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Molecular Detection and Risk Factor Analysis of *Babesia gibsoni* and *Babesia vogeli* in Naturally Infected Dogs in Andhra Pradesh, India

Jalajakshi Kopparthi, Sreedevi Chennuru, Chengalva Rayulu Vukka, Nalini Kumari Karumuri, Rani Prameela Devalam

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ABSTRACT

Background: Babesiosis in dogs is endemic in tropical countries like India because of presence of tick vectors as well as reservoir stray dog population round the year. There is paucity of information regarding the prevalence and molecular confirmation of *Babesia* species in dogs in Andhra Pradesh. The present study was aimed to detect *Babesia* species in naturally infected dogs in Andhra Pradesh.

Methods: During the one-year study *i.e*. from June 2019 to July 2020, blood samples were collected from 442 tick infected dogs showing clinical signs suggestive of babesiosis to determine the prevalence of *Babesia* species by microscopy and molecular technique. Factors associated with prevalence of babesiosis were analyzed by logistic regression models.

Result: Microscopic examination of stained blood smears revealed 11.8% prevalence of *Babesia* spp. infection in dogs in the study area, while 28.3% of dogs were found to be positive for *Babesia* spp. in the first *Babesia* genus-specific PCR. Further analysis of positive samples with species-specific PCR, *Babesia gibsoni* and *B. vogeli* were detected 19.9% and 8.6% of dogs, respectively. Risk factor analysis by binary logistic regression model revealed that the living condition of the dogs, gender, age, medium and tick infestation as risk factors for *B. gibsoni* and *B. vogeli* infection.

Key words: *Babesia gibsoni*, *B. vogeli*, Dog, PCR, Risk factors, Sequencing.

INTRODUCTION

Canine babesiosis, caused by apicomplexan parasites of the genus *Babesia* including *Babesia gibsoni, B. canis, B. vogeli* and *B. rossi* is an important tick-borne protozoan disease globally. Generally, the disease is characterized by fever, anaemia, lethargy, Jaundice and haemoglobinuria, however atypical clinical manifestation of acute babesiosis included neurological signs and pancytopenia (Maele *et al*., 2008). *Babesia gibsoni* and *B. vogeli* are the two main species causing natural infections in dogs in India. The brown dog tick, *Rhipicephalus sanguineus sensu lato*, has been reported as a competent or potential vector for canine babesiosis. Despite canine babesiosis is recognized as a tick-borne disease, transmitted by a variety of ixodid tick vectors around the world, results of previous studies indicated that *B. gibsoni* was transmitted by blood transfusion (Stegeman *et al*., 2003), transplacentally (Fukumoto *et al*., 2005) and bite wounds (Jefferies *et al*., 2007). Recently, Nimisha *et al*. (2019) also opined that either blood transfusion, bite wounds and, or transplacental transmission might be the reason for increased occurrence of *B. gibsoni* in Kerala as it was not detected from any of the tick samples examined.

Though canine babesiosis has been reported from different parts of India based on microscopic examination and molecular assays (Shaw *et al*., 2001; Senthil Kumar *et al*., 2009; Abd Rani *et al*., 2011; Laha *et al*., 2014; Manoj *et al*., 2020), there is paucity of information regarding the prevalence and molecular confirmation of *Babesia* species in dogs in Andhra Pradesh (AP), India. Keeping in view of the above information, the present study was aimed to detect *Babesia* species in naturally infected dogs in Andhra Pradesh, India by microscopy and conventional PCR and to determine possible factors associated with canine babesiosis in the study area.

College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517 502, Andhra Pradesh, India.

