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Research Article

INCIDENCE AND CLINICO-PATHOLOGICAL STUDIES ON CANINE BABESIOSIS IN AND AROUND AKOLA, MAHARASHTRA, INDIA

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ABSTRACT: Canine babesiosis is a tick-borne hemoprotozoan disease of canines. Two main infective agents of the disease are intra-erythrocytic *i.e. Babesia vogeli* and *Babesia gibsoni*. In the present study, a total of 145 numbers of dogs (90 male and 55 female) in and around Akola, Maharashtra were screened from January 2022 to September 2022 for Babesiosis and 18 animals (12.41%) were found clinically and cytologically positive. The incidence of babesiosis was higher in females (11/145, 7.58%) than in males (07/145, 4.83%). Out of 18 positive samples, 14 (9.65%) and 4 (2.75%) animals were found infected with small and large forms of *Babesia* spp., respectively. Further small form was confirmed as *B. gibsoni* and the large form was confirmed as *B. vogeli* by employing PCR assay. All the cases with babesiosis revealed moderate to severe regenerative anemia with occasional basophilic stippling and Howell jolly bodies with hemoglobin ranging from 7 to 11 g/dL. Characteristic clinical features were observed among 18 positive clinical cases. The present investigation reports the incidence of *B. vogeli* and *B. gibsoni* in canines in and around Akola of Maharashtra state of India.

Keywords: *B. gibsoni*, Maharashtra, PCR, Intra-erythrocytic.

INTRODUCTION

Canine babesiosis is an important vector-borne disease with worldwide distribution. It is caused by intra-erythrocytic protozoa belonging to the genus *Babesia*, order *Piroplasmida*, phylum *Apicomplexa* [1] Morphometric evaluation revealed large forms (2.5-5.0 µm) and small forms (1.0-2.5 µm) of the organism during blood smear examination. The large form consists of three species, *viz., B. canis*, *B. rossi*, and *B. vogeli*, and the small forms (1.0-2.5 µm) encompass the *B. gibsoni*, *B. conradae*, and *B. microti* [2]. *B. vogeli* and *B. gibsoni* are widely distributed in India amongst domestic canine populations causing fatal infections [3]. Ticks like *Dermacentor reticulatus*, *Rhipicephalus sanguineus*, *Haemaphysalis leachi* act as a vector for the transmission of protozoa which is usually specific to each species of *Babesia*. The distribution of vector species decides the occurrence of *Babesia* spp. in dogs in a particular area [4]. Furthermore, babesiosis can be transmitted between canines through bite [5] and transplacental route [6]. Canine babesiosis shows a wide range of clinical manifestations in the affected animals from sub-clinical to serious illnesses characterized by marked anemia, jaundice, depression, high fever, lymphadenopathy, splenomegaly, intravascular and extravascular hemolysis, hypoxic injury, systemic inflammation, and thrombocytopenia. After initial acute infection, the animal may become a chronic carrier [1]. Owing to the wide distribution of the vector population throughout the country, cases of canine babesiosis are frequently encountered in different places. The information on the occurrence of canine babesiosis in dogs in and around Akola is lacking. Hence present study was undertaken to estimate the occurrence of canine babesiosis and its clinical scenario in dogs in and around Akola.

MATERIALS AND METHODS

Collection of samples

A total of 145 blood samples were collected from dogs (90 male and 55 female) during January 2022 to

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September 2022 with clinical signs of high fever of 104°F, inappetence, anemia, lethargy, history of tick infestation, pale, icteric mucous membranes, lymphadenopathy, hematuria and epistaxis from different breeds, gender and age group of 1-12 years, which were presented to Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS) Akola, Maharashtra. Blood samples were collected in vials containing EDTA as an anticoagulant and stored at 4°C in the Department of Veterinary Pathology, PGIVAS, Akola for further processing.

Blood smear examination

Approximately 3-4 microliter of well-mixed (EDTA) fresh blood sample was used to prepare blood smear on a clean glass slide. Air-dried blood smears were fixed by methanol and stained with Giemsa and Leishman's stain following standard procedure. Well-stained slides were examined under 100x (oil immersion) objective of a microscope for the presence of intra-erythrocytic babesia organisms. *B. vogeli* and *B. gibsoni* were differentiated based on their size (large or small) and appearance inside the erythrocytes as per previous descriptions [7].

Hematology

Hematological parameters including hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), and total erythrocyte count (TEC) were estimated manually [8]. Leishman's stained blood smears were used to estimate differential leukocyte count and morphological alterations in the erythrocytes, platelets, and leukocytes.

Molecular confirmation

DNA Extraction: DNA was extracted from whole blood samples using Favorprep Genomic DNA mini kit (Favorgen) as per the manufacturer's protocol. DNA samples were stored at -20 °C, until further use.

