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Shameem Ara Begum
Assistant Professor, Department of Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Konkon Jyoti Dutta
Ph.D. Scholar, Department of Veterinary Pathology, CVSc, Khanapara, Ghy-22, Assam, India

Girin Hazarika
Assistant Professor, Department of Animal Biotechnology, CVSc, Khanapara, Ghy-22, Assam, India

Pallabi Devi
Assistant Professor, Department of Veterinary Parasitology, CVSc, Khanapara, Ghy-22, Assam, India

Anupam Brahma
Junior Scientist, Department of Veterinary Parasitology, AMMaC Core Lab-I. CVSc, Khanapara, Ghy-22, Assam, India

Lalit Deori
Assistant Commandant (Vety), 27 Bn, SSB, Howly, Assam, India

Corresponding Author:
Shameem Ara Begum
Assistant Professor, Department of Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Pathomorphological, parasitological and molecular diagnosis of *Babesia gibsoni* in a dog

Shameem Ara Begum, Konkon Jyoti Dutta, Girin Hazarika, Pallabi Devi, Anupam Brahma and Lalit Deori

Abstract
A 7 year old female German Shepered dog was presented with a primary complaint of inappetence and incoordination for 2 months. Physical examination revealed pale mucous membranes, enlarged pre-scapular lymph nodes, bilateral ocular discharges, pyrexia (41.2 °C), depression. History revealed that the dog was vaccinated and dewormed regularly. Following clinical examination, blood samples were collected for haematological, biochemical and parasitological analyses, and the results revealed the presence of intraerythrocytic Babesia parasites, normocytic and normochromic anaemia with extensive cellular damage. However, during the course of the investigation, the dog died. Post-mortem examination was carried out with appropriate consent. Gross examination revealed spleenomegaly, fatty change of liver, haemorrhage on the kidney and mucosal surface of stomach. Diffuse oedema with interstitial pneumonia in the lung, focal haemorrhage with cellular infiltration in the heart and coagulative necrosis, presence of urinary cast in the lumen of tubules, sub acute glomerulonephritis in the kidney were observed. Spleen revealed diffuse haemorrhage and stomach showed haemorrhage and congestion. Fatty changes associated with scattered haemoorhages were also recorded. Many of the observed clinicopathological alterations were consistent with chronic babesiosis.

Keywords: Canine babesiosis, haemtology, biochemical findings, histopathology, PCR

1. Introduction
Canine babesiosis, formally known as canine piroplasmosis, is a relatively common haemoprotozoan infection in dogs. Canine babesiosis occurs worldwide and results from infections with a variety of *Babesia* spp., tick-borne hemoprotozoa. There are now over 100 *Babesia* spp. reported in vertebrate hosts (El-Bahnasawy *et al*., 2011) [1] and it is thought that potentially all vertebrates, including people, can be infected with *Babesia*, largely depending on their suitability as hosts for tick vectors (Schnittger *et al*., 2012) [2]. The parasites *Babesia canis* and *B. gibsoni* are responsible for canine babesiosis throughout the world. Historically, the disease was discovered in the late 19th century by a Roman physician called Dr. Babes while analysing blood specimens from sheep and cattle (Anand *et al*., 2015) [3]. Members of the genus *Babesia* readily parasitize the red blood cells of dogs, causing progressive anaemia.
The *Babesia* genus belongs to the order Piroplasmida in the phylum Apicomplexa and can be seen as non-pigment forming pear or signet-ring shaped organisms in mammalian erythrocytes (Schnittger *et al*., 2012) [2]. They are mostly tick transmitted and are the second most common blood-borne parasites of mammals after the trypanosomes (Telford 1993) [4]. Asexual reproduction occurs in canine erythrocytes while the sexual phase occurs in a variety of ixodid ticks, which can transmit the organism transovarially. In the dog, *Babesia* was first described at the end of the 19th century and there are now four well-recognized canine species, *Babesia canis*, *Babesia vogeli*, *B. rossi* and *Babesia gibsoni*, and a number of less well recognized isolates. Initially, *Babesia* was classified according to its morphology in erythrocytes with "large" and "small" forms being recognized as *B. canis* and *B. gibsoni*, respectively

1.1 Case description
1.1.1 History
A 7 year old female GSD dog weighing 25 kg was presented at the Teaching eterinary Clinical Complex (TVCC), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22 with the complaints of inappetance, incoordination, depression and high fever.

