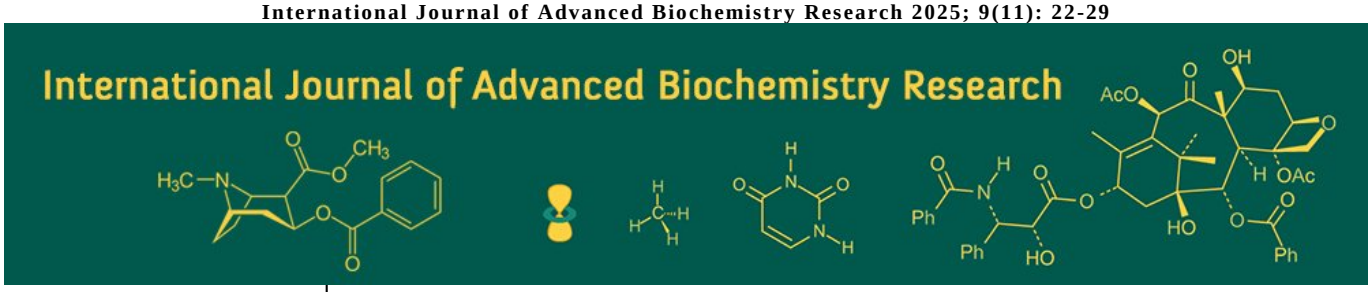

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Cerebral babesiosis in a calf: Diagnostic and Pathological findings

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Abstract
Babesiosis is a tick borne infectious disease caused by haemoprotozoan of genus *Babesia*. Occurrence of babesiosis is associated with the increased activity of the ticks during autumn and summer season. A carcass of one year old calf from Budaun was presented for necropsy examination with the history of dullness, high fever, tick infestation, and abnormal gait. All the visible mucus membrane were pale and yellowish discoloration of fascia was observed. Prescapular lymph nodes were enlarged and edematous and the cortical area had haemorrhages. Pericardial cavity was filled with about 20-30 ml of yellowish brown fluid and severe suffusive haemorrhages were seen in the endocardium of right ventricle. Anteroventral lobes of both the lungs were severely congested with patchy area of consolidation. Peritoneal cavity was filled with about 100-150 ml of straw colour fluid. Liver and spleen were enlarged and gall bladder was distended with about 60-80 ml of dark viscid yellowish-green bile. Brain showed cherry red discolouration with congested cerebral blood vessels. Urinary bladder was distended with about 80-100 ml reddish brown urine. Centrifugation of urine at 1500 rpm for 10 min revealed red coloured supernatant with no precipitate indicating haemoglobinuria. Microscopic examination of Giemsa stained blood smear from the peripheral blood vessels revealed piroplasm of *babesia*in more than 10-20% of RBCs under oil immersion. Histopathological examination of the lungs tissues revealed vascular congestion and alveolar edema alongwith desquamation of the bronchiolar epithelium. Heart showed myocardial degeneration and congested myocardial blood vessels. Centrilobular necrosis was seen in the liver and the sinusoids were dilated. Mild to moderate lymphoid depletion was seen in the lymph nodes and spleen. Severe tubular degeneration was seen in the kidney with medullary congestion and renal tubules were filled with eosinophilic homogenous fluid. Cerebral vessels were severely congested and satellitosis and neuronophagia was seen in the grey matter. Hemosiderin pigment accumulation was observed in Hematoxylin & Eosin stained. Tissue sections of liver, spleen, lymph node and lungs revealed brownish granular deposit of hemosiderin, which on Pearl's prussian blue staining were observed as bluish deposits. Microscopic examination of the tissues revealed piroplasms within erythrocytes in blood capillaries in the sections of lymph nodes and cerebrum. Based on the gross and microscopic examination the case was diagnosed as babesiosis. *Babesia*is associated with destruction of erythrocytes leading to hypoxia, anoxia and death.

Keywords: Anaemia, babesiosis, blood smear examination, cerebral congestion, haemoglobinuria

Introduction
Bovine babesiosis is a febrile, tick-borne disease of cattle and buffalo caused by intraerythrocytic protozoa of the genus *Babesia*, belonging to the family *Babesidae* and order *Piroplasmida*, within the phylum *Apicomplexa*. The organisms infect a wide range of domestic and wild animals and occasionally humans. The parasite reproduces asexually within the erythrocytes of the vertebrate host, producing two or more trophozoites. Infected red blood cells then release trophozoites, which invade and infect additional erythrocytes (Soulsby, 1882) [18].

