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Prevalence of Canine Hemotrophic *Mycoplasma* in Kannur District of Kerala, India

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A B S T R A C T

Hemotropic mycoplasmas in dogs, such as *Mycoplasma haemocanis*, have been described worldwide. Little data are available on the prevalence of haemoplasma infections in dogs of India. The objective of the study was to investigate the prevalence, hematological changes and risk factors of canine haemoplasmosis in Kannur district of Kerala where tick vectors are highly prevalent. To address this, dogs (N= 1,235) with tick infestation were studied during the period from November 2019 to June 2020. Microscopic blood smear examination was used to establish the prevalence and primary screening of canine haemoplasmosis. Thirty four dogs (2.75%) were positive for *Mycoplasma* organisms, of this 25 animals (74%) were found to be co-infected by *Babesia gibsoni*. Hematological analysis revealed that co-infection of dogs with *Mycoplasma* spp. and *B. gibsoni* had exacerbated the anemic status of the animal. It was also found that the host risk factors like age, breed and gender of dogs had insignificance in occurrence of *Mycoplasma* infection. The results from the present study reinforced the notion that canine haemotrophic *Mycoplasma* as a potential pathogen causing anemia in susceptible animals. Besides, co-infection with other blood parasites would complicate the anemic crisis and complicate diagnosis. The study further demands molecular investigation into the situation to probe the exact prevalence and epidemiology of the disease for exploiting effective control measures.

Introduction

Canine tick borne diseases (TBD) in Indian subcontinent causing wide range of clinical effects were *Hepatozooncanis, Ehrlichiacanis, Mycoplasma haemocanis, Anaplasmaplatys, Babesiavogeli* and *Babesia gibsoni* (Abd Rani *et al.*, 2011). Canine haemotropic mycoplasmosis or haemoplasmosis is an emerging epierythrocytic parasitic infection with a relative paucity of studies in India

(Dhanalakshmi *et al.,* 2017; Tresamol and Ameldev, 2018). Haemoplasmas are small, uncultivable, cell wall less bacteria causing chronic, asymptomatic conditions to severe hemolytic anemia in immunocompromised or splenectomised animals (Messick, 2003). Previous studies suggests that additional factors like immunosuppression or co-infections like mange, blood parasites etc. may be necessary to precipitate the conditions like fever, anorexia, anaemia and thrombocytopenia in hemoplasma-infected animals (Novacco *et al.,* 2010; Tresamol and Ameldev, 2018). Three different hemoplasma species,formerly named as *Haemobartonella*, have been recognized in dogs namely, *Mycoplasma haemocanis*, *Candidatus Mycoplasma haemominutum and Candidatus M. haematoparvum* (Zheng *et al.,* 2017). Natural means of transmission of canine hemoplasmas have not been largely explored; ectoparasites like mites, fleas and ticks were proposed to be the possible vectors. It was also suggested that transmission can occur during blood tranfusions, aggressive interactions, contaminated fomites and transplacentally. The brown dog tick, *Rhipicephalus sanguineus* has been considered as main vector for the transmission of *M. haemocanis* among dogs (Ravagnan *et al.,* 2017; Messick, 2003).

Microscopic examination of the stained peripheral blood smears revealing single, pairs or chain of organisms on the surface of erythrocytes were used for preliminary diagnosis of the condition. The clinical signs of canine tick borne diseases are often diffuse and overlapping, leading to a substantial diagnostic challenge to the veterinarians. Moreover, co-infections with two or more pathogens may enhance this problem further (Anderson *et al.,* 2017). The current protocol for treatment of haemotrophic mycoplasma infection includes the use of tetracycline antibiotics like oxytetracycline and doxycycline for three weeks (Chalker, 2005). Little data are available on the prevalence of hemoplasma infections in dogs of India. Hence, the present study was designed to investigate the occurrence and the potential host risk factors of canine haemotropic mycoplasmosis in Kannur district of Kerala using conventional diagnostic techniques.

