RESEARCH ARTICLE

Molecular Detection of Canine Babesiosis in and Around Bhubaneswar, India

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Abstract

Canine babesiosis, a hemolytic protozoan disease, represents an important veterinary problem caused primarily by large and small forms of piroplasms of *Babesia* spp. A molecular-based survey on the overall occurrence of natural *Babesia* infection in stray (n=98) and pet dogs (n=100) from Bhubaneswar and nearby areas using PCR technique targeting 18s RNA gene fragment along with genetic sequence analysis was carried out. A total of 38 (pet:22, stray:16) samples (19.19%) were found positive for babesiosis based on the amplification of 450 bp amplicon region of the gene, while 4 samples (0.02%) showed co-infection with *Hepatozoon canis*. The sequenced PCR products were submitted to NCBI, and on BLAST analysis, the isolates with accession no KT246303, KT246306, KT246307 showing similarity with *Babesia vogeli*, while KT246305 was identical to *B.gibsoni* isolates and KT246304 was identical to *Hepatozoon canis*. This is the first report on the molecular diagnosis of canine babesiosis in the state.

Keywords: Babesia vogeli, Babesia gibsoni, India, Molecular detection.

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Introduction

Canine babesiosis is an important tick-borne life-threatening haemo-protozoan disease caused by the intra-erythrocytic protozoan parasites belonging to the genus *Babesia*. The *Babesia* species mainly incriminated for causing disease in canids are the small form *(Babesia gibsoni, Babesia conrade*, *Babesia vulpes*) and large form (*Babesia canis, Babesia vogeli. Babesia rossi*) in different parts of the world (Solano-Gallego *et al*., 2016). The transmission occurs by *Dermacentor reticulatus* in Europe, *Rhipicephalus sanguineus* in tropical and subtropical regions, and *Haemaphysalis eliptica* in South Africa (Uilenberg, 2006). In Indian subcontinent, canids are infested mainly with *Rhipicephalus sanguineus* and *Haemaphysalis* spp. of ticks (Raut *et al*.,2006: Sahu *et al*., 2013: Ranju Ravindran *et al*.,2020).

Detection of *Babesia* spp. is usually achieved using microscopic examination of stained blood smears, but this technique is limited because of low sensitivity, chronic evolution of the disease, and the difficulty of distinguishing morphologically similar strains and species (Irwin, 2005). The serological test such as the immunofluorescent antibody test is useful but has poor specificity as a result of antigen cross-reactivity (Rani *et al*., 2011) and fails to identify current infection. The employment of recent biotechnological techniques like PCR, nested and semi-nested PCR, PCR-RFLP, and multiplex PCR has led to advancement in detecting this protozoan parasite. The true status of canine babesiosis is still not clear in India barring a few reports (Rani *et al*., 2011; Sarma *et al*.,2019) and in the region under study (Sahu *et al*.,2014), which was mostly based on microscopic examination of blood smears. The present study is a molecular-based detection of canine babesiosis in Bhubaneswar, Odisha.

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Materials and Methods

Blood samples from 198 dogs presented to Teaching Veterinary Clinical Complex, and Animal Birth Control Programme, Bhubaneswar (stray:98, pet:100) belonging to either sex and different age groups, showing tick infestation and clinical signs like pyrexia, anorexia or lethargy were selected for this study. Blood smear examination using Giemsa stain was conducted. The blood samples found positive or negative for *Babesia* piroplasms were preserved at -20°C for further study using molecular techniques. DNA was isolated from 200 µl of blood (with anticoagulant, EDTA) sampled from each dog using QIAamp DNA Mini kit (Qiagen, Germany) following the manufacturer's instructions. A genus-specific forward primer (PIRO A1 5′-AATACCCAATCCTGACACAGGG-3') and antisense PIRO-B (5′-TTAAATACGAATGCCCCCACC-3') were

used for PCR of extracted parasitic DNA (Laha *et al*., 2013). The amplification was performed in a thermal cycler (Gene Amp PCR System 9700, Applied Biosystem), and the amplified PCR products were analyzed on 1.5% agarose gel. According to the manufacturer's instructions, PCR products of the expected nucleotide size were purified with QIAquick PCR Purification Kit (Qiagen, Germany). The purified PCR products were sequenced by the automated DNA sequencer (310 Genetic Analyser, Applied Biosystems) using ABI Prism Dye Terminator kit and both forward and reverse primers, and the sequences obtained were subjected to BLAST analysis. Phylogenetic analysis was done by MEGA X using the Neighbour-joining tree method based on the partial gene sequences of 18S rRNA obtained in the study and reference sequences obtained from the NCBI GenBank database. The number at the node was the proportion of 500 bootstraps.