PCR Amplification: PCR was performed to amplify extracted DNA with the help of a specific primer, which targeted fragment of the mitochondrial cox 3 gene of *Babesia gibsoni* having sequence as BgX3F 5'- CAAAATGCCAATATGTACCTAAAC- 3' and BgX3R5'- TGTTAATCACAGTTGGTTTACAAC- 3' with amplicon size 164 bp [9]. For *Babesia vogeli* the primers were used with sequence BAB1F 5'- GTG AAC CTT ATC ACT TAA AGG- 3' and BAB4R 5'- CAA CTC CTC CAC GCA ATC G- 3' with amplicon size 600 bp [10]. The PCR reaction was carried out in 200 µL PCR tubes containing a reaction mixer of 12. 5 µL of Dream Taq Green Master mix (Takara Bio, India), 0.75 µL of forward primer and reverse primer each, 4 µL of extracted DNA and 7 µL of nuclease-free water. The reaction mixer tubes were then placed in a thermocycler and PCR cycling conditions were used for both *B. gibsoni* and *B. vogeli* with initial denaturation of 94°C for 2 min, followed by 35 cycles of [denaturation (94 °C, 30 s), annealing (47°C, 45 s for *B. gibsoni*; 56°C, 30 s for *B. vogeli*), extension on (72°C, 1 min)] and single cycle of final extension at 72°C, 1 min for *B. gibsoni*; 72°C for 5 min for *B. vogeli*. The 10 µL PCR products were electrophoresed with a 100 bp DNA ladder and 0.5 µg ethidium bromide-stained 1% agarose in 1X TAE buffer at 80 V for 1 h and viewed under UV transilluminator.

RESULTS AND DISCUSSION

In the present study, a total of 145 dogs (90 male and 55 female) in and around Akola were screened for the presence of small and large forms of the Babesia, only 18 animals (12.41%) were positive by blood microscopy. Similarly, Chaudhuri [11] and Selvaraj *et al.* [12] recorded 8.90% and 8.96% incidence of canine Babesiosis by blood microscopy, respectively. However, a higher incidence of the disease was also recorded in the literature [13, 14, 15]. The variations in the incidence of canine babesiosis could be attributed to the tick population and its distribution, season, immune status of the host, and other managemental practices.

Out of 18 positive samples in the present investigation, 14 (9.65%) and 4 (2.75%) animals were found infected with small (*B. gibsoni*) (Fig. 1) and large (*B. vogeli*) (Fig. 2) forms of *Babesia* spp., respectively. This is in line with the earlier findings indicating a higher incidence of *B. gibsoni* [14, 16]. Contrary to the present report earlier AbdRani *et al.* [17] and Kundu *et al.* [10] reported *B. vogeli* (large form) as the predominant *Babesia* spp. from Northern and Western India. Moreover, previous studies from India have confirmed the occurrence of *B. vogeli* (large form) and *B. gibsoni* (small form) as common babesia species by molecular methods, the former being the predominant species [10, 18].

The incidence of babesiosis was higher in females (11/145, 7.58%) than in males (07/145, 4.83%). Similarly, Das *et al.* [16] recorded a higher incidence of babesiosis in females vis-a-vis males. However, Mahalingaiah *et al.* [13] recorded a higher incidence in males compared to females. Age-wise incidence was 3.45% (5/145) in dogs with 1-2 years of age, 5.52% (8/145) in 2-4 years of age, and 3.45% (5/145) in 4 years and above age. Mahalingaiah *et al.* [13] recorded higher incidence in 1-2 years of dogs and Labrador (5.52%, 8/145) were found to be affected

Table 1. Hematological findings in cases of canine babesiosis.

Canine cases with Babesiosis	Hb (12-18 g/dL)	PCV (37-55 %)	TEC (5.5-8.5 $\times 10^6$ /cumm	TLC (6-14 $\times 10^3$ /cumm)	Differential Leucocyte Count (%)				
					N (60-73 %)	L (12-30 %)	M (3-10 %)	E (2-10 %)	B (0, Rare)
Case-1	7.4	22.2	3.1	20	82	12	4	2	0
Case-2	8	24	3.6	23	85	10	3	2	0
Case-3	8.4	25.2	3.6	29	79	18	2	1	0
Case-4	10	30	3.3	18	80	10	7	2	1
Case-5	7.8	23.4	3.2	10	68	25	5	2	0
Case-6	9.6	28.8	3.2	13	68	24	8	0	0
Case-7	9	27	3.8	8	59	28	7	5	1
Case-8	11	30	4.2	19	76	16	5	3	0
Case-9	10.2	28.8	3.4	18.2	75	20	1	4	0
Case-10	10.8	30	3.6	25	81	15	4	0	0
Case-11	8	24	4	24	82	10	6	2	0
Case-12	9	27	3.2	22	86	10	4	0	0
Case-13	8.4	25.2	3.6	20	82	10	4	4	0
Case-14	7.8	23.4	3.8	18	78	15	4	2	1
Case-15	7	21	3.4	18	78	20	2	0	0
Case-16	7.2	21.6	3.8	10	62	28	4	6	0
Case-17	8.2	24.6	4	19	75	20	5	0	0
Case-18	8	24	4	25	79	18	1	2	0

