Pathological observations on clinical *Anaplasma marginale* infection in cattle

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Abstract Gross and histopathological changes were recorded in a pregnant cattle died of clinical anaplasmosis, a tick transmitted economically important disease caused by *Anaplasma marginale*. Grossly emaciated carcass along with pale visible mucous membranes and pale serosal surface, splenomegaly and hepatomegaly was observed. Microscopically, in lungs variable extent of interstitial pneumonia, emphysema along with infiltration of mononuclear cells was seen. Spleen showed extensive increase in red pulp area with massive proliferation of lymphocytes. In liver marked thickening of capsule with fatty changes along with retention of bile was seen. Gall bladder showed congestion, glandular hyperplasia and thickening wall. Myocardium showed degeneration and necrosis.

Keywords *Anaplasma marginale* · Histopathology · Lungs · Heart · Liver · Gall bladder

Introduction

Bovine anaplasmosis caused by *Anaplasma marginale*, an intra erythrocytic rickettsial organism (Dumler et al. 2001)

transmitted by ticks, or mechanically by biting flies or blood-contaminated fomites. *A. marginale* invades and multiplies within mature erythrocytes and during acute anaplasmosis, rickettsemia levels may exceed 10^9 infected erythrocytes per ml (Kieser et al. 1990). Clinical signs include fever, anaemia, weakness, constipation, jaundice, loss of appetite, dehydration, depression, laboured breathing, abortion in pregnant animals and often death (Richey and Palmer 1990). Anaemia and icterus are usually observed without haemoglobinaemia and haemoglobinuria (Rymaszewska and Grenda 2008) as in place of intravascular haemolysis, extra vascular erthrophagocytosis occurs in disease. In severely affected animals urine often becomes dark brown due to the presence of bile pigments. While comparing parasitaemia with haemolytic indices, positive correlation has been observed in level of parasitaemia and mean corpuscular fragility (Nazifi et al. 2008) in naturally infected crossbred cattle. Degenerative changes are observed in different organs due to hypoxic conditions created by anemia. Further changes may be due to immunological reactions produced by parasite. Very few published reports are available in literature on histopathological changes in bovine anaplasmosis. Here in this communication gross and histopathological changes observed in various organs of an animal died of clinical anaplasmosis are recorded.

Materials and methods

Leishman stained thin blood smears were prepared and examined from pregnant cattle suspected for anaplasmosis based on history and clinical signs. Clinical examination of animal revealed presence of fever, pale mucus membranes, strenuous breathing and decrease in appetite. Later on

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animal died of disease. Systematic necropsy was performed on the animal and gross lesions were recorded. The tissue samples from various vital organs viz; lungs, heart, liver, kidneys and spleen were collected in 10 % neutral buffered formalin and processed for histopathological examination as per the standard procedure (Luna 1968). Thin sections of 5 µm thickness were prepared and stained with routine haematoxylin and eosin. Sections were thoroughly examined under light microscope for various histopathological changes.

Results and discussion

Microscopic examination of Leishman stained blood smears revealed presence of typical *A. marginale* organisms in the erythrocytes (Fig. 1).

Gross lesions

Gross lesions included pale mucus membranes with emaciated carcass. Visible mucous membranes and serosal surfaces of the abdominal organs had yellowish discoloration indicating icterus (Fig. 2).

Splenomegaly along with congestion and discoloration of spleen was seen. Splenomegaly may be due to excessive destruction of erythrocytes by phagocytic cells. In anaplasmosis, anaemia develops due to extensive erythrophagocytosis initiated by parasite-induced damage of red blood cells and anti-erythrocytic antibodies (DeVos et al. 2006). Lungs showed pulmonary congestion, haemorrhage, oedema and emphysema. Hepatomegaly (Fig. 3) with thickening of bile duct was an observation as also reported previously (Egbe-Nwiyi et al. 1997). Gall bladder was distended with dark colored bile (Fig. 4). Jaundice and anemia in anaplasmosis may be due to the destruction of the blood cells and their contents being released into the

blood stream. Dead foetus was seen in uterus of the animal presented in the study. Oxygen deprivation can result in death of foetus and abortion in pregnant cow (De-Whittier et al. 2007). Abortion has been reported previously in clinical anaplasmosis in cattle (Correa et al. 1978).

Histopathology

Lungs

Histopathological changes in the lungs showed variable extents of interstitial pneumonia (Fig. 5), marked thickening of inter alveolar septa characterized by proliferation of type-I pneumocytes and mild infiltration of mononuclear cells. The emphysema (Fig. 6) observed may be compensatory to terminal dyspnea. Progressively, these changes in the pulmonary tissues may cause respiratory distress which was evident clinically.

Fig. 2 Yellow discoloration of fascia with emaciated carcass. (Color figure online)

Fig. 1 Showing *Anaplasma marginale* in erythrocytes

Fig. 3 Hepatomegaly with distended gall bladder. Yellow discoloration of omentum. (Color figure online)

Heart

Histological examination of heart revealed disorientation of myocardial fibers. Mild to moderate multifocal myocardial degeneration and coagulative necrosis was seen which may be due to severe anaemia observed in the present case (Fig. 7).

Fig. 4 Gall Bladder distended with dark green coloured thick bile. (Color figure online)

Fig. 5 Lungs showing interstitial pneumonia H&E $\times$ 20X

Fig. 6 Lungs showing emphysema H&E $\times$ 10X

Spleen

Spleen showed increased red pulp with massive proliferation of lymphocytes. Red pulp contained numerous histiocytes showing erythrophagocytosis (Fig. 8). Splenic changes are similar to those observed in babesiosis except that there is generally more extensive accumulation of plasma cells in the red pulp in cases of anaplasmosis (DeVos et al. 2006).

Gall bladder

Gall bladder showed congestion and thickening of gall bladder wall with glandular hyperplasia. (Fig. 9) which may be due to retention of bile in the gall bladder.

Liver

Fatty changes were observed in the hepatocytes along with retention of bile which may be due to cholestasis (Fig. 10) along with mild infiltration of mononuclear cells in portal

Fig. 7 Myocardial degeneration and necrosis. H&E $\times$ 20X

Fig. 8 Section of spleen showing increased red pulp. H&E $\times$ 10 X. (Color figure online)

Fig. 9 Congestion and thickening of gall bladder wall with glandular hyperplasia. H&E $\times 10X$

Fig. 10 Section of liver showing retention of bile, mild fatty change and infiltration of mononuclear cells in portal triad along with stasis of bile in the bile ductule. H&E $\times 20X$

triad and marked thickening of glisson capsule or perihepatitis. Coagulative necrosis of the hepatocytes and the bile ducts hyperplasia has been reported earlier (Egbe-Nwiyi et al. 1997). Hepatic necrosis is a feature of this disease. Anderson and Hurtado (1989) also reported centrilobular hypoxic hepatic necrosis and splenic hemosiderosis which may be due to erythrocyte destruction. DeVos et al. (2006) also reported biliary retention in most cases of anaplasmosis as there is accumulation of haemosiderin in cells of mono-nuclear phagocytic system.

Necropsy findings revealed splenomegaly, hepatomegaly along with anemia and jaundice as prominent gross observations where as microscopic findings include bile duct hyperplasia, bile retention, increase in liver capsule size, fatty changes in liver, lungs emphysema and myocardial degeneration.

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