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Assessment of haemato-biochemical profile and successful medical management of *Babesia gibsoni* infection in five dogs: A case study

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Abstract
Canine Babesiosis is a clinically significant and geographically widespread haemoprotozoan disease of domesticated dogs and wild canids. The recent climatic changes and the humid climate prevailing in plain regions of Kerala have led to an increase in vector tick population resulting in greater incidence of canine babesiosis. The clinical manifestations range from mild illness to serious disease depending on the virulence of species, nutritional status, age and immune status. Uncomplicated clinical cases have been suggested to be a consequence of hemolysis while complicated cases have been suggested to be a consequence of the development of the systemic inflammatory response syndrome and multiple organ dysfunction syndrome. Typical symptoms include fever, anemia, pallor, jaundice, hemoglobinuria, splenomegaly and weakness. The diagnosis of Babesiosis is made by demonstrating *Babesia gibsoni* (1.5-2.5 micrometer, signet ring shaped) occur as a single piroplasm in infected erythrocytes. The primary therapeutic aim in the treatment of Babesiosis is the reversal of life-threatening anemia through blood transfusion and elimination of parasite with specific anti babesial drugs. The aim of this case study is to analyze the successful medical management of *Babesia gibsoni* infection in dogs.

Keywords: *Babesia gibsoni*, hemoglobinúria, anemia, Kerala

1. Introduction
Canine Babesiosis is a clinically significant and geographically widespread haemoprotozoan disease of domesticated dogs and wild canids. The large *Babesia canis* and small *Babesia gibson*i are two organisms commonly known to infect the dogs. Both organisms have Ixodid tick vectors and are found throughout Asia, Africa, Europe, Middle east and Northern America with *Babesia canis* being more prevalent. A typical babesial piroplasm is intraerythrocytic, pear shaped and often in pairs (*Babesia gibsoni* – signet ring shaped; occurs as single). The clinical manifestations range from mild illness to serious disease depending on the virulence of species, nutritional status, age and immune status [1]. Geographical occurrence of babesiosis is determined by the geographical and seasonal distribution of the tick vectors that transmit it. The recent climatic changes and the humid climate prevailing in plain regions of Kerala have led to an increase in vector tick population resulting in greater incidence of canine babesiosis. Canine babesiosis clinically classified as uncomplicated and complicated forms. Uncomplicated clinical cases have been suggested to be a consequence of hemolysis while complicated canine Babesiosis has been suggested to be a consequence of the development of the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). Typical symptoms include fever, anemia, pallor, jaundice, hemoglobinuria, splenomegaly and weakness. The diagnosis of Babesiosis is made by demonstrating *Babesia gibsoni* (1.5-2.5 µm, signet ring shaped) occur as a single piroplasm in infected erythrocytes [2]. The primary therapeutic aim in the treatment of Babesiosis is the reversal of life-threatening anemia through blood transfusion and elimination of parasite with specific anti babesial drugs. The aim of this case study is to analyze the successful medical management of *Babesia gibsoni* infection in dogs

2. Materials and Methods
In order to study the clinical management of *Babesia gibsoni* infection in dogs, five cases were investigated in Palakkad municipal area. Blood samples of five dogs showing clinical signs like fever, anorexia, anemia and hemoglobinuria (coffee colored urine) etc. were examined for identification of *Babesia gibsoni* in blood smear.

On the basis of clinical data collected, the blood smear of the suspected animals stained with Giemsa stain was observed under oil immersion objective. In positive cases babesial piroplasm were observed inside erythrocytes. Animals tested positive for *Babesia gibsoni* (via peripheral blood smear examination) was treated with Diminazene aceturate via intra-muscular route on first day, Imidocarb dipropionate via sub-cutaneous route on second day and Clindamycin for next seven days orally along with supportive medications. The blood smear, blood and urine of positive animals was examined after seven days.

3. Diagnosis

Blood and serum were collected for laboratory investigation. Blood smears revealed presence of *Babesia gibsoni.* in RBCs of all the smears (Figure 1). Hemogram revealed on average extremely low levels of Hb, PCV, TEC and platelets counts. Serum chemistry revealed hyperglycemia, hyperbilirubinemia, BUN, AST and hypoproteinemia. The values are presented in Table 1. Urine was coffee coloured and positive for hemoglobin, glucose and bile pigments in all the five dogs. From the history, clinical signs and microscopic examination the disease was confirmatively diagnosed as Babesiosis.

