

# Prevalence of Tick-Borne Haemoparasites in Dogs in Agartala, Tripura

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## ABSTRACT

Dogs usually suffer from numerous parasitic diseases and among which tick-borne haemoparasitic infection is one of the major concerns. Common tick-borne haemoparasites which cause disease in dogs are *Babesia canis*, *B. gibsoni*, *Hepatozoon canis* and rickettsial parasites viz. *Ehrlichia canis* and *Anaplasma platys*. In the present study, the prevalence of tick-borne haemoparasites was assessed in dogs in Agartala, Tripura. Blood samples were collected from a total of 1776 dogs irrespective of breed, age, sex and categories for a period of one calendar year starting from March 2022 to February 2023. The overall prevalence of tick-borne haemoparasites in dogs was found 59.46 % (1056/1776) with only 11 cases of mixed infection. The study on the basis of breed, sex, age, season and category showed the highest prevalence of tick-borne haemoparasites in German Spitz (77.20%), male dogs (63.57%), adult dogs (60.71%), monsoon season (73.83%) and stray dogs (68.45%). The high prevalence of tick-borne haemoparasitic infection in dogs in the study area demands the strategic control of tick population and effective management practices necessary to control tick-borne haemoparasitic infections.

**Keywords:** Agartala, Dogs, Prevalence, Tick-borne haemoparasites, Tripura.

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## INTRODUCTION

Now a day, dogs are considered as the most lovable and trustworthy companion animals of human being. However, they are often prone to various endo and ecto parasitic infestation. Among ectoparasites, ticks are considered as one of the important and harmful blood sucking parasites. They infect and transmit most of the pathogens than any other group of blood feeding arthropods of livestock and humans (Benelli *et al.*, 2016).

Common tick borne pathogens which cause disease in dogs are *Babesia canis*, *B. gibsoni*, *Hepatozoon canis* and rickettsial parasites viz. *Ehrlichia canis* and *Anaplasma* spp. Among the tick-borne haemoparasitic diseases, canine babesiosis caused by intra-erythrocytic protozoan parasites of the genus *Babesia* including *B. canis* and *B. gibsoni* is an important disease distributed world-wide. It is a life threatening tick-borne disease, characterized by fever, anaemia, gradual weight loss and lethargy. Whereas, canine hepatozoonosis, caused by *Hepatozoon canis* is considered to be one of the most prevalent canine vector-borne infection in the world including India (Otranto & Dantas-Torres, 2010; Singh *et al.*, 2017). Canine anaplasmosis, caused by *Anaplasma platys* infects the platelets and is the causative agent of infectious canine cyclic thrombocytopenia (ICCT). The infection is often mild or asymptomatic but can become fatal due to severe thrombocytopenia and co-infection with other vector-borne pathogens. Moreover, canine ehrlichiosis, caused by *Ehrlichia canis*, an obligatory intracellular pleomorphic rickettsia responsible for causing

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canine monocytic ehrlichiosis (CME) is also considered to be global importance in canines now. After first recognized in Algeria in 1935, day by day it has become a potentially fatal disease in dogs, which are mainly transmitted by brown tick,

*Rhipicephalus sanguineus* (Venkatesakumar *et al.*, 2018). These tick-borne haemoparasitic diseases if remained undiagnosed or not treated at the right time may result into the death of animal, which in turn is an emotional drain to the owner and also economical loss to the breeding industry.

The practice of keeping dog as pet is rising day by day in Agartala city which leads to the occurrence of many diseases more than the previous. Therefore, the present study was designed to explore the prevalence and identification of tick-borne haemoparasites in dogs in Agartala, Tripura, India.

## MATERIALS AND METHODS

The present study was conducted in and around Agartala. The study was conducted for one calendar year starting from March 2022 to February 2023. The study was conducted at the Department of Veterinary Parasitology, College of Veterinary Science & A.H., R. K. Nagar and the State Disease Investigation Laboratory, Abhoynagar, Agartala.

