

Seroprevalence of vector borne parasites in naturally exposed dogs of Assam, India

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Abstract

Aim: To determine the seroprevalence of vector(tick)-borne *Babesia canis*, *Babesia gibsoni*, *Ehrlichia canis* and *Borrelia burgdorferi* and mosquito-borne *Dirofilaria immitis* in naturally exposed pet, working, and street dogs in Assam, India.

Materials and Methods: Seroprevalence of *B. canis* and *B. gibsoni* was determined using Indirect Fluorescent Antibody Test (IFAT) kit and for *D. immitis*, *E. canis* and *B. burgdorferi* by Enzyme Linked Immunosorbent Assay (ELISA) in SNAP 3DX kit. The results along with blood microscopy findings were analyzed according to different categories of dogs.

Results: The present study revealed the presence of *Babesia canis*, *Babesia gibsoni*, *Dirofilaria immitis*, and *Ehrlichia canis* usually in mixed infections. Seropositivity against *B. gibsoni* and *B. canis* in IFAT was found comparatively higher in hospital dogs (84.0% and 22.0%) than the street dogs (73.3% and 10%), whereas evidence of *D. immitis* infection in SNAP 3DX ELISA was found higher in street dogs (37.5%) than the hospital group (10%). Antibody to *E. canis* was detected in hospital dogs (32.0%) only and none of the dogs was found positive for *Borrelia burgdorferi* in the same ELISA kit. Besides detection of *B. canis*, *B. gibsoni*, *D. immitis* and *E. canis* in single or mixed infections, *Hepatozoon canis* and *Anaplasma platys* were also recorded in 2.0% and 8.0% respectively in hospital dogs and 3.33% and 6.66% in street dogs during microscopic examination of blood.

Conclusion: High seroprevalence supported by microscopic findings indicate a very strong influence of ticks and mosquitoes as competent vectors in establishing an endemic situation for different species of *Babesia*, *Dirofilaria* and *Ehrlichia*.

Keywords: babesia, dirofilaria, dog, *Ehrlichia*, IFAT, lyme disease, tick, vector.

Introduction

Dogs are affected by a wide variety of vector-borne pathogens that include various species within the genus *Babesia*, *Hepatozoon*, *Dirofilaria*, *Ehrlichia*, and *Anaplasma*, which causes single or mixed infections [1]. Increasing dog population, their trading, and unabated movement within and out of the country have resulted in extension of zoogeographical ranges of vector-borne pathogens for which they are now gaining recognition worldwide [2]. In India sparse information available on vector-borne haemoparasitic diseases of dogs are mainly based on microscopically diagnosed case reports.

The objective of the present study was to determine the seroprevalence of *Ehrlichia canis*, *Babesia canis*, *Babesia gibsoni*, *Dirofilaria immitis* and of *Borrelia burgdorferi*, the etiological agent of Lyme borreliosis, in different categories of dogs in Assam.

Materials and Methods

Study area: The study was conducted in hospital dogs (privately owned pets and working dogs of several

defence organizations) at the Teaching Veterinary Clinical Complex, Assam Agricultural University, Guwahati, and in street dogs at a dog sterilization centre owned by "Just be Friendly", a non-governmental organization under Animal Welfare Board of India.

Ethical approval: The experiments comply with the guidelines laid down by the Institutional Ethical Committee and in accordance with the country law.

Study method: Blood samples, 2 ml each from hospital dogs with clinical problems and from the street dogs were obtained in vials containing EDTA anticoagulant. Antibody activity against *B. canis* and *B. gibsoni* was assessed in plasma of blood samples of 50 hospital dogs by Indirect fluorescent antibody test (IFAT) using *B. canis* IFA IgG antibody kit and *B. gibsoni* IFA IgG antibody kit (Fuller Laboratories, USA) as per recommended procedure. IFAT was similarly performed in sera separated from whole blood collected from 30 street dogs for detection of antibodies to *B. canis* and *B. gibsoni* using in-house antigen slides prepared [3] from the blood of a splenectomised bitch which had 10% parasitaemia with spontaneous *B. gibsoni*, FITC labelled rabbit anticanine conjugate, known *B. gibsoni* and *B. canis* positive and negative control sera.

Enzyme linked immunosorbent assay (ELISA) was performed in whole blood or sera of 50 hospital

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Table-1. Seroprevalence of *Babesia canis* and *Babesia gibsoni* in hospital (n= 50) and street (n= 30) dogs.

Test	Positivity to <i>Babesia gibsoni</i>				Positivity to <i>Babesia canis</i>			
	Hospital dog		Street dog		Hospital dog		Street dog	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
IFAT (1:50 dilution)	42	(84.0)	22	(73.3)	11	(22.0)	3	(10.0)
Microscopy	32	(64.0)	14	(46.6)	2	(4.0)	0	(0.0)

Table-2. Seroprevalence of *Ehrlichia canis*, *Dirofilaria immitis* and *Borrelia burgdorferi* in hospital (n= 50) and street (n= 8) dogs.

