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RESEARCH ARTICLE
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Seroprevalence of canine dirofilariosis, granulocytic anaplasmosis and lyme borreliosis of public health importance in dogs from India's North East

S. K. Borthakur, D. K. Deka, Kanta Bhattacharjee and P. C. Sarmah

Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.
Corresponding author: Dr. Kanta Bhattacharjee, email: kantabhatta@gmail.com, SKB: sanjoy_barthakur@rediffmail.com, DKD: dilipkumar.deka@rediffmail.com, PCS: pcsarmah@gmail.com
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Abstract

Aim: Vector-borne infections namely dirofilariosis, ehrlichiosis, anaplasmosis and lyme borreliosis are being recognized as emerging and/or re-emerging problems in dogs and man due to rapid extension of zoogeographical ranges of many causative agents through international tourism and increase mobility of dogs at national and international level towards meeting the demand for companion animals in the present day society. Anticipating such situation, a serological study was conducted in dogs from North East India to estimate the prevalence of zoonotically important *Dirofilaria immitis*, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* along with *Ehrlichia canis.*

Materials and Methods: Serological study was carried out using enzyme immunoassay in commercial SNAP 4DX® test kit (Idexx Laboratories, USA). The study was conducted in 191 dogs comprising 82 pets, 57 stray and 52 working dogs owned by defence organizations.

Results: The study revealed seroprevalence of mosquito-borne *D. immitis* (17.80%), tick-borne *E. canis* (22.51%) and *A. phagocytophilum* (4.71%) with an overall 41.88% prevalence of pathogens in single or co-infection. Serological evidence of tick-borne lyme borreliosis due to *B. burgdorferi* could not be established in dogs in the present study. Of the zoonotic species, highest prevalence of *D. immitis* was found in the stray dogs (22.80%) and that of *A. phagocytophilum* in pet dogs (6.09%).

Conclusion: The results of the present serological study serve as baseline information on the prevalence of *A. phagocytophilum* in dogs reported for the first time in India and reaffirmation on the high prevalence of *D. immitis* and *E. canis* in the North East India.

Keywords: *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *dog*, *Dirofilaria immitis*, *Ehrlichia canis*.

Introduction

Dog, a multipurpose highly demanding companion animal in the present day society suffers from several vector-borne diseases caused by bacterial, viral and parasitic pathogens. Majority of these infections in the changing environment have attained emerging/re-emerging and zoonotic status [1] due to rapid extension of their geographic distribution through travelling pets, working dogs owned by defence organizations and unabated dog trading at national and international levels [2,3]. This has warranted monitoring and surveillance of diseases for better information to veterinarians and pet owners about their prevalence, risk of spread and public health importance.

Therefore, the aim of this study was to determine the seroprevalence of mosquito-borne *Dirofilaria immitis* and tick-borne *Anaplasma phagocytophilum* and *Borrelia burgdorferi* of zoonotic importance besides *Ehrlichia canis* in dogs from the North East India.

Materials and Methods

Study population

The study was conducted in 191 dogs comprising pets (82), working (52) and stray dogs (57) of different breeds, ages more than 6 months and either sex. The pet dogs owned by private owners and the working dogs of several defence organizations were selected from the population of dogs that were presented with different clinical illness at the Teaching Veterinary Clinical Complex, College of Veterinary Science, Guwahati, Assam during the year 2011 and 2012. Selection was done on the basis of medical history and presenting clinical findings, which included lethargy, depression, anorexia, fever, lameness, hemorrhages, pale mucous membrane, tiredness and weight loss. The stray dogs included in the study were randomly chosen from the animals captured time to time by the animal welfare organizations for sterilization under birth control program.

Method

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid for enzyme linked immunosorbent assay in commercial SNAP 4DX® (Idexx Laboratories, USA) test kits for

qualitative detection of antibodies to *A. phagocytophilum*, *B. burgdorferi* and *E. canis* and *D. immitis* antigen. The test was performed as per manufacturer's protocol. Thin blood smears were prepared from the blood samples, stained with Giemsa and microscopically examined to correlate its findings with those of immunoassay for conclusive evidence.

Results

In the present study, overall 41.88% dogs tested positive for *D. immitis* antigen (17.80%) and antibodies to *E. canis* (22.51%) and *A. phagocytophilum* (4.71%). Exposure to single species pathogen was observed in 38.74% cases against 6.28% with dual infection (Figure-1). According to categories of dogs, highest exposure was recorded in stray dogs (52.63%), followed by working dogs (51.92%) and the least in the pet dogs (28.04%). Of the zoonotic species, highest record of *D. immitis* was found in stray dogs (22.80%) and that of *A. phagocytophilum* in pet dogs (6.09%). Evidence of *B. burgdorferi* was not recorded in any of the dog blood samples examined (Table-1).

Corresponding examination of Giemsa stained blood smears revealed lesser number of dogs positive to microfilariae of *D. immitis* and morulae of *E. canis* and *A. phagocytophilum* besides detection of *B. canis*, *Babesia gibsoni* in erythrocytes, *Hepatozoon canis* in neutrophils and *Anaplasma platys* inside platelets.

