Original Research Article

Clinical, Haemato-Biochemical and Molecular Findings of Babesiosis in Dogs

Juripriya Brahma[1*], D. Chandrasekaran[2], M.G. Jayathangaraj[1], S. Vairamuthu[3] and C. Soundararajan[4]

[1]Department of Veterinary Clinical Medicine, [2]Department of Clinics, [3]Department of Centralised Clinical Laboratory, [4]Department of Veterinary Parasitology, Madras Veterinary College, TANUVAS, Chennai-600007, India

*Corresponding author

A B S T R A C T

Canine babesiosis is a hemoprotozoan parasite affecting dogs. The goal of this study was to provide an overview of molecular examination of babesiosis and heamato-biochemical changes in canine babesiosis infected dogs. In this study, 8 cases infected with *Babesia* were confirmed by means of hematological, biochemical and multiplex PCR. The most common clinical signs were anorexia, pale or icteric mucous membranes, high rise of temperature and dark urine colour. The haematological and biochemical parameters showed decrease level of RBC, Hb, PCV, Platelets level and increase level of WBC, ALT, ALP, Total bilirubin, BUN and creatinine value.

Introduction

Babesiosis is a life-threatening disease of dogs that is caused by hemoprotozoan apicomplexan parasites of the genus *Babesia*. The disease is mainly caused by *Babesia gibsoni* (smaller piroplasms) and *Babesia canis* (larger piroplasms) and is transmitted by brown dog tick *Rhipicephalus sanguineus. Dermacentor reticularus, Dermacentor marginatus* and *Haemaphysalis leachi* also involved in the transmission of babesiosis (Filipe and Luciana, 2006).

Dogs become infected when ticks feed for 2-3 days and release sporozoites into the circulation (Taboada and Merchant, 1991, Taboada, 1998). Inside the host the organisms attach to the red cell membrane and are engulfed by endocytosis. In the cytoplasm, binary fission occurs, resulting in merozoites. Ticks become infected with merozoites during feeding and may remain infective for many generations through trans-stadial and transovarial transmission. Parasitaemia peaked at 1.9% to 6% by 4-6 weeks after infection. Easily detectable parasitaemia was present for 3 to 4 weeks. The severity of

2127

clinical signs was highly variable and developed approximately 1 to 2 weeks after infection (Meinkoth *et al.,* 2002).

After parasitaemia, the immune system does not totally eradicate the infection and chronic carrier state remains. Relapses may occur months to years later and long-term sequelae, such as glomerulonephritis or polyarthritis may develop (Conrad *et al.,* 1991, Wozniak *et al.,* 1997 and Lobetti 1998) with *Babesia gibsoni* infection, parasitemia is usually mild although anemia can be severe. Splenectomized animals may have more severe parasitemia and anemia.

Babesia gibsoni can cause hyperacute, acute and chronic infections. Acute infections are rare and primarily occur in puppies resulting in rapid death. These infections are presumed to be maternally acquired. Acute *B. gibsoni* infections are typically associated with fever, lethargy, thrombocytopenia and anemia. Chronic infections may be completely asymptomatic or may be characterized by intermittent fever, lethargy and weight loss.

Hemolytic anemia is the predominant feature of babesiosis and thrombocytopenia is also common in infected dogs. Anemia is attributed to extra and intravascular hemolysis. Mechanisms of RBC destruction include increased osmotic fragility, shortened RBC life span and erythrophagocytosis. Secondary immune-mediated destruction occurs because of parasite antigens on the RBC surface, parasite-induced membrane damage and possibly other membrane-associated antigens (Taboada and Merchant, 1991, Jacobson and Clark, 1994, Wozniak *et al.,* 1997, Taboada, 1998). Oxidative damage, impaired hemoglobin function, sludging and sequestration of erythrocytes also occur (Taboada and Merchant, 1991, Jacobson and Clark, 1994, Taboada, 1998).

In addition to tickborne transmission, vertical transmission is also suspected and infections have been identified in a dam and her 3-day old puppies. Transmission can also occur through transmission of infected blood.

Diagnosis *B. gibsoni* infection can be challenging because many animals are presumed to have idiopathic immune-mediated anemia or another tick borne disease. Detecting RBC auto-agglutination and positive results of a Coombs' test may complicate the diagnosis. Identifying the parasite through blood smear evaluation can be difficult because of the small size of the organism relatively low levels of parasitemia.