Bovine babesiosis is mainly caused by *Babesiabigemina*, *B. bovis*, and *B. divergens*, which are the three most common pathogenic species (Fakhar *et al.*, 2012) [6]. In cattle, *B. bigemina* and *B. bovis* are the most economically important (Bock *et al.*, 2004) [2]. Infection with *B. bovis* usually results in a more severe clinical disease than with *B. bigemina* (Gubbels *et al.*, 1999) [8]. Morphologically, *B. bovis* resides centrally in red blood cells, measuring approximately 1.1-1.5 × 0.5-1.0 µm, whereas *B. bigemina* is larger, often appearing as

paired, pear-shaped organisms measuring 1-1.5 µm in width and 3-3.5 µm in length (Soulsby, 1986; El Sawalhy, 1999) [19, 5].

The acute form of the disease is characterized by rapid parasitic proliferation in the bloodstream, severe erythrocyte destruction, and resultant anemia, icterus, hemoglobinuria, splenomegaly, and sometimes death (Deepak *et al.*, 2019) [4]. Economically, babesiosis has major significance in livestock production, leading to losses estimated at 57.2 million USD annually in India (McLeod and Kristjanson, 1999) [13]. In Indian cattle, *B. bigemina* is the predominant species, while *B. bovis* has been less frequently reported (Shastri *et al.*, 1991; Gautam and Chhabra, 1983; Muraleedharan *et al.*, 1984; Maharana *et al.*, 2016) [17, 7, 14, 12].

The major pathogenic mechanisms involve intravascular hemolysis and hemoglobinuria caused by *B. bigemina* infection, whereas *B. bovis* induces cerebral babesiosis primarily through excessive cytokine production—especially IL-6 and TNF-α. Although the thin blood smear remains the traditional and gold-standard diagnostic method for babesiosis and related blood protozoan infections, its sensitivity is limited (Kaur *et al.*, 2016) [10].

Cattle infected with *B. bovis* may exhibit sequestration of parasitized erythrocytes in cerebral capillaries, resulting in a severe neurological form of the disease.

Materials and Methods

Case History and Necropsy Examination

A one-year-old male non decript breed calf was submitted for post-mortem examination with a clinical history of high fever, lethargy, recumbency, nervous manifestations, and heavy tick infestation. The necropsy was carried out following standard veterinary diagnostic guidelines. External evaluation focused on body condition, mucosal appearance, and surface abnormalities prior to skin removal. After skinning, visceral organs were thoroughly inspected for gross lesions, vascular changes, and presence of abnormal fluid accumulations.

Tissue Sampling and Histopathological Processing

Representative tissue specimens from the liver, spleen, kidneys, lungs, heart, lymph nodes, and brain were collected immediately after necropsy. These samples were fixed in 10% neutral buffered formalin, processed through the paraffin-embedding technique, sectioned at 4-5 µm thickness, and stained with hematoxylin and eosin (H&E) for routine microscopic evaluation.

Blood and Urine Analysis

Peripheral blood was obtained from major blood vessels to prepare thin smears. The smears were air-dried, fixed in methanol, stained with Giemsa stain, and microscopically examined for intraerythrocytic piroplasms suggestive of *Babesia*species infection. Urine was aseptically aspirated from the urinary bladder, centrifuged at 15,000 rpm for 3 minutes, and the supernatant was evaluated for hemoglobinuria based on its coloration.

Special Staining Techniques

Selected tissue sections were subjected to special histochemical stains. Giemsa staining confirmed the occurrence of *Babesia*organisms within red blood cells in kidney and lymph node sections. Pearls' Prussian blue staining was performed on liver, spleen, and lung sections to demonstrate haemosiderin deposition.

Microscopic Evaluation

Histological slides were examined under a light microscope, and microscopic alterations were documented at varying magnifications. Observations under oil immersion (100×) were made to visualize intraerythrocytic *Babesia*organisms and assess cellular lesions in affected tissues.

Results

A 1 year old Non Descript male calf was presented for necropsy examination with the history of high fever, dullness, unable to stand (nervous signs) and tick infestation. External examination revealed fair carcass condition, good nutritional status, and rigor mortis was present in hind limb. Skinning of carcass showed yellowish discoloration of fascia on lateral abdomen area. Conjunctival and oral mucous membrane were pale and perianal area were soiled with mucoid diarrhoeic content.

Gross lesions revealed accumultion of 5-10ml straw colour fluid within the peritoneal cavity. Prescapular lymph nodes were found enlarged and oedematous with patechiation, diffuse haemorrhages and congestion within the cortex (Fig. 1). Urinary bladder was distended and filled with 2-3 litre brownish red urine and mucosa was congested (Fig. 2). Kidney showed severely congested cortical and medullary region on cut section (Fig. 3). Lungs displayed severely congested and consolidated anteroventral lobes (Fig. 4). Brain exhibit markedly pink red discolouration (Fig. 6). Heart depicted severe suffusive haemorrhages in endocardium of right ventricle (Fig. 5).