Results and Discussion

The most consistent clinical signs observed during the examination were elevated rectal temperature above 39.6°C, anorexia, pale mucous membrane, tick infestation, coughing, and vomition in 85.71, 68.57, 65.71, 57.14, 37.14, and 8.57% of dogs understudy respectively. A microscopical examination of stained blood smears piroplasms of Babesia spp. was detected in 8.08% (16/198) samples. The overall detection of babesiosis by conventional PCR technique was 19.19% (38/198) based on the presence of 450 bp amplicon (Fig. 1). Four samples showed double band after gel purification with 450 and 520 bp amplicon sizes indicating mixed infection with *Hepatozoon canis* (Fig. 2). The infection in pet dogs (22%) was higher than in stray dogs(16.23%).

The nucleotide sequence obtained after the sequencing of PCR product was submitted to GenBank and accession numbers KT246303, KT246304, KT246305, KT246306, KT246307 were assigned. BLAST analysis of GenBank revealed that the obtained nucleotide sequences of KT246303, KT246306, and KT246307 were 100% homologous to previously deposited 18S rRNA gene sequences of *B. vogeli*. The sequence of KT246305 showed the best matches (100%) with *Babesia gibsoni* sequences from different regions available in NCBI database. The sequence KT246304 showed 99% similarity with the previously deposited 18S rRNA gene sequences of *H.canis*.

On phylogenetic analysis (Fig 3), the sequences clustered into three distinct clades. *B. vogeli* isolates of the study (KT246303 and KT246307) were clustered together to form a well-defined group with other *B. vogeli* strains from different geographical regions (Brazil, China,Japan ,Thailand, Zambia). However, the isolate having accession number KT246305 showed close phylogenetic relationship with the *B. gibsoni*

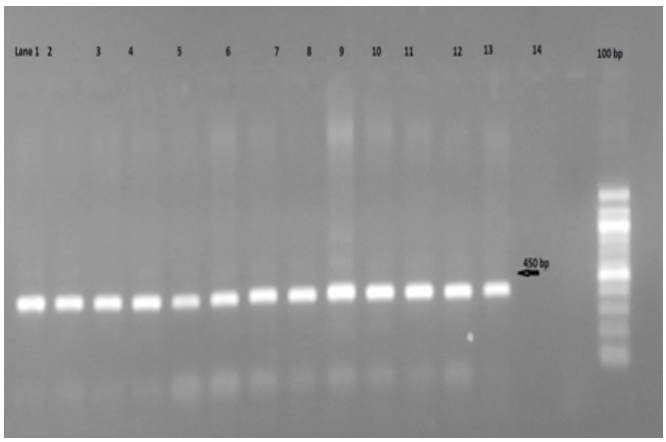

Fig. 1: Electrophoresis gel showing lanes from left to right, L1 to L13, PCR product showing positive for *Babesia spp.* (450 bp); 100 bp DNA ladder

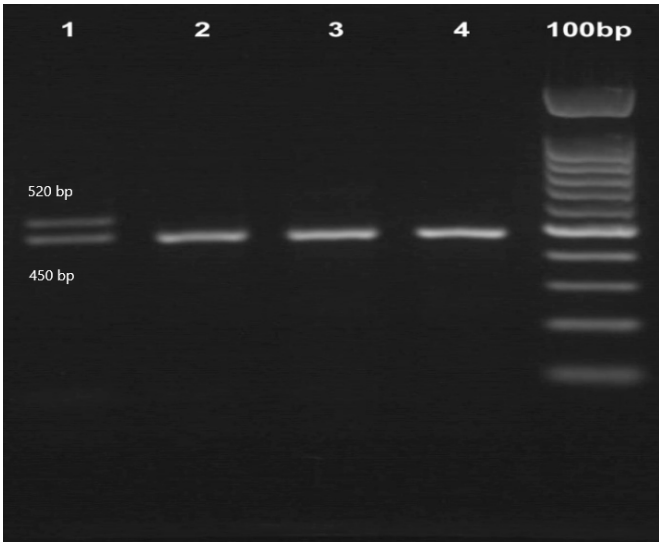

Fig. 2: Electrophoresis gel showing lanes from left to right, L1, a double band (450 bp and 520 bp) L2 to L4, PCR product showing positive for *B abesia spp.* (450 bp); L5,100bp DNA ladder

