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Role of babesiosis in canine pup mortality

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Abstract

Babesiois is one of the life threatening diseases in dogs, especially in pups. Three (3.89 per cent) babesiosis cases belonged to a 40 days old male crossbred, 20 days old male Labrador retriever and a 45 days old male Rottweiler pups were in 77 cases of pup mortality. Anorexia, dullness, depression and anaemic changes were observed. Heart blood/spleen impression smears revealed piroplasms in erythrocytes. Catarrhal gastroenteritis, hepatitis, pulmonary congestion, oedema and haemorrhage were observed. Thymic lymphoid cell depletion, lymphadenitis and phagocytosed parasitized erythrocytes in lymph node and cerebral capillaries were also observed. Brain revealed spongiosis.

Keywords: Canine babesiois, pup mortality, hematology, gross and histopathology

1. Introduction

Correa (1974) documented B. canis in pups that were 36 h old and B. gibsoni in 3 day old puppies and dam. Brikenheuer et al. (1999)^[2] documented B. gibsoni in puppies as young as 10 days of age and observed that the time interval was shorter than the prepatent period due to transplacental transmission of puppies. Muhlnickel et al. (2002)^[7] and Boozer and Macintire (2003) stated that young dogs were more susceptible to babesiosis and frequently had more severe infections and suffered from acute and hyperacute forms. Canine babesiosis, an important tick-borne infectious disease of dogs, has been described as an emerging veterinary problem worldwide (Duha et al., 2004; Irwin, 2009) ^[5]. Bashir et al. (2009) reported that the dogs less than two years of age were more likely to be infected with Babesia piroplasms than dogs of other ages. Jacobson (2006) stated that hepatopathy in babesiosis was defined by marked bilirubinaemia, bilirubinuria and icteric mucous membrane. Irwin (2007) reported that B. canis vogeli was capable of causing severe disease in suckling puppies and caused anaemia, marked thrombocytopenia, jaundice, renal failure and death. Anaemic changes included anisocytosis, poikilocytosis, hypochromasia, polychromasia, leptocytes, target cells, Howell Jolly bodies and nucleated erythrocytes. Differential leukocyte count revealed neutrophil (61%), lymphocyte (32%), monocyte (3%) and eosinophil (4%) (Balachandran et al., 2010) ^[1]. Lobetti (2000) noticed splenomegaly as a result of hyperplasia of mononuclear phagocytic system. Dvir et al. (2004) found heart haemorrhages and effusion. Balachandran et al. (2010) ^[1] reported that the gross findings in *B. canis* infected 10 day old pup were subepicardial petechial hemorrhages, congested lungs, intestine and meningeal blood vessels, enlarged pale liver, slightly enlarged dark reddish kidney and swollen haemorrhagic mesenteric lymph nodes. Irwin and Hutchinson (1991)^[6] reported histopathological changes in the spleen, lymph node, kidney, liver and lungs. Parasitized red blood cells were clearly seen in capillaries especially in the intestinal lamina propria, the renal cortex and the medulla and around pulmonary alveolae. A marked increase in red pulp area and reticuloendothelial hyperplasia was seen in most spleen. Lesions in the liver were variable with periacinar hepatocyte vacuolation, dilation of the hepatic sinusoids and distended lymphatics. Dvir et al. (2004) reported that heart revealed fibrin microthrombi, haemorrhage, necrosis and inflammatory infiltrate in babesiosis. Lobetti (2005) reported myocardial necrosis, inflammation, haemorrhage, fibrin microthrombi, usually in the left ventricle, which had the highest metabolic requirement. These changes were thought to be due to an inflammatory reaction to the pavementing of parasitised cells against the endothelium via adhesion molecules, coagulopathy or from myocardial hypoxia in babesiosis of dogs.

Irwin and Hutchinson (1991) ^[6], Conrad *et al.* (1991) ^[4], Yamane *et al.* (1993) ^[8] and Bohm *et al.* (2006) ^[3] stated that evaluation of smears prepared from capillary blood such as ear tip or nail bed enhanced the likelihood of organism detection because parasitized erythrocytes sludged in the capillaries. They were visualized well with Giemsa or field stains than quick stains.

2. Materials and Method

Puppies less than 6 months old, admitted to the Small Animal Clinics were subjected to a thorough clinical examination. Laboratory examinations like complete blood count, serum biochemistry were applied. Detailed post-mortem examinations of the dead puppies were conducted. Tissue samples from various organs were collected in 10 per cent formalin for histopathology. Spleen impression smear was collected Peripheral and heart blood smears, impression smears from various lesions, heart blood swabs and swabs from lesions, thoracic fluid, pericardial fluid, peritoneal fluid and urine were also collected and examined.