Blood was collected from peripheral blood vessels and a smear was made. Giemsa staining of the blood smear revealed that 10-20% of RBC were found to be infected with piroplasm *babesia*in 100x magnification (Fig. 7). Urine was collected from distended urinary bladder and was centrifuged at 15000rpm for 3min exhibited red supernatant indicating presence of haemoglobinuria (Fig. 8).

Histopathological examination of H&E stained tissue section showed lymphoid depletion in lymphatic nodule within cortex and severely congested blood vessels within medullary region of lymph node. Severe infiltration of neutrophils were found within the medullary sinus of lymph node (Fig. 9). Kidney revealed severe tubular degeneration with pink homogeneous fluid deposition within the tubules and severe vascular congestion in medullary region. Liver exhibited centrilobular hepatocyte necrosis and degeneration with dilated sinusoids containing neutrophils and mononuclear cells (Fig. 10). Massive vascular congestion and distension of alveoli with homogenous eosinophilic fluid deposition observed in lungs. Brain section showed satellitosis, perineuronal oedema, dilated and severely congested cerebral blood vessles within the grey matter (Fig. 11, 12, 13). Spleen exhibited mild lymphoid depletion in white pulp with markedly congested red pulp containing mononuclear cells and neutrophils (Fig. 14). Microscopic examination under oil immersion of H&E stained tissue section revealed presence of *babesia*like organism within RBC in lymph node and brain (Fig. 15 and 16). Liver, lymph node, lungs showed positive Pearls Prussian blue staining with blue colored haemosiderin pigment deposit within the macrophages (Fig. 17).

Diagnosis

Morphological diagnosis revealed enlarged and hemorrhagic superficial lymph nodes, Subepicardial & endocardial patechiae, marked cystomegaly with reddish brown urine, marked congestion of various organs, cherry red discolouration of brain. Blood smear examination confirms the etiological agent as *Babesia*. Since brain was infected and exhibited marked lesions, this case can also be referred as cerebral babesiosis.

Discussion

Natural infections and experimental inoculations with *Babesiabovis* in calves have been shown to produce neurological symptoms accompanied by significant pathological alterations in the nervous system (Wright *et al*., 1979) [24]. In cerebral babesiosis, the grey matter of the brain often exhibits a distinctive cherry-pink coloration. This hue results from vasodilation, vascular stasis, and hypotension, associated with changes in the kallikrein-kinin system during infection (Bhikane *et al.*, 2001) [1]. These vascular disturbances promote erythrocyte sequestration within capillaries of multiple organs to varying extents (Rogers, 1971; Wright *et al.*, 1979) [16, 24]. In the acute form of *B. bovis* infection, there is rapid parasite multiplication in the bloodstream, leading to extensive destruction of erythrocytes, severe anemia, jaundice, hemoglobinuria, splenomegaly, and often death (Deepak *et al.*, 2019) [4].

Acute infections typically present with pronounced organ congestion and membrane-associated hemorrhages, whereas chronic infections are characterized predominantly by anemia and jaundice. Hypoglycemia has also been reported in terminal stages of the disease (Wright, 1971) [23]. In severe cases, excessive inflammatory responses or "cytokine storms" contribute to pathogenesis (Clark and Jacobson, 1998; Hemmer *et al.*, 2000; Krause *et al.*, 2007) [3, 9, 11]. Overproduction of TNF-α and IL-1 by Th1 cells and macrophages during high parasitemia can precipitate acute respiratory distress syndrome (ARDS) and multi-organ failure (Hemmer *et al.*, 2000) [9]. High virulence strains of *B. bovis* exhibit increased cytoadherence potential *in vitro*, which heightens susceptibility in cattle (O'Connor *et al.*, 1999) [15]. Elevated parasitemia can result in vascular occlusion, tissue hypoxia, and neuronal death due to adhesion of parasitized erythrocytes in cerebral microvasculature. Erythrocyte lysis during merozoite release further drives fever, anemia, jaundice, hemoglobinemia, hemoglobinuria, tissue hypoxia, and renal impairment (Vannier and Krause, 2012; Vannier *et al.*, 2015) [22, 21].

The clinical history in the present case—high fever, nervous signs, tick infestation, and pallor of mucous membranes—matches the classic presentation of cerebral babesiosis as documented in earlier reports, including lethargy, anemia, and motor incoordination resulting from cerebral involvement (Wright *et al.*, 1979; The Cattle Site, 2022) [24, 20]. The yellowish discoloration of fascia and mucosa is indicative of icterus, a manifestation of massive hemolysis and erythrocyte destruction (Deepak *et al.*, 2019) [4]. The gross necropsy findings, particularly the accumulation of straw-colored fluid in body cavities, hemorrhagic lymphadenopathy, marked congestion of the kidneys and lungs, and cherry-pink discoloration of the brain, reflect severe vascular compromise and sequestration of parasitized erythrocytes, leading to hypoxia and widespread tissue injury (Bhikane *et al.*, 2001; Rogers, 1971) [1, 16].

