocytopenia. There was no statistically significant difference between seropositivity with age or gender of the dogs.

KEYWORDS: Sri Lanka, Anaplasmosis, thrombocytopenia, seroprevalence, Western Province

INTRODUCTION

Canine Anaplasmosis is caused by *Anaplasma phagocytophilum (A. phagocytophilum)* and *Anaplasma platys (A. platys)* which are tick borne, Gram negative, obligatory intracellular rickettsial organisms that belong to the family Anaplasmataceae (Sykes and Foley, 2014). The disease caused by *A. phagocytophilum* is called canine granulocytic anaplasmosis whereas the disease caused by *A. platys* is referred to as infectious canine thrombocytopenia (Sykes and Foley, 2014).

A. phagocytophilum commonly infects neutrophils and rarely affects eosinophils while *A. platys* infects platelets (Carrade *et al*., 2009; Sykes and Foley, 2014). *A. phagocytophilum and A. platys are transmitted by Ixodes ricinus and Rhipicephalus sanguineus* ticks (brown dog tick) respectively (Carrade *et al*., 2009; Sykes and Foley, 2014). Dogs become infected after exposure to infected nymphs or adult ticks (Sykes and Foley, 2014) or sometimes through blood transfusions (Sainz *et al*., 2015). Due to shared arthropod vectors, co-infections with other pathogens like *Ehrlichia canis* (*E. canis*) may also occur complicating the clinical picture (Sykes and Foley, 2014; Sainz *et al*., 2015).

Often, clinical signs of anaplasmosis in dogs are non-specific including lethargy, inappetence/ anorexia, fever, anaemia, lameness, gastrointestinal signs including vomiting, diarrhoea, lymphadenopathy, infrequent cough, surface bleeding (melena, petechiae, epistaxis), tense abdomen and rarely neurological signs (Greig and Armstrong, 2006; Kohn *et al*., 2008; Eberts *et al*., 2011). Due to the non-specificity of clinical signs, canine anaplasmosis is underdiagnosed in clinical practice. The haematological and biochemical changes caused by *Anaplasma spp* infection include thrombocytopenia, lymphopenia, non-regenerative mild to moderate normocytic, normochromic anaemia, neutropenia, neutrophilia, hyperglobulinemia, hypoalbuminemia, and a mild increase in hepatic enzymes (Kohn *et al*., 2008; Carrade *et al*., 2009; Sykes and Foley, 2014).

A. phagocytophilum can infect humans and several animals other than dogs including cats, sheep, goats, cows, equines, rodents, roe deer, deer, other wild mammals and birds (Sainz *et al*, 2015). *A. platys* primarily infects dogs, but DNA of *A. platys* was recently found in cats in non- European continents (Sainz *et a*l., 2015).

Anaplasmosis is an emerging zoonotic disease and a number of studies conducted in different regions of the world have reported the occurrence of both *A. phagocytophilum* and *A. platys* in humans (Dumler *et al*., 2005; Li *et al*., 2011; Maggi *et al*., 2013; Arraga-Alvarado *et al*., 2014). Compared with *A. phagocytophilum* which has been extensively studied, studies related to *A. platys* are rare and conducted only in endemic regions (Yuasa *et al*., 2017). Even though *Anaplasma spp.* has a worldwide occurrence, there are only a limited number of studies conducted in Sri Lanka on the occurrence of these organisms in dogs.

Diagnosis of anaplasmosis is traditionally done by the detection of morulae within the neutrophils and platelets in blood smears. Recently, serological testing and PCR assays are also used to confirm infections (Gaunt *et al*., 2010; Silva, 2016; Beall *et al*., 2018). As molecular and serological testing are laborious and time consuming, patient-side test kits have been developed to diagnose these infections. Serologic tests, which detect the presence of serum antibodies produced against *Anaplasma spp.* indicate whether the animal has previously been infected with the organism. Determining seroprevalence is important to understand the true magnitude of the diseases in a particular geographical location. Seroprevalence of anaplasmosis in different regions of the world range between 3-57% (Khatat *et al*., 2021). A previous study conducted in Sri Lanka has reported the seroprevalence of *A. platys* among client-owned dogs and stray dogs in Colombo as 18% and 12% respectively (Bennett *et al*, 2005).

