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Predisposing effect of immature amphistomiasis in the incidence of haemorrhagic septicaemia in cross bred dairy cattle

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Abstract

A Holstein Friesian cross bred dairy cow was brought to Veterinary College and Research Institute hospital with the history of severe watery diarrhoea and bloat. Blood and serum samples of the animal revealed severe anaemia, hyponatremia and hypokalemia. In spite of the treatment, the animal died on the same day. The carcass was referred to Department of Veterinary Pathology for post-mortem examination. Grossly, there were ecchymotic haemorrhage in epicardium, marbling of lungs due to severe edema and fully packed rumen with whitish flukes. The gravid uterus contained five months old female foetus and ecchymotic haemorrhage seen over neck and thigh regions of the foetus. The heart blood and lung swabs of both dam and foetus were positive for *Pasteurella multocida* and were further confirmed by biochemical tests. The worms collected from the rumen were identified as immature amphistomes based on the morphometry and morphological characters. Histopathologically, severe edema in the inter alveolar and inter lobular septa of lungs, portal fibrosis, bile duct hyperplasia and necrosis of hepatocytes were noticed.

Keywords: Immature amphistomes, stress, *Pasteurella multocida*, septicemia

Introduction

Amphistomes, a trematode parasite has recently emerged as an important cause for productivity loss and mortality^[1] in cattle and has a wide geographical distribution in subtropical and tropical areas^[2]. The ruminants affected with amphistomiasis usually exhibit severe bloat, anorexia, diarrhoea, anaemia and jowl edema^[3]. Hemorrhagic septicemia (HS) is an acute, fatal and septicemic disease of cattle and buffaloes caused by a gram negative bipolar bacterial organisms namely *Pasteurella multocida* (*P. multocida*)^[4]. The healthy cattle harbors small numbers of *P. multocida* and it cause outbreaks during periods of stress like adverse climatic conditions (high temperature and humidity), concurrent infections, parasites, poor nutrition and excess productive or work stress. Immature amphistome flukes are more dangerous as they attached to the intestinal wall which causes sloughing of the intestinal mucosa, diarrhoea, loss of condition and death. It leads to mortality as high as 80% among sheep and cattle population^[5]. The loss of vitality and immune depletion invite various pathogens and secondary bacterial infection. This paper deals about the predisposing effect / stress induced effect of parasitic amphistomiasis in the incidence of haemorrhagic septicaemia in cross bred dairy cattle.

Materials and Methods

A Holstein Friesian cross bred cow was brought from kolli hills (hilly area at Namakkal district, Tamil Nadu, India) to Veterinary College and Research Institute hospital with the history of severe watery diarrhoea and bloat. Blood and serum samples were collected for haemogram and serum biochemistry respectively. In spite of the treatment, the animal died on the same day. The carcass was referred to Department of Veterinary Pathology for post-mortem examination.

Detailed necropsy was conducted and the gross lesions were recorded. Aseptically prepared smear from heart blood was subjected to Leishman's staining to screen the bacterial organisms. Heart blood and lung swab were collected for isolation and identification of bacterial etiology by cultural examination^[6].

Likewise, heart blood swab and lung pieces were also collected from the foetus which was present in the gravid pregnant uterus. In brief, the samples from the dead animal as well as foetus were inoculated directly on Brain Heart Infusion (BHI) broth and incubated overnight at 37°C. After that, over night culture was streaked onto Blood agar (BA), BHI agar and MacConkey agar plates. The suspected colonies were identified by morphological characters by using Gram's stain and Leishman's stain. Then it was further confirmed by different biochemical tests such as catalase, oxidase, Methyl red (MR), Voges-Proskauer (VP), Indole production, sugar fermentation and motility tests^[7].

The organs showing lesion were collected in 10 per cent neutral buffered formalin for histopathological examination. The processed tissue specimens were embedded in paraffin and 5 micron cut sections were subjected to Haematoxylin – Eosin (H&E) staining for microscopical evaluation^[8].

Fleshy worms from the rumen and duodenum content were collected, washed, filtered and then fixed in 10 per cent formalin. The parasites were identified based on the morphometry and their morphological characters.

Results

1. Clinical signs and clinical pathology

The clinical examination of the animal revealed severe bloat, dehydration, dullness, pale mucus membrane, sunken eyeballs and pasted rectum with foul smelled diarrhoea. The haematological parameters revealed severe anaemia (Hb – 5 g/dl; PCV – 16% and RBC – 2.7×10^6 /cu.mm. Serum biochemical values showed hyponatremia (sodium – 86 mmol/L) and hypokalemia (2.2 mEq/L) when compared to the normal level of sodium (126 -144 mmol/L) and potassium (3.6 to 5.8 mEq/L) respectively. In spite of the treatment the animal died on the same day.