Histopathological lesions—including lymphoid depletion and neutrophil infiltration in lymph nodes, renal tubular degeneration, centrilobular hepatic necrosis, pulmonary congestion with eosinophilic fluid in alveoli, and neuronal damage characterized by satellitosis and neuronophagia—are consistent with systemic inflammatory responses and focal ischemic injury induced by *Babesia*infection (Clark and Jacobson, 1998; Hemmer *et al.*, 2000) [3, 9]. Detection of *Babesia*organisms within RBCs in lymphatic and brain tissues confirms parasite dissemination and multi-organ involvement.

Pearls' Prussian blue staining positively identified haemosiderin deposits in macrophages of the liver, spleen, and lungs, confirming ongoing erythrocyte destruction and iron sequestration, a typical feature of hemolytic diseases like babesiosis (Vannier and Krause, 2012) [22]. Severe hemoglobinuria observed in the urine corroborates intravascular hemolysis, liberating free hemoglobin—a hallmark of acute *B. bovis* infection with renal complications.

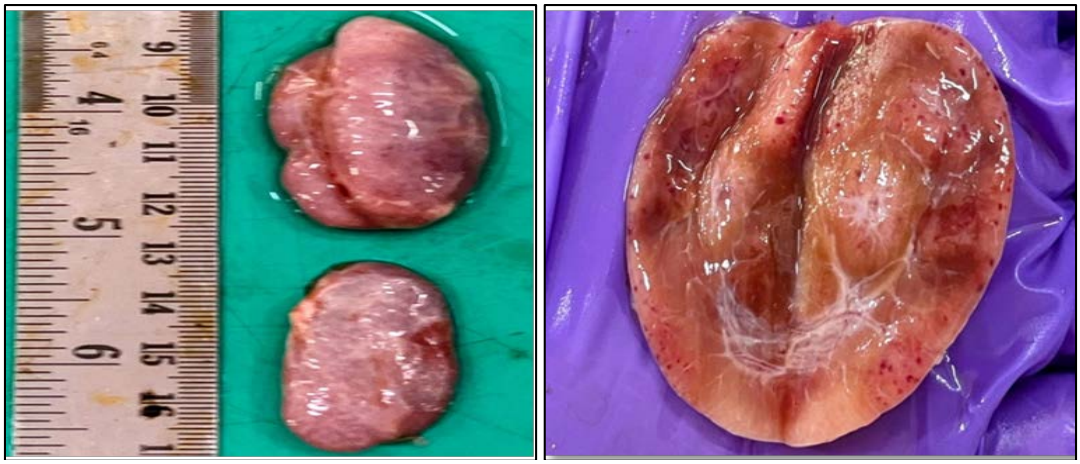

Fig1: Prescapular lymph node: Enlarged and oedematous wih cortex showing patechial and diffuse haemorrhages and Congestion

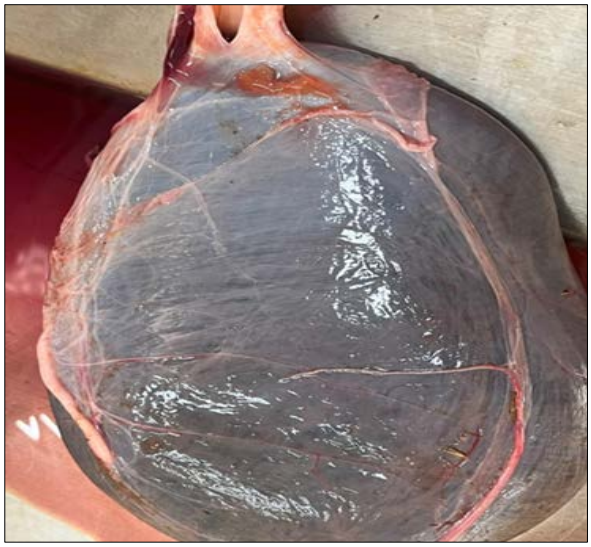

Fig 2: Urinary bladder distended and filled with brownish red urine and mucosa was seen mildly congested