The objectives of the present study were to determine the seroprevalence of anaplasmosis among the dogs in the Western Province of Sri Lanka and report clinical and laboratory findings of seropositive dogs.

MATERIALS AND METHODS

A prospective cross-sectional study was conducted from 01st July 2018 to 27th February 2020, using 450 client-owned dogs presented to the "Suwana" Pet Care Animal Hospital, 4th Lane, Nagoda, Kalutara South, Sri Lanka. Dogs with pale mucous membranes, lethargy, loss of appetite and haematocrit of less than 30% were included in the study. Informed consent was obtained from dog owners in written form prior to enrolling in the study. From each dog 1ml of peripheral blood was collected into an EDTA tube and signalment and disease history were also recorded. The blood samples were tested with IDEXX SNAP 4DX rapid ELISA test kit (IDEXX Laboratories, Westbrook, Maine, USA) following the manufacturer's instructions to determine the presence or absence of *Anaplasma spp*. Further haematological and biochemical analyses namely full blood count, serum creatinine and blood urea nitrogen were also performed using a veterinary haematology analyzer and a semi-automated analyzer (Biotoo, SMART HA-5000Vet, China). SNAP 4Dx test simultaneously detected antibodies against *E. canis*, A. *phagocytophilium, A. platys. B. burgdorferi* and *D. immitis*.

RESULTS

Of the 450 dogs tested, 266 (59%) were seropositive for *Anaplasma spp.* as shown in Figure 1. The test kit simultaneously detected the antibodies for *E. canis*. Accordingly, out of 266 dogs who were seropositive for anaplasmosis, 174 were seropositive for *E. canis*.

Figure1: SNAP 4DX Test kits positive for *Anaplasma spp.* and *E.canis*

Out of the 266 dogs positive for anaplasmosis, 154 were form Kalutara district (57.8%), 80 were from Colombo district (30%) and 32 were from Gampaha district (12%). It was evident that anaplasmosis was detected in dogs from different geographical locations in the Kalutara district with the highest cases reported from Panadura, Kalutara and Beruwala Divisional Secretariats (DS). The majority of affected dogs in the Colombo district were from Moratuwa (n=39) and of the 32 positive dogs from the Gampaha district, the majority were from Gampaha DS.

Figure 2: The three Divisional Secretariats from which the dogs were used for the study

The age range of the affected dogs varied between 4 months to 12 years. Of the 218 male animals and 232 female animals tested, 47.3% and 51.5% were seropositive for anaplasmosis respectively. The seropositivity for *Anaplasma spp*. or *Anaplasma spp*. and *E. canis* were not statistically significantly different between male and female dogs or young and adult dogs. The dogs used in the study represented 15 different breeds including local mongrels and crossbred dogs. As the number of animals belonging to Pitbull, Boxer, Dachshund, Belgian Shepherd and American Bulldog breeds were relatively small, they were categorized as other breeds for the purpose of analysis (Table 1).

Table 1. Distribution of the dog population (n=450) according to age, sex and breed

Category	Number tested	Number positive	% (positive/tested)
Age			
< 1 year	233	143	61
≥ 1 year	217	123	57
Sex			
Male	218	137	63
Female	232	129	56
Breed			
German Shepherd	129	92	71
Mongrel/local	91	51	56
Rottweiler	62	40	64
Labrador Retriever	54	30	55
Doberman	14	9	64
Pomeranian	15	7	46
Beagles	14	7	50
Terry	9	5	55
Crossbred	33	15	45
Other Breeds	29	10	34

It was observed that German Shepherds are frequently seropositive for *Anaplasma spp.* than other breeds. Further, in this study, the majority of dogs that became seropositive for *Anaplasma spp.* were purebred dogs.