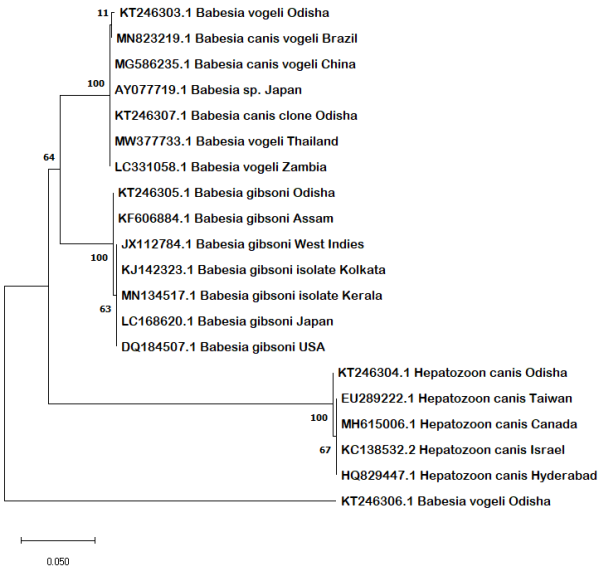

Fig. 3: Phylogenetic Analysis using MEGA X by Neighbour-joining tree method based on the partial gene sequences of 18S rRNA

isolate from India (eastern and southern India) and other regions (Japen, USA, West Indies) with a high bootstrap value. The *B. vogeli* isolate (KT246306) of the present investigation represented a separate clade. *Hepatozoon canis* isolate (KT246303) showed a close phylogenic relationship with isolates from different parts of the world (China, Taiwan, Israel)

The overall prevalence rate of babesiosis on the basis of PCR assay ranged from 4.8 - 56.75% as per reports from different parts of India (Laha *et al*.,2013; Singh *et al*.,2014; Sarma *et al*.,2019) and 2.4 - 88.3% from around the globe (Bastos *et al*., 2004; Foldvari *et al*. 2005; and Corali *et al*.,2018) . These differences might have occurred because of the differences in diagnostic techniques, the population sampled in the study, and climatic as well as management factors in locations where the research was conducted. A moderate rate of incidence observed during the present research might be due to the state's hot and humid environmental condition, which favors the survival of tick vectors and a higher availability of stray dogs that act as transporting medium of the parasites due to their uncontrolled movements.

A higher presence of *Babesia vogeli* recorded in our study could be due to the dominating prevalence of *Riphicephalus sanguineus*, among canids of Bhubaneswar (Sahu *et al*.,2013). *Riphicephalus sanguineus* is a natural vector for *B.vogeli* and *H.canis* (Dantas-Torres, 2008). The present investigation also detected the presence of *Hepatozoon canis* along with *Babesia* sp, which corroborates with earlier reports from Northeast India (Sarma *et al*.,2019). This might be due to Piro A1 and Piro B primers, which are specific to *Babesia* spp. but could also detect *Hepatozoon* species. The use of primers Babesia F and Babesia R by Oyamada *et al*., (2005) has also revealed cross-reaction between *Babesia and Hepatozoon* species.

Based on sequencing results and BLAST analysis, it was also observed that one sequence (KT246305) showed maximum homogeneity with sequences of *B.gibsoni* with a stray match with *B.canis*. In India, *B.canis* is yet to be reported in any molecular diagnosis possibly due to absence of potential vector. *B.gibsoni* infection in dogs has been reported earlier in blood smear examination from Bhubaneswar (Sahu *et al*.,2014) while molecular detection have been reported in different regions of India (Singh *et al*.,2014; and Sarma *et al*.,2019). The molecular identification of *B. gibsoni, B. vogeli* and *H.canis* from canines of Punjab has been previously described (Singla *et al*., 2016). On phylogenetic analysis the present three isolates showed an affiliation with other *B. vogeli* isolates from different geographical regions. But one of the isolates showed no affiliation with other *B. vogeli* isolates though it was confirmed to be *B. vogeli* from Blast analysis, which might have originated from a different strain. *B.gibsoni* infected dogs exhibit varying clinical manifestations ranging from subclinical to fatal depending on the host body condition. Though B.vogeli is less pathogenic exhibiting moderate symptoms in adult dogs, they cause severe conditions in puppies splenectomized dogs (Wang *et al*., 2019). The study clearly re-established reliability of PCR as a technique over microscopy. Therefore, molecular diagnosis can facilitate pertinent treatment and control regimen.

Conclusion

Traditionally, the presumptive diagnosis is based on fever, anemia and thrombocytopenia, while the microscopic examination still remains the most rapid confirmatory method for diagnosis of canine babesiosis under field conditions. PCR assay has been found to be a more specific method, could detect even in carrier state where there were no clinical signs and symptoms while co-infection with other vector-borne agents were also recorded. Further studies utilizing more sensitive tests and species-specific primers and a larger number of clinical samples need to be analyzed to get insight into the epidemiological patterns of canine babesiosis and devising an effective control program.