Fig 3: Kidney showed severely congested cortical and medullary region of kidney

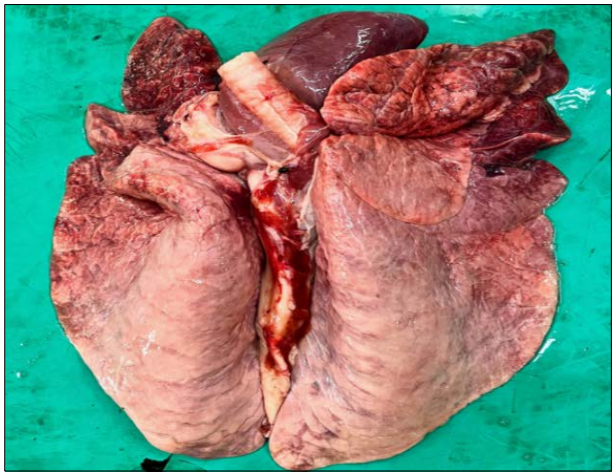

Fig 4: Lungs showed Severely congested and consolidated anteroventral lobes of lungs

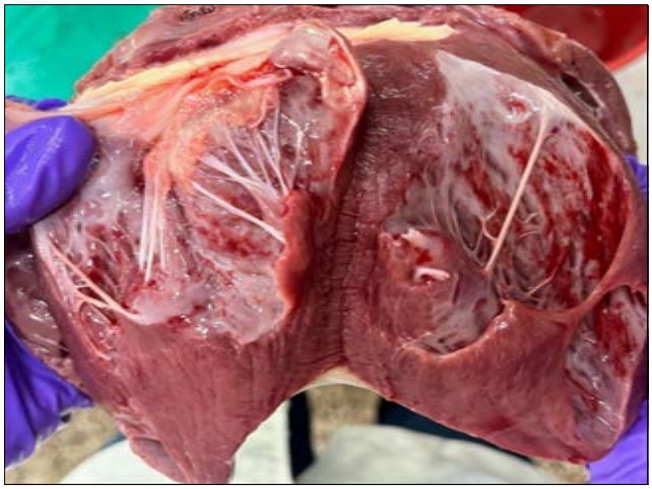

Fig 5: Heart: Showing severe suffusive haemorrhages in the endocardium of right ventricle

