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Canine babesiosis in a male Boerboel: Hematobiochemical and anatomic pathological changes in the cardiorespiratory and reproductive organs

Azubuike S. Ubah[a], Samson E. Abalaka[b], Idoko S. Idoko[b], Sunday S. Obeta[c], Charles E. Ejiofor[c], Philip P. Mshelbwala[d], Jude N. Omeje[d], Itopa E. Ajayi[e],*

[a] Department of Theriogenology, University of Abuja, Abuja, Nigeria
[b] Department of Veterinary Pathology, University of Abuja, Abuja, Nigeria
[c] Department of Veterinary Parasitology and Entomology, University of Abuja, Abuja, Nigeria
[d] Department of Veterinary Medicine, University of Abuja, Abuja, Nigeria
[e] Department of Veterinary Anatomy, University of Abuja, Abuja, Nigeria

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ABSTRACT

A 9-month old male Boerboel was presented at the University of Abuja Small Animal Clinic with a primary complaint of inappetence and micturition with brownish-yellow urine. Physical examination revealed pale mucous membranes, enlarged pre-scapular lymph nodes, bilateral ocular discharges, pyrexia (41.2 °C), depression and the presence of ticks of the genus *Rhipicephalus sanguineus* on the body of the animal. History revealed that the dog was recently vaccinated against canine distemper, hepatitis, leptospirosis, parainfluenza, parvovirus (DHLPP) and rabies six days prior to the onset of clinical signs, and had been anorexic. Following clinical examination, blood and faecal samples were collected for 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. With appropriate consent, a post-mortem examination was carried out. Diffuse oedema with interstitial pneumonia in the lung, focal haemorrhage with cellular infiltration in the heart and progressive necrosis of epithelial cells within the seminiferous tubules of the testicles were observed. Many of the observed clinicopathological alterations were consistent with complicated babesiosis. However, in the current case, some unique systemic complications such as testicular degeneration, which has not been previously observed in the Boerboel are discussed.

1. Introduction

Canine babesiosis, formally known as canine piroplasmosis, is a relatively common haemoprotozoan infection in dogs (Köster, Lobetti, & Kelly, 2015; Solano-Gallego, Sainz, Roura, Estrada-Peña, & Miró, 2016). 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, Anand, & Kashyap, 2015). However, over 100 species of Babesia have since been reported in vertebrate hosts and a significant amount of infections found in dogs all around the world (Gray, 2004). In dogs, the disease is caused by numerous *Babesia* species including *Babesia canis, Babesia gibsoni, Babesia rossi* and *Babesia vogeli* (Ayoob, Hackner, & Prittie, 2010; Solano-Gallego et al., 2016) with infections that could be transmitted horizontally through tick bites, blood transfer from a dog bite, a blood transfusion, or vertically, across the placenta from an infected bitch to puppies (Köster et al.,

2015).

Ticks are the most popular vectors for the *Babesia spp.*, and they provide a conducive environment for sexual conjugation and the sporogony stages of their life cycle. These stages of the life cycle typically occur within the intestinal lumen and subsequently in the haemocoel of the tick (Solano-Gallego et al., 2016). Following a bite from an infected tick, an animal is infected with sporozoites from the tick's salivary gland. The later stages of the life cycle of the protozoan are then completed by asexual replication within erythrocytes (Mehlhorn & Schein, 1985; Shortt, 1973; Solano-Gallego et al., 2016), where the parasites appear as merozoites and are easily detectable under the light microscope. Rapid replication of merozoites within the red blood cells often results in lysis and consequently signs of anaemia, septicaemia and pyrexia amongst other symptoms (Solano-Gallego et al., 2016), to produce a disease syndrome that could be fatal. All the mentioned symptoms were observed in the current case but with the unique

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* Corresponding author.
  E-mail address: i.ajayi@uq.edu.au (I.E. Ajayi).

peculiarities of damage to reproductive organs and cardiorespiratory involvements, which forms the novelty of the current report.

