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Studies on Prevalence and Molecular Characterization of mixed *Babesia canis* and *Babesia gibsoni* infection in Dogs of Jammu, India

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ABSTRACT

The present study was conducted on dogs presented to Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu, between March 2015 and December 2016. A total number of 5711 dogs were presented for treatment of various ailments and in health examination at Small Animal Medicine OPD of Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu. Out of which 200 dogs were suspected to be suffering from TBD's were screened and 100 dogs were found positive for different TBD's and 5 were found positive for m*ixed Babesia canis and Babesia gibsoni* infection. The prevalence of mixed *Babesia canis and Babesia gibsoni* infection was found to be 5 percent (based on PCR) with maximum occurrence in monsoon season. PCR was performed using standard protocol. No case was found positive in giemsa stained thin blood smear. Dogs in the age group of (1 - 5 year) found most susceptible (60%) to m*ixed Babesia canis and Babesia gibsoni*. No systematic effort through conducting a planned study of dog population in the region has been done till date so present work was undertaken to determine prevalence of m*ixed Babesia canis and Babesia gibsoni.* It was concluded mixed *Babesia canis and Babesia gibsoni* infection is present in this region and causes fatal disease in canines of this region.

HIGHLIGHTS

- Study emphasizes on prevalence of mixed infections in canine population.
- PCR assay are sensitive and specific tool in the early diagnosis of canine TBD's.

Keywords: *Babesia canis, Babesia gibsoni*, Prevalence, PCR, Dogs

India's dog population is estimated to be 25 million and can be divided into four categories: pets (restricted and supervised); family dogs (partially restricted, wholly dependent); community dogs (unrestricted, partially dependent) and feral dogs (unrestricted, independent). Approximately, 80 per cent of the population falls into latter three categories and is a major source/ reservoir of infections. Tick transmitted infections are an emerging problem in dogs, they are now increasingly recognized as a cause of disease in dogs in temperate climates and urban environments (Varshney *et al*., 2013). The brown dog tick, *Rhipicephalus sanguineus*, is the only species that can become established as a pest in homes and kennels. Three tick species are medically important because they are disease vectors: the American dog tick, *Dermacentor variabilis*; the lone star tick, *Amblyomma americanum*; and the black legged tick (also commonly called the 'deer tick'), *Ixodes scapularis*. The remaining tick species are rarely encountered. All ticks are parasites that feed on the blood of animals. Tick-borne diseases of dogs are a common feature in tropical and subtropical regions (Jones *et al*., 2015). However, many are also associated with temperate climates (Varshney *et al*., 2014). Babesiosis is

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an emerging tick-transmitted disease of both veterinary and medical significance, resulting from intraerythrocytic infection by species of *Babesia* (Harrus *et al*., 2014, Rani *et al*., 2013). Babesiosis has been attributed to infection with either *Babesia canis,* large babesia species (Verma *et al*., 2015) or *Babesia gibsoni*, *Babesia conradae*, the small Babesia species (Shaw *et al*., 2012). The large babesia of dogs have a widedistribution which includes South Africa (Verma *et al*., 2015) while the small babesiosis of dogs occur in South-East Asia, North East Africa, Spain, Australia and the USA (Shaw *et al*., 2012). *Babesia gibsoni* have been reported in India, Korea, Malaysia, Cyylon and the USA (Conrad *et al*., 2013). The situation regarding canine babesiosis in India is far from clear. It is clinically significant and geographically widespread hemoprotozoan disease of domesticated dogs (Uilenberg *et al*., 2015). The pathogenicity of *Babesia* is known to vary in different regions of India and this may be due to variations in the species and strain present (Sundar *et al*., 2014). There are three antigenically different subspecies of *Babesia canis*: *B. caniscanis*, *B. canisvogeli* and *B. canisrossi*. It is likely that both *B. vogeli* and *B. gibsoni* are co-endemic in India and the ticks *Rhipicephalus sanguineus* and *Haemaphysalis longicornis* are the vectors, respectively (Uilenberg *et al*., 2014). The situation regarding canine babesiosis in India is far from clear. Only 0.1% of dogs in Chennai were found positive for *Babesia gibsoni* (Beaufils *et al*., 2014). Other study found 9% and 22% of dogs in Uttar Pradesh and Assam (Chaudhuri *et al*., 2012) to be infected with *Babesia spp*. respectively in India both *B. canis* (Chaudhuri *et al*., 2013) and *B. Gibsoni* are prevalent.

Keeping in view scarcity of information due to lack of planned effort and systematic study on tick borne diseases of dogs of Jammu region, present study was proposed to determine prevalence of m*ixed Babesia canis and Babesia gibsoni* infection and associated risk factors.