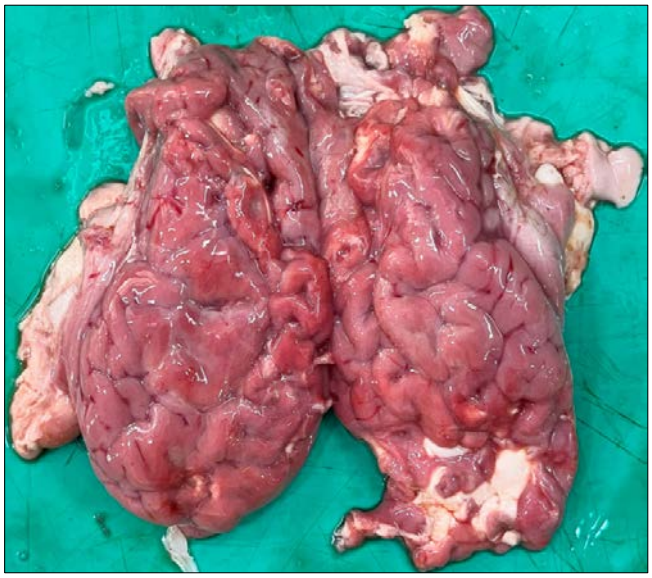

Fig 6: Brain: showing markedly pink red discolouration

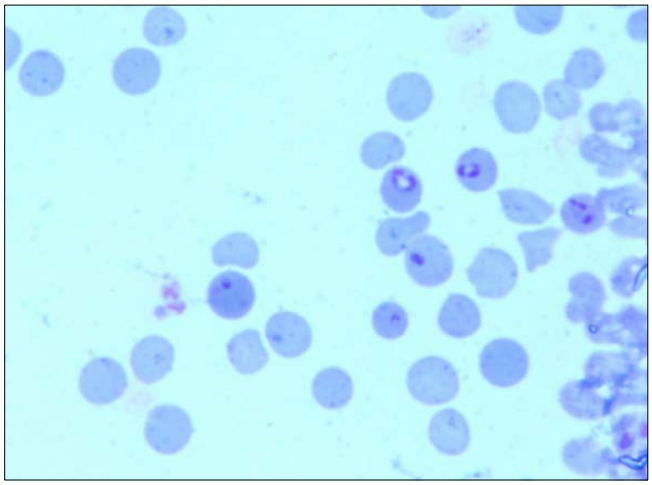

Fig 7: Upto 10-20 % of rbc were found infected with Piroplasm *Babesia*in 100X

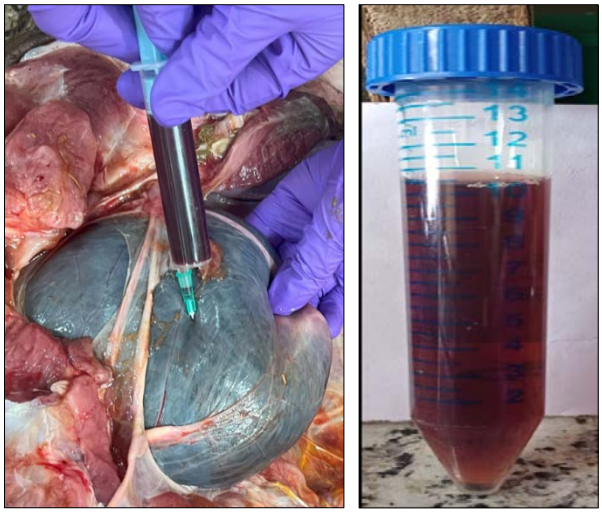

Fig 8: Reddish brown discoloured urine was collected from distended urinary bladder; Red supernatant after centrifugation indicating presence of haemoglobinuria

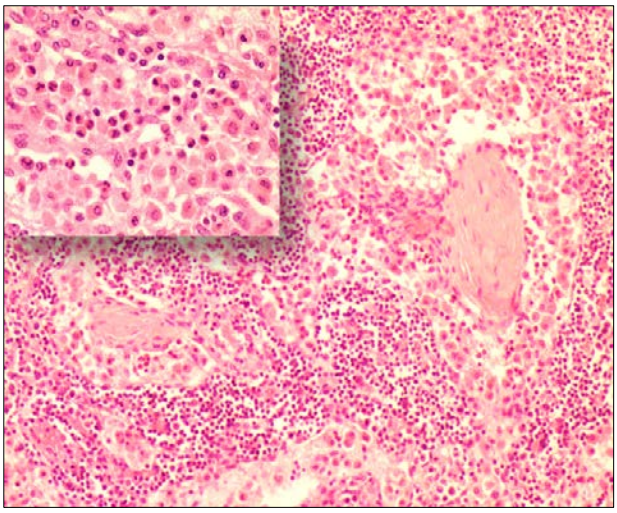

Fig 9: Lymph node: Showing accumulation of Neutrophils in the medullary sinus. 10X, 40X H&E

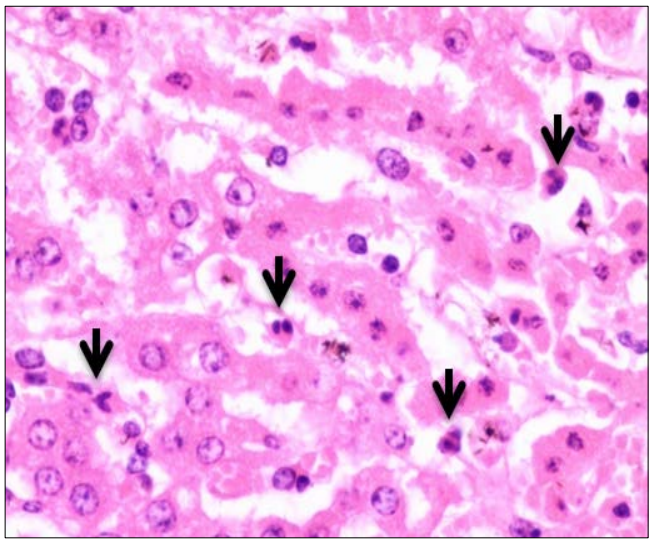

Fig 10: Liver: Showing centrilobular hepatocyte necrosis and degeneration with dilated sinusoids containing neutrophils and mononuclear cells 40X H&E.