2. Case description

2.1. History

A 9-month old male Boerboel weighing 55 kg was presented at the Small Animal Clinic of the Veterinary Teaching Hospital, University of Abuja, Abuja, Nigeria, with the complaints of being off feed and depressed for approximately 3 days. History further revealed that the dog was vaccinated with 1 ml of DHLPP® subcutaneously (SC) and 1 ml of rabies vaccine intramuscularly (IM) six days prior to the onset of clinical presentation.

2.2. Clinical examination

On clinical and physical examination, the dog had pyrexia (41.2 °C), pale ocular mucous membranes, bilateral epiphora, bilateral enlargement of pre-scapular lymph nodes, a heavy tick infestation and it voided brownish-yellow urine. Blood and faecal samples were collected and sent for haemoparasitic, haematological and biochemical evaluations, respectively on the fifth day of presentation. The ticks were also collected and sent to the Department of Veterinary Parasitology and Entomology, University of Abuja, Abuja, Nigeria, for identification. A summary of the samples collected, the quantity collected and the purpose of the collection is presented in Table 1. The dog was reportedly treated with Piroxicam (0.3 mg/kg, IM), Penicillin-Streptomycin (200,000 IU and 200 mg, IM) and Vitamin B-Complex (2 ml, IM) for the first three days followed by Ceftriaxone or Ceftron-Vet® (25 mg/kg, IM), 10% Dextrose saline (200 ml, IV), Vitamin B-Complex (0.5 ml, IM) and Iron dextran (2 ml, IM) for another 3 days.

Based on the history, signalment and initial clinical examination, a tentative diagnosis of Canine Babesiosis was made. However, our differential diagnoses were Ehrlichiosis and Hepatozoonosis both of which are haemoparasitic infections that present similar signs, are transmitted by tick vectors and are endemic in tropical Africa (Kelly, 2000; Klag, Dunbar, & Girard, 1991; Schnittger, Rodriguez, Florin-Christensen, & Morrison, 2012). Thus, it became important to conduct laboratory examinations to isolate the specific disease.

2.3. Laboratory examination

Three (3) ml of blood was collected from the left cephalic vein of the dog. Thin blood smears were made and stained with Giemsa for haemoparasitic evaluation. The remaining blood sample was analysed for total red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV) and haematological indices (MCV, MCH and MCHC), as well as white blood cell (WBC), counts and differential WBC counts as described by Dacie and Lewis (1991). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, sodium (Na), potassium (K), chloride (Cl) and bicarbonate (HCO3) contents of the serum were also analysed using Standard Randox assay kits (Randox Laboratory Ltd., United Kingdom BT29 4QY). Gamma-glutamyl transferase (GGT) was determined colourimetrically using a commercial kit from Sigma-Aldrich

Co., St. Louis, USA.

Despite symptomatic therapy, the dog died six days later as it was being prepared for Imidocarb dipropionate administration at 5.0 mg/kg IM twice 14 days apart based on the laboratory result. Consequently, the carcass was presented for a detailed post-mortem examination.

2.4. Post-mortem examination

With appropriate consent, a post-mortem examination was carried out immediately after death. During the examination, cardiorespiratory and reproductive organs were assessed based on the gross lesions observed. Tissue samples from the lungs, heart and testes were harvested and fixed in 10% neutral buffered formalin before preparation and processing according to standard histologic procedures (Slaoui & Fiette, 2011) in order to determine histopathological changes within them under a light microscope at varying magnifications.

2.5. Outcome

Microscopic examination: Giemsa stained thin blood smears showed the presence of intraerythrocytic *Babesia parasites* with an approximate 5% degree of parasitaemia.

Haematology: The haematological result revealed the presence of normocytic normochromic anaemia as presented in Table 2. The ALT, AST, ALP, GGT and urea levels were elevated but serum creatinine, Na and Cl levels were decreased whereas total serum bilirubin, K and HCO3 were within the normal range as shown in Table 3.

Post-mortem: At post-mortem examination, gross signs of pulmonary oedema, hepatomegaly, kidney damage, and splenomegaly were observed (Fig. 1). Inflammation of the intestines was also observed with patches of haemorrhages in the mesentery and cardiac musculature.