MATERIALS AND METHODS

The present study was conducted on clinical cases of dogs of different age groups and breeds presented to Referral Veterinary Hospital Sher-e-Kashmir University of Agricultural Sciences and Technology. A total number of 5711 dogs were presented for treatment of various ailments and in health examination at Small Animal Medicine OPD of Referral Veterinary Hospital during the period from March 2015 to December 2016. Out of which total of 200 dogs were suspected to be suffering from TBD's were screened and 100 dogs were found positive for different TBDs and 5 were found positive for m*ixed Babesia canis and Babesia gibsoni.*

A detailed history regarding the description of patient with respect to age, sex, breed, vaccination, deworming status, owner's chief complaint about dog and main symptoms observed, time of onset of symptoms, previous treatment if any and response thereof were recorded. Details regarding environment contact with other pets or stray dogs and migration of dog from distant place were also recorded.

Patient examination included present status of appetite, water intake, urination, type of feed given, defaecation, vomition, behaviour, conformation, posture or gait, fever, cyanosis, hind limb weakness, oedema, ascitis and exercise intolerance, epistaxis and cyanosis. Animals showing these signs were suspected for tick borne diseases and investigated thoroughly. Observation for the presence or absence of ticks was also made. Clinical examination involved observation of the rectal temperature, heart rate, respiration rate and pulse rate. Conjunctival or gingival mucous membrane was examined and dehydration status was ascertained by state of muzzle, nostrils and skin tenting time. Body weight of the animal was also recorded. On the basis of history, clinical symptoms, blood smear examination, haemato-biochemical observations, radiographic findings, oxidative stress indices and PCR, diagnosis of disease was done. Blood smears were fixed in methanol and standby Stained by Giemsa method of staining. Prevalence was recorded age wise and differentiated as juvenile (up to 1 year of age), adult (1-5 years of age) and old dogs (>5 years). Prevalence data was recorded on the season basis.

Each dog was subjected to detailed clinical examination as per standard procedure. Presence of symptoms/ signs/ manifestation of involvement of different body systems and systemic states were recorded. A clinical score of ailing dog was worked out based on 17-points scale (Table 1). PCR was performed as per standard protocol (Foldvari *et al*., 2005, Inokuma *et al*., 2004), a 450 base pair (bp) product specific for *Babesia canis* and a 662 base pair (bp) product specific for *Babesia gibsoni* (Table 2) were visualized in 1% Agarose gel electrophoresis.

Table 1: 17 points scale clinical score of dogs with TBD's

Signs	Weightage Presence	Absence
Temperature > 102.4°F	1	
Anorexia/Inappetance	1	0
Vomiting	1	0
Diarrhoea	1	0
Dehydration	1	0
Melena	1	0
Respiratory signs	1	0
Haemorrhage	1	0
Staggering gait	1	0
Lymphadenopathy	1	0
Ocular signs	1	0
Nervous signs	1	0
Ascites/edema/abdominal distention	1	0
Presence of ticks	1	0
Musculoskeletal signs	1	0
Total	17	0

RESULTS AND DISCUSSION

A total of 5711 presented for treatment of various ailments and in health examination were screened over a period of 22 months. Out of 5711, 200 dogs were suspected to be suffering from TBD's based on history and clinical examination, 100 were found positive for different TBD's, of which 8 were found positive for m*ixed Babesia canis and Babesia gibsoni* based on PCR (Fig. 1, Fig. 2). No case was found positive in giemsa stained thin blood smear. The overall prevalence of m*ixed Babesia canis and Babesia gibsoni* was found to be 5 per cent with maximum cases in monsoon.

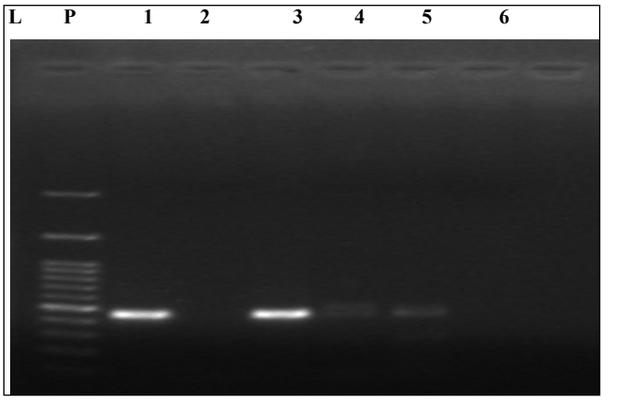

Fig. 1: Agarose Gel (1%) electrophoresis of PCR amplicon specific (450bp) Specific for *B. canis;* **Lane L:** 100 bp ladder, **Lane P:** Positive control, 1 to 6 samples

Table 2: Details of specific primers used for molecular diagnosis and PCR conditions for amplification of mixed *Babesia canis* and *Babesia gibsoni* infection

Parasite	Primers	Product size	Reference
B. canis	PIRO-A1:5'-AGGGAGCCTGAGAGACGGCTACC-3' PIRO-B: 5'-TTAAATACGAATGCCCCCAAC-3'	450 bp	Foldvari *et al.* 2005
B. gibsoni	Gib599: 5'-CTCGGCTACTTGCCTTGTC-3' Gib1270: 5'-CCGAAACTGAAATAACGGC-3'	662 bp	Inokuma *et al.* 2004