2. Gross lesions

On post-mortem examination, severe ecchymotic haemorrhage in the subcutaneous tissue and blood tinged fluid in the pericardium, thoracic and abdominal cavity were observed. Epicardium revealed severe ecchymotic haemorrhage (Fig. 1) and the cardiac chambers contained clotted blood. Trachea and bronchi contained blood tinged frothy exudate. Lungs were highly edematous, severe distension of interlobular septa with edema fluid and shown typical marbling pattern (Fig. 2). The cut surface of the lung parenchyma revealed blood tinged frothy exudate. Liver was pale yellowish in colour and gall bladder was distended with yellow green bile. The cortical surface of kidney revealed petechiae with focal necrosis.

Rumen was severely distended with partially digested coarse ingesta and the contents were fully mixed with whitish to pale fleshy flukes (Fig. 3) measuring about 3 mm length and 1.5 mm diameter. Abomasum contained digested feed material and the mucosa showed severe congestion. The duodenum had foul smelling watery intestinal content and its mucosa revealed severe necrosis, haemorrhage, congestion and few fleshy worms as that of rumen. Gravid uterus contained five months old female foetus and ecchymotic haemorrhage seen over neck and thigh regions of the foetus (Fig. 4).

3. Confirmation of etiological agent

a) Amphistomiasis

There were numerous fleshy flukes packed with the ruminal content and were collected and processed for species

identification. The flukes were white in colour (Fig. 5) and were subjected to microscopic examination to study the morphometry. These flukes revealed the presence of oral sucker, ventral sucker and intestinal caeca (Fig. 6) without any reproductive organs. Based on the morphological characters the worms were confirmed as immature amphistomes which might have migrated recently from duodenum.

b) *Pasteurella multocida* (Haemorrhagic septicaemia)

Leishman's stained heart blood smear from the animal and foetus revealed typical bipolar organisms suggestive of *Pasteurella sp.* In cultural examination, small, glistening, mucoid, dew drop like colonies were found on BA and BHI plates after overnight incubation at 37 °C. The bacteria were found to be non-hemolytic on BA and did not grow on McConkey's agar. Likewise, isolated organism in this study was found to be gram-negative cocco-bacillary in Gram staining and bipolar characteristics in Leishman's staining. The organism was found to be non-motile when examined under microscope by hanging drop technique.

The isolated organisms were positive for catalase, oxidase and indole test and negative for MR and VP tests. This organism fermented dextrose, sucrose and mannitol but not maltose and lactose. These fermented sugars producing acid without gas formation. The cultural, staining and biochemical characters clearly illustrated the presence of *Pasteurella multocida* in the heart blood and lung tissues of both dam and foetus.

4. Histopathology

Severe necrosis of cardiac myofibers and separation of cardiac fibers due to severe haemorrhage were noticed. Lung section showed massive edema in the inter alveolar and inter lobular septa (Fig. 7), alveolar haemorrhage and infiltration of polymorphs. Liver exhibited portal fibrosis (Fig. 8), bile duct hyperplasia and necrosis of hepatocytes. There was severe congestion, inter tubular haemorrhage and severe necrosis of renal tubules noticed. The mucosa of rumen and abomasum showed severe necrosis and desquamation of mucosal epithelium along with submucosal congestion. Duodenal mucosa revealed loss of villi and severe haemorrhage in the submucosa.

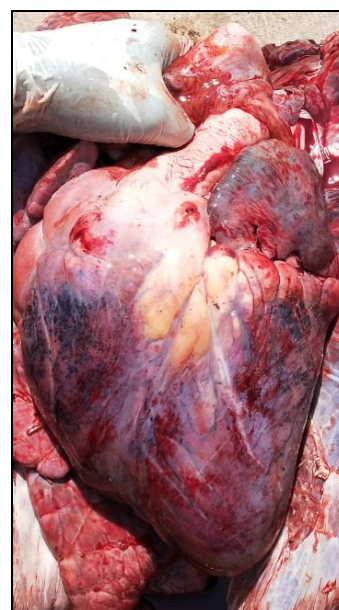


Fig 1: Heart – Epicardium showing severe ecchymotic haemorrhage

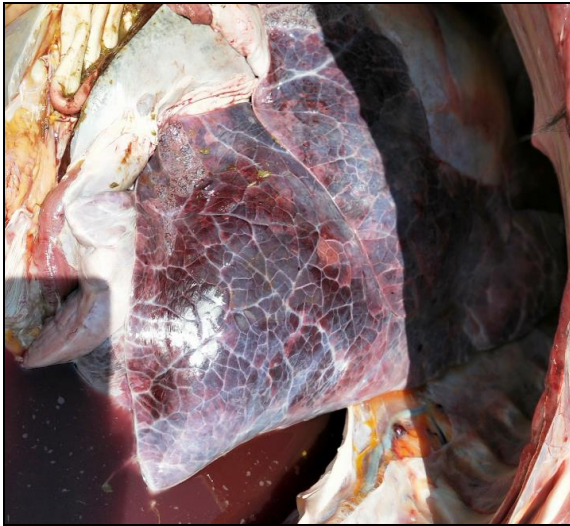


Fig 2: Lung – Severe distension of interalveolar septa exhibiting marbling pattern and haemothorax