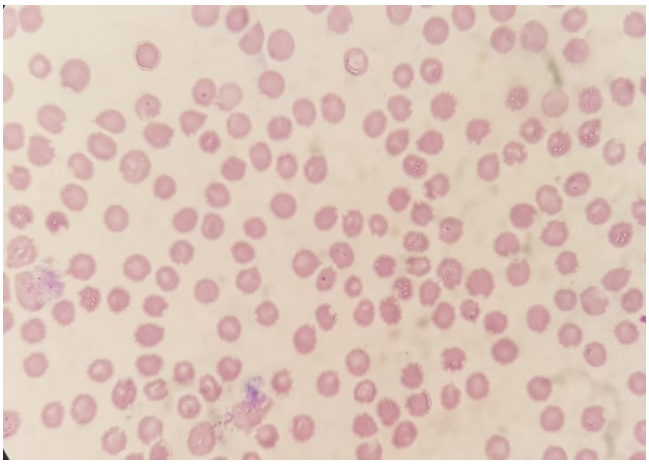

Fig 1: Presence of *Babesia gibsoni* within RBCs of Giemsa-stained blood smear

Table 1: Mean haemato-biochemical values and urine analysis values of dogs under study

Parameters	Apparently healthy dog values	Avg Pre- treatment values	Avg Post- treatment values
Hemoglobin (g/dL)	12 – 18	7.5 ± 1.22	13.3 ± .66
PCV (%)	35 - 57	22 ± 2.53	36 ± 1.4
TEC X 10^6/µL	5 – 8	3.3 ± 1.31	5.2 ± 1.22
TLC X 10^3/µL	5 – 14	4.25 ± 1.6	10.3 ± 1.21
MCV (fL)	66 - 77	47.23 ± 2.3	62.7 ± 1.72
MCH (pg)	21 - 26	15.54 ± 1.56	21.2 ± 2.16
MCHC (g/dL)	320 - 363	232.1 ± 32.72	311.1 ± 27.53
Platelets / µL	211000 - 621000	157000 ± 1529	192000 ± 2782
Neutrophils (%)	58 - 85	40 ± 3.06	52 ± 2.73
Lymphocytes (%)	8 - 21	28 ± 1.46	18 ± 1.82
Monocytes (%)	2 – 10	2 ± 0.34	3 ± 0.47
Eosinophils (%)	2 – 9	7 ± 0.18	3 ± 0.36
Basophils (%)	0 – 1	-	-
Serum Biochemical Values			
AST (U/L)	8 - 49	67 ± 1.23	34 ± 2.32
TP (g/dl)	5.5 – 7.2	8.3 ± 1.58	6.1 ± 2.22
BUN (mg/dl)	8-30	41 ± 2.45	26 ± 1.29
Tot. Bilirubin (mg/dl)	0.1 - 0.6	0.9 ± 0.21	0.56 ± 0.11
Glucose (mg/dl)	60 - 111	110 ± 7.28	75± 3.56
Creatinine (mg/dl)	0.5 – 1.5	2.1± 0.26	0.9± 0.11
Urine analysis			
Blood (Hb)	-	+++	-
Glucose	-	±	-
Bile pigments	-	++	±

4. Treatment

The animals were treated with Diminazine accurate @ 3.5 mg/kg Bwt i/m on first day and Imidocarb dipropionate @ 6.6 mg/kg Bwt on second day. Clindamycin tablet (@ 11 mg/kg Bwt orally), Pantoprazole tablet and Hematinic oral suspension was advised from third day onwards for next 7 days. Animals were brought after 1 week of medication. All the four cases recovered successfully with treatment of diminazine aceturate in combination with imidocarb dipropionate and clindamycin. Upon blood smear examination, no hemoprotozoans could be detected

5. Discussion

Babesiosis is a common haemoprotozoan disease among dogs. It is most commonly associated with *Babesia canis*, *Babesia rossi*, *Babesia vogeli*, *Babesia gibsoni* and *Babesia conradae*. These protozoa parasitize RBCs and are most frequently associated with development of anaemia. The worldwide distribution, vectors and virulence of Babesia species varies. *Babesia rossi* is transmitted by *Haemophysalis elliptica* and is most pathogenic. *Babesia canis* is transmitted by *Dermacentor* species and *Rhipicephalus* species and is moderately pathogenic; *Babesia vogeli* is the least pathogenic and is transmitted by *Rhipicephalus sanguineus* [1].