History further revealed that the dog was detected with babesiosis and treatment was given accordingly but unfortunately the dog expired. The dog was vaccinated and dewormed regularly.

2. Materials and Methods

2.1. Clinical examination

The animal was examined clinically and temperature, pulse and respiration were recorded. The colour of visible mucous membrane was also recorded.

2.2. Haemoparasitic examination

Thin blood smears were made and stained with Giemsa for haemoparasitic evaluation as per standard protocol (Luna 1968) [5].

2.3. Haematological study

Five (5) ml of blood was collected from the dog by puncturing the left cephalic vein. Out of which, 2 ml was transferred into ethylene diamine tetra acetic acid (EDTA) vacutainer and 3 ml in clot activator vacutainer. Blood samples were immediately brought to the laboratory and was analyzed for haemoglobin (Hb) concentration, packed cell volume (PCV), total red blood cell (RBC) count, haematological indices (MCV, MCH and MCHC), platelet count, white blood cell counts (WBC), as well as and differential leucocytic count (DLC) with automated haematological cell counter manufactured by Melet Schloesing Lab (France) MS 4e model.

2.4. Biochemical study

Blood collected in clot avtivator vacutainer was centrifuged at 3000 rpm for 15 minutes and serum was separated and transferred into an eppendorf tube. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, total bilirubin, direct and indirect bilirubin, total protein and glucose contents of the serum were analysed using Standard BeneSphera ™ kit (Avantor Performance Materials, Netherlands). Despite symptomatic therapy, the dog died three days later. Consequently, the carcass was presented to Department of Veterinary Pathology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022 for a detailed post-mortem examination.

2.5. Pathological study

2.5.1. Gross pathology

A detailed post-mortem examination was carried out immediately after death. Before opening the carcass the whole body was thoroughly examined for the presence of any external lesion. Then all the organs were examined and the visible gross lesions were recorded systematically. Representative tissue samples of 4-7 mm thickness were collected from lungs, heart, stomach, liver, spleen and kidneys in 10% formalin at the ratio of 1:10 for further histopathological examination.

2.5.2. Histopathologcal examination

After proper fixation, the tissue samples were processed and sections were made with a rotary microtome at 4-5 micron thickness. The sections were stained with Haematoxylin and Eosin (H&E) (Luna 1968) [5] and examined under microscope to detect tissue alterations.

2.6. PCR

For molecular detection of Babesia, the blood samples were collected in EDTA vials. The genomic DNA was extracted from blood using the "DNeasy® Blood & Tissue Kit" (Qiagen, Germany). The extracted DNA were subjected to species specific PCR using primers of *B. gibsoni* described by Inokuma *et al*. (2004) targeting 18S rRNA. The forward primer F-5' CTCGGCTACTTGCCTTGTC 3'and reverse primer R 5'GCCGAAACTGAAATAACGGC 3' were used for the amplification of 671 bp product size DNA fragment specific for *B. gibsoni*. The extracted gDNA from blood was used as the template DNA to prepare PCR reaction mixture. A 2 µl volume of template DNA was added to 12.5 µl (2X) Dream Taq PCR Master mix (Thermo scientific) with 1 µl of Forward primers (10 picomol/µl) and 1 µl of Reverse primers (10 picomol/µl). The final volume of the reaction mixture was made up to 25 µl with nuclease-free water. The reaction mixture was properly mixed by vortexing and PCR tubes with all components were placed in a thermal cycler (Applied Biosystem, USA) under the optimized PCR condition of Initial denaturation 95 °C for 5min, denaturation 95 °C for 30sec, annealing 55 °C for 30sec and extension 72 °C for 30sec with 35 cycles and final extension for 72 °C/5min. The PCR product were run in a 1.5% agarose gel for 60 minutes at 80 volts and the gel was viewed under the Gel Documentation System (Bio-Rad) and the result was recorded.