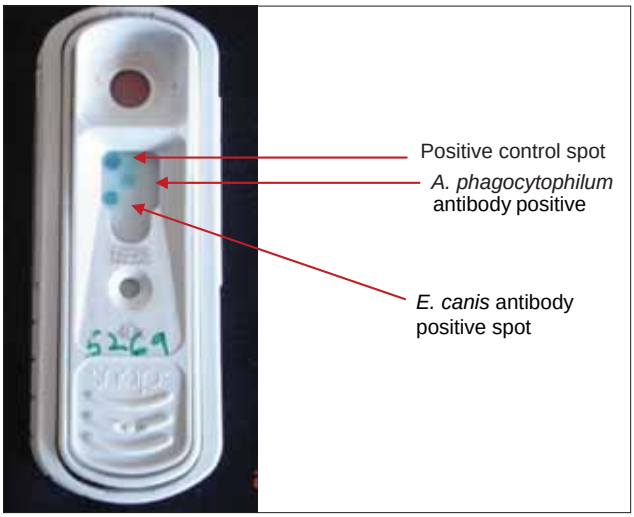

Figure-1: SNAP 4DX kit showing a dog seropositive to *Ehrlichia canis* and *Anaplasma phagocytophilum* antibodies.

Discussion

The present study was conducted to understand the epidemiology of mosquito borne *D. immitis* and tick-borne *E. canis*, *A. phagocytophilum* and *B. burgdorferi* infection in dogs from the North East India to increase awareness among veterinary practitioners and the pet owners about their prevalence and risk of human exposure. Serological test based on enzyme immunoassay technology in a commercial kit SNAP 4DX® with proven high degree of sensitivity and specificity [4,5] employed in the present study could detect 41.88% dogs positive to *D. immitis* antigen and antibodies against *E. canis* and *A. phagocytophilum*. A handful of studies conducted in different countries have revealed prevalence of these pathogens, including *B. burgdorferi* at varying rates according to geographical location [5-8].

Canine vector-borne diseases in India are reported to be far from clear [9]. Limited studies conducted at different places revealed prevalence of *Trypanosoma evansi*, *B. canis*, *B. gibsoni*, *D. immitis*, *Dirofilaria repens*, *H. canis*, *E. canis* and *A. platys* [10,11]. Present findings also confirmed the existence of these pathogens except *T. evansi* in this study region. However the rates of prevalence of *D. immitis* and *E. canis* in all categories of dogs were found much higher than those reported from other parts of the country. This might suggest maximum confinement of heartworm infection in India's North East [9] and use of SNAP 4DX® which is the most efficient kit for serodiagnosis of *E. canis* compared to detection of morula by microscopy [5]. Seroprevalence of *A. phagocytophilum* recorded in this region provides a new insight since the pathogen has not been reported earlier from India. Evidence of lyme borreliosis due to *B. burgdorferi* in dog could not be established in the present investigation although its prevalence in human population has been recorded in India including the North east region [12].

There is ample literature to suggest serological assay as an efficient tool that provides more accurate result compared with microscopy in diagnosis of hidden or past infections [13,14]. Serological findings supported by microscopic detection of corresponding parasite stages in Giemsa stained blood smear also helped to ascertain the disease status by overcoming the complexity of diagnosis from clinical symptoms,

Table-1: Seroprevalence of *D. immitis*, *E. canis*, *A. phagocytophilum* and *B. burgdorferi* in dogs from North-East India.

Category (No.)	*Number of seropositive*	Number of seropositive to			
		D. immitis	*E. canis*	*A. phagocytophilum*	*B. burgdorferi*
Pet dog (82)	23 (28.04)	10[a,b]	11[a,c]	5[b,c]	0
Working dog (52)	27 (51.92)	11[a]	17[aa,c]	2[c]	0
Stray dog (57)	30 (52.63)	13	15	2	0
Overall (191)	80 (41.88)	34 (17.80)	43 (22.51)	9 (4.71)	0

Figures in parenthesis indicate percentage value. [a]One dog seropositive to *D. immitis* and *E. canis*, [b]One dog seropositive to *D. immitis* and *A. phagocytophilum*, [c]One dog seropositive to *E. canis* and *A. Phagocytophilum*. *D. immitis=Dirofilaria immitis*; *E. canis=Ehrlichia canis*, *A. phagocytophilum=Anaplasma phagocytophilum*, *B. burgdorferi=Borrelia burgdorferi*

which were not pathogen specific and mimic each other as reported elsewhere [4,15]. Consistent with earlier reports made from this region [16,17] on the high rates of prevalence of haemoparasites, the present findings of serology supported by microscopic study suggest that this region has ideal biotope for the vector-borne pathogens. Endemic situation with mosquito-borne canine dirofilariasis in this region warrants a thorough investigation in the human population for the risk of zoonosis, which has been reported in India and elsewhere [18-20].

Conclusion

The results of the present serological study serve as baseline information on the prevalence of *A. phagocytophilum* in dogs reported for the first time in India and reaffirmation on the high prevalence of *D. immitis* and *E. canis* in the North East India.

Authors' Contributions

SKB: Collection of blood, procurement of SNAP 4 DX ELISA kit, testing of sera; DKD: Procurement of SNAP 4 DX ELISA kit, testing of sera, presentation of data; KB: Collection of blood samples and photography; KB and PCS: Detailed microscopic examination of blood, conception, preparation and revision of the article. All the 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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