Canine babesiosis is caused by two species of babesia viz; *Babesia canis* and *Babesia gibsoni*, which are morphologically differentiated on the basis of their size. *Babesia canis* – large form (4-5 µm long) of Babesia., pyriform in shape, pointed at one end and round at the other end. In a single RBC, during multiple infection, more than

one organism may be found. *Babesia gibsoni*- small form (1.5-2.5 µm long), lack usual pyriform shapes, trophozoites are annular or oval; and signet ring forms may occur. *Babesia canis* is found in Europe, *Babesia vogeli* in northern Africa, North America and South Africa and *Babesia rossi* in southern Africa. *Babesia gibsoni* is found in Asia, Australia, North America and northern and eastern Africa.

5.1 Life cycle

Babesia infection occurs when an infective tick bites the host and releases sporozoites into the bloodstream. The organisms attach to the red cell membrane inside the host and are engulfed through endocytosis. The parasites remain directly inside the cytoplasm when the membrane disintegrates, where they undergo binary fission, resulting in two or more merozoites. Ticks become infected with merozoites during feeding on the host. Schizogony occurs inside the ticks with the formation of polyploid kinetes (large merozoites) in the salivary glands, gastrointestinal cells, and oocytes. Secondary Schizogony occurs in the oocytes in successive cycles, allowing the ticks to remain infective for multiple generations. Kinetes are converted into multinucleated stages in the salivary glands of ticks (sporogony). The sporozoites are formed when these are broken up. Except for *Babesia bigemina*, sporozoite growth normally begins only after the infected tick attaches to the vertebrate host. When the tick feeds, the sporozoites infect the host. The different routes of transmission of disease are tick - vector transmission, through bite wound and via blood transfusion [2, 3].

5.2 Pathogenesis

Rather than the parasite directly destroying the erythrocyte, many pathogenic mechanisms are the outcome of the host's immunological reaction to the organism. Although parasitemia seldom surpasses 10 percent, severe anaemia is a common complication. Babesiosis is caused by parasites invading and replicating in the erythrocyte, resulting in regenerative anaemia, haemoglobinaemia, haemoglobinuria, and bilirubinuria. Direct parasite damage to the erythrocyte membrane, splenic evacuation of damaged and parasitized erythrocytes, complement activation, and the existence of anti-erythrocyte antibodies all contribute to erythrocyte destruction, resulting in secondary immune-mediated haemolytic anaemia [1]. Pyrexia can also occur as a result of endogenous pyrogens released during erythrolysis, parasite elimination, and the activation of inflammatory mediators. Splenomegaly can develop as a result of hyperplasia of the mononuclear phagocytic system. The resulting haemolytic crisis causes anaemic hypoxia, anaerobic metabolism, and metabolic acidosis [3]. Furthermore, the residual haemoglobin, particularly at the tissue level, does not function adequately, exacerbating the anaemic hypoxia. Babesiosis that is not anaemic causes an immediate, overwhelming inflammatory response that is driven by cytokines, nitric oxide, platelet activating factors, and eicosanoids. Non-anemic babesiosis causes severe azotemia, electrolyte and acid-base abnormalities, and limited leukocyte responses or even leukopenia in animals. A large leukocyte bone marrow response and the haemolytic condition may not have time to develop due to the severity of the inflammatory response [4].

5.3 Clinical Manifestation

Clinical symptoms include anaemia, thrombocytopenia, lethargy, anorexia, splenomegaly, hemoglobinuria, bilirubinuria, fever, and jaundice, which can range from preclinical to clinical. The anaemia is initially normocytic, normochromic, and non-regenerative, but eventually progresses to a macrocytic, hypochromic, regenerative anaemia with reticulocytosis. Acute renal failure, acute respiratory distress syndrome, disseminated intravascular coagulation, icterus, hepatopathy, and a neurological disease known as cerebral babesiosis can all occur in severe cases. Symptoms include posterior paresis, muscular tremor, nystagmus, anisocoria, paddling, sobbing, changed state of awareness, limb oedema, and altered state of consciousness. Posterior paresis, muscular tremor, nystagmus, anisocoria, paddling, sobbing, and altered state of consciousness are all indicators of this disease. Oedema of the limbs and scrotum, as well as secondary pneumonic signs, are also common in field conditions.