3. Results

3.1. Clinical examination

On clinical and physical examination, the dog had pyrexia (41.2 °C), pale ocular mucous membranes, bilateral enlargement of pre-scapular lymph nodes and it voided brownish-yellow urine. Blood samples were collected and sent for haemoparasitic, haematological and biochemical evaluations, respectively. Based on the history, initial clinical examination and haemoparasitic examination, a tentative diagnosis of Canine Babesiosis was made. The dog was reportedly treated with inj. Meloxicam @ 0.5 mg/kg body weight with supportive therapy 0.9% NS followed by 5% DNS@10ml/kg body weight. Parentral administration of Clindamycin 20mg/kg body weight 24 hrsly, inj Tribivet (1ml), two doses of iron d overcom @ 2ml weekly twice. After confirmation of *B. gibsoni*, a single dose of inj. diminazineaceturate @ 5 mg/kg body, IM, was given. Inj Avilin (1ml) was given IM before administration of inj. diminazineaceturate to prevent adverse reaction. Daily supplementation of aRBC pet syrup @ 5 ml BID was given as general supplementation. The case was in chronic stage and remained untreated when it presented initially. So, the case was not responded to the therapy and died on the 7th day of treatment.

3.2. Haemoparasitc examination

Giemsa stained thin blood smears showed the presence of intraerythrocytic Babesia parasites (Fig 1) with an approximate 5% degree of parasitaemia.

3.3. Haematological study

The hematological parameters are tabulated in Table 1. There was decrease in RBC count, PCV and haemoglobin level in the affected dog. There was no variation in the lymphocyte, monocyte, eosinophil, and basophil percent. The haematological result revealed the presence of normocytic normochromic anaemia.

Table 1: The haematological profile of a 7 year old German Shepherd diagnosed with canine babesiosis

Sl No	Haematological parameters	Values	Reference range Coles 1986
1	Red blood cell (RBC) count	4.05 $\times 10^6$/µl	6-9 $\times 10^3$/µl
2	Packed cell volume (PCV)	32%	37-54%
3	Haemoglobin (Hb) concentration	10.8 g/dl	12-18 g/dl
4	Mean corpuscular volume (MCV)	71.0 fl	60-77 fl
5	Mean corpuscular haemoglobin (MCH)	24.1 pg	20-25 pg
6	Mean corpuscular haemoglobin concentration (MCHC)	34.0 g/dl	31-34 g/dl
7	White blood cell count (WBC)	11.40 $\times 10^3$/µl	6-17 $\times 10^3$/µl
8	Neutrophil	68.5%	60-77%
9	Eosinophil	0.2%	2-10%
10	Basophil	0.4%	Rare
11	Lymphocyte	28.9%	12-30%
12	Monocyte	2.0%	3-10%
13	Thrombocyte	176 $\times 10^6$/µl	$\times 10^3$/µl

3.4. Biochemical study

The ALT, AST, BUN and RBS levels were elevated but serum creatinine and TSP levels were decreased whereas total serum bilirubin, direct bilirubin and TSP were within the normal range as shown in Table 2.

Table 2: The biochemical profile of a 7 year old German shepherd diagnosed with canine babesiosis

Sl No	Biochemical parameters	Values	Reference range Kaneko *et al.*, 2008
1	Aspartate aminotransferase (AST)	90.2 IU/L	23-66 IU/L
2	Alanine aminotransferase (ALT)	212.1 IU/L	21-102 U/L
3	Direct bilirubin	0.3 mg/dl	0.06-0.12 mg/dl
4	Total bilirubin	0.5 mg/dl	0.10-0.50 mg/dl
5	Blood urea nitrogen (BUN)	51.2 mg/dl	10-20 mg/dl
6	Creatinine	1.0 mg/dl	0.50-1.50 mg/dl
7	Total serum protein (TSP)	3.0 gm/dl	5.40-7.10 mg/dl
8	Random blood sugar (RBS)	109.7 mg/dl	65-118 mg/dl

3.5. Pathological study

3.5.1. Gross pathology

On gross examination lungs revealed consolidation of apical and diaphragmatic lobes. Heart showed congested blood vessels on the epicardial surfaces. Liver was found to be enlarged and icteric Gall bladder was distended with bile. Stomach revealed haemorrhage throughout the mucosal surface. Intestine was dilated and filled with icteric faeces. Diffuse haemorrhages were noticed throughout the surfaces of both the kidneys (Fig. 2). Urinary bladder showed petechial haemorrhage on its serosal surface. Spleen was found to be highly enlarged (Fig. 3).