Materials and Methods

Study population

The present study was conducted in 1,235 dogs from different parts of Kannur district enrolled in outpatient ward, District Veterinary Centre, Kannur (Kerala) during the period from November 2019 to June 2020. Sampling was performed only from dogs with history of tick infestation with no restrictions regarding age, gender and breed. Clinical health status like healthy or sick, based on the dog's history and clinical examination, and epidemiological information like age, sex, breed and demographic area of all animals were also obtained. The study area is Kannur district which is a coastal city in the northern part of south Indian state of Kerala. It is located on the western coast of the country which lies in the latitude of 11° 52' 28.1172" N and longitude of 75° 22' 13.3284" E with an elevation of 20 meters height. The tropical bioenvironment in Kannur district remarkably facilitate the tick infestation and their by transmission of various tick borne pathogens as observed by Preena *et al.,* (2019).

Light microscopic examination

Peripheral blood smears were prepared from ear margins, air-dried and fixed in 100% ethanol and evaluated microscopically using Giemsa method, for the presence of piroplasms (*Babesia* spp.), inclusion bodies (*Anaplasma* spp. or *Ehrlichia* spp.), or small basophilic structures on the periphery of

erythrocytes as indication of hemotropic *Mycoplasma* infections under oil immersion (100X).

Haematological examination

Around 2 ml of blood was collected from either the cephalic vein / recurrent tarsal vein in EDTA vials. Complete blood count analysis was performed on an automatic haematology analyzer (Exigo EOS, Sweden).

Statistical analysis

The data obtained were represented as mean ± standard deviation. All haematological parameters were evaluated statistically using one-way ANOVA with Duncan's multiple range test. The association of the hemoplasmosis and various risk factors were tested for statistical significance using Chi-square test. The statistical Package for Social Sciences (SPSS Version 20.0.0) was used in the study. Variables with $p<0.05$ were considered as statistically "significant".

Results and Discussion

The first documented case of canine mycoplasmosis was in 1934 (Shoetensack, 1934). *Mycoplasma* infections were known to cause anaemia in certain cases by deformation and autoantigen exposure of erythrocytes leading to clearance of these infected RBCs by host immune response through spleen. Deformation of erythrocytes occurs during penetration of hemoplasma fibrils into its membrane for getting nutrition causing the increased permeability and fragility of the membrane leading to haemolytic anaemia (Yang *et al.,* 2007). Canine haemotrophic *Mycoplasma* are cosmopolitan in occurrence, however in India, scare reports are available. Hence, the occurrence, distribution and frequency of *Mycoplasma* spp. in 1235 dogs were determined in this study. The dogs with history of tick infestation were selected in the study as it was previously stated by Abd Rani *et al.,* (2011) that dogs infected with ticks were likely to positive for at least one or more canine TBD pathogen than dogs without tick infestation. Spherical or ring-shaped microorganisms were detected at the periphery of erythrocytes by microscopy in 34 (2.75%) dogs out of a total of 2,345 examined (Figure 2). Similarly, presence of small coccoid organisms in the periphery of RBCs in a severe anemic dog had been described by Tresamol and Ameldev (2018). Among the *Mycoplasma* positive animals, 25 animals (74%) were found to be co-infected by *Babesia gibsoni.* A similar study has also been reported by Anderson *et al.,* (2017) wherein a dog with *B. gibsoni* infection also harboured *M. haemocanis*.