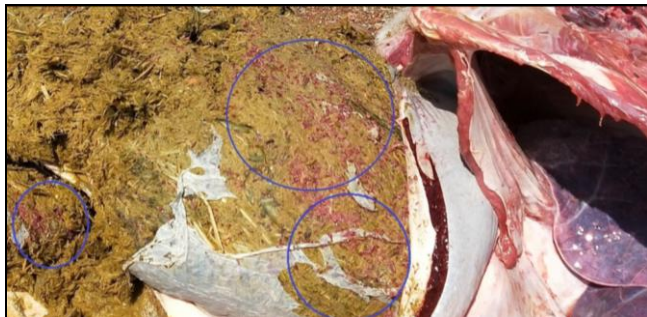


Fig 3: Rumen content exhibiting bunches of immature amphistome flukes



Fig 4: Dead foetus revealing ecchymotic haemorrhage in neck, hip and thigh region



Fig 5: Numerous whitish immature amphistomes measuring about 3 mm length and 1.5 mm in diameter.



Fig 6: Immature amphistomes exhibiting oral and ventral sucker without reproductive organs

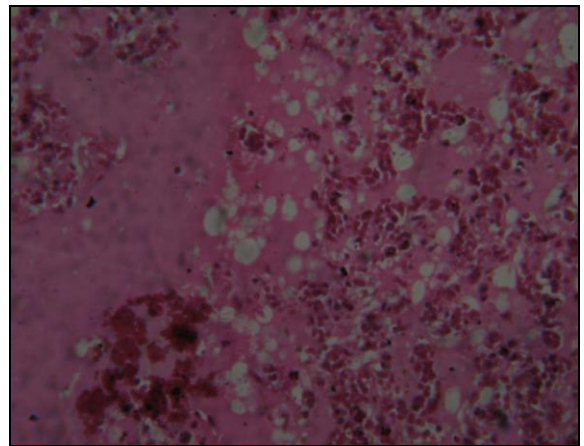


Fig 7: Lung section showing severe edema and haemorrhage (H&E, 400)

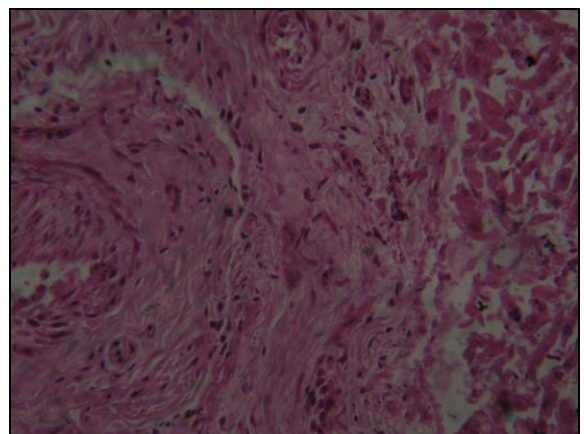


Fig 8: Liver section revealing severe portal fibrosis and necrosis of hepatocytes (H&E, 400)

Discussion

Clinical examination of the animal revealed severe bloat, dehydration, dullness, pale mucous membrane and foul smelling diarrhoea. Similar type of symptoms was also recorded in amphistomiasis cases by earlier authors^[9, 10]. The amphistome flukes are plug feeders as they have strong oral suckers might have caused severe diarrhoea. Bloat noticed in the present case might be due to the obstruction around the cardiac region by

numerous amphistomes. Similar type of clinical signs was also observed by Yokeshpriya *et al.*^[10].

The postmortem observation revealed numerous amphistome flukes attached to the wall of the ruminal mucosa. The gross lesions were very well correlated with the clinical signs noticed in this study. This observation is concurrent with the findings of Eslam *et al.*^[11] who also found the pear shaped, pink to red flesh worms attached to the lining of the rumen.

Leishman's stained heart blood smear from the animal and foetus revealed typical bipolar organisms suggestive of *Pasteurella sp.* Small, glistening, mucoid, dew drop like colonies on BA and BHI plates confirmed the presence of *Pasteurella multocida*. Similar cultural characters for *Pasteurella multocida* were also recorded by Levy *et al.*^[12]. It was further confirmed by the biochemical characters. These biochemical reactions were consistent with the findings of earlier authors^[12, 13].

Microscopic picture of portal fibrosis and bile duct hyperplasia in the liver section shown the migratory route of immature amphistomes. These histopathological lesions were in accordance with the earlier findings of Ahmedullah^[14].

The immature flukes in the duodenum and migration towards rumen might have contributed stress and thereby the emergence of pathogenic type of *Pasteurella multocida*. Based on the epidemiological study, the cattle might have contracted the amphistomes infection from the water bodies due to the existence of snails which act as an intermediate host for amphistomiasis.

Conclusion

The amphistome worm load caused severe stress and anemia which resulted in the emergence of pathogenic type of *P. multocida*. These pathogenic bacteria caused severe septicemia and typical septicemic lesions in dam as well as in foetus. Hence, the present study clearly exemplifies that the predisposing effect / stress induced effect of parasitic amphistomiasis bring about the incidence of haemorrhagic septicaemia by *Pasteurella multocida* and caused death in the cattle.

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