The majority of dogs had non-specific clinical signs such as anorexia (98.1%), lethargy (80%) and weakness (62.4%). Splenomegaly which is a common finding of dogs affected with haemoparasites was detected in 40% of dogs. Clinical signs which could be associated with low platelet counts as a consequence of anaplasmosis or ehrlichiosis namely epistaxis, petechiae and malena were observed only in less than 10% of dogs. Nervous signs, pulmonary oedema and abortion were detected in less than 5% of dogs.

Low packed cell volume and thrombocytopenia were observed in most dogs (90 %). Leukocytosis was found in 65 % of dogs and around 30 % of the affected dogs showed low haemoglobin concentration, increased blood urea nitrogen and increased serum creatinine. Further, it was observed that within 45 days after treatments, 6% of the dogs who were seropositive for *Anaplasma spp*. died, in contrast to 10% of the dogs who were seropositive for both *Anaplasma spp*. and *E.canis*.

DISCUSSION

In the present study, we tested the presence of serum antibodies specific for *Anaplasma spp.* and *E. canis* in 450 clinically anaemic dogs presented to a private veterinary practice at Kalutara. These dogs were mainly from Kalutara and Colombo districts and a small proportion was from Gampaha district. Of the tested dogs, around 59% were seropositive for *Anaplasma spp*. Similar studies done in Germany, Portugal and Iran have reported around 50% seropositivity for *Anaplasma spp.* among dogs presented to respective veterinary practices **(**Barutzki *et al*., 2006; Santos *et al*, 2009; Hamidinejat *et al.*, 2019). In contrast, a study conducted in Egypt reported a seroprevalence of 6% for *Anaplasma spp*. (Selim *et al.*, 2021). Further, the same study identified living outdoors, German Shepherd breed, tick infestation, irregular sanitation and not using ectoparasiticides as major risk factors associated with anaplasmosis. The seroprevalence of *Anaplasma spp*. reported in this study is higher than the 18% seroprevalence reported in a previous study from Sri Lanka (Bennett *et al.*, 2005) and in other Asian

countries; China- 13. % (Cui *et al*., 2017), Korea-18% (Lim *et al*., 2010), Malaysia- 4% (Koh *et al*., 2016), India-4% (Borthakur *et al*., 2014), Taiwan-21% (Yuasa *et al*., 2017).

A study conducted in the USA has shown that the prevalence of *Anaplasma spp.* increases with increasing precipitation and forest coverage and decreases with increasing temperature, population density, relative humidity, and elevation (McMahan *et al*., 2016). Even though anaplasmosis occurs throughout the year, its prevalence was high with the ending of south-west monsoon season that occurs from May to September. This finding is consistent with a study conducted in India proving that the prevalence of ticks was highest in the monsoon season as the hot and humid environmental conditions in the monsoon are most conducive for the development of various developmental stages of ticks (Singhand Rath, 2013).

In this study, the majority of the seropositive dogs were German Shepherds, but it is not appropriate to conclude that this breed is more susceptible to the disease as it is one of the commonest dog breeds in Sri Lanka. When compared to purebred dogs, the percentage of local mongrels presented to the animal hospital was less. Most often mongrels are not presented for veterinary examinations unless suffering from a severe disease or met with an accident. Therefore, it is difficult to conclude that purebred dogs are at a high risk of contracting anaplasmosis than mongrel or crossbred dogs. However, there is a higher chance for seropositivity for *Anaplasma spp.* and *E. canis* among mongrel dogs than purebred dogs as these dogs are often reared outdoors, increasing their chances of exposure to ticks. In addition, tick control measures are not routinely done for mongrel dogs. However, it is also important to be aware that due to natural resistance against haemoparasites, these dogs may remain asymptomatic and provide a continuous source of infection to purebred dogs.