B. gibsoni is approximately 1x2.5 μm in size and signet, rod or cocci shape. Giemsa or Wright's stained of fresh blood smears are recommended. The organisms are found in the peripheral portion of the blood smear. The serological assays IFA (Immunofluorescent antibody) and ELISA (Enzyme-linked immunosorbent assay) are also been used to detect infection.

PCR (Polymerase chain reaction) is used for identifying the infective species, detecting low levels of parasitaemia, recognizing subclinical infections and monitoring response to therapy.

Materials and Methods

Thirty four dogs of different breeds, ages naturally infected with canine *Babesia* were selected from the Madras Veterinary College Teaching Hospital under this study. Blood samples was collected from animals exhibiting clinical symptoms of fever, pale mucous membrane, loss of appetite, depression, haemoglobinuria and tick infestation. Blood was collected in EDTA-anticoagulated for complete blood counts, smear observations and PCR analysis.

Complete blood count (CBC) was assessed with an automatic cell counter (Mindray BC Vet 2800). Parameters assessed were: red blood cell count (RBC), hemoglobin (Hb), PCV, PLT count, white blood cell count (WBC), WBC differential count including neutrophils, lymphocytes, monocytes, eosinophil.

Blood smears were prepared, air dried and stained with Giemsa solution. Smears were examined under oil immersion objective (100X) microscope to detect the piroplasms and the results obtained were compared with PCR assay.

Regarding estimation of biochemical parameters 2 ml of blood was collected without anticoagulant vial. The serum concentration of alanine amino transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), blood glucose, albumin, creatinine, total bilirubin and direct bilirubin were determined by automated serum biochemistry analyser (A-15 Biosystem) by using standard kits. Results obtained were expressed as means ± standard error.

DNA extraction

DNA isolation kits (QIAamp DNA Mini Kit®, Qiagen) was used for the extraction of parasite DNA from 200µl of blood sample collected in EDTA vacutainers according to the manufacturer's instructions. Genomic DNA isolated from the whole blood of healthy dogs was used as negative control.

Multiplex PCR amplification

Multiplex PCR for amplification of the 16s rRNA gene fragment of genus *Babesia* and VirB9 of *E. canis* was employed by using the procedure of Kledmanee *et al.,* (2009). The following primers were used (Table 1).

Thermocycling consisted initial denaturation step of 15 minutes at 94°C followed by 30 cycles of 45 second at 94°C, 45 second at 65°C and 90 second at 72°C with final extension step of 10 min at 72°C. The amplicons were separated by electrophoresis using 1.5% agarose gel in 40 mM Tris-acetic acetate (pH 8.4), 1 mM EDTA stained with ethidium bromide (0.5 µg/ml) after that visualized under UV light.

Results and Discussion

A total of 8 dogs infected with canine babesiosis were diagnosed by clinical examination and observation of intra erythrocytic piroplasm within blood smears. Giemsa-stained blood smears shows the presence of small pear-shaped (Plate 1) parasites.

Animal infested with tick was observed (Plate 2). The most prevalent clinical abnormalities were anorexia, Pale or icteric mucous membranes (Plate 3a, 3b), lethargy (Plate 4), fever, dark urine and ecchymosis on ventral aspect of abdomen (Plate 5).

Table.1 PCR primers

Pathogen	Primer	Sequence (5'-3')	Product Size
Babesia spp.	Ba103F	CCAATCCTGACACAGGGAGGTAGTGACA	
	Ba721R	CCCCAGAACCCAAAGACTTTGATTTCTCTCAAG	619 bp
E. canis	Ehr1401F	CCATAAGCATAGCTGATAACCCTGTTACAA	
	Ehr1780R	TGGATAATAAAACCGTACTATGTATGCTAG	380 bp

Table.2 Hematological parameters and biochemical parameters in 8 dogs
infected with Canine babesiosis