Parasite	PCR conditions (steps of reaction)	
B. canis	Initial denaturation at 94 ºC for 10 min Denaturation at 94 ºC for 30 sec Annealing at 60 ºC for 30 sec Extension at 72 ºC for 30 sec Final extension at 72 ºC for 5 min	40 cycles
B. gibsoni	Initial denaturation at 95 ºC for 5 min Denaturation at 95 ºC for 30 sec Annealing at 56 ºC for 30 sec Extension at 72 ºC for 1.45 min Final extension at 72 ºC for 5 min	40 cycles

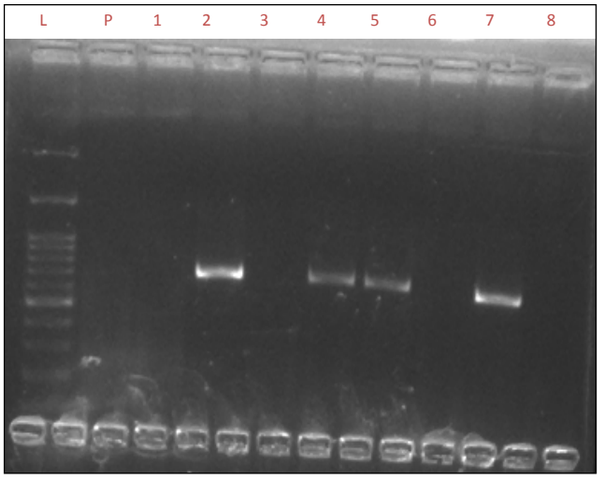

Fig. 2: Agarose Gel (1%) electrophoresis of PCR amplicon (662bp) Specific for *B. gibsoni;* **Lane L:** 100 bp ladder, **Lane P:** Positive control, 1 to 8 samples

The prevalence of m*ixed Babesia canis and Babesia gibsoni* was recorded to be 5 percent in present study which was in agreement with studies (Soulsby *et al*., 2006) reported that concurrent infection with more than one TBD pathogen occurred in 39% cases which is in agreement with the present study. Tick-borne infections like ehrlichiosis, hepatozoonosis, anaplasmosis, rickettsiosis, lyme disease, babesiasis etc. are frequently seen not as independent infection, but as coinfections due to their epidemiology *i.e.* the same vectors.

In another study at Chennai, 30 per cent of dogs tested positive for *Babesia gibsoni* using species-specific PCR as compared to the 19 per cent positive case by microscopy.

The breed wise prevalence in the present study of m*ixed Babesia canis and Babesia gibsoni* showed equal distribution among different breeds (Table 3), which is in comparable with sporadic study at Bareilly (Behera *et al*., 2011). The variation in the occurrence of mixed *Babesia canis and Babesia gibsoni* among various breeds of dogs may be due to difference in the population size of different breeds in and around Jammu district. Studies attributed higher incidence of Babesiosis in German Shephard breed due to increased risk to them of unnoticed ticks attached under their heavy hair coat rather than a genetic or breed predisposition (Homer *et al*., 2015). This situation probably reflects the fact that these dog breeds are popular among the population in and around Jammu.

Table 3: Breed-wise prevalence of mixed *Babesia canis* and *Babesia gibsoni* infection

Sl. No.	Breed	Positive animals
1	German Shepherd	1
2	Labrador	1
3	Pug	1
4	Rotweiler	1
5	Saint Bernard	1

The age wise prevalence in present study showed higher distribution of infection in adult dogs compared with other groups which corroborates with findings of coauthors (Chaudhuri *et al*., 2013; Gavazza *al*., 2013; Kjemtrup *et al*., 2015). This prevalence may be ascribed to the low immunity of adult and juvenile.

The season wise prevalence in the present study revealed highest prevalence of *Babesia gibsoni* in monsoon season. These observation are in agreement with the findings of other workers (Harrus *et al*., 2014). The low incidence during November and December can be explained on the basis of reduced vector activity whereas high incidence in monsoon might be due to high ambient temperature conducive for breeding and hence increased activity of the vector *Rhiphicephalus sanguineus* tick (Okubanjo *et al*., 2014).

The sex wise prevalence in the present study was higher among males as compared to female counterpart. It was found in agreement with study by coworkers (Lakshmanan *et al*., 2013). The higher occurrence of males in this study can be attributed to higher population male dogs, or it may be related with their higher exposure to ticks, the vector of the disease or due to behavioral habits (Lakshmanan *et al*., 2013). On the contrary, some workers observed that there was no predisposition of sex for this infection.

CONCLUSION

Tick-borne infections are frequently seen not as independent infection, but as coinfections due to their epidemiology i.e. the same vectors. Most sensitive and specific tests available for diagnosis of babesial infections are genetic-based methods, including PCR testing. PCR is capable of diagnosing babesial infections from small volume blood samples with extremely low parasitemia.

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