3. Results

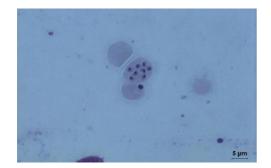
Out of 77 cases, three (3.89 per cent) were positive for *Babesia* spp. These were a 40 days old male crossbred a 20 days old male Labrador retriever and a 45 days old male Rottweiler. In all three cases, anorexia, dullness and depression were observed.

3.1 Laboratory diagnosis

Peripheral blood smears revealed anisocytosis, poikilocytosis, hypochromasia, polychromasia, presence of Howell Jolly bodies, nucleated erythrocytes and piroplasms in all the three cases. Heart blood smears and spleen impression smears also revealed piroplasms in all the three cases. In one case, intestinal contents revealed *A. caninum* and *T. canis* eggs. Out of three heart blood swabs and one intestinal swab taken, one heart blood swab revealed *Staphylococcus* spp., and *E. coli* and another swab revealed *Staphylococcus* spp., alone.

3.2 Gross pathology

Body condition was poor in all the three cases. Visible mucous membranes were blanched in two cases and icteric in one. Ticks were noticed in two cases and bilateral nasal mucus discharge in one case. In all the three cases, subcutaneous fat was poor and axial and prescapular lymph nodes were enlarged, oedematous and haemorrhagic. On incision, the haemorrhagic areas obscured the line of demarcation between the cortex and medulla. Stomach in one case, was distended and contained liquid brown contents mixed with numerous *T. canis* worms. In the second case, it was distended and contained white curdled milk of about 40 mL. In the third case, stomach had semisolid mucus mixed contents.



Blood smear examination - numerous piroplasms



Severe icteric internal organs

In one case, numerous T. canis worms were present in the duodenum mixed with liquid brown contents and mucus. The contents in the jejunum were liquid red-brown mixed with numerous A. caninum Dark red-brown, semisolid scanty contents in one case and dark red-brown contents with prominent Peyer's patches in one case. In two cases, liver was mildly enlarged and pale brown. In the third case, it was yellow-brown, mottled and showed 3 distinct irregular areas of subcapsular ecchymotic haemorrhages on the parietal surface of the quadrate lobe $(2 \times 3 \text{ cm})$, right central $(0.7 \times 1 \text{ cm})$ and left central lobes (0.5×0.6 cm). On incision, the haemorrhagic areas did not extend into the parenchyma Pancreas was pale. Pericardial sac contained about 5 mL of serosanguineous fluid in one case. Heart showed a few scattered haemorrhagic streaks on the ventricular epicardium in one case. In the second case, multifocal petechiae and paint brush haemorrhages were noticed on the epicardium and endocardium of the left and right ventricles. It was diffusely pale in one case. Trachea contained frothy fluid in all the three cases. The third case showed copious fluid up to the terminal bronchioles. In one case, the lung was dirty pink interspersed with irregular brown areas and oedematous and in another it was diffusely oedematous and slightly congested. Small pieces of lung sank in water. In the third case, it was diffusely pale with multifocal ecchymotic haemorrhages in all the lobes. Mesenteric lymph nodes were enlarged, oedematous and haemorrhagic in all the three cases. Spleen showed marked uniform enlargement with rounded borders and smooth surface in all three cases. On incision, blood oozed out in two cases and prominent white pulp was observed in one case. In all the three cases, kidney showed mild enlargement. Cortical surface was grey-brown in one case and yellow-brown in other two cases. Meningeal blood vessels were congested in two cases.

3.3 Histopathology

Stomach showed mild, focal desquamation of mucosal epithelium and submucosal congestion in all the three cases. Increased goblet cells activity was noticed in the intestine in one case. Intestine showed desquamation and necrosis of the villi in two cases. Eosinophilic infiltration with necrosis, dilatation of crypts, increased goblet cells activity, haemorrhage and blunting of intestinal mucosa and cut section of *A. caninum* were noticed in one case. Liver showed complete loss of architecture, diffuse vacuolar degeneration and necrosis of hepatocytes and infiltration of lymphocytes around periportal and periductular areas in all the three cases. Zonal to bridging necrosis, extensive focally diffuse subcapsular haemorrhages with hemosiderin pigments were observed. Two cases, showed stasis of bile and hyperplasia of bile duct. Pancreas in all three cases showed focal to

multifocal areas of mild congestion, moderate infiltration of lymphocytes and plasma cells with mild fat necrosis. Myocardium showed loss of striation, and vacuolar degeneration in two cases and multifocal congestion, haemorrhage and mild plasma cells infiltration in two other cases.