Parameters	Mean±SE
Hb (g/dl)	4.29±1.63
PCV (%)	13.03±0.58
RBC ($\times 10^6$/mm^3)	2.12±0.08
WBC (10^3/mm^3)	8514.23±580.13
Platelets (10^5/mm^3)	139750 ± 20490.39
Neutrophil %	73.88±0.44
Lymphocyte %	17.5±0.54
Monocyte %	6.25±0.36
Eosinophil %	2±0.07
BUN (mg/dl)	69.88±12.16
Creatinine (mg/dl)	1.6±0.30
Calcium (mg/dl)	10.23±0.22
Phosphorus (mg/dl)	5.05±0.09
ALT (U/L)	113.76±11.44
ALP (U/L)	280.25±33.89
Total bilirubin (mg/dl)	0.96±0.12
Direct bilirubin (mg/dl)	0.49±0.036
Total protein (g/dl)	5.93±0.22
Albumin (g/dl)	1.71±0.07
Glucose (mg/dl)	88.88±1.97
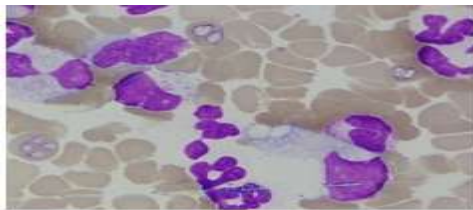
Plate1 : Picture showing intra erythrocytic pear-shaped form of *Babesia canis*.

Plate 2: Tick infestation

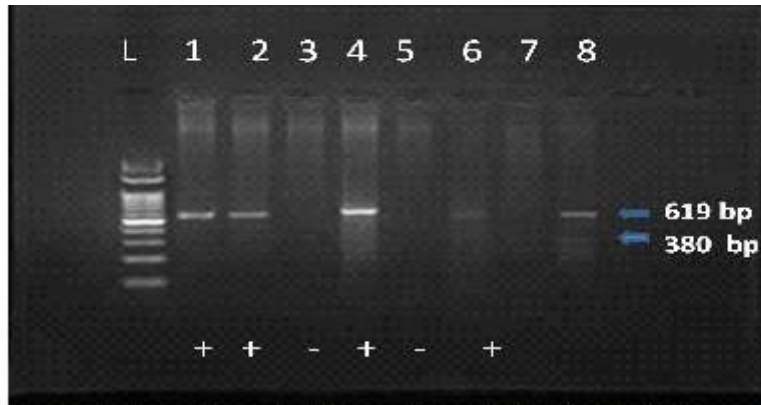Plate 3a: A dog showing pale and icteric oral mucous membrane

Plate 3b: A dog showing pale conjunctival mucous membrane

Plate 4: A dog showing weak and lethargy

Plate 5: Ecchymosis on ventral aspect of abdomen

L= Ladder, sample 8= positive control, 9= negative controlPositive for *Babesia spp.* in sample no. 1,2, 4,6

Plate 6: *Babesia spp.* positive in multiplex PCR

The finding revels that 8 dogs had the RBC, Hb, PCV and platelets values below the reference values and increase level of WBC value. In biochemical examination, ALT, ALP, Total bilirubin, BUN, Creatinine values are increase than the reference values (Table 2). As shown in (Plate.6) the primer Ba103F and Ba721R successfully amplified an approximately 619 bp DNA fragment from all 8 dogs infected with canine babesiosis.

The goal of this study was to provide an overview of molecular examination of babesiosis and haematobiochemical changes.

In the present study, 8 cases infected with *Babesia* were confirmed by mean of clinical history, physical examination, hematological, biochemical and multiplex PCR. Anemia and thrombocytopenia were the most common hematological alterations observed in the present study which concurred with the report of (Birkenheuer *et al.,* 1999, Ayoob *et al.,* 2010, Irwin *et al.,* 2005). The destruction of circulating RBC by auto antibodies which are directed against infected and non-infected red cell membranes resulting in intravascular and extravascular haemolysis. According to Taboada and Lobetti, (2006), direct parasitic damage contributes to anaemia. A low RBC count, PCV and Hb concentration define anemia. Severe microcytic-hypochromic anemia may have been initiated by antibody mediated cytotoxic destruction of erythrocytes and/or by

auto-antibody directed against components of the membranes of infected and uninfected erythrocytes which has also been reported previously in *B. gibsoni* infection (Aysul *et al.,* 2013).