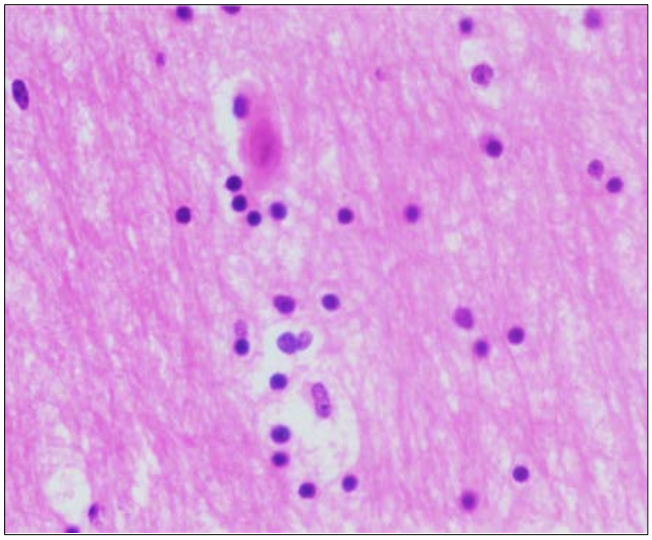

Fig 11: Brain: Grey matter showing satellitosis and neuronophagia 40X H&E

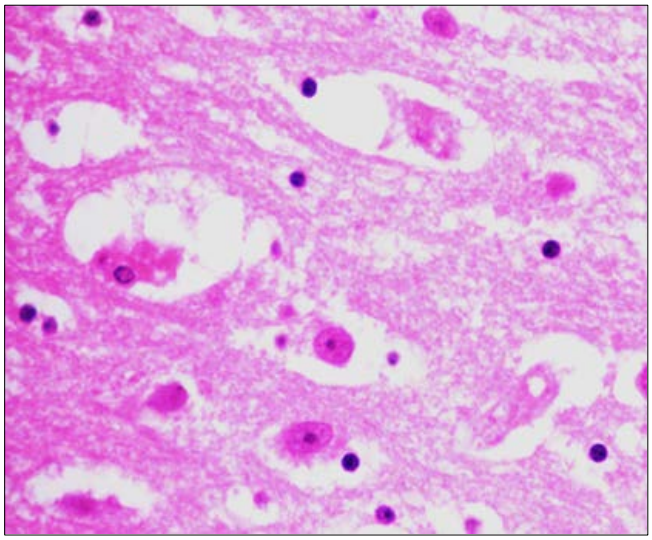

Fig 12: Brain: Grey matter showing perineuronal oedema 40X H&E

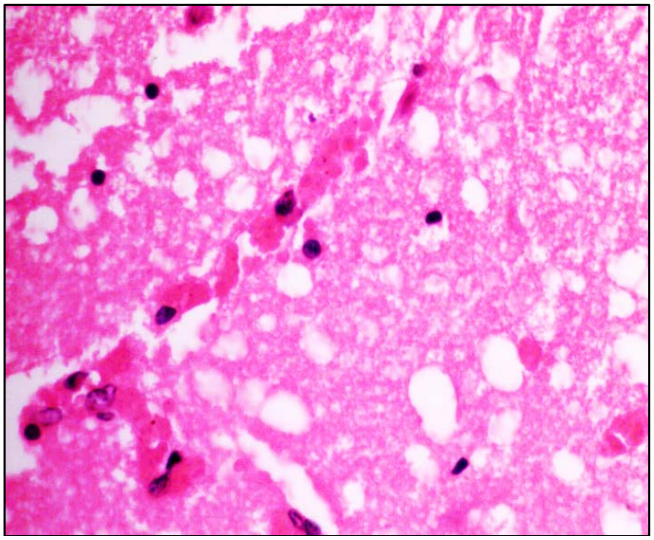

Fig 13:1 Brain: dilated and severely congested cerebral blood vessles. 40X H&E

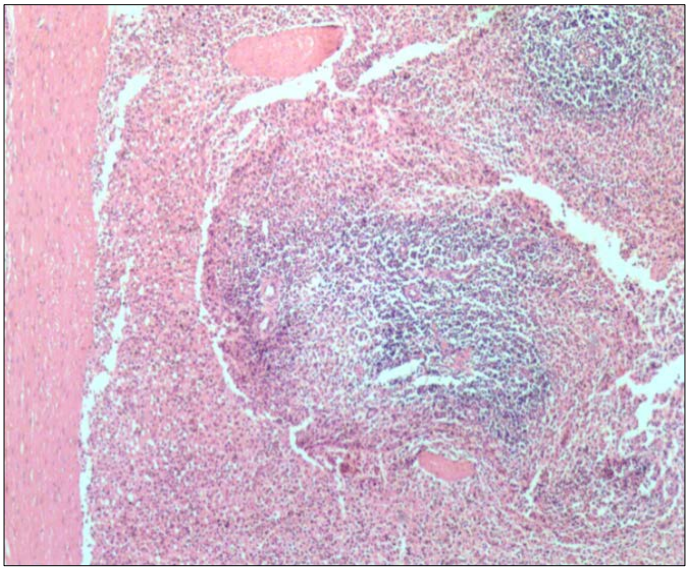

Fig 14:2 Spleen: Showing mild lymphoid depletion in white pulp with markedly congested red pulp. 10X H&E

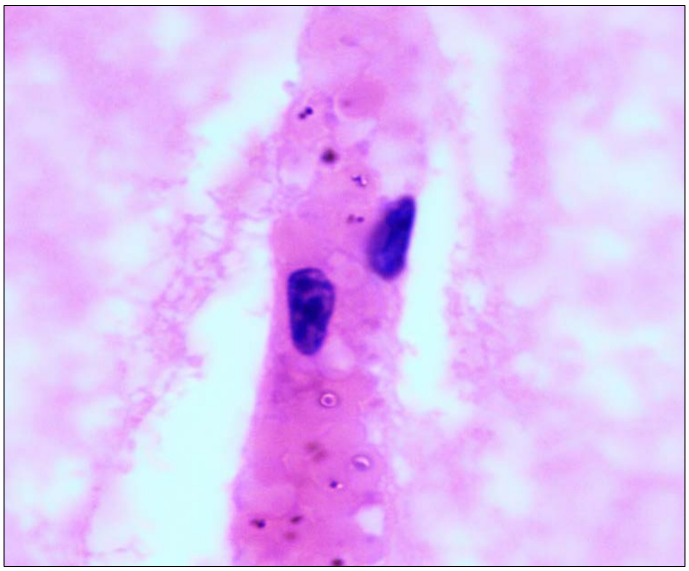

Fig 35: Brain: showing dilated and congested capillaries with RBCs containing piroplasm *Babesia*within it. 100X H&E