Corresponding Author: Jalajakshi Kopparthi, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517 502, Andhra Pradesh, India. Email: jalajakshikopparthi@gmail.com

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MATERIALS AND METHODS

Blood samples were collected aseptically from the cephalic vein of the dogs (n=442) presented to the Veterinary Polyclinics and Veterinary Hospitals of AP with tick infestation

and/or showing clinical signs suggestive of babesiosis, for one year period from June 2019 to July 2020 in the Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati. A demographic data for each dog was obtained through a questionnaire that contained questions regarding age, sex, breed, living condition of the dog, presence/absence of ticks, acaricidal history and origin to determine risk factors associated with babesiosis. Thin blood smears were prepared from whole blood, air-dried, fixed in pure methanol and stained with Giemsa for microscopic examination (Soulsby, 1982). Blood samples were further preserved at -20°C until DNA extraction.

PCR assay

DNA was extracted from individual blood samples (n=442) using the QIA amp DNA Blood Mini Kit (Qiagen) as per the manufacturer's protocol. The 16S rRNA gene of *Babesia* spp. of dogs was amplified at specific loci using the primers and cycling conditions as per Kledmanee *et al*. (2009). Samples positive for *Babesia* spp. were further subjected to species-specific PCR. The 18S rRNA gene of *B. gibsoni* and *B. vogeli* were amplified at specific loci using the species-specific primers and cyclic conditions of Inokuma *et al*. (2004) and Duarte *et al.* (2008), respectively.

PCR was carried out in a final reaction volume of 25 µL containing 5µLgenomic DNA, 12.5 µL of master mix (Genei, Bengaluru), 2.5 pmol of each forward and reverse primers and 5.5 µL of nuclease free water. A negative control was run along with the samples at every PCR setup. PCR amplicons (5 µL each) were analyzed on 1.5% (w/v) agarose gel in 1x TAE buffer at 100 V for 55 min and visualized on UV trans-illuminator. The PCR positive products were sequenced by Sanger's method and compared with the already reported sequences from other geographic regions available in GenBank using the BLAST program (www.ncbi.nlm.nih.gov/ blast/).

Statistical analysis

The chi-square test was used for comparison of the frequencies by SPSS Statistics base 20. The role of risk factors *viz*. age group, gender, breed, living condition, tick infestation and season on the frequency and type of *Babesia* spp. infections was identified by binary logistic regression models using SPSS Statistics base 20.

RESULTS AND DISCUSSION

Microscopic examination of stained blood smears revealed 11.8% (52/442) prevalence of *Babesia* spp. infection in dogs in the study area, while 28.3% (125/442) of dogs were found to be positive for *Babesia* spp. in the first *Babesia* genus-specific PCR. The genus specific PCR amplified 619 bp partial 16S rRNA of *Babesia* spp. (Plate 1). The microscopic examination of stained blood smear is the conventional method used for the specific differentiation between *B. vogeli* and *B. gibsoni*, which is based on the morphometric characteristics of each species. This is a rapid and low-cost method with high specificity, but lacks sensitivity to detect low-level parasitemia. Recently, molecular methods including the polymerase chain reaction (PCR) and sequence analysis have proven effective in some epidemiological studies of *Babesia* infection in dogs. Out of these, the phylogenetic analysis of fragments of the SSU rRNA gene has been frequently applied for molecular studies of *B. canis* and *B. gibsoni* isolates (Inokuma *et al*., 2004; Duarte *et al*., 2008).

Further analysis of babesiosis positive samples with species-specific PCR, *Babesia gibsoni* and *B. vogeli* was conducted. Of 125 samples subjected to species-specific PCR, 87 (69.6%) were found to be positive for *B. gibsoni* infection using primers Gib 599 and Gib 127. Thirty eight dogs (30.04%) were positive for *B. vogeli*-specific PCR using primers BAB1 and BAB4. The parasite specific PCR amplified 546 bp partial and 671 bp partial 18S rRNA of *B. vogeli* and *B. gibsoni* (Plate 2), respectively. Overall, the prevalence of *B. gibsoni* and *B. vogeli* in Andhra Pradesh was 19.9% (87/442) and 8.6% (38/442), respectively indicating higher percentage of *B. gibsoni* infection in dogs under study. Even though the virulence of this *Babesia* spp. varies among species, *B. gibsoni* is known to cause highly severe disease (Irwin and Hutchinson, 1991) and may represent a serious threat for dogs living in Andhra Pradesh. *Babesia vogeli* is considered less virulent than its two sibling