### Collection of Samples

A cross sectional study was conducted on 1776 dogs of different breeds, age groups (below 1 year and above 1 year), sex and categories to determine the prevalence as well as identification of tick-borne haemoparasites in the study area. In the classification of categories of dogs, the dogs which were kept by people at home for companionship were grouped under the 'pet dogs'. Dogs which were kept without enclosure or proper shelter and remained free-ranging were grouped under category 'stray dogs' and those used to perform practical tasks like detection, search and rescue etc. were grouped under the category 'working dogs'. Blood samples were collected from dogs which were found suspicious for tick-borne haemoparasitic infection on the basis of their case history (fever, depression, inappetence/anorexia, lethargy, pale mucous membrane and history of tick attachment) provided by their owner or attendant. During the collection of blood sample, the details of owner and dog, history of illness, ongoing treatment details, travel history if any, were properly recorded. Blood samples were collected in vials containing EDTA, properly labeled and brought to the laboratory for parasitological examination. Microscopic examination was performed on the same day of blood collection.

### Blood Smear Examination for Detecting Tick-Borne Haemoparasites

Thin blood smear was prepared from each properly labeled anticoagulated blood sample and stained with commercial Giemsa stain diluted with buffered water for 30-40 min. After that the slides were washed thoroughly under running tap water and air dried. Stained smear was later examined by conventional light microscopy under oil immersion objective (100X) for detection of tick-borne haemoparasites. The parasites were identified on the basis

of their characteristic morphology (Soulsby, 1982). Failure to detect any haemoparasite in the blood smear after evaluating at least 50 oil immersion fields was declared as microscopically negative sample.

### Statistical analysis

Results were expressed as the percentage. A difference with value  $p < 0.05$  was considered statistically significant. Chi-square test was performed to determine presence or absence of significant difference in parameters among the different groups using the statistical package for social sciences, Version 17.0.1 software (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

During the present study, out of 1776 blood samples, 1056 (59.46 %) were found to be positive for different tick-borne haemoparasitic infection. The high prevalence of tick-borne haemoparasites in the present findings correlates with the findings of Aktas and Ozubek (2017) in Turkey, Sarma *et al.* (2019) and Bhattacharjee & Sarmah (2013) in North-east India. The higher prevalence of tick-borne haemoparasites in the present finding might be due to the favourable climatic conditions for optimum propagation of the vector. The warm and humid climate in the present study area probably played a key role in the growth and multiplication of ticks which in turn influenced the higher occurrence and spread of the disease.

A total of five different species of tick-borne haemoparasites were identified microscopically by giemsa staining method in the present study. Highest prevalence was recorded in case of *Babesia gibsoni* 814 (45.83 %) (Fig. 1) followed by *Hepatozoon canis* 115 (6.47 %) (Fig. 2), *B. canis* 67 (3.77 %) (Fig. 3), *Anaplasma platys* 54 (3.04 %) (Fig. 4) and *Ehrlichia canis* 06 (0.33 %) (Fig. 5). In the present study, a total 11 cases of mixed infection were recorded. Among those, mixed infection with *B. gibsoni* and *B. canis* ( $n=6$ ) was found to be the highest followed by *B. gibsoni* and *H. canis* ( $n=4$ ) and *B. gibsoni* with *A. platys* ( $n=1$ ). Earlier Bhattacharjee and Sharma (2013), Laha *et al.* (2014) and Devi (2022) also recorded mixed infection of tick-borne haemoparasites in their study. Co-infection of different tick-borne haemoparasites might be due to availability of such vector in the study area which can act as the transmitting agents of different pathogen.

