Journal of Animal Research: v.8 n.6, p. 1121-1124. December 2018

DOI: 10.30954/2277-940X.12.2018.29

SHORT COMMUNICATION

Prevalence and Molecular Detection of *Babesia canis* in Dogs of Jammu Region

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

Division of Veterinary Medicine, F.V.Sc. & A.H., SKUAST-J,R.S. Pura, Jammu J&K, INDIA

**Corresponding author: Himalini; Email: himalini.12@gmail.com*

Received: 26 July, 2018 **Revised:** 08 Oct., 2018 **Accepted:** 15 Oct., 2018

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 of 5711 dogs were presented for treatment in 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 a total of 200 dogs suspected to be suffering from various Tick borne diseases (TBD's) were screened and 100 were found positive for different TBD's and 11 were found positive for *Babesia canis*. The prevalence of *Babesia canis* was found to be 11 percent (based on PCR) with maximum occurrence in summer. PCR was performed using standard protocol. No case was found positive in giemsa stained thin blood smear. Males were more affected than female with highest prevalence in Labrador breed. Dogs in the age group of (1 - 5 year) were found most susceptible (72.27%) to *Babesia canis*. No case was recorded among juvenile dogs. 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 the prevalence of *Babesia canis*. It was concluded that *Babesia canis* infection is present in dogs of Jammu region and may be a possible factor causing disease in dogs of the region.

Keywords: *Babesia canis*, Prevalence, TBD's, PCR, Dogs

Diverse agroclimatic zones in India allow wide range of vectors and pathogens of medical and veterinary importance to multiply and spread diseases among human beings and animals. India's dog population is estimated to be 25 million (Lakshmanan, 2001) 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. Among the various economically important canine diseases, vector-borne haemoprotozoan infections such as babesiosis, trypanosomiasis, ehrlichiosis, hepatozoonosis and anaplasmosis are recognized as a cause of severe clinical illness in canids in tropical and subtropical regions of country. Significant economic losses due to high morbidity, mortality and drug usage have been reported due to TBD's. Canine babesiosis is a tick borne diseases caused by intraerythrocytic piroplasms of the genus *Babesia,* has been attributed to infection with either *Babesia canis,* large babesia species (Uilenberg *et al*., 1989) or *Babesia gibsoni*., *Babesia conradae*, the small Babesia species (Kjemtrup *et al*., 2006). The large babesia of dogs have a wide distribution which includes South Africa (Uilenberg *et al*., 1989) while the small babesiosis of dogs occur in South-East Asia, North East Africa, Spain, Australia and the USA (Kjemtrup *et al*., 2006). *Babesia gibsoni* have been reported in India, Korea, Malaysia, Cyylon and the USA (Jain *et al*., 1991). Brown dog tick, *Rhipicephalus sanguineus*, is the major tick infesting canine and acts as a vector of several agents such as *Anaplasma platys, Babesia canis vogeli, Babesia gibsoni, Ehrlichia canis* (*E. canis*), spotted fever group Rickettsia spp. *and Hepatozoon canis* (*H. canis*). Interactions among various parasitic agents unquestionably affect the organisms individually and alter their effects on the host. Concomitant tick borne infections are very common in endemic areas but clinical reports are scarce.

Keeping in view the paucity of information as no systematic effort through conducting a planned study of dog population in the Jammu region has been done till date and importance of tick borne diseases in dogs, present work was undertaken to determine the prevalence of *Babesia canis* and associated risk factors in dogs.

The present study was conducted on dogs presented. A total of 5711 dogs were presented for treatment in 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 between March 2015 and December 2016. Out of which a total of 200 dogs suspected to be suffering from various Tick borne diseases (TBD's) were screened and 100 were found positive for different TBD's and 11 were found positive for *Babesia canis*.

A comprehensive history comprised of 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 and PCR, diagnosis of disease was done. Blood smear were fixed in methanol and standby stained by Giemsa method of staining (Jain, 1986). Prevalence was recorded as age wise and classified 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 each ailing dog was worked out based on 17-points scale (Jones, 1998) (Table 1). PCR was performed as per standard protocol given by Foldvari *et al.* 2005 (Table 2) and 450 base pair (bp) product specific for *Babesia canis* was 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

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 11 were found positive for *Babesia canis* based on PCR. PCR was performed as per standard protocol given by Foldvari *et al.* 2005 and 450 base pair (bp) product specific for *Babesia canis* was visualized in 1% Agarose gel electrophoresis (Fig. 1). No case was found positive in giemsa stained thin blood smear. The overall prevalence of *Babesia canis* was found to be 11 per cent with maximum (2 cases; 18.1%) during April, May, June, 2015 and May 2016 and mimimum (1 case; 9.09%) in