3.5.2. Histopathological study

Lungs showed presence of diffuse edematous fluid (Fig. 4) in the alveolar lumen. Blood vessels were congested with focal haemorrhage in the parenchyma. Mild interstitial pneumonia characterized by thickening of interalveolar septa (Fig. 5) with mononuclear cell infiltration was also observed. Heart revealed no significant lesion, except focal areas of cellular infiltration in the myocardium. Liver showed fatty change, mild capillary congestion and scattered haemorrhages in the parenchyma. The stomach showed haemorrhage and congestion in the mucosal layer. A haemorrhage throughout the splenic parenchyma was also noticed. Kidneys revealed congestion and haemorrhage in the intertubular areas. There was degeneration of the tubular epithelium. Presence of epithelial, protein, hyaline (Fig. 6) as well as haemoglobin casts were also noticed in the lumen of some of the renal tubules. Some focal areas of coagulative necrosis were also evident. There were focal areas of subacute glomerulonephritis (Fig. 7) and interstitial nephritis.

3.6. PCR

For molecular detection of *B. gibsoni*, the PCR product was run in 1.5% agarose gel and observed the result for amplification of 18S ribosomal RNA gene of *B. gibsoni*. The result of electrophoresis showed that there is a presence of a specific single band of product size 671bp using ethidium bromide solution (stock conc. 10 mg/ml) for visualization of the bands (Fig. 8). There is also amplification of 18S ribosomal RNA gene of *B. gibsoni* with product size 671bp in case of positive control use this present study where as there is any amplification in Negative control where we used all the components of PCR except DNA Template when compare with 100bp ladder (NEB).