Histopathology: Histopathological examination of the lung showed enlarged and oedematous alveoli, which were mostly fused with the presence of marked inter-septal cellular infiltration (Fig. 2d). The myocardium had focal haemorrhage with cellular infiltration (Fig. 2b). Similarly, the testes revealed diffuse and progressive necrosis of the seminiferous tubular epithelia of the affected dog (Fig. 2c).

Parasitology: The ticks were identified as *Rhipicephalus sanguineus*.

A definitive diagnosis of acute canine babesiosis complicated with multiple organ involvement was, therefore, made primarily based on the demonstration of intra-erythrocytic *Babesia parasites* in the thin blood smears of the dog (Fig. 2a), the size and shape of the organisms observed, clinical manifestations and pathological changes.

3. Discussion

Canine babesiosis is a life-threatening tick-borne haemoparasitic disease of dogs caused by intraerythrocytic organisms of the genus *Babesia* and reported worldwide (de Marco et al., 2017; Laha et al., 2014). The current case is reported from Nigeria, West Africa although the dog species is not indigenous to the region. However, the dog belonged to an F4 generation of a breeding line that has existed for approximately ten years in the West African region. Thus, the animal, including its pedigree have had significant exposure to endemic diseases in the region. Suffice to say that the medical records of the dogs'

Table 1
Samples collected from the 9-month old Boerboel that was suspected of carrying haemoparasites.

S/no.	Sample type	Quantity collected	Purpose of examination
1.	Blood	3 ml	Thin blood smear and biochemical analysis
2.	Faeces	200 g	Assessments for the presence of gut parasites
3.	Whole ticks	5	Identification
4.	Tissue samples (testes, cardiac muscles and lungs)	200–300 g	Tissue processing for histopathological analysis

Table 2
The haematological profile of a 9-month old Boerbeol diagnosed with complicated canine babesiosis.

S/no.	Haematological parameters	Values	Reference range (Blood et al., 2007)
1.	Red blood cell (RBC) count	2.90×10^{12} g/L	$6.00{-}9.00 \times 10^{12}$ /L
2.	Packed cell volume (PCV)	0.20 L/L	0.37–0.55 L/L
3.	Haemoglobin concentration (Hb)	67.00 g/L	120.00–180.00 g/L
4.	Mean corpuscular volume (MCV)	68.90 fL	60.00–77.00 fL
5.	Mean corpuscular haemoglobin (MCH)	23.10 pg	20.00–25.00 pg
6.	Mean corpuscular haemoglobin concentration (MCHC)	335.00 g/L	320.00–360.00 g/L
7	White blood cell (WBC) count	8.90×10^{9} /L	$6.00{-}17.00 \times 10^{9}$ /L
8.	Neutrophil	7.12×10^{9} /L	$3.00{-}11.50 \times 10^{9}$ /L
9.	Lymphocyte	1.78×10^{9} /L	$1.00{-}4.80 \times 10^{9}$ /L
10.	Eosinophil	0.00×10^{9} /L	$0.10{-}1.30 \times 10^{9}$ /L
11.	Basophil	0.00×10^{9} /L	Rare
12.	Monocyte	0.00×10^{9} /L	$0.20{-}1.40 \times 10^{9}$ /L

filial contain reports of successful treatments of babesiosis. However, in the current instance, there was an apparent overwhelm of the organ-systems beyond the immune system threshold.

The mode of transmission/infection of the organism in the present case was unclear. However, the typical signs of canine babesiosis such as pyrexia, anorexia, depression, pallor of ocular membranes and lymphadenopathy (Nalubamba et al., 2015) were exhibited. Some other lesions that could be associated with babesiosis including icterus, hepatomegaly, splenomegaly, pulmonary oedema, acute respiratory disease syndrome and coagulopathy (Jacobson & Clark, 1994) were also observed at post-mortem. However, the presence of the parasite in the intraerythrocytic membrane aided a confirmatory diagnosis. Respiratory and cardiovascular lesions were also identified, and suspected as potential causes of the respiratory disease syndromes and the septicaemic shock that was observed. It is also important to note that the observed testicular damage in the current case suggests that canine babesiosis may be a significant contributor to male infertility in dogs and therefore, a need for the current report.