Table 2: Details of specific primer used for molecular diagnosis and PCR conditions for amplification of *B. canis*

Parasite	Primers	Product size	Reference
B. canis	PIRO-A1:5'-AGGGAGCCTGAGAGACGGCTACC-3'	450 bp	Foldvari *et al.* 2005
	PIRO-B: 5'-TTAAATACGAATGCCCCCAAC-3'		
Parasite	**PCR conditions (steps of reaction)**		
B. canis	Initial denaturation at 94ºC for 10 min		
	Denaturation at 94ºC for 30 sec		
	Annealing at 60ºC for 30 sec		40 cycles
	Extension at 72ºC for 30 sec		
	Final extension at 72 ºC for 5 min		

July, August September 2015, April, June, October and November 2016.

The prevalence of *Babesia canis* was recorded to be 11 percent in present study which is in agreement with work done by Dhanakar *et al*. (2011) who found 11.35% dogs positive for *Babesia canis* in Haryana and Delhi states. From South-Western regions the republic of Korea (South Korea) *Babesia canis* was found in 6.1% of the screened canine blood smears by Lim *et al*. (2007). In another study at Chennai, 50 per cent of dogs tested positive for *Babesia canis* using species-specific PCR as compared to the 19 per cent positive case by microscopy (Lakshmanan *et al*., 2007).

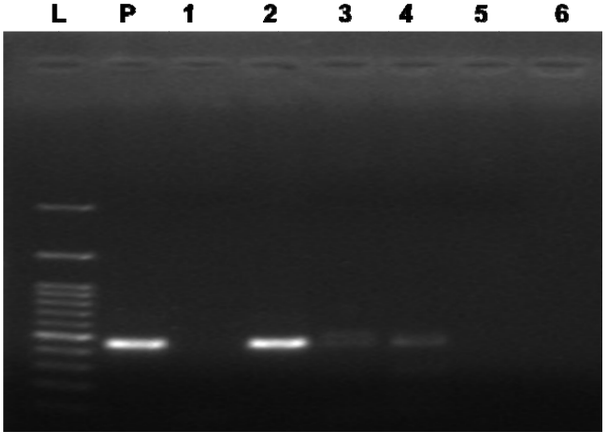

Fig. 1: Agarose Gel (1%) electrophoresis of PCR amplicon (450bp) Specific for *B. canis*. Lane L: 100 bp ladder; Lane P: Positive control; Lane: 1 to 6 samples

The breed wise prevalence in the present study of *Babesia canis* was found to be higher in Labrador Retriever (45.4%) followed by German Shephard (27.27%) and lowest in Pug (9.09%), Pitbull (9.09%), Rottweiler (18.18%) (Table 3), which is in comparable with sporadic study at Bareilly (Behra, 2011). The variation in the occurrence of *Babesia canis* 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.* (2006) attributed higher incidence of Babesiosis in Labrador breed due to increased risk to them of unnoticed ticks attached under their heavy hair coat rather than a genetic or breed predisposition.

The age wise prevalence in the present study was found higher (72.72%) in adult dogs as compared to old dogs (27.27%). No case was recorded among juvenile group. Age wise analysis of data to study the distribution of *Babesia canis* revealed presence of infection in almost in adult and older dogs which was in agreement with Harrus *et al*. (1997b) and Harikrishnan *et al*. (2001). The higher incidence was observed in the adult group (1- 5 years) which corroborates with findings of Mundim *et al*. (1994), Gavazza *et al*. (2003) and Chaudhuri, (2006). This prevalence may be ascribed to the low immunity of adult animals.

Table 3: Breedwise prevalence of *Babesia canis*

Sl. No.	Breed	Positive animals
1	Labrador	5
	German Shepherd	3
3	Pug	1
4	Pitbull	1
5	Rottweiler	1

The month wise prevalence in the present study revealed highest prevalence of *Babesia canis* in the month of May and April and least number of cases in August, October, December and September. These observation are in agreement with the findings of other workers (Harrus *et al*., 1997 a,b; Greene, 2006). The low incidence during November and December can be explained on the basis of reduced vector activity whereas high incidence in summer might be due to high ambient temperature conducive for breeding and hence increased activity of the vector *Riphicephalus 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.* 2003. 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 behavioural habits (Okubanjo *et al.,* 2014).

CONCLUSION

It may be concluded canine babesiosis results in severe fatal disease in dogs. earlier fewer cases of the disease have been reported , molecular biology provides a powerful method not only in subspecies (genotype) identification, but also in cases when symptoms and/or blood smears do not provide definitive diagnostic information.

