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CASE REPORT

Laboratory diagnosis of Babesiosis in a Rottweiler dog

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ABSTRACT

A three and half years old female Rottweiler dog was brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli for diagnosis and treatment with a history of anorexia since a week. Physical examination of the ailing animal revealed pyrexia and splenomegaly. Haematological estimation showed anaemia, thrombocytopenia and eosinophilia. Microscopic examination of peripheral blood smears revealed the presence of intra erythrocytic parasites, suggestive of *Babesia gibsoni*. Blood picture showed polychromasia and presence of nucleated red blood cells (RBC) and Howell Jolly bodies. Biochemical studies revealed increased levels of serum alkaline phosphatase (ALP), alanine transaminase (ALT) and blood urea nitrogen (BUN). Polymerase chain reaction (PCR) assay was carried out using the suspected blood sample confirmed the presence of *Babesia gibsoni* infection. Based on the present laboratory findings, diagnosis was confirmed as babesiosis and the affected animal was treated successfully. The animal became active and alert with normal feeding habits after 10 days of post treatment.

Keywords: *Babesia gibsoni*, blood smear examination, PCR, Rotweiller

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Babesiosis is one of the most common vector borne hemoprotozoan diseases, caused by various *Babesia* species affecting both domestic as well as wild animal species including cattle, buffaloes, sheep, goats, dogs etc. Canine babesiosis is caused by two most common species include *Babesia vogeli canis* and *Babesia gibsoni*, of which, the former being the larger one measuring 3-5 µm in diameter, while the latter being smaller, measuring 1-2 µm in diameter[1]. Both the forms are transmitted by Ixodid ticks notably *Rhipicephalus* and *Hemophysalis*. Transmission occurs through transovarian and transtadial route and also by transfusion of blood infected with *Babesia* spp.[2,3,4]. The above two vectors responsible for transmission of disease are widely distributed in Asia, Europe and African countries. The organism proliferates within the erythrocytes and gets released into the blood stream, which in turn eventually produces disease with characteristic clinical signs namely pyrexia, splenomegaly, hemoglobinuria, ascites, azotemia, liver dysfunctions and jaundice[5]. These haemoprotozoan parasites are easily demonstrated in the peripheral blood smears. However, detection of smaller forms of *Babesia* spp. such as *B. gibsoni* in the blood smears is difficult, for which application of molecular techniques such as Polymerase chain reaction (PCR) will be of valuable tool in confirmation of disease[6,7]. Thus, the present study reports a case of *Babesia gibsoni* infection, which was confirmed both by peripheral blood smear examination as well as by PCR assay in a Rotweiller dog.

A three and half years old female Rottweiler dog (Fig. 1) was brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli for diagnosis and treatment with a history of anorexia since a week. A thorough physical examination was carried out on the ailing animal and blood samples were collected from the saphenous vein for haematobiochemical studies. For haematological studies, blood samples were collected in ethylene diamine tetra acetic acid (EDTA) containing tubes and subjected to detailed analysis of various parameters namely Haemglobin (Hb), Packed cell volume (PCV), total erythrocyte count, total leukocyte count and thrombocyte count using automatic hematology analyser (Mindray - BC-2800). For biochemical estimations, blood samples were collected in clot activator tubes and serum was separated after centrifuging the clotted samples @ 300 rpm for 5 minutes. The separated serum was subjected to various estimations namely urea nitrogen, creatinine, ALP and ALT using automatic biochemical analyser (A15 Biosystems). In addition, blood smears prepared

from the ear vein were stained with Leishman and Giemsa (LG) and subjected to microscopic examination for presence of hemoprotozoan parasites.

For molecular confirmation of hemoprotozoan infections, blood samples were subjected to PCR assay using primers sequence as described[8]. The analysis was performed at Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu, India. The procedure were as follow, 200 µL of EDTA anticoagulant blood taken and genomic DNA was isolated using (QIAGEN DNeasy® Blood & Tissue Kit, Germany) by following manufacturer's protocol. The PCR cyclic conditions, including initial denaturation at cycling conditions involved initial denaturation at 95^{O}C for 5 minutes, followed by denaturation at 95^{O}C for 30 seconds, annealing at 55^{O}C for 30 seconds and extension at 72^{O}C for 90 seconds for 35 cycles. A final extension step was performed at 72^{O}C for 5 minutes. The 18S rRNA gene *B. gibsoni* target sequences that amplified positively were examined using 1.5% agarose gel electrophoresis, seen under a UV transilluminator and recorded.

Table 1. Haematological profile.

Parameters	Unit	Value	Normal range[14]	Result
Hb	g/dl	5.2	12-18	Anemia
RBC	10^6/µL	2.6	5.5-8.5	Anemia
PCV	%	21.6	37-55	Anemia
WBC	10^3/µL	4.7	6-17	Normal
Platelet	10^5/µL	33,000	2-9 lakhs	Thrombocytopenia
Neutrophil	%	63	60-70	Normal
Eosinophil	%	14	2-10	Eosinophilia
Lymphocyte	%	18	12-30	Normal
Monocyte	%	5	3-10	Normal

Table 2. Biochemical profile.