Haematological attributes of *Mycoplasma* infected dogs, *B. gibsoni* co-infected dogs and healthy dogs are described in Table 1. There was significant difference in Hb and RBC values in *Mycoplasma* infected dogs and *B. gibsoni* co-infected dogs. Severity in anemia may be due to fact that co-infection in dogs with more than one pathogen may exacerbate clinical manifestations leading to unpredictable incubation period, clinical outcome and prognosis (Otranto *et al.,* 2009). Haematological analysis revealed thrombocytopenia, decrease in neutrophil count, increase in lymphocyte count and monocyte count in infected animals when compared to the healthy dog population as similarly described by Preena *et al.,* (2019). In the figure 1, polychromatophilic RBCs were portrayed which are bluish coloured immature anucleated erythrocytes indicating regenerative anaemia due to the hemolytic effects of the pathogen. Distribution of *Mycoplasma* spp. among the dog population studied with relation to various risk factors has been depicted in Table 2. In the study, it was found that the age, breed and gender of

dogs has insignificance in occurrence of *Mycoplasma* infection in concordance with the previous studies of Roura *et al*., (2010); Ravagnan *et al.,* (2017) and Aquino *et al.*, (2014), who also found no association between hemoplasma prevalence and other potential risk factors like clinical status, anaemia, ectoparasites, prophylaxis etc. However, it was found that young dogs were more frequently infected to hemoplasma which may be due to higher exposure rate to tick vectors. Moreover, it was postulated that the living conditions and climate were casually linked with the increased risk of hemoplasma infection rather than the breed of the dogs (Novacco *et al.*, 2010).

Figure.1 Geographical location of Kannur district of Kerala where the study was conducted

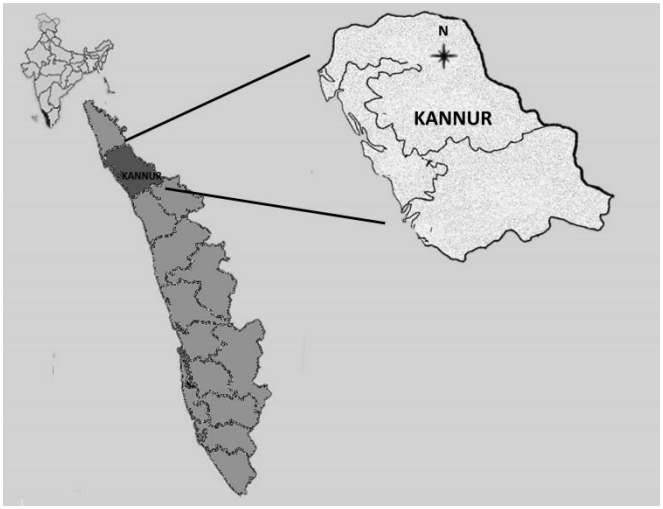

Fig.2 Photomicrograph of Geimsa stained blood smear from a dog in which numerous small black dot-like *Mycoplasma* infected erythrocyte (red arrowhead) co-infected with signet ring shaped *B. gibsoni* (black small arrow). Two polychromatophils (P) could also be observed which are immature erythrocytes indicating regenerative anemia

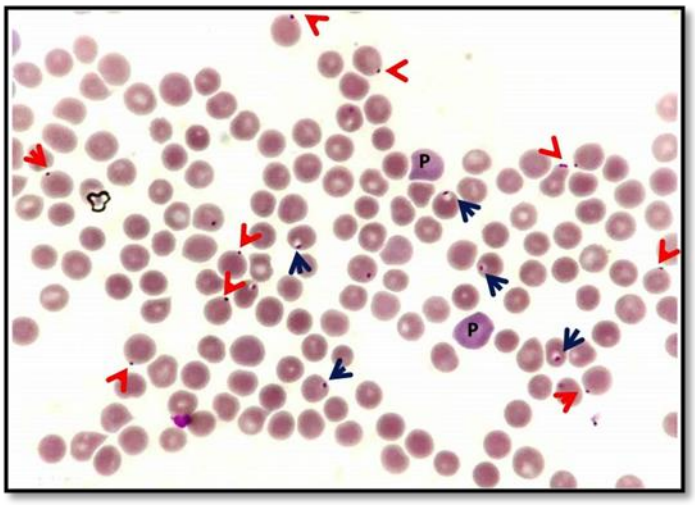

Table.1 Haematological values of affected and healthy dogs (Mean ± Standard deviation)