Testicular necrosis is a striking novelty (Fig. 2c) and an indication that canine babesiosis is a potential infertility risk factor in male dogs. The mechanisms that resulted in the observed testiculopathy are unclear. However, the authors infer that the observed haemolytic anaemia could have generated a situation of oxidative stress arising from either tissue iron overload, disruptions of the testicular microcircuitry or inflammatory responses. Of course, activated neutrophils play crucial roles in the production of reactive oxygen species, proteases and elastases, and these enzymes possess the capacity to result in dysfunctions of endothelial cells, microvasculopathies (Lee, Quinn, Jennings, & Ng, 2003) and consequently, tissue necrosis. Moreover, a reduction in blood flow is capable of instigating necrosis and consequently impairing the process of spermatogenesis (Wei, Yan, & Zhou, 2011). Perhaps future *in vivo* studies could be designed to investigate these theories further and establish a link between the potential causes of tissue necrosis and other observed systemic disorders.

As part of the syndromes of respiratory disease is pulmonary

oedema, which is a severe and frequent complication of canine babesiosis, and is characterised by dyspnoea and nasal discharges. 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). 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). 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), while the observed necrotic destruction of the spermatogonia and primary spermatocytes could significantly affect spermatogenesis and therefore, the reproductive ability and capacity of male dogs infected with *Babesia* parasites. Similar haemorrhagic, necrotic and inflammatory responses have been reported in the hearts of dogs diagnosed with babesiosis (Dvir, Lobetti, Jacobson, Pearson, & Becker, 2004).

In addition to the histologic lesions, our 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) and Furlanello, Fiorio, Caldin, Lubas, and Solano-Gallego (2005) about complicated 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.

The elevated levels of ALT, AST, ALP and GGT were indicative of hepatocellular damage, cholestasis or biliary obstruction. Furlanello et al. (2005) reported similar elevated ALT, AST and ALP levels in natural canine babesiosis. Also, the elevated ALT and AST may be due to the observed haemolysis as these enzymes are important

Table 3
The blood chemistry profile of a 9-month old Boarbeol diagnosed of complicated canine babesiosis.