Parameters	Unit	Value	Normal range[15]	Inference
Blood urea nitrogen	mg/dl	40.4	5-30	Elevated
Creatinine	mg/dl	0.9	0.7-1.8	Normal
Total protein	g/dl	6.1	5-8	Normal
Albumin	g/dl	2.8	2.5-3.7	Normal
Total bilirubin	mg/dl	3.1	0.07-0.61	Normal
Calcium	mg/dl	9.2	9-11	Normal
Phosphorus	mg/dl	3.1	3.0-5.9	Normal
Alkaline phosphatase	IU/dl	312	20-200	Elevated
Alanine transaminase	IU/dl	210	10-109	Elevated

Physical examination of the ailing dog revealed pyrexia (103.5^{O}C), lymph node enlargement, pale mucous membrane and splenomegaly. The results of the hematobiochemical studies are presented in Table 1 and 2. Haematological examination showed anaemia, thrombocytopenia and eosinophilia, while biochemical studies revealed increased levels of serum alkaline phosphatase (ALP), alanine transaminase (ALT) and blood urea nitrogen (BUN). Microscopic examination of peripheral blood smears revealed the presence of numerous intra erythrocytic inclusions of variable sizes and shapes (Fig. 2) comprising both signet ring (Fig. 3 & 4) and comma shaped organisms suggestive of *Babesia gibsoni*. Blood picture showed polychromasia (Fig. 5), presence of nucleated red blood cells (RBC) (Fig. 6) and Howell Jolly bodies. These cells are immature RBCs, produced from bone marrow and appear in response to a high requirement for red blood cells due to increased destruction or loss (hemolytic anemia).

Polymerase chain reaction (PCR) assay (Fig. 7) confirmed the presence of *Babesia gibsoni* infection (671 bp to amplify 18s RNA using forward primer Gibb 599 (5′ CTCGGCTACTTGCCTTGTC 3′) and reverse Primer (5′ GCCGAAACTGAAATAACGGC 3′)[8]. Enlargement of spleen noticed during the present study was in accordance with earlier worker, who also recorded similar findings in a dog infected with *Babesia gibsoni.* Splenomegaly in babesiosis is due to more amounts of RBCs destruction occur. These cells are engulfed and removed by splenic macrophages during phagocytosis, which increases the cellularity of the red pulp and makes it harder to distinguish between the white and red pulp regions. The present haematological findings namely anemia, thrombocytopenia and eosinophila observed due to hemolysis of RBCs in the present case was in agreement with that of[9,10] who also recorded similar findings in *Babesia gibsoni* infected dog. The anemia noticed during the present study could be attributed to the cause of oxidative damage caused by *Babesia* organisms[11]. The present blood picture which revealed polychromasia and presence of nucleated RBC and Howell jolly bodies could be due to intra and extra vascular hemolysis[12].

Biochemical examination which revealed elevated levels of serum ALP and ALT might be attributed to cause of liver damage especially necrosis due to hypoxia and cytokines while increased levels of urea nitrogen (BUN) might be due to renal dysfunction due to slowing done of blood flow caused by refractory hypotension. Similar