In the present study elevation in mean alanine aminotransferase (ALT) was noticed, which was correlated with Aysul *et al.,* (2013) findings. Aysul *et al.,* (2013) reported that in dogs suffering from babesiosis, elevation of bilirubin, ALT and alkaline phosphatase levels could be seen with hepatic hypoxia.

Acute renal failure in canine babesiosis (Schoeman, 2009) might have resulted into increase BUN and creatinine both *B. gibsoni* and *B. canis* infected dogs. According to Amie (2009) increased non-insulin mediated glucose consumption believed to be induced by inflammatory mediators, more especially in macrophage-rich tissues like the spleen, liver and the lungs was the cause of hypoglycaemia in the affected dogs and at the same time regarded it as a poor prognostic indicator.

Elevation of bilirubin, ALT and AKP are indicative of hepatic hypoxia and increase BUN and creatinine are indicative of degenerative changes in kidneys. Results of this study suggest that haemato-biochemical changes could be beneficial in determination of the severity of babesiosis in dogs.

The use of molecular characterization to identify *Babesia* species highlights the value of procedure of PCR as an adjuvant to current diagnostic methodology.

References

Amie, K., 2009. Hypoglycemia. *Small anim. Crit. Care Med*. 69:295–299.

Ayoob, A.L., Hackner, S.G. and Prittie, J. 2010. Clinical management of canine babesiosis. *J. Vet. Emerg. Critical Care.* 20(1): 77-89.

Aysul, N., Ural, K., Ulutas, B., Eren, H. and Karagenc, T. 2013. First detection and molecular identification of *Babesia gibsoni* in two dogs from the Aydin province of Turkey. *Turkish J. Vet. Anim. Sc,* 37(2): 226-229.

Birkenheuer, A.J., Levy, M.G., Savary, K.C.M., Gager, R.B. and Breitschwerdt, E.B. 1999. *Babesia gibsoni* infections in dogs from North Carolina. *J. Am. Anim. Hosp. Assoc.* 35(2): 125-128.

Conrad, P., Thomford, J., Yamane, I., Whiting, J., Bosma, L., Uno, T., Holshuh, H.J. and Shelly, S.1991. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. *J. Am. Vet Med. Assoc*.199 (5):601-5.

Filipe, D.T. and Luciana, A.F. 2006. Canine babesiosis: A Brazilian persepective. *Vet.Parasit.* 141(3-4): 197-203.

Irwin, P.J., 2005. Babesiosis and Cytauxzoonsis. Arthropode-Borne Infectious Diseases of Dogs and Cats,1st edition. *Manson Publishing Ltd*., Barcelona, Spain,

Jacobson, L.S. and Clark, I.A. 1994. The pathophysiology of canine babesiosis: new approaches to an old puzzle. *J. South Afr. Vet. Assoc*. 65(3): 134-145.

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 detection of *Ehrlichia canis*, *Babesia* spp and *Hepatozoon canis* in canine blood. *South Asi. J. Trop. Med. Public Health.* 40: 35–39.

Lobetti, R.G., 1998. Canine babesiosis. *Compend Contin. Educ. Pract. Vet*. 20:418-430.

Meinkoth, J.H., A.A.Kocan, S.D. Loud. and M.D.Lorenz. 2002. Clinical and hematologic effects of experimental infection of dogs with recently identified *Babesia gibsoni*-like isolates from Oklahoma. *J. Am. Vet. Assoc.* 220:185-189.

Schoeman, J.P., 2009. Canine babesiosis. *Onderstepoort. J. Vet. Res.* 76:59-66.

Taboada, J. and S.R.Merchant. 1991. Babesiosis of companion animals and man. *Vet. Clin. North Am. Small Anim. Pract.* 21:103-123.

Taboada, J., 1998. Babesiosis. In: Greene CE (Ed): Infectious diseases of the dog and cat. Philadelphia, *WB Saunders*. Pp. 473-481.

Taboada, J. and Lobetti, R. 2006. Babesiosis. In: *Infectious Diseases of the Dog and Cat*. Greene, C.G. (Ed.), *Elsevier,* 3rd edition.

Wozniak, E.J., Barr, B.C., Thomford, J.W., Yaamanel, I., McDonough, S.P. Moore, P.F., Naydan, D., Robinson. T.W. and Conrad, P.A. 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*canis familiaris*). *J. parasitol.* 83(4): 692-699.