| S/no.	Blood chemistry parameters	Values	Reference range (Blood et al., 2007)
1.	Alanine aminotransferase activity (ALT)	261.0 U/L	17.0–69.0 U/L
2.	Aspartate aminotransferase activity (AST)	58.0 U/L	12.0–37.0 U/L
3.	Alkaline phosphatase activity (ALP)	79.0 U/L	5.0–73.0 U/L
4.	Gamma-glatamyl transferase (GGT)	17.0 IU/L	0.0–11.0 U/L
5.	Total bilirubin	6.0 μmol/L	0.0–7.0 μmol/L
6.	Urea	20.1 mmol/L	2.5–7.5 mmol/L
7.	Creatinine	49.0 μmol/L	62.0–141.0 μmol/L
8.	Sodium (Na)	72.0 mmol/L	137.0–149.0 mmol/L
9.	Chloride (Cl-)	53.0 mmol/L	102.0–117.0 mmol/L
10.	Potassium (K)	3.9 mmol/L	3.7–5.6 mmol/L
11.	Bicarbonate (HCO_3^{-})	17.0 mmol/L	17.0–24.0 mmol/L
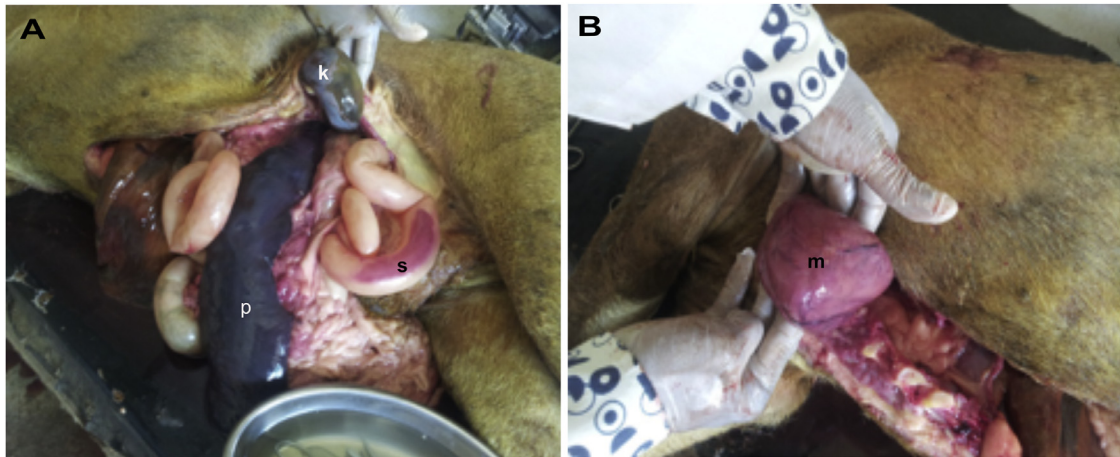
Fig. 1. Representative pictures of the post mortem examination of the 9-month old Boerboel infected diagnosed with canine babesiosis. The pictures indicate multiorgan involvement including kidney damage (k) and splenomegaly (p). Inflammation of the intestines was also observed with evidence of disseminated intravascular coagulation (s), patches of haemorrhages in the mesentery and cardiac musculature (m).
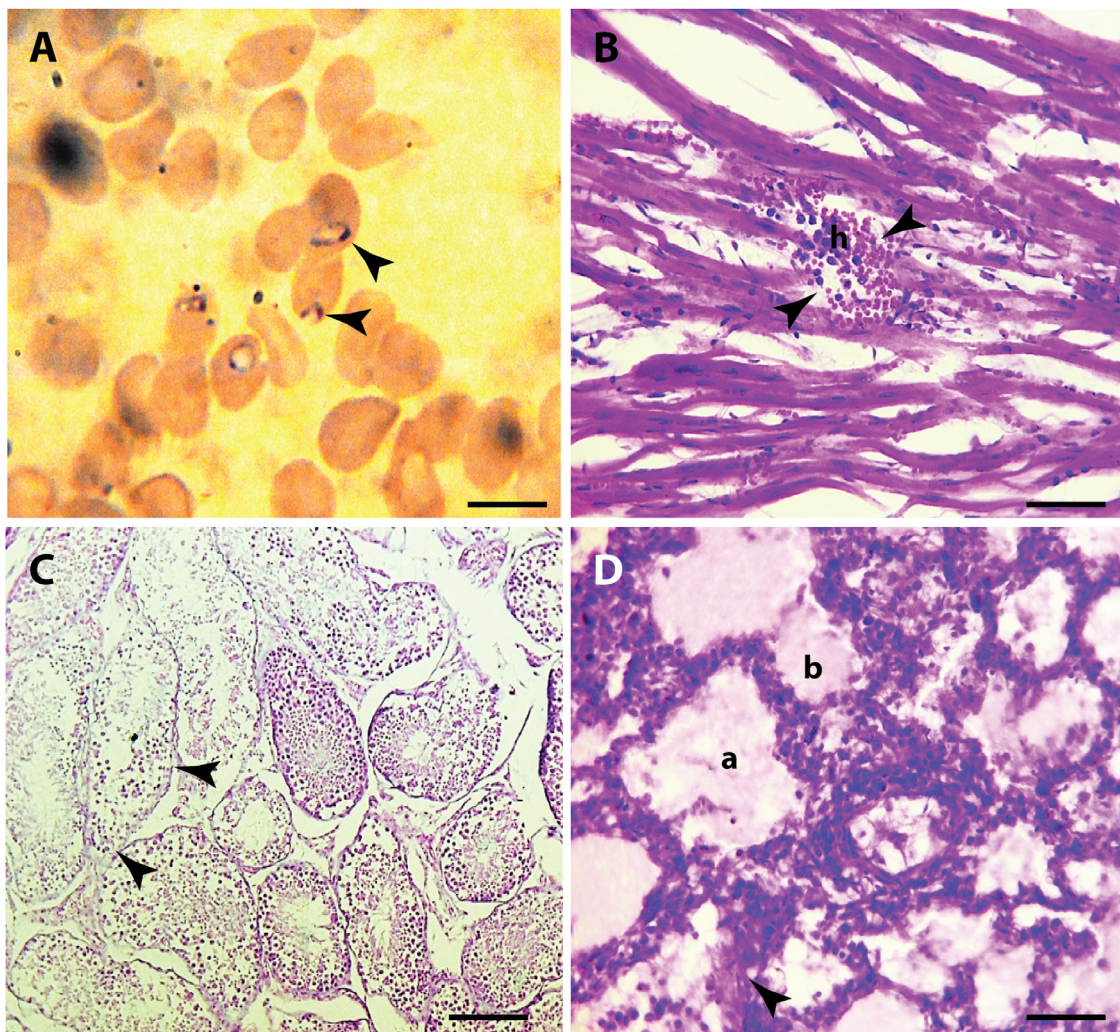