Test	Positivity to <i>Ehrlichia canis</i>				Positivity to <i>Dirofilaria immitis</i>				Positivity to <i>Borrelia burgdorferi</i>			
	Hospital dog		Street dog		Hospital dog		Street dog		Hospital dog		Street dog	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
SNAP 3DX	16	(32.0%)	0	(0.0%)	5	(10.0%)	3	(37.5%)	0	(0.0%)	0	(0.0%)
Microscopy	5	(10.0%)	0	(0.0%)	2	(4.0%)	2	(25.0%)	-	-	-	-

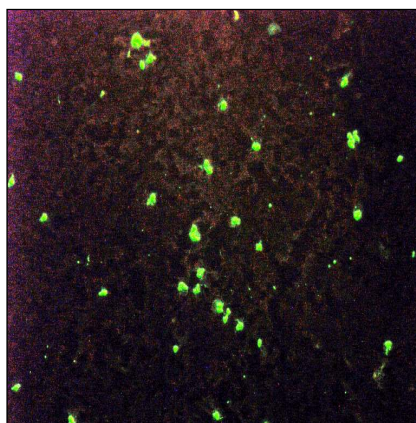
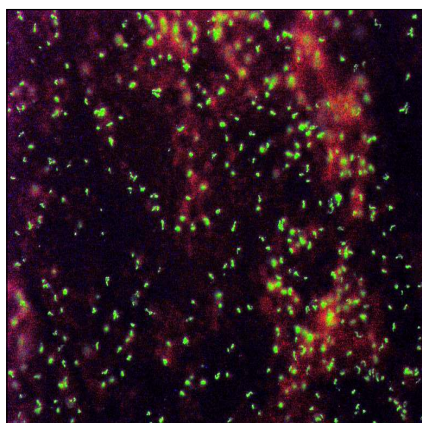


Figure-1. *Babesia gibsoni* IFAT positive at 1: 50 dilution plasma (40X)

Figure-2. *Babesia canis* IFAT positive at 1: 50 dilution plasma (40X)

Figure-3. SNAP 3DX test showing *E. canis* Ab and *D. immitis* Ag positive sample

dogs and 8 street dogs by SNAP 3DX kit, a commercial product of Idexx Laboratories, USA for simultaneous detection of antibodies to *E. canis* and *B. burgdorferi* and *D. immitis* antigen.

Thin blood smears prepared from blood samples of all dogs and stained with Giemsa were microscopically examined for detection of haemoparasites and compared the findings with those of serological tests.

Results

Results of IFAT and microscopy presented in Table-1 revealed 84.0% of hospital and 73.3% of street dogs are seropositive to *B. gibsoni* antibody at 1:50 dilution of test samples (Figure-1), while microscopy could detect 64.0% in hospital and 46.6 % in street dogs. With respect of *B. canis*, 22.0% and 10.0% of hospital and street dogs, respectively, were antibody positive (Figure-2), while microscopy detected only 4.0% *B. canis* in hospital dogs. The street dogs were negative to *B. Canis* with microscopy.

The results of SNAP 3DX test (Figure-3) are presented in Table 2. *E. canis* antibody was detected in 32.0% compared to only 10.0% detection of morula in blood of hospital dogs tested. Street dogs were however found negative to *E. canis* in both the tests. Evidence of *D. immitis* infection was recorded in both street and hospital dogs. However, street dogs showed higher detection by microscopy (25%) and SNAP 3DX (37.5%) than 4-10% detection in hospital dogs.

Blood samples of dogs in the study population tested by SNAP 3DX were all antibody negative to *B. burgdorferi*.

Among all, IFAT and SNAP 3DX tests conducted simultaneously in 10 microscopically pre-examined blood samples revealed evidence of co-infection with all four parasite species (*B. gibsoni*, *B. canis*, *D. immitis* and *E. canis*) in a dog, three species (*B. gibsoni*, *B. canis* and *D. immitis* or *E. canis*) in five dogs and three animals with both the species of *Babesia*. Additionally, *Hepatozoon canis* and *Anaplasma platys* were also detected in blood of 2.0% and 8.0% respectively in hospital dogs and 3.33% and 6.66% street dogs during microscopic examination.

Discussion

Higher prevalence of *Babesia* infection recorded in IFAT than microscopy in both domesticated and street dogs of the present study might suggest sub-clinical carrier status during which parasitaemia is too low to be detectable by microscopy as opined by Baneth et al and Irwin P.J. [4,5]. Similar results showing high prevalence of *Babesia* infections have also been recorded by Senthilkumar et al [6].

Better performance of SNAP 3 DX test in diagnosis of canine monocytic ehrlichiosis have been reported by Mircean et al and Ndip et al [7,8] indicating false negative result of microscopic diagnosis in serologically confirmed positive cases.

In the present study, street dogs showed greater positivity to *D. immitis* than hospital dogs indicating higher risk of exposure to *D. immitis* in outdoor street dogs than the dogs housed indoor as recorded by other workers [9,10]. Sensitivity of serological test is higher than that of microscopic diagnosis in the present study, which is in agreement with Sun et al and Bahadori et al [9,11].

Lyme borreliosis in dogs has been reported from United States [12], Europe [13], Africa and Asian countries [4]. In India, occurrence of this zoonotic disease is established among human population [14, 15]. Present investigation conducted in dogs revealed no evidence of disease in dogs although a sizeable number of dogs tested were engaged by the security agencies in the forested area of the North East from where the disease has been reported earlier from human [15].

The present findings on detection of *Babesia* sp, *D. immitis*, *E. canis*, *H. Canis*, and *A. platys* bear similarity with previous reports published elsewhere [2,6].

Conclusion

Serological investigations supported by blood microscopy revealed the prevalence of *B. gibsoni*, *B. canis*, *D. immitis*, and *E. canis* usually as mixed infections apart from microscopic detection of *H. canis* and *A. platys*. These findings indicate a very strong influence of the competent vectors in establishing an endemic situation.

Authors' contributions

KB: Collection of blood samples and photography; KB, PCS: Detailed microscopic examination of blood, serological tests, conception, preparation and revision of the article; NNB: Works relating to IFAT study and photography. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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