The seroprevalence of E. *canis* in dogs has been reported in several countries: Italy 6.4% (Solano-Gallego *et al.*, 2006), France 0.33% (Pantchev *et al*., 2009a), U.S. 0.33–0.6% (Bowman *et al*., 2009), Mexico 44.1% (Rodriguez-Vivas *et al*., 2005), Iran 14.63% (Akhtardanesh *et al*., 2010), and Korea 6.1% (Lim *et al*., 2010). This study reports a high prevalence of *E. canis* infections (39%) compared to the previously reported prevalence of 14% in dogs in the Western Province in Sri Lanka 9.9% (Rajakaruna *et al*., 2021). This could be because in the present study, the dogs tested for seropositivity of *Anaplasma spp*. were sick dogs that are presented for treatments unlike in the previous studies.

Anorexia, lethargy, pale mucous membranes, weakness and splenomegaly were the most common clinical and physical findings of dogs who were seropositive for anaplasmosis. Common haematological parameters recorded in the majority of dogs included thrombocytopenia, low packed cell volume, and leukocytosis. These findings are consistent with experimental and natural infections of canine anaplasmosis (Antognoni, 2014; Lara *et al*., 2020). However, it is important to note that the seropositivity may or may not be due to an active infection. Dogs who were previously exposed to the organisms and recovered from the disease also have antibodies specific to these pathogens. Therefore, it is important to examine the peripheral blood smears of those dogs and perform appropriate molecular tests such as PCR to confirm active infections before initiating the treatment.

Due to serological cross reactivity between *A. platys* and *A. phgocytophilium*, results presented here indicated exposure to *Anaplasma spp*. and it is necessary to perform molecular tests for species-level identification.

A statistically significant differences were not detected between age groups and between males and females who are seropositive for *Anaplasma spp*., which agrees with the previous studies (Sainz *et al*., 2015; Dahmani *et al*., 2015; Movilla *et al*., 2016; Rajakaruna *et al*., 2021). Due to the zoonotic nature of *Anaplamsa spp.* diagnosis, treatment and control of the disease are important for both animal and human health (Maggi *et al.*, 2013; Arraga-Alvarado *et al*., 2014). As seropositivity was detected in the dogs who had clinical signs suggestive of *E. canis* infection, affected dogs were treated with imidocarb dipropionate (I/M injection) and doxycycline was prescribed once a day for a period of 45 days. Dogs who had mild clinical signs and symptoms had a good prognosis and it indicates the importance of early therapeutic intervention for canine anaplasmosis.

The findings of this study corroborate the previous studies that report a high co-infection rate of *Anaplasma spp.* and *E. canis* among dogs (Sainz *et al*., 2015; Selim *et al*., 2021). Both these diseases are transmitted by the same tick (*R. sanguineus)*, and hence, chances of co-infections are high (Liyanaarachchi *et al*., 2014). Moreover, the two organisms may mutually enhance each other's pathogenicity complicating the clinical picture and resulting in poor prognosis (Skyes and Foley, 2014; Sainz *et al*., 2015) which is also evident through this study. Therefore, identifying co-infection is important for a successful treatment schedule avoiding treatment failures.

The design of this study does not facilitate the determination of the true prevalence as it only included dogs with clinical signs of anaemia and due to the lack of data on the dog population of this area which is a limitation of this study. Therefore, it is important to do an appropriately designed study to determine the true prevalence of *Anaplasma spp*. In addition, seropositivity only indicates the exposure to a pathogen, but not necessarily the clinical disease. Therefore, it would be important to run other tests such as PCR that detect the presence of the pathogen to identify the dogs that have clinical disease in a future study.

CONCLUSION

The present study reported 59% seroprevalence of *Anaplasma* spp. in a population of dogs with clinical signs of anaemia from three areas of Western Province, Sri Lanka. This study suggests that the clinical picture of dogs seropositive for *Anaplasma spp.* is highly variable and a proportion of dogs that are seropositive for *Anaplasma spp.* have co-infection with other haemoparasites such as *E. canis*. The present findings highlight the importance of further investigations to determine the seroprevalence of *Anaplama* spp. and clinical disease caused by *Anaplasma* spp. in dogs in Sri Lanka.

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