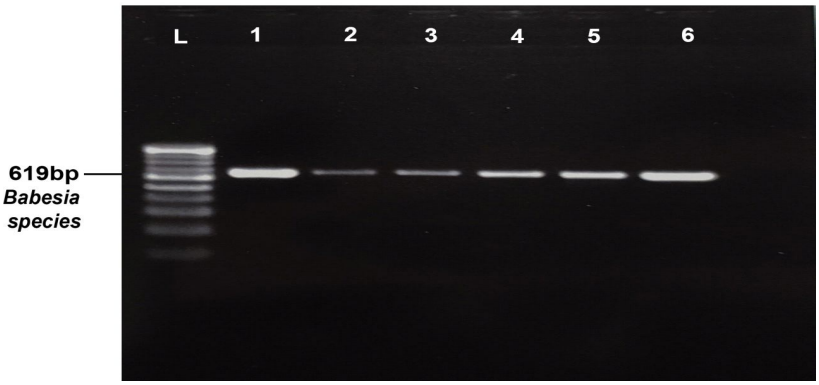

Plate 1: PCR products of *Babesia* species.
L: 100bp DNA ladder; 1 to 6: Amplicons of 16S rRNA gene of *Babesia* spp.

species (*B. canis* and *B. rossi*) and tends to produce subclinical disease, except in young or immunosuppressed dogs (Penzhorn, 2011). Muhammad *et al*. (2018) first reported optimization of loop-mediated isothermal amplification (LAMP) for the diagnosis of *B. felis* in cats.

Babesiosis in dogs is endemic in tropical countries like India because of round the year presence of tick vectors and reservoir stray dog population (Abd Rani *et al*., 2011). Some of the contemporary studies in India using microscopy and molecular techniques have shown that incidence of babesiosis due to *B. gibsoni* infection among dogs is increasing (Vairamuthu *et al*., 2014; Singh *et al*., 2014; Mahalingaiah *et al*., 2017; Jain *et al*., 2018). Absence of accurate diagnosis, lack of knowledge among field veterinarians regarding the pathogen, poor prognosis and absence of effective treatment has made the disease more problematic (Mittal *et al*., 2019). Contrary the prognosis remains good for *B. vogeli* infections, the large form of canine *Babesia* infecting dogs in India (Solano-Gallego *et al*., 2016). However, Laha *et al*. (2014) and Manoj *et al*. (2020) reported higher prevalence of *B. canis/B. vogeli* infection (54.05% and 10.00%, respectively) in dogs in Assam and Tamil Nadu, respectively, compared to that of *B. gibsoni* infection (48.64% and 0.4%, respectively) using conventional PCR. Gautam Patra *et al*. (2020) observed no significant difference in rate of prevalence of *B. gibsoni* (1.71%) and *B. canis* (1.52%) in different regions of Northeastern India. *Babesia canis* reported from different parts of India is presumably *B. vogeli.B.gibsoni* is the common cause of babesiosis in India. so, its diagnosis by conventional methods results in false negative results particularly in low parasitemic cases. (Kushwaha *et al*., 2018). Presence of large pleomorphic