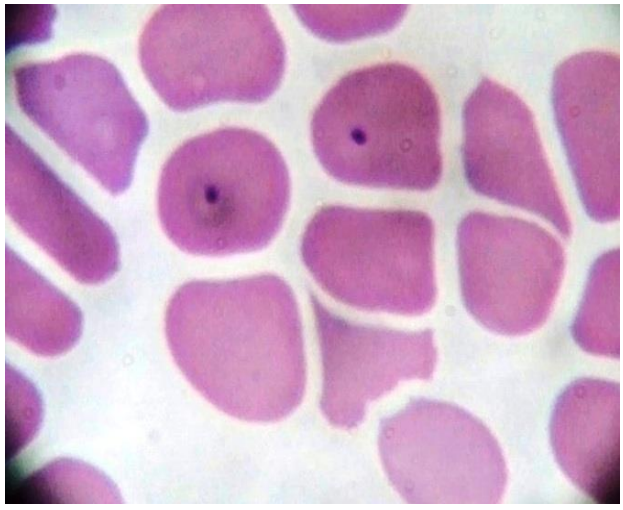

Fig 1: Blood smears showing the presence of intraerythrocytic Babesia parasites

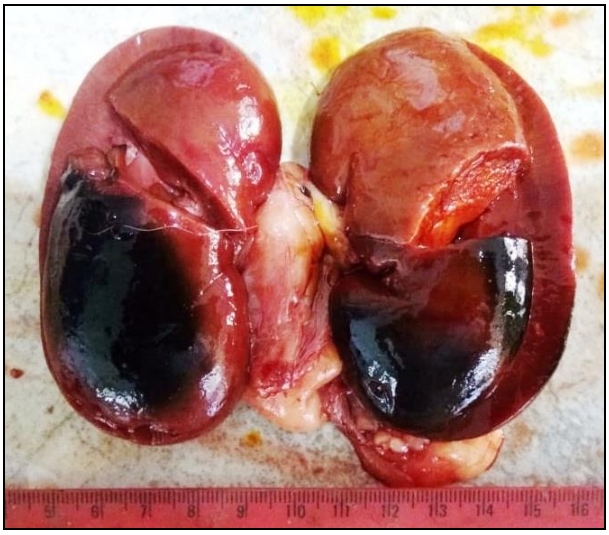

Fig 2: Kidneys showing diffuse haemorrhage throughout the surface

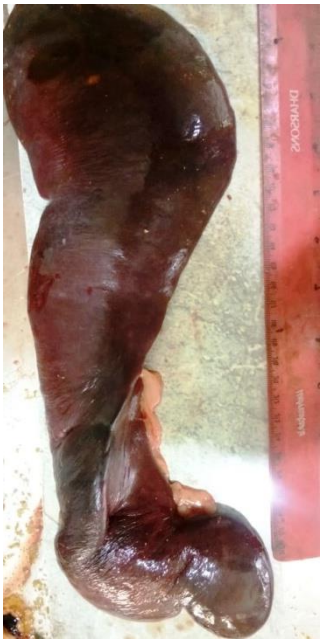

Fig 3: Enlarged spleen

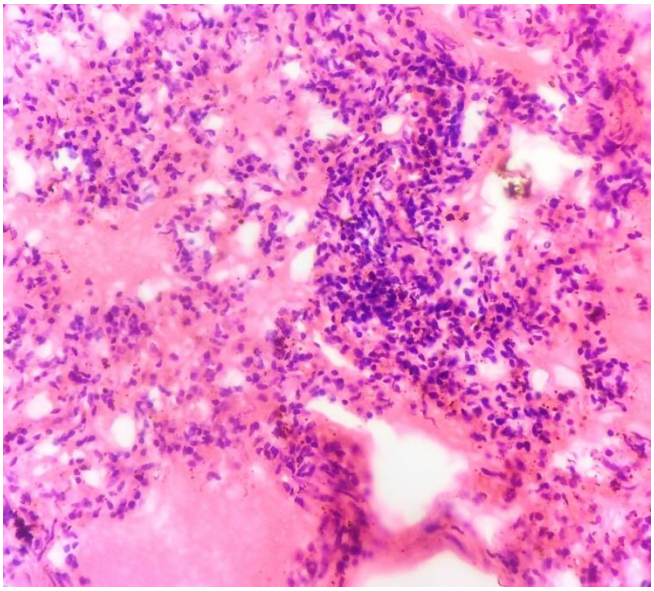

Fig 4: Lungs showing edema (H&E × 40X)

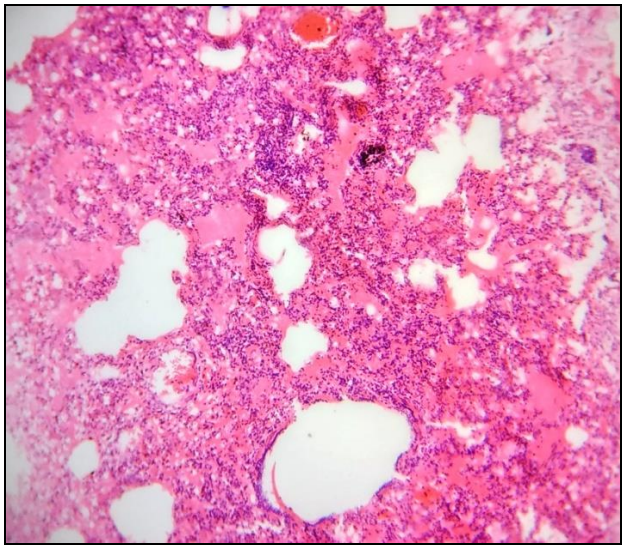

Fig 5: Lungs showing interstitial pneumonia (H&E × 10X)

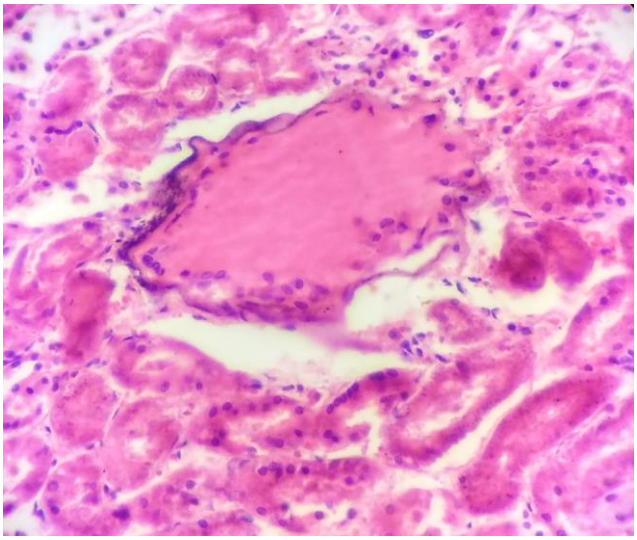

Fig 6: Kidneys showing hyaline cast (H&E × 40X)