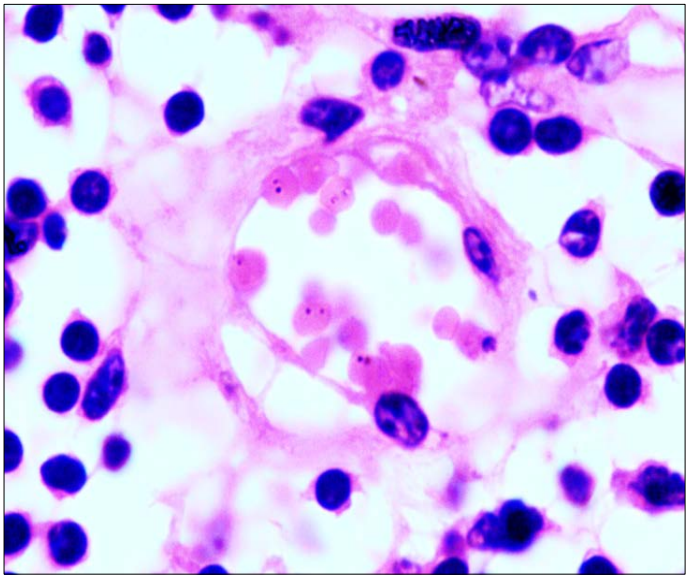

Fig 46: Lymph node (medulla): showing infected RBCs containing piroplasm *babesia*organism in it. 100X H&E

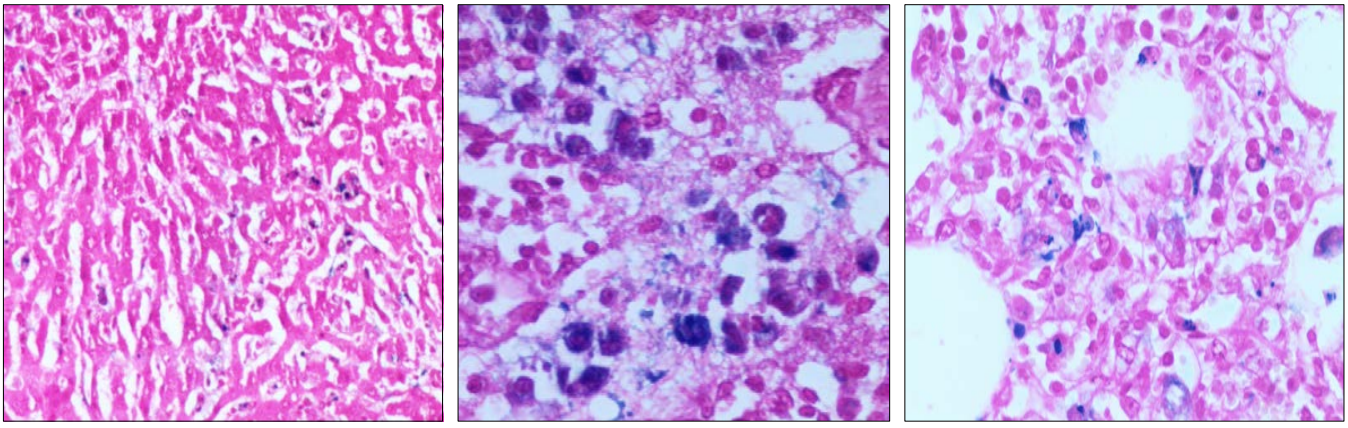

Fig 17: Liver, lymph node, lungs showing positive Pearls Prussian blue staining respectively with blue colored haemosiderin pigment deposit within the macrophages

Conclusion

In summary, the case represents a classical presentation of cerebral babesiosis compounded by systemic hemorrhagic and hemolytic pathology. The coherence between clinical signs, gross and microscopic lesions, and parasitological findings supports the diagnosis and parallels descriptions of *B. bovis* pathogenesis in literature, including vascular pathology, immune-mediated damage, and organ failure linked to parasite proliferation, red blood cell adhesion to microvascular endothelium, and cytokine-mediated tissue injury. This underscores the necessity for early diagnosis, effective tick control measures, and integrative clinicopathological approaches to mitigate disease severity.

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