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Septicemic Mannheimiosis in Sahiwal Cattle

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Septicemic Mannheimiosis in Sahiwal Cattle

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I. Introduction

Respiratory tract infections are common in farm animals throughout the world. Pneumonic mannheimiosis is a highly contagious disease among intensively reared cattle especially beef cattle and results in high morbidity and mortality. This disease is highly infectious with severe clinical symptoms and is often fatal [1].The incubation period of the disease ranges from 3 to 5 days, however, the onset usually occurs after stress like transport [2]. It is widespread in prevalence in ruminants and results in severe economical losses to the livestock industry [3]. The disease is caused by *Mannheimia haemolytica,* a pathogenic Gram-negative, non-motile coccobacillus belonging to the family *Pasteurellaceae,* is a normal inhabitant of the upper respiratory tract as commensal. These opportunistic bacteria can multiply exponentially producing leukotoxin in stressed animals leading to pneumonia and death. The outbreaks of mannheimiosis can occur throughout the year in the endemic areas [4]. Though usually mannheimiosis is recorded as an outbreak in farm conditions, we report an isolated case which was presented for postmortem to identify the cause of sudden death.

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a) Case Details

An organized dairy farm housing around 100 native cattle of different breeds maintained under intensive system of rearing reported sudden death of a Sahiwal cow aged 9 years. The animal was anorectic with fever prior to death. Gross examinations showed subcutaneous adipose tissue gelatinisation and oedema with streaks of haemorrhage in the submaxillary space, pharyngeal region, neck, dewlap and brisket region. Fibrinous adhesions between the pericardium of heart and ventral aspect of lungs. Thoracic cavity revealed yellow fluid with fibrin shreds.Pericardial sac showed thickening with thick dirty white fluid. Epicardium was congested.Tracheal mucosa was congested and lumen contained frothy exudate extending up to the bronchi of both lungs. Kidneys were congested and intestinal mucosa showed haemorrages. Lungs revealed generalized congestion along with massive haemorrhages. The interlobular septa were prominent, widened and massively filled with either fibrinous or fibrino-purulent exudation. The lesions were most severe in the cranial and middle lung lobes. On cut section, blood tinged frothy exudate oozing-out from small bronchi and bronchioles was evident. Other organs such as kidneys, spleen, liver, heart, lymph nodes, stomach and intestines revealed mild to moderate congestion or occasional haemorrhages.

Peripheral blood smear, smear and swab from oedematous fluid, heart blood, trachea, lungs, pericardium were collected for bacteriological studies. Tissue samples were collected in 10% buffered formalin for histological studies.

II. Discussion

a) Isolation and Identification

The swab materials were streaked onto different media viz.Brain heart infusion agar, Mac Conkey agar, Blood agar, Anaerobic agar, Herrolds egg yolk medium after processing the samples. After incubation at 37°C overnight, the plates showed the presence of circular, slightly raised colonies with an entire margin in blood agar from heart blood, trachea, lungs, pericardial fluid, liver and kidney swabs. The surface of the colonies is smooth, shiny and non- transparent with a greyish tinge. A diameter of approximately 2 mm colony with a narrow zone of ß-haemolysis is observed. Pure colonies were obtained by subculturing and subjected to phenotying as per [5]. Biochemical characterization of the isolates showed they belong to *Mannheimia haemolytica*.

b) Mannheimia Haemolytica Specific PCR

Suspected single colonies of bacteria were lysed by incubating in 40µl of TE buffer at 98°C for 5 min. The lysate was centrifuged at 13,000x g for 5 min, after which 2µl of supernatant was added to the PCR reaction as template DNA. To confirm M. haemolytica, species-specific primers were used as described by [6] targeting O-sialoglycosidase endopeptidase. Following PCR, 10µL of product was visualized on 1.0% (w/v) agarose gel containing ethidium bromide (Fig.I). A product size of 267bp was noticed.

c) Multiplex PCR

Multiplex PCR was used to enable specific identification and differentiation of Mannheimia haemolytica from other species of the genus as described by [7]. Following PCR, 10µL of product was visualized on 2.0% (w/v) agarose gel containing ethidium bromide (Fig.2). The primer set consisted of four primers specific for 16 s rDNA gene, Lkt- region of the Leukotoxin gene specific for *M. haemolytica and M.glucosida*, Lkt 2 –region of Leukotoxin gene specific for *M.glucosida* and HP – region of unknown hypothetical protein specific for Mannheimia *haemolytica.* An amplicon of 304, 206 and 90 bp specific for Mannheimia haemolytica was observed.