Among various breeds of dogs, the prevalence of tick-borne haemoparasites was recorded highest in German Spitz (77.20%) followed by Mongrel/Local (65.61%) and least in Siberian huski (16.66%) (Table 1). The present finding correlates to the finding of Tsegay *et al.* (2016) and Ezema *et al.* (2021) who recorded higher prevalence in exotic dogs. Highest prevalence of tick-borne haemoparasites in German Spitz might be due to the fact that long hair of these dogs enables ticks to remain unnoticed to the owner for a long period of time which enhances the chances of establishment and transfer of tick-borne haemoparasitic diseases in



to them. Higher prevalence in the Mongrel/local dogs might be due to the fact that they mainly remain outdoor, free-roaming and some of them are stray in nature which might have caused them in acquiring tick infestation and spreading the tick-borne haemoparasites easily to a large population of dogs.

Male dogs had higher prevalence for all the tick-borne haemoparasites (63.57 %) than the females (54.13 %) (Table 2). The present finding correlates with the findings of Mehta *et al.* (2020) and Patra *et al.* (2020). However, Devi (2022) found higher prevalence in females. The higher prevalence of tick-borne haemoparasites in male dogs might be due to the fact that male dogs are used to perform many outdoor works in police forces, paramilitary, CRPF etc which may also cause them to acquiring tick infestation easily resulting into higher tick-borne haemoparasitic infection. However, certain hormonal factors such as higher testosterone level also predispose male dogs to tick infestation (Sahu *et al.*, 2013) which may also in turns results in higher tick-borne haemoparasitic infection.

Age-wise, prevalence of different tick-borne haemoparasites was found slightly higher in adult dogs (> 1 year dogs) (60.71%) than the young ones (< 1 year dogs)

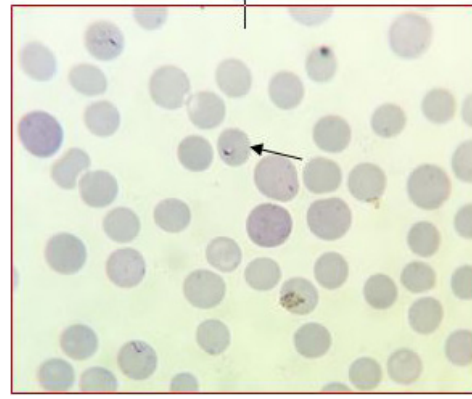
(57.29%) (Table 2). Higher prevalence in adult dogs in the present study also correlates with the findings of Ezema *et al.* (2021) and Kumar *et al.* (2009). However, Bashir *et al.* (2009) recorded higher prevalence of babesiosis in younger dogs than the older age groups. Adult dog shows increase scavenging and wandering habit and also get less attention from their owner compared to the young ones for which the ecto-parasites might get longer time to flare up disease in them.

Season-wise highest prevalence of different haemoparasites was observed in Monsoon (73.83 %) followed by post monsoon (60.98 %), pre monsoon (53.76 %) and winter (37.18 %) (Table 2). The present finding of higher prevalence in monsoon season correlates with the findings of Devi (2022) and Kumar *et al.* (2009). Lower prevalence of different tick-borne haemoparasites in winter season correlates with the findings of Sahu *et al.* (2014) and Vatsya *et al.* (2010). Higher prevalence in monsoon might be on account of increased activity of ticks and other vectors responsible for spreading of parasitic infection (Soulsby, 1982; and Radostits *et al.*, 1994). On the contrary, lower prevalence of different tick-borne haemoparasites in winter might be due to lower availability of ticks.