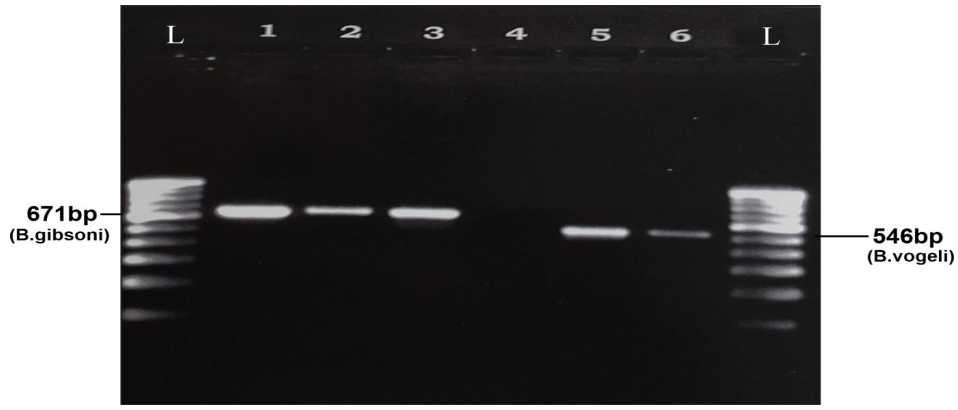

Plate 2: Specice specific PCR products of *Babesia* spp.
L: 100bp DNA ladder; 1 to 3: Amplicons of 18S rRNA gene of *B. gibsoni* (1 to 3) and *B. vogeli* (5 to 6).

Table 1: Prevalence of *Babesia gibsoni* and *B. vogeli* according to host factors and other variables.

Host factors/other variables	*Babesia gibsoni*	*Babesia vogeli*	Total
Age			
≤ One year (n=172)	46(26.74)	25(14.53)**	71(41.27)
≥ One year (n=270)	41(15.18)	13(4.81)	54(20.0)
Gender			
Male (n=234)	62(26.49)*	26(11.11)*	88(37.60)
Female (n=208)	25(12.02)	12(5.77)	37(17.79)
Breed			
Pure breed (n=352)	65(18.46)	27(7.67)	92(26.13)
Mongrels (n=90)	24(26.66)*	9(10)*	33(36.67)
Medium			
Urban (n=276)	57(20.65)	31(11.23)	88(31.88)
Rural (n=166)	30(18.07)	7(4.21)	37(22.3)
Living conditions			
Kennel dogs (n=36)	15 (41.66)**	10(27.77)**	25(69.44)
Pet dogs (n=335)	67 (20.00)	23(6.86)	(0(26.86)
Stray dogs (n=71)	5(7.04)	5(7.04)	10(14.08)
Season			
Winter (n=86)	19(22.09)	9(10.46)	28(32.56)
Summer (n=188)	51(27.13)**	11(5.85)	62(32.98)
Rainy (n=168)	17(10.11)	18(10.71)*	35(20.83)

single, paired and multiple pear shaped forms of *B.canis* in the erythrocytes of peripheral heart and blood smears collected from a ten day old female Great Dane pup carcass, stained with LandG stain revealed by Balachandran *et al*. (2010).

In the present study no dogs were found to be co-infected with either *B. gibsoni* and or *B. vogeli*. But concurrent infections of *B. canis* and *B. gibsoni* was noticed in dogs in Chennai (Vairamuthu *et al*., 2014), Guwahati (Laha *et al*., 2014) and Bengaluru (Mahalingaiah *et al*., 2017). The present study supports and confirms that canine babesisois is endemic in different States of India including Andhra Pradesh, with a significantly higher proportion of animals harboring *B. gibsoni*, the small form of *Babesia* piroplasm. The sequence analysis of Andhra Pradesh *B. gibsoni* and *B. vogeli* isolates formed a single major group with other Indian isolates and with other geographical isolates outside India. Studies conducted in different parts of India also indicated that Indian *B. gibsoni* and *B. vogeli* strains clustered into a single major group with other Indian isolates and with Asian countries isolates (Mittal *et al*., 2019; Betgiri *et al*., 2019; Lavanya *et al*. 2019a; Lavanya *et al*., 2019b).