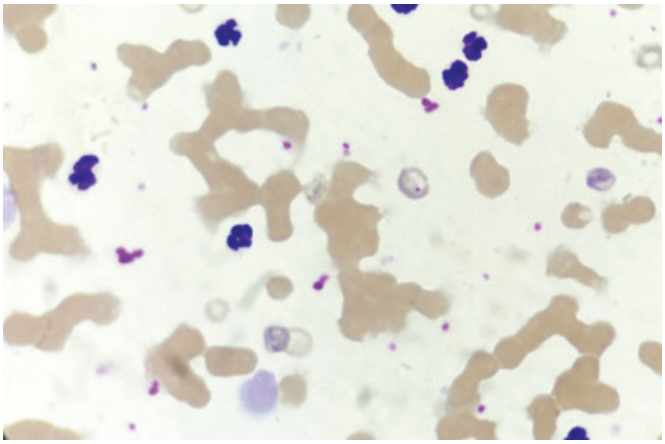

Fig. 1. Photomicrograph of the canine blood smear showing intraerythrocytic piroplasm of *B. gibsoni*, polycromatophilic RBCs and numerous nuetrophils (oil immersion 100X, Geimsa Stain).

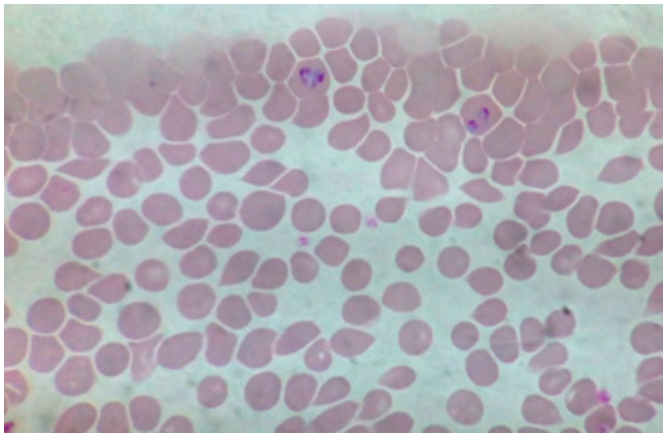

Fig. 2. Photomicrograph of the canine blood smear showing pear shaped intraerythrocytic piroplasms of *B. vogeli* (oil immersion 100X, Leishman's Stain).

more frequently followed by non-descript dogs (4.14, 6/145), German shepherd (2.07%, 3/145) and Pomerian breed (0.69%, 01/145). Variations in the incidence of babesiosis could be attributed to the population and distribution of transmitting vectors in the specific region and the immune status of the host.

All the cases with babesiosis revealed moderate to severe regenerative anemia with occasional basophilic stippling and Howell jolly bodies.TEC, hemoglobin, and PCV of positive cases ranged from 3.1 to 4.2 x 106/cumm, 7 to 11 g/dL, and 22 to 30 % respectively. Leishman's stained blood smear examination revealed neutrophilia in 77.77 % (14/18) cases along with mild to moderate shift to left and toxic changes in the neutrophils (Table I). These results were in line with the previous reports [12, 19, 20]. In the majority of babesiosis cases, platelets were granular and aggregated as clumps on the blood smear. Morphologically anemia was characterized as microcytic hypochromic with regenerative response as

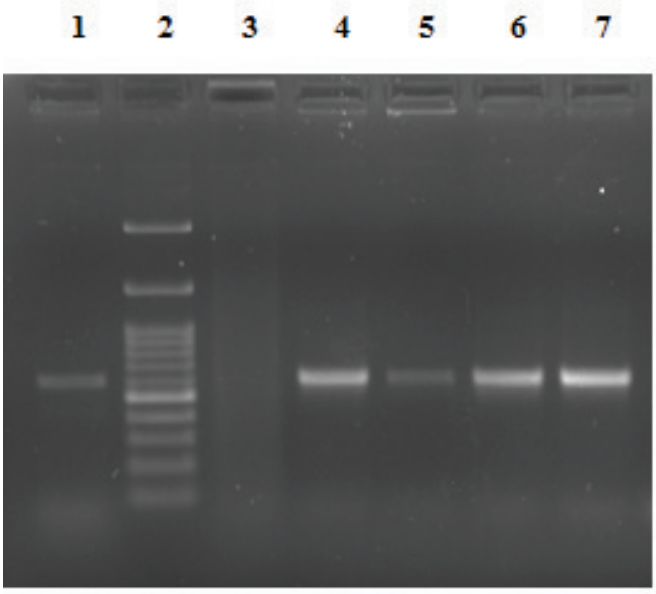

Fig. 3. Agarose gel electrophoresis [showing, lane 4, 5, 6, 7- positive sample for *B. vogeli* (600bp), lane 3- negative control, lane 2- 100 bp ladder and lane 1- positive control].