**Table 1:** Breed-wise prevalence of tick-borne haemoparasites in dogs in Agartala, Tripura

Breed of dog	Number of dogs examined	Total number of positive cases of haemoparasites	Prevalence (%)	Tick-borne haemoparasites				
				<i>Babesia gibsoni</i>	<i>Babesia canis</i>	<i>Hepatozoon canis</i>	<i>Anaplasma platys</i>	<i>Ehrlichia canis</i>
German Spitz	487	376	77.20	307	22	28	18	01
Mongrel/Local	445	292	65.61	197	32	47	14	02
German Shepherd	109	68	62.38	50	07	09	01	01
Cocker Spaniel	67	37	55.22	29	01	06	01	00
Lhasa Apso	11	5	45.45	04	00	01	00	00
Pug	41	20	48.78	14	02	02	02	00
Cross	242	112	46.28	98	02	11		01
Labrador Retriever	241	109	45.22	87	01	04	16	01
Doberman Pinscher	09	4	44.44	1	00	02	01	00
Beagle	19	7	36.84	6	00	01	00	00
Rottweiler	15	4	26.66	2	00	02	00	00
Siberian Huski	06	1	16.66	1	00	00	00	00
Golden Retriever	52	13	25.00	10	00	02	01	00
Bull mastiff	04	1	25.00	1	00	00	00	00
Saint Bernard	04	1	25.00	1	00	00	00	00
Dachshund	04	2	50.00	2	00	00	00	00
Dalmatian	09	2	22.22	2	00	00	00	00
Pomeranian	11	2	18.18	2	00	00	00	00
<b>Total</b>	<b>1776</b>	<b>1056</b>	<b>59.46</b>	<b>814</b>	<b>67</b>	<b>115</b>	<b>54</b>	<b>6</b>

Category-wise prevalence of tick-borne haemoparasites was found to be highest in stray dogs (68.45 %) followed by pet dogs (59.20 %) and working dogs (51.92 %) (Table 2). Similar findings of higher prevalence of tick-borne haemoparasites in stray dogs were also reported by Bhattacharjee and Sarmah (2013) and Gadahi *et al.* (2008). Stray dogs mainly remain homeless, ownerless; live in an unhygienic condition, remain malnourished, run here and there which increases the chances of gaining and spreading of ticks that result in the higher occurrence of disease to them. Prevalence of infection in working dogs and pet dogs was less than stray dogs due to health concerns particularly in controlling the vectors from time to time and also due to their ideal care and managerial practices followed by their owner/attendants.



**Fig. 1:** Giemsa stained blood smear of dog showing piroplasms of *Babesia gibsoni* inside RBC (x100).

**Table 2:** Prevalence of tick-borne haemoparasites in dogs in Agartala, Tripura according to different epidemiological factors

Epidemiological factor	Number of dogs screened	Number of dogs infected	Prevalence (%)	p value	No. (%) positive for tick-borne haemoparasites				
					<i>Babesia gibsoni</i>	<i>Babesia canis</i>	<i>Hepatozoon canis</i>	<i>Anaplasma platys</i>	<i>Ehrlichia canis</i>
<b>Sex</b>									
Male	1002	637	63.57	<0.001*	492 (77.23%)	42 (6.59%)	61 (9.57%)	37 (5.80%)	05 (0.78%)
Female	774	419	54.13		322 (76.84%)	25 (5.96%)	54 (12.88%)	17 (4.05%)	01 (0.23%)
<b>Season</b>									
Pre monsoon	473	257	54.33		191 (74.31%)	16 (6.22%)	32 (12.45%)	17 (6.61%)	01 (0.38%)
Monsoon	642	474	73.83	<0.001*	352 (74.26%)	39 (8.22%)	51 (10.75%)	28 (5.90%)	04 (0.84%)
Post monsoon	364	222	60.98		175 (78.82%)	10 (4.50%)	29 (13.06%)	7 (3.15%)	01 (0.45%)
Winter	277	103	37.18		96 (93.20%)	2 (1.94%)	3 (2.91%)	2 (1.94%)	00 (0%)
<b>Category</b>									
Pet dogs	1076	637	59.20		542 (85.08%)	31 (4.86%)	42 (6.59%)	19 (2.98%)	03 (0.47%)
Stray dogs	336	230	68.45	<0.001*	127 (55.21%)	28 (12.17%)	49 (21.30%)	24 (10.43%)	02 (0.86%)
Working dogs	364	189	51.92		145 (76.71%)	08 (4.23%)	24 (12.69%)	11 (5.82%)	01 (0.52%)
<b>Age</b>									
Young (<1year)	651	373	57.29	> 0.05 <sup>NS</sup>	312 (83.64%)	22 (5.89%)	21 (5.63%)	16 (4.28%)	02 (0.53%)
Adult (>1year)	1125	683	60.71		502 (73.49%)	45 (6.58%)	94 (13.76%)	38 (5.56%)	04 (0.58%)
<b>Total</b>	<b>1776</b>	<b>1056</b>	<b>59.46</b>		<b>814</b>	<b>67</b>	<b>115</b>	<b>54</b>	<b>6</b>