Fig. 2. Representative photomicrographs of the histopathological changes observed in the 9-months old Boerboel diagnosed with canine babesiosis. An initial diagnosis was made from a thin blood smear (A) that showed the presence of intraerythrocytic merozoites and morphologically malformed or irregularly shaped erythrocytes, which is an indication of the early stages of anaemia and a potential sequela to parasitic activities within the red blood cells. Focal haemorrhages (h) were also identified in the histological slide of the cardiac muscle (B) alongside cellular infiltration (arrow) within the myocardium. In the testes (C), note the diffuse and progressive necrosis of the epithelial cells of the seminiferous tubules (arrows) while the lungs (D) showed diffuse oedema within an enlarged alveolus (a) and fused alveoli (b) with interseptal cellular infiltration (arrow). Scale bar = 100 μ.

components of erythrocyte membranes, and subsequent degradation of haemoglobin evidenced by marked icterus in the dog (Furlanello et al., 2005). 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). Also, catabolic states such as starvation and severe infection with the intra-erythrocytic *B. canis* could be predisposing factors to tissue damage and could account for the observed decreased serum creatinine level (Homer, Aguilar-Delfin, Telford, Krause, & Persing, 2000). de Scally, Leisewitz, Lobetti, and Thompson (2006) reported similar disproportionally raised urea to creatinine levels in canine babesiosis. Suffice to say that, unlike the result obtained in this case, Button (1979) reported increased HCO3- and Cl- with decreased K in acute canine babesiosis, which suggests an interplay of other physiological factors in the pathophysiological dynamics of canine babesiosis.

4. Concluding remarks

The prevalence of canine babesiosis in Nigeria is on the increase (Takeet, Oyewusi, Abakpa, Daramola, & Peters, 2017), and there is limited information on the pathology of complicated cases within the country. This report thus aimed to describe the clinicopathological changes associated with a complicated case of canine babesiosis in dogs in Nigeria. Following thorough investigations of the current case, we infer that the prognosis of an acute and complicated case of canine babesiosis is poor. If a dog survives the infection, canine babesiosis is a potential cause of male infertility.

Moreover, we suspect that the stress of vaccination could contribute to the progression of acute babesiosis in dogs. Thus, we recommended that domesticated dogs be maintained in a tick-free environment while complimenting vaccination procedures with antioxidant-containing vitamins to minimise a potential oxidative stress sequelae. The authors also recommend scientific investigations into the pathophysiological mechanisms of the observed testiculopathy and in-depth experimental histopathological analysis of babesiosis-induced testicular lesions in domesticated animals. Such investigations would enhance current knowledge of canine babesiosis and inform steps towards early mitigation of the disease.

5. Ethical approval

The clinical case reported in the current article was presented to the Veterinary Teaching Hospital of the University of Abuja, Nigeria. The procedures followed were in accordance with institutional clinical practice protocols and in alignment with that of standard textbooks of Veterinary Medicine.

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Author contribution

ASU, SEA, ISI, SSO handled the clinical case, specimen acquisition and processing. ASU conducted the post-mortem examination. SEA, SSO and ASU produced and interpreted the photomicrographs. ASU, SEA, ISI, IEA, PPM, JNO and CEE interpreted the laboratory results. SEA and ISI wrote the first draft of the manuscript. IEA, SEA, ISI, edited and revised the manuscript.

Conflicts of interests

No conflicts of interest to declare.

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Supplementary materials

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