Lung showed mild multifocal congestion, emphysema, oedema, haemorrhage with perivascular and interstitial infiltration of lymphocytes with occasional macrophages in all the three cases. Focal areas of interstitial thickening in two cases and hyperplasia with desquamation of bronchiolar epithelium in one case were observed. Thymus showed multifocal congestion, depletion of thymocytes and prominent Hassel's corpuscles. Lymph nodes showed severe haemorrhage and connective tissue proliferation in all the three cases. Phagocytosed erythrocyte with piroplasms was evident in one case. Spleen showed multifocal areas of congestion in all the three cases. Kidney showed severe, diffuse vacuolar degeneration, necrosis and sloughing of the proximal tubular epithelial cells, a few atrophic glomeruli and cortico-medullary congestion in all the three cases. A few tubules showed casts in one case. Brain revealed multifocal areas of congestion and spongiosis of the cerebral cortex in all the three cases amongst one, most red blood cells showed parasitized erythrocytes 4-5 µm in diameter, in blood vessels

4. Discussions

Out of 77 cases, three (3.89 per cent) babesiosis cases belonged to a 40 days old male crossbred, 20 days old male Labrador retriever and a 45 days old male Rottweiler pups. Correa (1974) documented B. canis infection in pups that were 36 h old pup and B. gibsoni infection in 3 day old puppies while Birkenheuer et al. (1999)^[2] reported B. gibsoni infection in 10 days old puppies and observed that the time interval was shorter than the prepatent period due to transplacental transmission of puppies. Young dogs were more susceptible to babesiosis and frequently had more severe infections and suffered from acute and hyperacute forms (Muhlnickel et al., 2002; Boozer and Macintire, 2003) [7]. Anorexia, dullness and depression and anaemic changes of anisocytosis, poikilocytosis, polychromasia, presence of Howell Jolly bodies, nucleated erythrocytes and piroplasms were observed. Heart blood smears and spleen impression smears also revealed piroplasms in erythrocytes (Irwin and Hutchinson 1991; Conrad et al. 1991; Yamana et al. 1993; Bohm et al. 2006; Balachandran et al., 2010) ^[6, 4, 3, 1]. In one case, concurrent ancylostomiasis and toxocariasis were recorded. Cultural examination of swab revealed Staphylococcus spp., E. coli (n=1) and Staphylococcus spp., (n=1) alone.

4.1 Pathology

Body condition was poor in all the three cases. Visible mucous membranes were blanched in two cases and icteric in one case (Irwin, 2007). Toxocariasis in one case, subepicardial petechial hemorrhages, congestion of lungs, intestine and meningeal blood vessels, enlarged pale liver, slightly enlarged dark reddish kidney, splenomegaly and swollen haemorrhagic mesenteric lymph nodes were observed (Dvir *et al.* 2004; Lobetti 2000; Balachandran *et al.*, 2010)^[1] Catarrhal gastroenteritis was noticed in a case (CFSPH, 2005). Diffuse vacuolar degeneration and necrosis of hepatocytes, infiltration of lymphocytes around periportal and periductular areas in all the cases, zonal to bridging necrosis, extensive focally diffuse subcapsular haemorrhages with

hemosiderin pigments biliary stasis and hyperplasia in two cases, pancreatitis, pancreatic fat necrosis and myocardial degeneration and necrosis in two cases observed were in accordance with the findings of earlier workers Dvir et al., (2004) and Lobetti (2005). Pulmonary congestion, oedema, haemorrhage with perivascular and interstitial infiltration of lymphocytes with occasional macrophages and focal interstitial pneumonia (n=2) were observed. Thymic congestion, depletion of thymocytes and prominent Hassal's corpuscles in 12 cases, lymphadenitis in all the three cases, phagocytosed parasitized erythrocytes in one case, splenic congestion in all the three cases, severe, diffuse vacuolar degeneration, necrosis and sloughing of the proximal tubular epithelial cells, in all the three cases with a few tubular casts in one case observed were also reported by Irwin and Hutchinson (1991)^[6]. Brain revealed multifocal areas of congestion and spongiosis of the cerebral cortex in all the three cases with piroplasms seen in the capillary erythrocytes.

5. Conclusion

Pups are highly vulnerable to a whole range of adverse environmental conditions because of the immaturity. Poorly developed thermoregulatory mechanisms lead to hypothermia and this favours many kind of infections, among which babesia is the most important one. Out of 77 cases, three (3.89 per cent) babesiosis cases belonged to a 40 days old male crossbred, 20 days old male Labrador retriever and a 45 days old male Rottweiler pups. Anorexia, dullness, depression and anaemic changes were observed. Heart blood/spleen impression smears revealed piroplasms in erythrocytes.

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