Effect of host factors and other variables on the prevalence of *B. gibsoni* and *B. vogeli* (Table 1) indicated that the prevalence of *B. gibsoni* (P>0.05) was more in young dogs (≤ one year) than in adults (≥ one year). Infection with *B. gibsoni* was more common (P<0.05) in male dogs than in female dogs. High prevalence of babesiosis in male is attributable to increased levels of testosterone associated with immunosuppression and increased susceptibility to parasites (Zuk and McKean, 1996). Kalaivanan *et al*. (2018) also noted higher prevalence of *B. gibsoni* in male dogs in Namakkal, Tamil Nadu. The prevalence of *B. gibsoni* and *B. vogeli* was significantly (P<0.05) high in mongrels than in pure breed. Similarly, pure breed dogs were less prone for infection in Lahore, Pakistan (Bashir *et al*., 2009) agreeing the present findings. The prevalence of *B. gibsoni* and *B. vogeli* was non-significantly (P>0.05) high in urban dogs compared to that of rural dogs. The prevalence of *B. gibsoni* and *B. vogeli* was high in kennel dogs compared to that of pet and stray dogs. Due to close contact of dogs in kennels probably there may be the higher chance of transmission of babesiosis through ticks and bite wounds. The prevalence of *B. gibsoni* and *B. vogeli* was significantly high during summer (P<0.01) and rainy (P<0.05) seasons respectively, which might be due to high abundance of ticks in these seasons of the year (Soulsby, 1982).

Risk factor analysis by binary logistic regression model revealed that the living condition of the dogs (kennel dogs),

Table 2: Risk factor analysis of *Babesia gibsoni* by logistic regression model.

Risk factor	Chi square value	Odds ratio	95% CI		Wald *P* value
			Lower	Upper	
Age					
≥ One year	3.531	1			
≤ One year		1.745	0.971	3.136	0.060
Gender					
Male	5.694	1			
Females		2.057	1.127	3.755	0.017
Breed					
Pure breed	2.912	1			
Mongrels		1.756	0.914	3.372	0.088
Medium					
Urban	5.694	1			
Rural		2.057	1.127	3.755	0.017
Living condition					
Stray dogs		1			
Kennel dogs	13.035	8.400	2.411	29.268	<0.001
Pet dogs	9.189	0.322	0.150	0.691	0.002
Season					
Winter		1			
Summer	0.465	1.295	0.616	2.723	0.495
Rainy	3.507	0.432	0.176	1.061	0.061
Ticks					
Present	110.625	1			
Absent		0.035	0.015	0.080	<0.001
Tick control measures					
Followed	5.013	1			
Not followed		0.490	0.260	0.924	0.025

Logistic regression model *P<0.05, **P<0.01, ***P<0.001.

Table 3: Risk factors analysis of *B. vogeli* by logistic regression model.

Risk factor	Chi square value	Odds ratio	95% CI		Wald *P* value
			Lower	Upper	
Age					
≥ One year	32.858	1			
≤ One year		39.453	5.266	295.616	<0.001
Gender					
Male	1.858	1			
Females		1.804	0.764	4.260	0.173
Breed					
Pure breed	0.490	1			
Mongrels		1.408	0.538	3.680	0.484
Medium					
Urban	1.858	1			
Rural		1.804	0.764	4.260	0.173
Living condition					
Stray dogs		1			
Kennel dogs	6.974	8.500	1.391	51.945	0.008
Pet dogs	0.067	1.218	0.273	5.426	0.796
Season					
Winter		1			
Summer	2.019	0.440	0.138	1.405	0.155
Rainy	0.017	0.934	0.333	2.618	0.897
Ticks					
Present	12.833	1			
Absent		0.187	0.068	0.516	<0.001
Tick control measures					
Followed	1.324	1			
Not followed		1.299	0.431	3.916	0.254

Logistic regression model *P<0.05, **P<0.01, ***P<0.001.

gender (male), medium (urban) and tick infestation as risk factors for *B. gibsoni* infection (Table 2). The variables such as living condition of the dogs (kennel dogs), age (young age) and tick infestation were identified as risk factors for *B. vogeli* infection (Table 3). Kennel dogs were 5.2 (OR=5.17; P<0.01) and 5.4 (OR=5.429; P<0.01) times more prone for *B.gibsoni* and *B.vogeli* infection respectively, than stay dogs. Young dogs (≤ one year) were 39.5 (OR=39.453; P<0.001) times more likely to be infected with *B.vogeli* compared to that of adult dogs (≥ one year). In young animals immune system is immature and are less probably to have prior exposure to many infections, they are expected to be more susceptible.