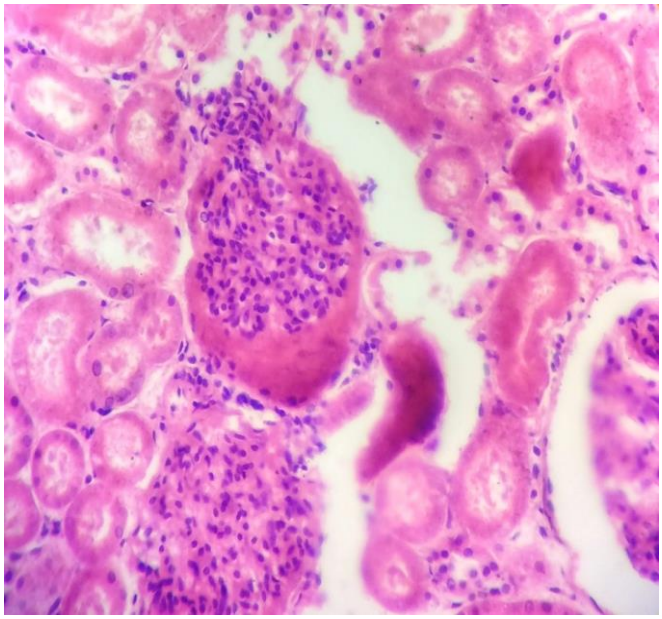

Fig 7: Kidney showing subacute glomerulonephritis (H&E × 40X)

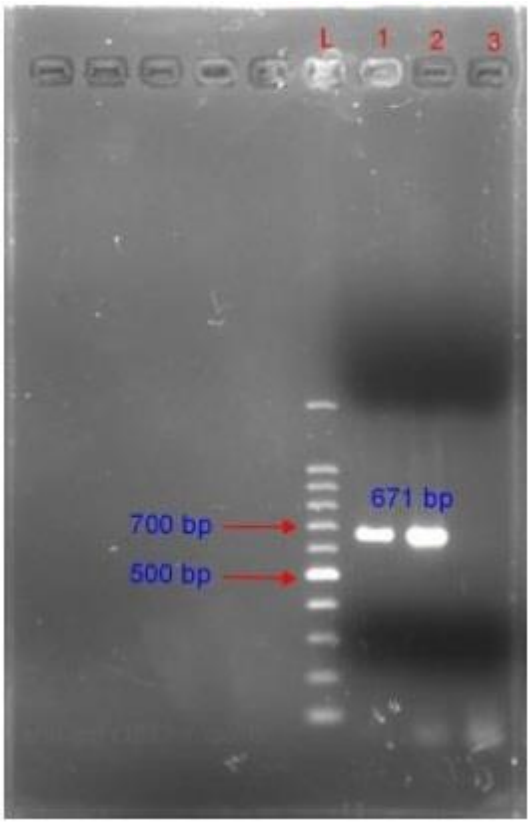

Fig 8: PCR amplified products of 18S rRNA gene of *B. gibsoni* in 1.5 per cent agarose gel showing 671 bp product size bands. (L=100 bp Ladder; 1= Clinical sample; 2=Positive control; 3=Negative control)

4. Discussion

The affected dog showed typical signs of canine babesiosis such as pyrexia, anorexia, depression, pallor of ocular membranes and lymphadenopathy which were similar to the findings of Nalubamba *et al*., 2015 [9]. It is thought that the clinical signs are the result of tissue hypoxia following the anaemia and a concomitant systemic inflammatory response syndrome caused by marked cytokine release (Lobetti 2006) [10].

The presence of the parasite in stained blood smear was detected by light microscopy, though the technique is being the simplest, reasonably sensitive during infections (Matsuu *et al*., 2005) [11] and this is similar to the finding of Bhattacharjee and Sarmah 2013 [12] who recorded a very low prevalence of 47.72 per cent for *B. gibsoni* in dogs in in Guwahati (Assam) by microscopic examination of peripheral blood smears. Diagnosis of acute cases infected with *Babesia.* is based on the classic clinical presentation and the demonstration of the parasites within red blood cells on Diff-quick stained, thin capillary blood smears. *Babesia gibsoni*, the small babesia of dogs, is typically found as single, annular bodies measuring 1 x 3.2 µm. The recognition of these small parasites require good staining technique and considerable practice, as many red blood cells in anaemic dogs are vacuolated and pitted. Blood smears are usually taken from the ear margin. However, the presence of the parasite in the intraerythrocytic membrane aided a confirmatory diagnosis.