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References

Bastos, C.V., Moreira, S. M., and Passos, L.M.F. (2004). Retrospective study (1998-2001) on canine babesiosis in Belo Horizonte, Minas Gerais, Brazil. Annals New York Academy of Sciences, New York . 158-160.

Corali, C.A., Gabrielli, S., Zahirovic, A., Nikola, M., Milardi, G.L., Jazic, A., Zuko, A., Camo, D., and Otasevic, S. (2018). First molecular detection of *Babesia canis* in dogs from Bosnia and Herzegovina . *Ticks and Tick-borne Diseases*, *9*,363–368.

Dantas-Torres, F. (2008). Causative agents of canine babesiosis in Brazil. *Preventive Veterinary Medicine*, *83*, 210-211.

Foldvari, G., Hell, E. and Farkas, R. (2005). Babesia canis canis in dogs from Hungary, detection by PCR and sequencing. *Vet Parasitology*, *127*(3-4), 221-6.

Irwin, P.J. (2005). Babesiosis and Cytauxzoonsis; Arthropode-BorneInfectious Diseases of Dogs and Cats, Manson Publishing Ltd.Barcelona, Spain, 1st edition.

Laha, R., Bhattacharjee, K., Sarmah, P.C., Das, M., Goswami, A., Sarma, D., and Sen, A.(2013). Babesia infection in naturally exposed pet dogs from a north-eastern state (Assam) of India, detection by microscopy and polymerase chain reaction. *Journal of Parasitic Diseases*, *38*, 45-49.

Oyamada, M., Bernard, D. B., Boni, M., Dereure, J., Bucheton, B., Hammad, A., Itamoto, K., Okuda, M., and Inokuma, H. (2005). Detection of *Babesia canis rossi*, *B. canis vogeli* and *Hepatozoon canis* in Dogs in a Village of Eastern Sudan by Using a Screening PCR and Sequencing Methodologies. *Clinical and Diagnostic Laboratory Immunology*, *12*, 1343-1346.

Porchet, M. J., Sager, H., Muggll, L., Oppliger, A., Muller, N., Frey, C., and Gottstein, B. A. (2007). Descriptive epidemiological study of canine babesiosis in the Lake Geneva region. *Schweiz Arch Tierheilkd*, *149*, 457-465.

Rani, P., Irwin, P.J., Coleman, G.T., Gatne, M., & Traub, R.J. (2011). A survey of canine tick-borne diseases in India. *Parasites and Vectors*, *4*,1-8.

Ranju Ravindran, M.S., Roberta, I., Maria Stefania, L., Loredana, C., Muthusamy, R., Vito, C., and Domenico, O. (2020). Canine vector-borne pathogens from dogs and ticks from Tamil Nadu, India. *Acta Tropica*, *203*, 105308.

Raut, P.A., Maske, D.K., Jayraw. A.K. , and Sonkusale, V.G. (2006). Ectoparasitism in dogs from the eastern zone of Maharashtra state. *Journal of Parasitic Diseases*, *30* (2), 138–141.

Sahu, A., Mohanty, B., Panda, M.R., Sardar, K.K., and Dehuri, M. (2013). Prevalence of tick infestation in dogs in and around Bhubaneswar. *Veterinary World*, *6*, 982-985.

Sahu, A., Mohanty, B., Panda, M., and Sardar, K.K. (2014). Incidence of haemoprotozoan parasites in dogs in and around Bhubaneswar. *Indian Vet.Journal*, *91*, 93-95

Sarma, K., Nachum-Biala, Y., and Kumar, M. (2019). Molecular investigation of vector-borne parasitic infections in dogs in Northeast India. *Parasites and Vectors*. *12*, 122.

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. *BioMed Research International*. *1*(2014), 1-5.

Singla, L.D., Sumbria, D., Mandhotra, A., Bal, M.S., and Kaur, P. (2016). Critical analysis of vector-borne infections in dogs, *Babesia vogeli*, *Babesia gibsoni*, *Ehrlichia canis* and *Hepatozoon canis* in Punjab India. *Acta Parasitologica*, *61*, 697-706.

Solano-Gallego, L., Sainz, A., Roura, X., Estrada Pena, A., and Miro, G. (2016). A review of canine babesiosis, the European perspective. *Parasite and Vectors*. *9*,336.

Uilenberg, G., (2006). Babesia - a historical overview. *Veterinary Parasitology*. 1383-10.

Wang, J., Liu, J., Yang, J., Liu, Z., Wang, X., Li , Y., Guan, G., and Yin, H. (2019). Molecular detection and genetic diversity of *Babesia canis canis* in pet dogs in Henan Province, China. *Parasitol International*, *71*, 37-40.