Female dogs were 2.1 (OR=2.057; P<0.05) times less prone for *B. gibsoni* infection than male animals. The increased immune reactivity in females due to estrogens helps produce an effective resistance to infection and therefore females are less susceptible to infections (Taneja, 2018). The prevalence of *B. gibsoni* was 2.1 (OR=2.057; P<0.05) times less in dogs living in rural areas than in dogs living in urban area that could be due to the low population size of dogs in rural area where infections cannot be continued for prolonged period (Acosta-Jamett *et al*. 2010).

Dogs without tick infestation were 28.6 (OR=0.035; P<0.001) and 5.3 (OR=0.187 P<0.001) times less likely to be infected with *B. gibsoni* and *B. vogeli* respectively, than dogs with tick infestation. Dogs infected with *B. canis* were found to be infested with ticks in Nigeria (Konto *et al*. 2014) indicating tick infestation as risk factor for babesiosis. The prevalence of *B. gibsoni* was two (OR=0.490; P<0.05) times more in dogs that were not treated for tick infestation previously than in dogs treated for tick infestation. Other variables such as gender, breed and season were not identified as risk factors for *B. vogeli* infection. In accordance, no variables were identified to be significant risk factors for testing positive *via* PCR for *Babesia* spp. in Nigeria, but dogs with ticks found on physical examination were 3.6 times more likely to test positive for hemoplasmas (Happi *et al*. 2018).

CONCLUSION

The present study reveals the higher prevalence of *B. gibsoni*, the virulent form of *Babesia* in dogs of Andhra Pradesh. The risk factors discussed above significantly associated with the prevalence of babesiosis. The PCR based assays will be of great help in species-specific discrimination of pathogens.

REFERENCES

Abd Rani, P.A.M., Irwin, P.J., Coleman, G.T., Gatne, M. and Traub, R.J. (2011). A survey of canine tick-borne diseases in India. Parasites and Vectors. 4 (141): 1-7.

Acosta-Jamett, G., Cleaveland, S., Cunningham, A. and Bronsvoort, M., (2010). Demography of domestic dogs in rural and urban areas in coquimbo region of Chile and its implication for diseases transmission. Preventive Veterinary Medicine. 94: 272-281.

Balachandran, C., Sridhar, R., Pazhanivel, N. and Anoopraj, R. (2010). Anote on the incidence of Babesia canis in a 10 day old pup on postmortem examination in Chennai, Tamil Nadu. Indian Journal Animal Research. 44(1): 73-75.

Bashir, I.N., Chaudhry, Z.I., Ahmed, S. and Saeed, M.A. (2009). Epidemiological and vector identification studies on canine babesiosis. Pakistan Veterinary Journal. 29(2): 51-54.

Betgiri, A.A., Jadhav, S.N., Pawde, M., Shukla, A., Mote, C., Pawar, P.D., Shanmugam, D. and Kundu, K. (2019). Mitochondrial cytochrome oxidase c subunit III (cox3) gene as a sensitive and specific target for molecular detection of *Babesia gibsoni* infection in dogs. Experimental Parasitology. 206: ISSN 0014-4894.

Duarte, S.C., Linhares, G.F.C., Romanowsky, T.N., Neto, O. and Borges, L.M.F. (2008). Assessment of primers designed for the subspecies-specific discrimination among *Babesia canis canis*, *Babesia canis vogeli* and *Babesia canis rossi* by PCR assay. Veterinary Parasitology. 152(1-2): 16-20.

Fukumoto, S., Hiroshi, S., Igarashi, I. and Xuan, X. (2005). Fatal experimental transplacental *Babesia gibsoni* infections in dogs. International Journal for Parasitology. 35(9): 1031-1035.