5.4 Diagnosis

Tentative diagnosis can be done based on history and clinical signs. Confirmatory diagnosis is done depending upon the laboratory findings. Upon complete blood count examination, thrombocytopenia and anaemia are the most common feature regardless of the *Babesia* spp. Leukogram is highly variable [1]. In Serum biochemistry profile, hyperglobulinemia, hyperbilirubinemia, increased liver enzyme activities, azotemia (*Babesia canis*, *Babesia gibsoni*, *Babesia annae*) and hypoalbuminemia are clinically indicative of canine babesiosis. Upon urinalysis, bilirubinuria, hemoglobinuria, and proteinuria are positive indicators of the disease. On blood smear examination, presence of the organisms in RBCs detected by Wright or Giemsa stains on thin blood smear. Capillary blood is preferred source. For large Babesia species: 3–7 µm long single or pair tear-drop intra-erythrocytic forms are visible while 1–3 µm long signet-ring form forms are diagnostic of small Babesia species [5, 6]. Indirect fluorescent antibody (IFA) testing cannot differentiate among Babesia species; titres of ≥1:64 support exposure. It is most often used test for surveillance and export certification. Polymerase chain reaction (PCR) testing has high specificity and sensitivity. It can determine species or subspecies with specific PCR assay or DNA sequencing. False-negative results are possible with low numbers of circulating parasites. False-negative results are also possible if primers are “too specific” (that is will only amplify *Babesia gibsoni* and will not detect *Babesia canis*. A nested PCR to amplify Babesia small subunit ribosomal RNA is extremely sensitive and able to detect a parasitemia of 0.0001%. These can detect and differentiate Babesia species, and are particularly useful in carriers [1, 2].

5.5 Treatment

Species of Babesia vary in their susceptibility to babesicidal treatments. In general, small Babesia are considered more resistant to treatment [1].

- Imidocarb dipropionate: A single dose of 7.5mg/kg or a single dose of 6mg/kg given the day after the dose of diminazene (3.5mg/kg) has also been shown to clear infections. Imidocarb is not effective for clearing *B. gibsoni* infections, but is effective at reducing morbidity and mortality.
- Diminazene aceturate (3.5–7mg/kg IM q1–2wk) is effective against *B. canis*, but are not capable of clearing *B. gibsoni.*

5.6 Combination therapy

- Atovaquone (13.3 mg/kg PO q8h) and azithromycin (10 mg/kg PO q24h) combination therapy (for 10 days) has effectively cleared *Babesia gibsoni* infections. Atovaquone should be given as liquid suspension with a fatty meal to ensure adequate absorption.
- Clindamycin (25 mg/kg PO q12h), metronidazole (15 mg/kg PO q12h), and doxycycline (5 mg/kg PO q12h) have been associated with clearance of *Babesia.gibsoni.*

Aggressive supportive care and monotherapy with Clindamycin (25 mg/kg PO q12h) for 7 to 21days has been recommended if the specific antibabesial drugs are not available. At this dosage anaemia and other clinical findings have resolved [7]. With regard to the supportive therapy, IV fluids shall be given for correction of dehydration and hypovolemia and assisted respiration is done in case of pulmonary oedema due to acute respiratory distress syndrome. Antioxidants and steroids can also be given if necessary.

5.7 Prevention

Tick control and vaccination are the main strategies to be implemented with regard to prevention of canine babesiosis. Vaccination is currently not practiced in India. Subcutaneous administration of Ivermectin @ 200 µg/kg and use of Flumethrin pour-on solution are the methods practiced for prevention all over the world depending upon the situation and availability of drugs.

6. Conclusion

The present study was conducted on five clinical cases of dog, presented at District veterinary centre, Palakkad, during a period of forty five days in the month of May-June 2021.Most of these animals showed clinical symptoms such as high fever, anorexia, anaemia, lymphadenopathy, splenomegaly and haemoglobinuria. Peripheral blood smears stained with Giemsa stain, revealed piroplasm of *Babesia gibsoni* in the erythrocytes. The combination therapy with Diminazene aceturate, Imidocarb dipropionate and clindamycin was found to be effective in curing the clinical disease. Based on several clinical case studies conducted in our country before, babesiosis is very much prevalent in dogs, especially the stray dogs. Hence, stake holders should take appropriate measures to control the tick population and also institute the chemoprophylactic measures. This study throws light upon further research work to be conducted on canine babesiosis for effective containment of the disease.

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