



Studies on Prevalence and Molecular Characterization of *Babesia gibsoni* in Dogs of Jammu Region

Himalini*, R. Singh, R.K. Bhardwaj and A.K. Gupta

Division of Veterinary Medicine, Sher-e-Kashmir University of Agricultural Sciences and Technology of Sciences and Technology of Jammu Jammu and Kashmir, INDIA

*Corresponding author: Himalini; E-mail: himalini.12@gmail.com

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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 8 were found positive for *Babesia gibsoni*. The prevalence of *Babesia gibsoni* was found to be 8 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. Equal prevalence among juvenile and adult dogs with highest prevalence in German Shephard breed. Dogs in the age group of (1 - 5 year) and juvenile dogs were found most susceptible (37.5%) to *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 *Babesia gibsoni*. It was concluded *Babesia gibsoni* infection is present in this region and possible factor causing disease in this region.

HIGHLIGHTS

- ① Study emphasizes on prevalence of *Babesia gibsoni*.
- ① PCR assay are sensitive and specific tool in the early diagnosis of canine TBD's.
- ① *Babesia gibsoni* infection is present in dogs of Jammu region.

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

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 (Shaw *et al.*, 2012). 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 (Irwin and Jefferies, 2010). However, many are also associated with temperate climates (Shaw *et al.*, 2012). Babesiosis is an emerging tick-transmitted disease of both veterinary and medical significance, resulting from intraerythrocytic infection by species of *Babesia* (Homer *et al.*, 2010). It is clinically significant and geographically widespread

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hemoprotozoan disease of domesticated dogs (Irwin, 2010). 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 (Rani et al., 2011). There are three antigenically different subspecies of *Babesia canis*: *B. canis canis*, *B. canis vogeli* and *B. canis rossi*. 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 (Irwin and Jefferies, 2010). The situation regarding canine babesiosis in India is far from clear. Only 0.1% of dogs in Chennai were found positive for *Babesia gibsoni* (Sundar et al., 2014). Other study found 9% and 22% of dogs in Uttar Pradesh and Assam (Choudhuri and Varshney, 2007) to be infected with *Babesia spp.* respectively in India both *B. canis* (Varshney et al., 2010) and *B. gibsoni* (Varshney et al., 2010) are prevalent. The diseases has been reported from various states of India such as Assam (Saud and Hazarika, 2010), West Bengal (Mitra et al., 2009), Bihar (Verma et al., 2009), Delhi (Farwell et al., 2012), Jammu & Kashmir (Gupta et al., 2012), Tamil Nadu (Kumar et al., 2009) and Uttar Pradesh (Varshney et al., 2010). *Babesia spp.* are transmitted to dogs by a wide variety of ixodid ticks including *Haemaphysalis longicornis*, *H. leachi*, *Rhipicephalus sanguineus* and *Dermacentor marginatus* (Shaw et al., 2012), although infection from blood transfusions, transplacental transmission (Fukumoto et al., 2012) and direct transmissions through bite wounds (Jefferies et al., 2007) have been reported. The situation regarding canine babesiosis in India is far from clear.

Keeping in view scarcity of information due to lack of systematic effort and planned study on tick borne diseases of dogs of Jammu region, present study was ventured to determine prevalence of *Babesia gibsoni* 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 8 were found positive for *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, vomiting, 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 smear were fixed in methanol and standby Stained by Giemsa method of staining. Prevalence was recorded age wise and differentiated as juvenile (upto 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 (Jones, 1994). 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 (Jones, 1994) (Table 1). PCR was performed as per standard protocol given by Inokuma et al. (2004) (Table 2) and a 662 base pair (bp) product specific for *Babesia gibsoni* was visualized in 1% Agarose gel electrophoresis.

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 *Babesia gibsoni* based on PCR(Fig.1). No case was found positive in giemsa stained thin blood smear. The overall prevalence of *Babesia gibsoni* was found to be 8 per cent with maximum cases in monsoon.