REFERENCES

Behera, S.K. 2011. Canine ehrlichiosis: Clinico-epidemiology and therapeutic considerations with special reference to evaluation of immunomodulatory activity of homeopathic drugs. *Ph.D. Thesis. Indian Veterinary Research Institute. Izatnagar*, Bareilly, India.

Chaudhuri, S. 2006. Studies on clinico-therapeutic aspects of babesiosis in dogs**.** In *MVSC Thesis*. Indian Veterinary Research Institute, Bareilly, India.

Conrad, P., Thomford, J., Yamane, I., Whiting, J., Bosma, L., Uno, T., Holshuh, H.J. and Shelly, S. 1991. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. *J. Am. Vet. Med. Assoc.,* **199**: 601–605.

Dhanakar, P. R., Hafiz, A., Bora, S., Phukan, A., Baishya, B. C and Kalita, D. N. 2011. Prevalence of canine diseases in Guwahati city. *Indian Vet. J.*, **90:** 103–104.

Foldvari, G., Hell, E. and Farkas, R. 2005. *Babesia canis canis* in dogs from Hungary: detection by PCR and sequencing. *Vet. Parasitol.,* **127:** 221–226.

Gavazza, A., Bizetti, M. and Papini, R. 2003. Observations on dogs found naturally infected with *Hepatozoon canis* in Italy. *Revue. Med. Vet.,* **154**: 565-571.

Greene, C.E. 2006. *Infectious Diseases of the Dog and Cat*. 3rd edition. New York: Elsevier Health Sciences.

Harrus, S., Aroch, I., Lavy, E. and Bark, H. 1997a. Clinical manifestations of infectious canine cyclic thrombocytopenia. *Veterinary Record,* **141**: 247–250.

Harrus, S., Waner, T. and Bark, H. 1997b. Canine monocytic ehrlichiosis: an update. *Comp. Cont. Edu. Pract. Vet.,* **19**:431-447.

Harikrishanan, T.J., Chellappa, D. J., Pazhanivel, N., Sreekumar, C., Anna, T., Raman, M. and Rajavelu, G. 2001. Epizootiology of canine ehrlichiosis in Chennai. *Indian J. Anim. Sci.*, **71:** 133-135.

Hornok, S, Edelhofer, R. and Farkas, R. 2006. Seroprevalence of canine babesiosis in Hungary suggesting breed predisposition**.** *J. Parasitol. Res.,* **99**: 638-642.

Jain, N.C. 1986. *Schlam's Vet. Haematology*, *4th edn*. Lea and Febriger, Philadelphia. USA.

Jones, C.H., Smye, S.W., Newstead, C.G., Will, E.J. and Davison, A.M. 1998. Extracellular fluid volume determined by bioelectric impedance and serum albumin in CAPD patients. *Nephrol. Dial. Transplant*., **13**: 393–397.

Kjemtrup, A.M. and Conrad, P.A. 2006. A review of the small canine piroplasms from California: *Babesia conradae* in the literature**.** *Vet. Parasitol.*, **138**: 112-117.

Lakshmanan, B. 2001. Diagnosis of canine ehrlichiosis using polymerase chain reaction. *MVSc Thesis. The Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.* Lakshmanan, B., John, L., Gomathinayagam, S. and Dhinakarraj, G. 2007. Molecular detection of *Ehrlichia canis* from blood of naturally infected dogs in India. *Vet. Arhiv.*, **77**: 307-312.

Lim, Y.Y., Lim, T.T. and Tee, J.J. 2007. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.,* **103(3)**: 1003-1008.

Mundim, A.V., Mundim M.J.S., Jensen, N.M.P. and Arau, J. S.F. 1994. *Hepatozoon canis*: estudo retrospectivo de 22 casos de infeccao natural em caes de Uberlandia, MG. *Rev Cent. Cienc. Biomed. Univ. Fed. Uberlandia.,* **10**: 89–95.

Okubanjo, K., Pyle, R.L. and Reddy, A.M. 2014. *Rickettsia canis* in Hyderabad. *Indian Vet. J.,* **35**: 63-68

Soulsby, E.J.L. 2006. *Helminths, Arthropods and Protozoa of domesticated animals*, *7th edn.,* Elsevier, New Delhi, India.

Uilenberg, G., Franssen, F.F., Perie, N.M. and Spanjer, A.A. 1989. Three groups of *Babesia canis* distinguished and a proposal for nomenclature**.** *Vet. Rec.,* **11**: 33-40.