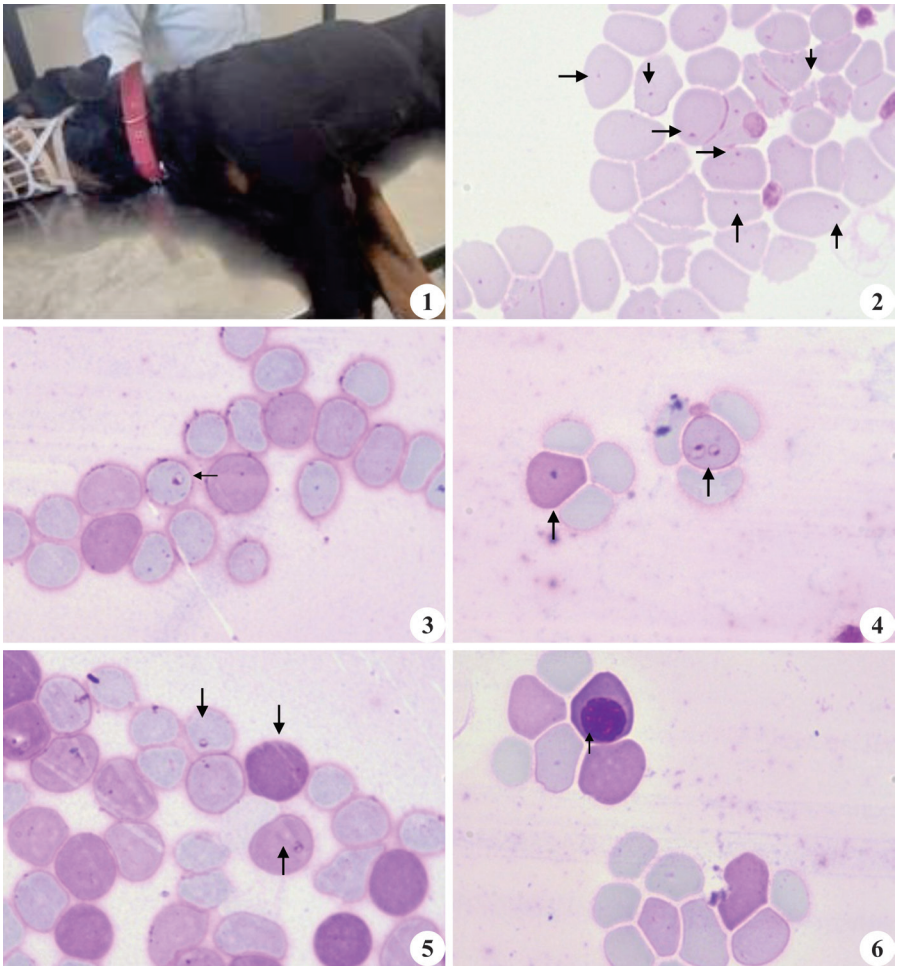

Fig. 1. A female Rottweiler dog affected with Babesiosis; **Fig. 2.** Peripheral blood smears revealed the presence of numerous intra erythrocytic inclusions of variable sizes and shapes (L&G staining x1000); **Fig. 3.** Blood smear of a dog showing the presence of numerous intra erythrocytic signet ring shape merozoite suggestive of *B. gibsoni* (L&G staining x1000); **Fig. 4.** Blood smear of a dog showing the presence of numerous intra erythrocytic double signet ring shaped inclusions suggestive of *B. gibsoni* (L&G staining x1000); **Fig. 5.** Blood smear of a dog infected with *B. gibsoni* showing polychromasia RBC (L&G staining x1000); **Fig. 6.** Blood smear of a dog infected with *B. gibsoni* showing nucleated RBC (L&G staining x1000).

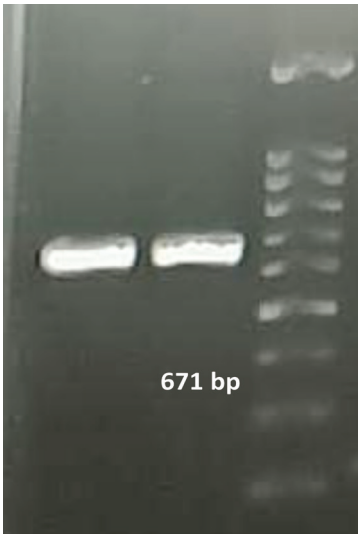

Fig. 7. PCR assay showing positivity of *B. gibsoni*. Lane 1: 100 bp ladder, Lane 2: Positive Control, Lane 3: Sample.

findings were also recorded[11,12,13] in dogs infected with babesiosis. The present microscopic findings of intra erythrocytic inclusions of variable sizes and shapes which were suggestive of *Babesia gibsoni* were in agreement with that of[10,9] who also reported similar morphological appearance in dog infected with *Babesia gibsoni*.

Blood smear examination provide less cost, simple, quick and easy way to identification of the blood protozoan parasite in the infected blood and enabling timely diagnosis and therapy. It can assist in determining the severity of infection. PCR assay which is more sensitive, confirmative and also can assist in distinguishing from plasmodium infection. Other common reliable methods like detecting antibodies against babesia infection in the blood, using procedures like enzyme linked immune sorbent assay (ELISA), indirect immunofluoresent antibody test (IFA) and complement fixation test (CFT), not suited for acute infection as antibodies may not be

present in adequate amounts until later in the infection. Triple therapy was initiated in this case with Clindamycin @ 25 mg/kg b.w., intravenously, BID, Metronidazole @ 15 mg/kg b.w., intravenously, BID and Doxycycline @ 5 mg/kg b.w., intravenously, BID, for 15 days on the date of presentation of this case. Also, the animal had given supportive treatment. Advised the owner with syrup LIV 52 and aRBCe @ 5 mL, Orally, BID and syrup THROMBOFIT @ 10 mL, Orally, BID. For the management of valvular heart dysfunction, Tablet. Envas @ 10 mg, sig. 1 tab, Orally, BID and Tablet. Lasilactone @ 50 mg, sig. 2 tab, Orally, BID was prescribed for 15 days and for supportive liver function, Tablet. Lisybin large, sig. 2 tab, Orally, SID for 10 days and Tablet. Ursodeoxycholic acid @ 300 mg, sig 1 tab, Orally, SID for 10 days were prescribed.

On day 15 following treatment, a peripheral blood smear stained with leishman and Giemsa stain showed the absence of *Babesia gibsoni*. Significant improvements were made to the haematobiochemical parameters. With a remarkable response and a smooth recovery, the dog made a full clinical recovery. In conclusion, microscopic examination of peripheral blood smear of a dog suspected for hemoprotozoan infection revealed intra eryhtrocytic inclusions suggestive of *B. gibsoni* which was further confirmed by molecular technique namely PCR assay.

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