Categories	*Mycoplasma* positive animal	Co-infected animals	Healthy dogs
Hb (g/dl)	9.7 ± 2.13^{a}	7.47 ± 2.61^{b}	14.49 ± 1.63^{c}
RBC ($\times 10^{6}$ /µl)	4.27 ± 1.12^{a}	2.97 ± 1.14^{b}	6.39 ± 0.63^{c}
Platelet ($\times 10^{3}$ /µl)	1.22 ± 1.30^{a}	0.64 ± 0.50^{a}	2.93 ± 0.63^{b}
TC ($\times 10^{3}$ /µl)	9.69 ± 2.89^{ac}	13.39 ± 4.56^{b}	10.99 ± 2.87^{bc}
NP (%)	55.89 ± 9.3^{a}	56.15 ± 10.13^{a}	73.57 ± 5.47^{b}
LC (%)	32.44 ± 8.76^{a}	32.35 ± 9.92^{a}	17.93 ± 3.83^{b}
MC (%)	11.67 ± 2.12^{a}	11.5 ± 3.19^{a}	8.5 ± 2.79^{b}

a,b,c: Means ± SD with different letter as super scripts within a row differ significantly

Table.2 Distribution of *Mycoplasma* spp. among dog population studied

Variables	No. (%) positive for *Mycoplasma* spp.
Gender:	
Male	20 (58.82)
Female	14 (41.18)
Age group:	
Young (< 1 year)	16 (47.06)
Adult (> 1 year)	18 (52.94)
Breeds:	
Dalmatian	3 (8.82)
Dachshund	1 (2.94)
Doberman	3 (8.82)
Golden retriever	2 (5.88)
German Shepherd	3 (8.82)
Labrador	7 (20.59)
Pug	3 (8.82)
Rottweiler	4 (11.76)
Spitz	5 (14.71)
Other	3 (8.82)
Infection:	
***Mycoplasma* infection**	9 (26)
***Mycoplasma* &*B.gibsoni* co-infection**	25 (74)

In the present study, conventional methods were utilized to assess the prevalence of canine hemotrophic mycoplasmosis. In addition, the hemoplasma infected dogs in the present study did not exhibit clinical signs and hematological variations clearly attributing it to canine hemoplasma infection and differentiating it from commonly prevalent

babesiosis in the study area. Only *B. gibsoni* was found to be the infection concurrently seen with the *Mycoplasma* which was found to aggrevate the clinical condition of the affected dogs complicating the diagnosis. This also warrants the requirement of an elaborated study on the prevalence or effects of co-infection of other prevalent infections like *Babesiacanis, Ehrilichiacanis,* mange and dirofilariasis which are highly prevalent in the area. Bacterial infection with *Mycoplasma* spp. occurred in a substantial number of dogs studied indicating a rather high rate of transmission of the pathogen in the dog population in Kannur.

There are some limitations in the present study, which still haveto be mentioned. The specificity of blood smear examination may be poor due to subclinical or chronic conditions. In addition, false positive results may be obtained in case of inappropriate staining or fixation methods and intraerythrocytic inclusions like Howell-Jolly bodies (Ameldev and Tresamol, 2018). Hence, advanced molecular methods are essential to find the real prevalence of the condition and the factors associated with concurrent infection. Genomic sequencing of haemoplasma organism is essential for identifying the transmission, virulence factors, protein antigens and genetic variability for better prevention control of the condition.

Vector-borne diseases are one of the most complex of all infectious diseases to diagnose, mitigate, control and prevent. In this work, we presented the high prevalence of *Mycoplasma* infection in dog from Kannur district suggesting a potential role of causing anemia in susceptible animals. Henceforth, this study warrants the need for investigation of epidemiological patterns, vector distribution and economic impact of the condition in canine populations of the country.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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