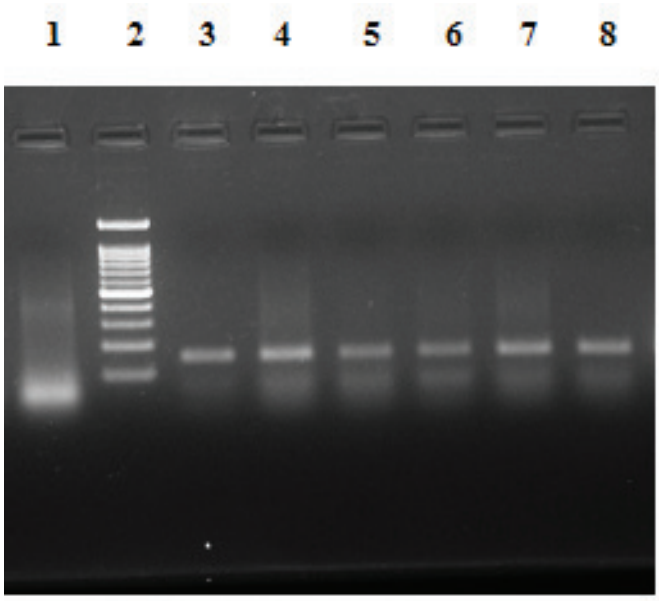

Fig. 4. Agarose gel electrophoresis. [Showing, lane 4, 5, 6, 7 and 8 - positive sample for *B. gibsoni* (164bp), lane 3 - positive control, lane 2 - 100bp ladder and lane 1 - negative control].

numerous polychromatophilic and nucleated RBCs were recorded in the blood smear.

Serum biochemical investigation of babesiosis cases revealed increased levels of total bilirubin, blood urea nitrogen (BUN), creatinine, AST, and Alkaline phosphatase in 100% (18/18), 61.11% (11/18), 33.33% (06/18), 61.11% (11/18) and 33.33% (6/18) cases respectively, when compared with normal reference range values. An abrupt increase of total bilirubin in cases of babesiosis is due to hepatic hypoxia caused by babesiosis [21]. Acute renal failure in canine babesiosis elevated blood urea nitrogen (BUN) and creatinine [22]. The present findings of increased AST in 11 cases are in agreement with previous findings [23]. Increased AST activity may be caused as a result of escaping from parenchymal liver cells that have undergone necrosis or have changed membrane permeability, both of which signify hepatic dysfunction [24]. The increased Alkaline phosphatase in 6 out of 18 cases showed a similar finding to the study of Shah *et al.* [19]. Hypoalbuminemia exhibited in 4 present cases was corroborated with the previous findings [25]. Increased levels of globulin were observed in 3 out of 18 cases, in accordance with the previously described report [26]. Characteristic clinical features observed among 18 positive clinical cases were fever/pyrexia (15/18, 83.33%), anorexia (12/18, 66.66%), tick infestation (18/18, 100%), lymphadenopathy (8/18, 44.44%), depression and lethargy (9/18, 50%), pale mucous membrane (13/18, 72.22%), respiratory distress (6/18, 33.33%), weakness (10/18, 55.55%), ocular discharge (2/18, 11.11%), nasal discharge (03/18, 16.5%), lameness (03/18, 16.5%), vomiting (2/18, 11.11%), diarrhea (03/18, 16.5%), icteric mucous membrane (6/18, 33.33%), haematuria (5/18, 27.77%) and epistaxis (2/18, 11.11%). A similar type of clinical manifestation has been reported earlier from the canine babesiosis cases [27, 28] and it may range from subclinical to severe or fatal disease, critically depending on the piroplasm species involved, on factors associated with the hosts (*e.g.*, age and immune status) and importantly host-parasite interplay towards the insult [4].

Canine babesiosis is a significant disease of veterinary importance with an incidence of 12.41% in canines in and around Akola with *B. gibsoni* as a predominated species. Control of vectors is very important, many of them can also spread other viral diseases in dogs [29]. The present findings will help clinicians with differential diagnosis in cases of anemia and to start proper treatment of the ailing canines.

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