Gautam Patra, Ana, S., Ghosh, S., Behara, P., Borthakur, S.K., Biswas, P., Apurba. D. and Seikh, A. (2020). Prevalence of tick-borne pathogens in domestic dogs in North-Eastern region of India. Biological Rhythm Research. 51(2): 1-10.

Happi, A.N., Toepp, A.J., Ugwu, C.A., Petersen, C.A. and Sykes, J.E. (2018). Detection and identification of blood-borne infections in dogs in Nigeria using light microscopy and the polymerase chain reaction. Veterinary Parasitology: Regional Studies and Reports. 11: 55-60.

Inokuma, H.Y., Yoshizaki, Y., Matsumoto, M., Okuda, T., Onishi, K., Nakagome, R., Kosugi. and Hirakrwa, M. (2004). Molecular survey of *Babesia* infection in dogs in Okinawa, Japan. Veterinary Parasitology. 121: 341-346.

Irwin, P.J. and Hutchinson, G.W. (1991). Clinical and pathological findings of *Babesia* infection in dogs. Australian Veterinary Journal. 68: 204-209.

Jain Jose, K., Lakshmanan, B., Hitaishi, V., Nagaraj, Praveena. J.E., Syamala, K. and Aravindakshan, T. (2018). Detection of *Babesia canis vogeli*, *Babesia gibsoni* and *Ehrlichia canis* by multiplex PCR in naturally infected dogs in South India. Veterinarski Arhiv. 88(2): 215-224.

Jefferies, R., Ryan, U.M., Jardine, J., Broughton, D.K., Robertson, I.D. and Irwin, P.J. (2007). Blood, bull Terriers and Babesiosis: Further evidence for direct transmission of *Babesia gibsoni* in dogs. Australian Veterinary Journal. 85(11): 459-463.

Kalaivanan, M., Saravanan, S. and Ponnudurai, G. (2018). Identification of *Babesia gibsoni* in dogs from Namakkal region by polymerase chain reaction. Journal of Entomology and Zoology Studies. 6 (5):1146-1148.

Kledmanee, K., Suwanpakdee, S., Krajangwong, S., Chatsiriwech, J., Suksai, P., Suwannachat, P., Sariya, L., Buddhirongawatr, R., Charoonrut, P and Chaichoun, K. (2009). Development of multiplex polymerase chain reaction for the detection of *Ehrlichia canis*, *Babesia* spp. and *Hepatozoon canis* in canine blood. South Asian Journal of Tropical Medicine and Public Health. 40(1): 35-39.

Konto, M., Biu, A.A., Ahmed, M.I., Mbaya, A.W and Luka, J. (2014). Clinico-biochemical responses of dogs to experimental infection with *Babesia canis*. Veterinary World. 7(3): 113-118.

Kushwaha, N., Mondal, D., Singh, K.P. and Mahapatra, R.R. (2018). Comparative evaluation of different diagnostic tests for *Babesia gibsoni* in dogs. DOI:10.18805/ijar.3413.

Laha, R., Bhattacharjee, K., Sarmah, P.C., Das, M., Goswami, A., Sarma, D. and Sen, A. (2014). *Babesia* infection in naturally exposed pet dogs from north-eastern state (Assam) of India: Detection by microscopy and polymerase chain reaction. Journal of Parasitic Diseases. 38: 389-393.

Lavanya, K.V., Puttalakshmamma, G.C., Yogisharadhya, R., Mohan, H.V., Lakkundi, J.N., Manjunatha Reddy, G.B. (2019a). Development of cytochrome b based PCR and epidemiology of *B. gibsoni* in dogs. Journal of Experimental Biology and Agricultural Sciences. 7(4): 411-417.

Lavanya, K.V., Puttalakshmamma, G.C., Dhanalakshmi, H., Ananda, K.J., Mohan, H.V., Yathish, H.M., Manjunatha reddy, G. (2019b). Prevalence and molecular phylogenetic analysis of *Babesia vogeli* from dogs in Karnataka. Indian Journal of Veterinary Pathology. 43(2): 104.