The pathogenesis of the anaemia recorded is incompletely understood; intravascular and extravascular haemolysis take place, but other mechanisms such as poor bone marrow response are thought to play a role as well. (Jacobson *et al*., 1996) [13]. Laboratory investigations revealed the presence of normocytic and normochromic anaemia and marked cellular damage in some organs consistent with the reports of Irwin and Hutchinson (1991) [14] and Furlanello *et al*., 2005 [15]about canine babesiosis. The observed normocytic and normochromic anaemia might be due to intravascular and extravascular haemolysis resulting from direct parasite induced damage, increased osmotic fragility of infected RBCs, oxidative injury and activity of a secondary immune-mediated process. This in part might have caused the weakness, which could have been responsible for the progressive depression observed in the dog. *Babesia gibsoni* infection is characterized by fever, lethargy, haemolytic anaemia, thrombocytopenia, lymphadenopathy and splenomegaly (Conrad *et al*., 1991) [16]. These observations are similar to the findings of the present case.

Further laboratory findings include elevation of liver enzymes such as ALT and AST reflecting the concomitant hepatopathy in these cases. The elevated levels of ALT and AST were indicative of hepatocellular damage, cholestasis or biliary obstruction. Furlanello *et al*. (2005) [15] reported similar elevated ALT and AST levels in natural canine babesiosis. Also, the elevated ALT and AST may be due to the observed haemolysis as these enzymes are important haemoglobin evidenced by marked icterus in the dog (Furlanello *et al*., 2005) [15]. The increased serum urea level, as observed in the current case report, may have been due to hepatobiliary leakages owing to degenerative or necrotic changes, decreased renal elimination or urinary tract obstruction associated with advanced renal disease and reduction in glomerular filtration rate (GFR).

Gross pathological findings recorded in the case were similar to the findings of Sastri, 2008 who recorded marked spleenomegaly, hepatomegaly and haemorrhage on the mucosa of stomach.

There is pulmonary oedema, which is a severe and frequent complication of canine babesiosis. In the current case, pulmonary oedema was observed histologically by the presence of diffuse oedema of enlarged and fused alveoli with massive interseptal cellular infiltration. These are typical characteristics of interstitial pneumonia (Antoniou *et al*., 2014) [17]. Interstitial pneumonia may have been caused by either of two factors: Increased alveolar capillary permeability or increased hydrostatic pressure as a consequence of acute respiratory distress syndrome of complicated canine babesiosis (Jacobson & Clark, 1994) [18]. The observed focal haemorrhage and cellular infiltration within the myocardium of the affected dog might be due to the inflammatory response and anaemic hypoxia associated with canine babesiosis (Reyers *et al*., 1998) [19]. Similar haemorrhagic, necrotic and inflammatory responses have been reported in the hearts of dogs diagnosed with babesiosis (Dvir, Lobetti, Jacobson, Pearson, & Becker, 2004) [20]. Pathological changes recorded in the kidneys were similar to those reported by Sastri, 2008 who reported degenerative changes of tubular epithelium along with presence of haemoglobin, proteinacious and epithelial casts in the lumen. The increase in the level of BUN recorded in the case correlated well with the histopathological alterations observed in the kidneys.

In comparison to microscopy, PCR is a promising tool for detection and diagnosis of *B. gibsoni* in dogs with specificity and sensitivity of 100.0 per cent [Martin *et al*., 2006]. This finding is in agreement with that of [Abd Rani *et al*., 2011] who recorded a very low prevalence of 0.2 per cent for *B. gibsoni* by PCR in dogs in India, where as [Laha *et al*., 2014]

recorded a high prevalence of 48.64 per cent by PCR for *B. gibsoni* in dogs in India. 18S rRNA gene-based PCR assay was employed for detection of *B. gibsoni* in dogs [Lemos *et al*., 2012; Terao *et al*., 2015] [25, 26].