d) Histopathology

Microscopically, tissue sections obtained from lungs revealed massive dilatation and engorged blood vasculature. The inter-alveolar spaces were completely filled with red blood cells. Numerous haemorrhages were observed in lung parenchyma. The alveolar spaces were diffusely filled with sero-fibrinous exudates admixed with neutrophils and red blood cells. Interlobular septa were thickened and markedly distended with fibrinous exudation (Fig.3). The alveoli and bronchioles were massively packed with variable proportions of neutrophils, macrophages, fibrin, exudation, erythrocytes and necrotic cellular debris (Fig.4). The heart revealed fibrovascular thickening of the pericardium (Fig.5). Liver showed extensive congestion of veins and sinusoids with diffuse mild fatty changes in hepatocytes. The intestinal mucosa revealed congestion and haemorrhages in mucosa with infiltration of lymphocytes in submucosa. Necrosis of intra-alveolar leukocytes mainly neutrophils leading to appearance of oat shaped inflammatory cells was conspicuous finding. The cranio-ventral distribution of lesions, massive fibrinous/suppurative exudation, necrotic changes in lung parenchyma including necrosis of inflammatory leucocytes with the formation of characteristic oat shaped cells are strongly suggestive of pneumonic mannheimiosis [8,9].

In domestic animals, *M. haemolytica* is responsible for causing various pathological conditions and respiratory disease is an important manifestation. Harsh climatic conditions, stocking density, onset of other viral or bacterial diseases act as predisposing factors. However, the manifestation of mannheimiosis is also reported without the involvement of any predisposing factor [10]. The manifestation of the disease occurs possibly due to the transfer of *Mannheimia* microorganisms from the nasopharynx into the lungs by draining along the trachea and settling into the bronchi, bronchioles and alveoli. This consequently results in release of *Mannheimia* endotoxins which infect lung lobules and causes thromboses along with occlusion of lymphatics, capillaries, and veins; thereby causing ischemic necrosis [11]. In spite of face to face housing, isolated case of Septicemic mannheimiosis suggests that native breeds of cattle are more resistant to Mannheimia infection. Screening of all the animals in the farm for *Mannheimia haemolytica* revealed 10% of the healthy animals harbored this organism in the upper respiratory tract (Data not shown). Hence the possible reasons attributed for the isolated case could be the age of the animal and extreme summer stress which could have led to mannheimiosis.

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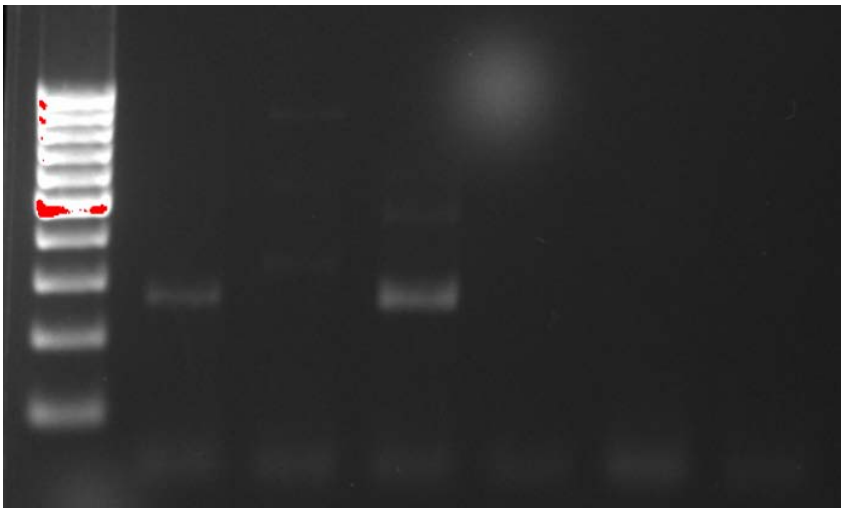