Maele, I.V.D., Bataille, I., Karine, K.S., Gielen, I., Daminet, S. (2008). An unusual form of canine babesiosis. The Canadian Veterinary Journal. 49: 283-6.

Mahalingaiah, M.K.C., Asoor, M., Thimmaiah, R.P., Narayanaswamy, H.D. and Mukartal, S.Y., Elattuvalappil, A.M., Chikkahonnaiah, N., Gupta, S., Singh, S. (2017). Prevalence of canine babesiosis in different breeds of dogs in and around Bangaluru. Advances in Animal and Veterinary Sciences. 5(3): 140-144.

Manoj, R.R.S, latta, R. Latrofa, M.S., Capozzi, L., Raman, M., Colella, V. and Otranto, D. (2020). Canine vector-borne pathogens from dogs and ticks from Tamil Nadu, India. Acta Tropica. 203: 105308.

Mittal, M., Kundu, K., Chakravarti, S., Mohapatra, J.K., Singh, V.K., Raja Kumar, B., Thakur, V., Churamani, C.P. and Kumar, A. (2019). Canine babesiosis among working dogs of organised kennels in India: A comprehensive haematological, biochemical, clinicopathological and molecular epidemiological multiregional study. Preventive Veterinary Medicine. 1: 169: 104696. doi: 10.1016/j.prevetmed.2019.104696.

Muhammad, A.S., Raheela, A,.,Muhammad, L., Muhammad, I.R, Haroon, A., Wasim, S.,Fareeha, A., Saher, I., Shahid, H.F. and Uzma, F.D. (2018). First report on optimization of loop-mediated isothermal amplification (LAMP) for the diagnosis of *Babesia felis*. Indian Journal of Animal Research. 52: 401-404.

Nimisha, M., Devassy, J.K. and Ravindran, R. (2019). Ticks and accompanying pathogens of domestic and wild animals of Kerala, South India. Experimental and Applied Acarology. 79: 137-155.

Senthil Kumar, K., Vairamuthu, S. and Kathiresan, D. (2009). Prevalence of haemoprotozoans in canines in Chenni city. Tamilnadu Journal of Veterinary and Animal Sciences. 5(3): 104-108.

Shaw, E.S., Michael, J.D., Birtles, R.J., Edward, B.B. (2001). Tick-borne diseases of dogs. Trends in Parasitology. 17(2): 74-80.

Singh, A., Singh, H., Singh, N.K., Singh, N.D. and Rath, S.S. (2014). Canine babesiosis in northwestern India: Molecular detection and assessment of risk factors. Bio-Med Research International. 2014: 1-5.

Solano-Gallego, L., Sainz, A., Roura, X., Estrada-Peria, A. and Miro, G. (2016). A review of canine babesiosis: The European perspective. Parasites and Vectors. 9: 336-354.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Demesticated Animals. 7th ed. ELBS, Bailliers Tindall and Cassel, London.

Stegeman, J.R., Birkenheuer, A.J., Kruger, J.and Breitschwerdt, E. (2003). Transfusion-associated *Babesia gibsoni* infection in a dog. Journal of the American Veterinary Medical Association. 222(7): 959-963.

Taneja, V. (2018). Sex hormones determine immune response. Frontiers in Immunology. 9: 1931.

Penzhorn, B.L. (2011). Why is southern african canine babesiosis so virulent? An evolutionary perspective. Parasites and Vectors. 4: 51.

Vairamuthu, S., Ranju, R.S., Latha, B.R., Dhivya, B. and Balachandran, C. (2014). A six year (2006-2011) retrospective study of haemoprotozoan parasites affecting dogs in Chennai, Tamil Nadu, India. Journal of Parasitic Diseases. 38(2): 193-195.

Zuk, M. and McKean, K.A. (1996). Sex differences in parasitic infections: Patterns and processes. International Journal of Parasitology. 26: 1009-1024.