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

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

Parasite	Primers	Product size	Reference
<i>B. gibsoni</i>	Gib599: 5'-CTCGGCTACTTG	662 bp	Inokuma <i>et al.</i> (2004)
	CCT TGTC-3'		
	Gib1270: 5'-CCGAAACTGA		
	AATAACGGC-3'		
Parasite	PCR conditions (steps of reaction)		
<i>B. gibsoni</i>	Initial denaturation at 95 °C for 5 min		
	Denaturation at 95 °C for 30 sec		
	Annealing at 56 °C for 30 sec		
	Extension at 72 °C for 1.45 min		
	Final extension at 72 °C for 5 min		

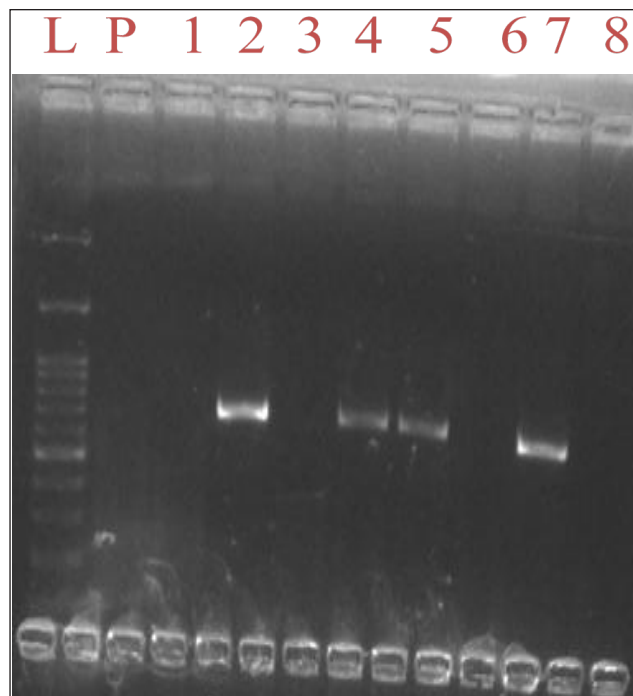


Fig. 1: PCR amplicon (662 bp) Specific for *B. gibsoni* (Lane L: 100 bp ladder; Lane P: Positive control Lane: 1 to 8 samples)

The prevalence of *Babesia gibsoni* was recorded to be 8 percent in present study which was in agreement with work done by Behra *et al.* (2011) who found 8.40% dogs positive in a sporadic study at Bareilly. *Babesia gibsoni* in dog was reported with a prevalence of 0.1% in Chennai (Sundar *et al.*, 2004), in Uttar Pradesh 9% (Chaudhuri, 2010) and 22% in Assam (Saud and Hazarika, 2010). 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 (Lakshmanan *et al.*, 2009).

The breed wise prevalence in the present study of *Babesia gibsoni* was found to be higher in German Shephard (62.50 percent) followed by Labrador Retriever (25 percent) and lowest in Pug (12.5 percent (Table 3), which is in comparable with sporadic study at Bareilly (Behra, 2011). The variation in the occurrence of *Babesia gibsoni* among various breeds of dogs may be due to difference in the population size of different breeds in and around Jammu district. Hornok *et al.* (2010) 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. This



situation probably reflects the fact that these dog breeds are popular among the population in and around Jammu.

Table 3: Breedwise prevalence of *Babesia gibsoni*

Sl. No.	Breed	Positive animals
1	German Shepherd	5
2	Labrador	2
3	Pug	2

The age wise prevalence in present study showed equal distribution (37.5%) of infection in adult and juvenile dogs which corroborates with findings of Gavazza *et al.* (2008) and Chaudhuri, (2010). This prevalence may be ascribed to the low immunity of adult and juvenile animals.

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. 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 *Rhipicephalus sanguineus* tick (Soulsby, 2006).

The sex wise prevalence in the present study was higher among males as compared to female counterpart. It was found in agreement with Okubanjo *et al.* 2014 and Gavazza *et al.* 2008. 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 (Okubanjo *et al.*, 2014).

CONCLUSION

It may be concluded that *Babesia gibsoni* is present in dogs of Jammu region and results in severe fatal disease. Fewer cases have been reported earlier, molecular diagnosis based on sensitive and specific PCR assays is helpful especially in the early diagnosis of canine TBD's along with identification of the infecting species, thereby helping with the taxonomic classification, identification of subclinical cases which are/or not diagnosed by routine blood smear examination.

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