5. References

1. El-Bahnasawy MM, Khalil HH, Morsy TA. Babesiosis in an Egyptian boy aquired from pet dog, and a general review. Egypt Soc. Parasitol. 2011; 41:99-108.
2. Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: a world emerging. Infect Genet Evol. 2012; 12:1788-1809.
3. Anand KP, Anand A, Kashyap AS. Dr. Victor Babes, discoverer of Babesia. J Assoc. Physicians India. 2015; 63:80-81.
4. Telford SR. Reservoir competence of white-footed mice for *Babesia microti*. Spielman A J Med. Entomol. 1993; 30:223-227.
5. Luna LG. Histologic staining methods of the Armed Forces Institute of Pathology. 3rd edn. Mcgraw Hill Book Co., New York, 1968.
6. Inokuma H, Yoshizaki Y. Molecular survey of Babesia infection in dogs in Okinawa, Japan. Vet parasitol. 2004; 121:341-346.
7. Coles EH. Veterinary Clinical Pathology, 4th edn. W. B. Saunders Company, Piladelphia, London, 1986.
8. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals, 6th edn. Academic press, Cambridge, United states, 2008
9. Nalubamba KS, Mudenda NB, Namwila MW, Mulenga CS, Bwalya EC, M'kandawire E *et al*. A study of naturally acquired canine babesiosis caused by single and mixed Babesia species in Zambia: Clinicopathological findings and case management. J Parasitol. Res. 2015; 985015. https://doi.org/10.1155/2015/985015.
10. Lobetti RG. Babesiosis, in Infectious diseases of the dog and cat, 3rd edn. W. B. Saunders, Philadelphia, 2006.
11. Matsuu A, Ono S, Ikadi H, Uchide T, Imamura S, Onuma M. Development of a SYBR green real-time polymerase chain reaction assay for quantitative detection of *Babesia gibsoni* (Asian genotype) DNA. J Vet Diagnostic Investigation. 2005; 17:569-573.
12. Bhattacharjee K, Sarmah PC. Prevalence of haemoparasites in pet, working and stray dogs of Assam and North-East India: A hospital based study. Vet World. 2013, 874-878.
13. Jacobson LS, Reyers F, Berry WL, Viljoen E. Changes in haematocrit after treatment of uncomplicated canine babesiosis: a comparison between diminazene and trypan blue, and an evaluation of the influence of parasitaemia. J South African Vet Assoc. 1996; 67:77-82.
14. Irwin PJ, Hutchinson GW. Clinical and pathological findings of *Babesia* infection in dogs. Australian Vet J. 1991; 68:204-209.
15. Furlanello T, Fiorio F, Caldin M, Lubas G, Solano-Gallego L. Clinicopathological findings in naturally occurring cases of babesiosis caused by large form *Babesia* from dogs of Northeastern Italy. Vet Parasitol. 2005; 134:77-85.
16. Conrad P, Thomford J, Yamane I, Whiting J, Bosma L, Uno T *et al*. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. J American Vet Med Assoc. 1991; 199:601-605.
17. Antoniou KM, Margaritopoulos GA, Tomassetti S, Bonella F, Costabel U, Poletti V. Interstitial lung disease. European Respiratory Review. 2014; 23:40-54. https://doi.org/10.1183/09059180.00009113.
18. Jacobson LS, Clark IA. The pathophysiology of canine Babesiosis: New approaches to an old puzzle. J South African Vet Assoc. 1994; 65:134-145.
19. Reyers F, Leisewitz AL, Lobetti RG, Milner RJ, Jacobson LS, Van Zyl M. Canine Babesiosis in South Africa: More than 1 disease. Does this serve as a model for Falciparum malaria? Annals of Tropical Med and Parasitol. 1998; 92:503-511.
20. Dvir E, Lobetti RG, Jacobson LS, Pearson J, Becker PJ. Electrocardiographic changes and cardiac pathology in canine babesiosis. J Vet Cardiol. 2004; 6:15-23. https://doi.org/10.1016/S1760-2734(06) 70060-1.
21. Sastri GA. Veterinary Clinical Pathology, 3rd edn. CBS Publishers and Distibutors, Delhi, India, 1989.
22. Martin AR, Dunstan RH, Roberts TK, Brown GK. *Babesia canis* Vogeli: A novel PCR for its detection in dogs in Australia. Exp Parasitol. 2016; 112:63-65.
23. Abd Rani PAM, Irwin PJ, Coleman GT, Gatne M, Traub RJ. Survey of canine tick-borne diseases in India. Parasites and Vectors. 2014; 4:1-8.
24. Laha R, Bhattacharjee K, Sarmah PC, Das M, Goswami A, Sarma D. Babesia infection in naturally exposed pet dogs from a northeastern state (Assam) of India: detection by microscopy and polymerase chain reaction. J Parasitic dis. 2014; 38:389-393.
25. Lemos TD, Cerqueira AMF, Tomas HK, Silva AV, Correa RGB, Paludo GR. Detection and molecular characterization of piroplasms species from naturally infected dogs in Southeast Brazil. Revista Brasileira de Parasitologia Veterinaria. 2012; 21:137-142.
26. Terao M, Akter S, Yasin G, Nakao R, Kato H, Zahangir M. Infection, Genetics and Evolution Molecular detection and genetic diversity of *Babesia gibsoni* in dogs in Bangladesh, Mymensingh**.** Infection, genetics and evolution. 2015; 31:53-60.