Fig. 1 : Mannheimia haemolytica specific PCR

Lane 1 DNA ladder; Lane 2 – 6 samples; Lane 7 – Negative control

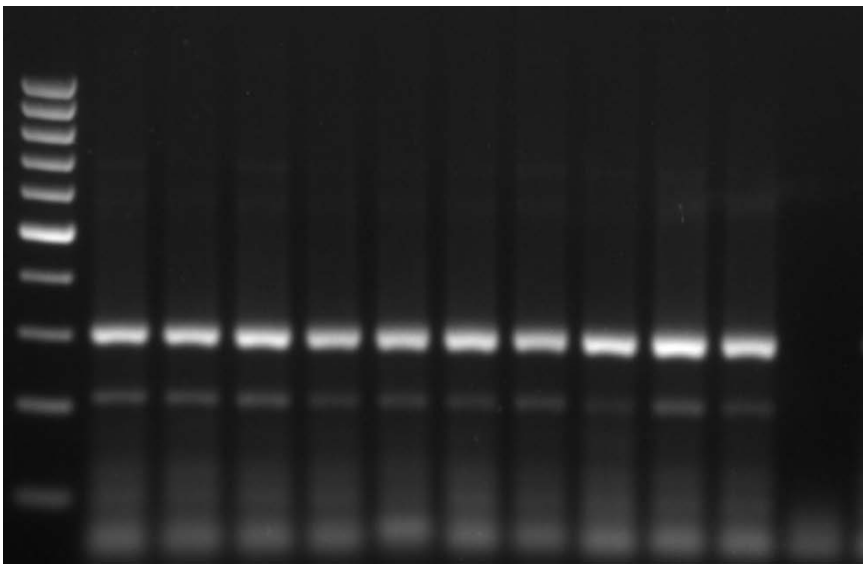

Fig. 2 : Multiplex PCR for Mannheimia haemolytica

Lane 1 – DNA ladder; Lane 2- 11 – samples from heart, trachea, lung, liver and kidney in duplicate. Lane 12 – Negative control

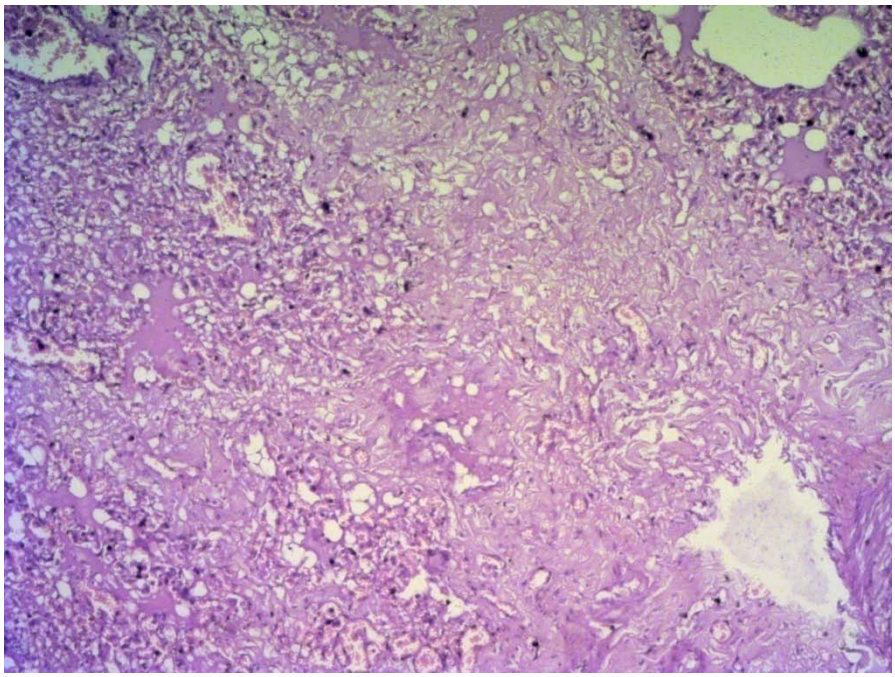

Fig. 3 : Haemotoxylin Eosin Staining.