\*Denotes statistically highly significant p value ( $p < 0.001$ ), Chi square statistic

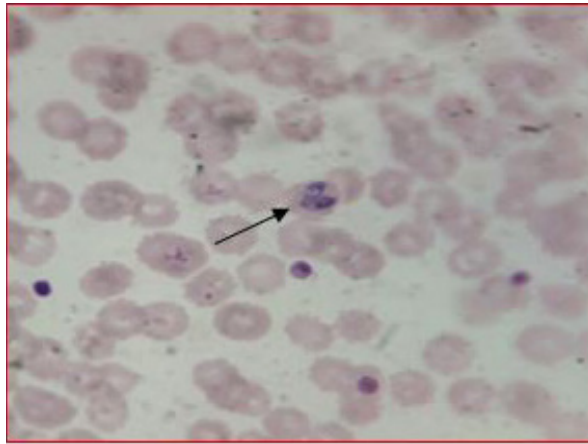
<sup>NS</sup>Denotes statistically non significant p value ( $p > 0.05$ ), Chi square statistic



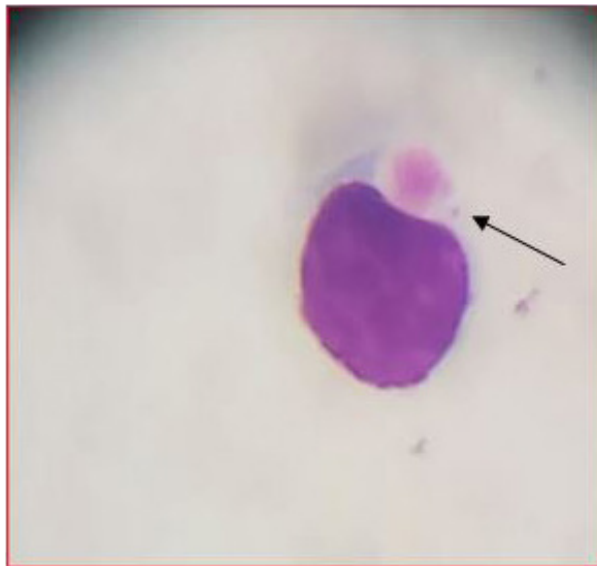
**Fig. 2:** Giemsa stained blood smear of dog showing mature gametocyte of *Hepatozoon canis* inside cytoplasm of neutrophil (x100).



**Fig.5:** Giemsa stained blood smear of dog showing morula of *Anaplasma platys* inside platelet (x100).



**Fig. 3:** Giemsa stained blood smear of dog showing piroplasms of *Babesia canis* inside RBC (x100).



**Fig. 4:** Giemsa stained blood smear of dog showing morula of *Ehrlichia canis* inside cytoplasm of monocyte (x100)

## CONCLUSION

Present study revealed a considerable high (59.46%) prevalence of tick-borne haemoparasites in dogs in Agartala, Tripura, India. Tick-borne haemoparasites were recorded throughout the year in which highest prevalence was observed in monsoon. The breed, sex, age and category wise study revealed highest prevalence of tick-borne haemoparasites in German spitz breed, in male dogs, adult dogs (> 1 year dogs) and in stray dogs, respectively. The observed high prevalence of tick-borne haemoparasites in the study region warrant strategic intervention to reduce the tick infestation in the dog population so as to reduce the haemoparasitic infections. The data generated in the present study can also be used for further studies on prevalence of haemoparasitic infections in dogs in other areas of the State.

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