Lung- Interlobular septa thickened with fibrinous exudation\

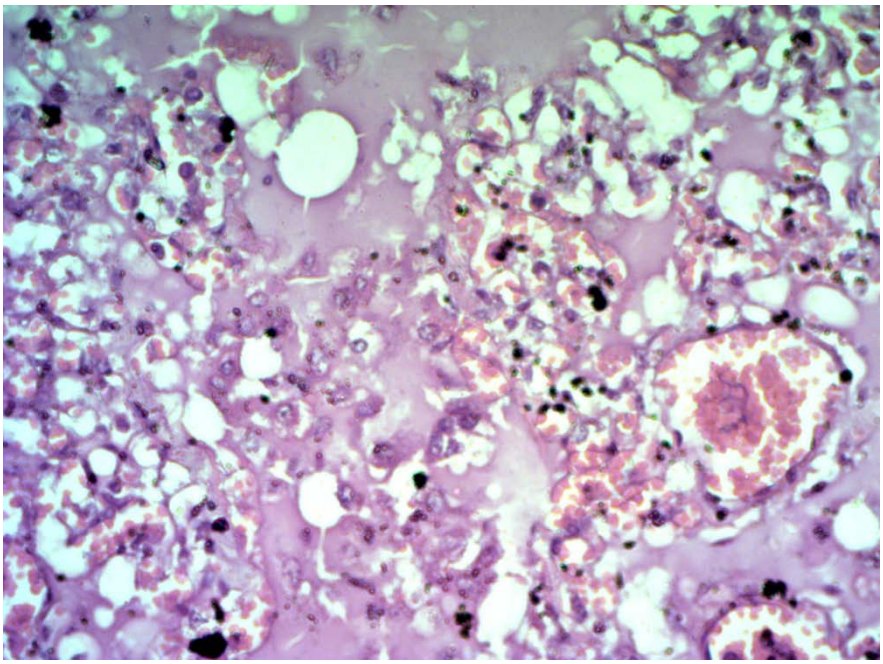

Fig. 4 : Haemotoxylin Eosin Staining.

Alveoli and bronchioles– infiltrated with neutophils, macrophages, fibrin

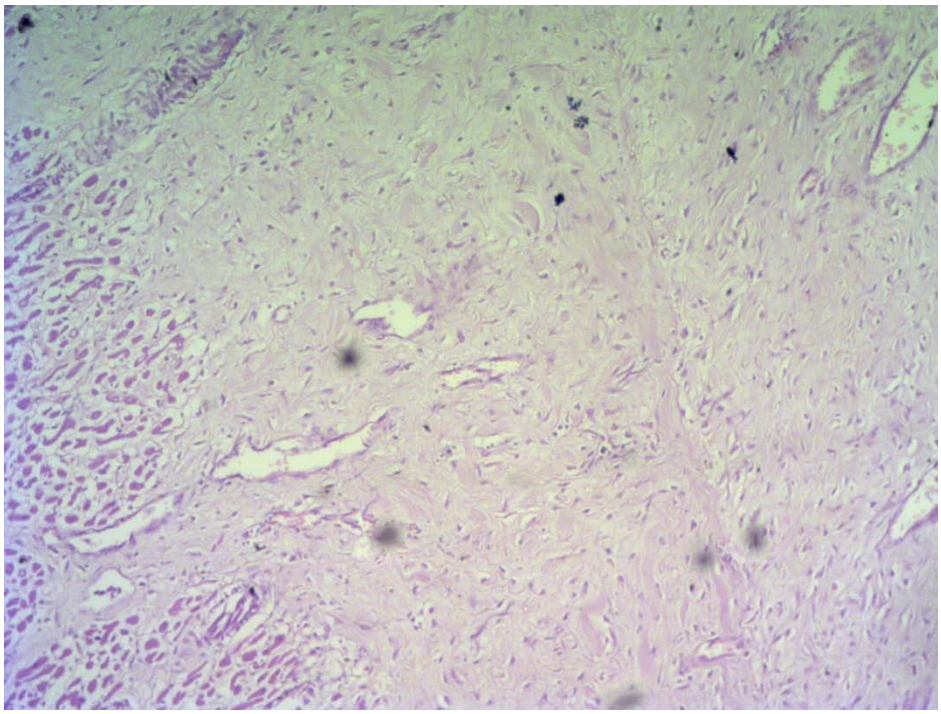

Fig. 5 : Haemotoxylin Eosin Staining.

Heart- Fibrovascular thickening of the pericardium

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