Chapter

Pathological Changes Associated with Natural Outbreak of Swine Pasteurellosis

Mamta Choudhary, Binod Kumar Choudhary and Ratan Chandra Ghosh

Abstract

Swine pasteurellosis is usually observed in descript as well as nondescript pigs imparting in huge economic losses to the pig producers. The disease is characterized by pyrexia, dullness, staggering gait, anorexia, serous nasal discharge and dyspnoea. Case fatality rate may as high as 95% in adult animals and 100% in piglets. Typical lesions of oedematous swellings may remarkably visible in the pharyngeal region, these swellings spread to the ventral cervical region and brisket of pigs. Gross lesions include severe pneumonia and haemorrhages in lungs, petechial haemorrhages on serous membranes and other visceral organs. Lymph nodes usually get enlarged, oedematous and haemorrhagic. The blood smears from heart blood and tissue impression smears reveal teaming numbers of bipolar organisms indicating the presence of *Pasteurella* spp., the etiological organism. The bacteriological isolation and characterization of causative agent should be ruled out to identify by Gram' staining for purity and bipolar morphology and biochemical characterization of the organisms. Molecular characterization necessitates to confirm *Pasteurella multocida* along with capsular types of the organism. Histopathological examination of lungs usually reveals typical fibrinous bronchopneumonia, multifocal suppuration and pleural thickening. Heart of some pigs may show presence of thrombi, haemorrhages and necrosed myocardium.

Keywords: Swine Pasteurellosis, *Pasteurella multocida*, Haemorrhagic septicaemia, capsular types, fibrinous bronchopneumonia

1. Introduction

Pasteurella multocida is of substantial economic significance in the livestock industry [1]. Infections by *Pasteurella multocida* have been reported in all the animals and fowls [2]. It is an important principal animal pathogen for over a century and is becoming crucial as human pathogen [3] leading to a disease process termed Pasteurellosis. *Pasteurella multocida* B:2, which causes haemorrhagic septicaemia (HS) of ruminants, is believed to enter the host via respiratory and oral routes. While the role of respiratory route of infection has been established, *Pasteurella multocida* is one of the most fascinating Gram-negative bacteria and is a commensal of the upper respiratory tract of many animal species as the

organism is also a primary or secondary pathogen and responsible for a wide range of economically important diseases in domesticated animals throughout the world. Pasteurellosis is an infection of cattle, buffalo, swine and other species of animals caused by Gram-negative coccobacillary bipolar organism, *Pasteurella multocida*. It is OIE list B disease of ruminants in the tropical countries. *Pasteurella multocida* strains express a polysaccharide capsule on their cell surfaces and the antigenic specificity of the capsule determines the serogroups: A, B, D, E or F [4]. It has long been recognized that there is relationship exist between the capsular type and disease predilection [5], which suggests that the capsular polysaccharide type plays a role in host and disease specificity. For example, the majority of cases of fowl cholera are caused by capsular type A strains. Progressive atrophic rhinitis (PAR) of pigs is associated predominantly with capsular type D isolates, bovine and porcine pneumonia are associated mainly with capsular type A strains and haemorrhagic septicaemia of cattle and water buffaloes is caused exclusively by capsular type B and E isolates [6].

Capsular types A and D cause economic losses in swine because of their association with progressive atrophic rhinitis and enzootic pneumonia [7]. Its association with acute septicemic pasteurellosis in pigs has been recognized. *Pasteurella multocida,* a part of the commensal flora in the upper respiratory tract of pigs is shown to appear intermittently in the nasopharynx and subsequently shed in nasal secretions [8]. During this period, the carrier animal act to become a source of infection for in-contact susceptible animals. The role of pig as a reservoir of *Pasteurella multocida* for the transmission of the disease between pigs and cattle has been suggested. *Pasteurella multocida* is an important pathogen of pigs. It causes pneumonic pasteurellosis and is characterized by pneumonia, purulent bronchopneumonia and pleurisy. Affected pigs may have fever of up to 106^0F, are anorectic and disinclined to move. They show significant respiratory distress, often breathing through the mouth. Death is common after a clinical course of 4–7 days. There is a marked tendency of the disease to become chronic, resulting in reduced weight gains and frequent relapses. On post mortem examination there is a chronic bronchopneumonia with abscessation. Pleuritis is common and there may also be pericarditis. Peracute cases show an acute necrotizing fibrinous bronchopneumonia. Septicaemic disease with death asymptomatic acute deaths may occur within 12 hours in piglets. In India, it is associated with infection by capsular serotype B. The disease occurs in all ages of pigs including adults and is manifested by fever, dyspnoea and congestion on serosal surfaces.

Yet, Pasteurellae have been shown to be a common microflora of the upper respiratory tract in normal animals [9]. The organisms more often than not act as secondary invaders in animals with concurrent diseases or suffering from debilitating stressful conditions. HS is a peracute disease and is considered to be one of the most economically important diseases in Asia particularly in South and South East Asia leading to huge economic loss in livestock industry. *Pasteurella multocida* type B:2 assumed to be transmitted between the animals by aerosol infection and ingestion of contaminated river water or material with *P. multocida* especially during the HS outbreak. The clinical indication of this disease is often characterized by rapid course of high fever, respiratory distress, dullness, depression followed by death [10]. Pathogenesis of *P. multocida* is a complex interaction between host specific factors and specific bacterial virulence factors; therefore, understanding the disease pathogenesis is complex and depends on the bacterial strain, the animal model and their interactions. The key virulence factors identified in *Pasteurella multocida* include capsule, lipopolysaccharides, surface adhesions, iron regulated and iron acquisition proteins [11].

2. Epidemiology

The scope of epidemiology in modern animal husbandry practice is continuously widening. Epidemiological data provide information on various diseases which are pre-requisite for planning, execution and monitoring of disease control programmes. It is an important requirement for assessing economic impact of a disease and also for developing disease forecasting system. The disease is usually associated with wet, humid weather and increased incidence is recorded during wet, humid weather and during wet seasons. In countries where systemic epidemiological studies have been carried out, it has become evident that outbreaks do occur throughout the year but those occurring during wet seasons tend to spread presumably due to the longer survival of the organism under moist conditions [8]. Zhao *et al*. [12] examined one hundred and sixty-four clinical isolates of *Pasteurella multocida* recovered from two swine herds in Minnesota. The isolates were characterized by restriction endonuclease analysis (REA) and rRNA gene restriction fragment length patterns. They concluded that these genomic fingerprinting techniques were highly discriminatory and that capsular serotyping in combination with REA or ribotyping was an appropriate technique for epidemiological studies of *Pasteurella multocida* of swine origin.

3. Cultural, Phenotypic and Biochemical Characterization

Pasteurella multocida is one of the most fascinating bacterial pathogens. It is a small, Gram-negative rod or coccobacillary, non-motile, non-spore forming, facultative anaerobe belonging to the family *Pasteurellaceae.* The organism can be identified on the bases of cultural, morphological and biochemical characteristics. The organism is a Gram-negative rod with bipolar staining characteristics, which is non-haemolytic on sheep blood agar, aerobic to facultatively anaerobic and produces indole, oxidase, catalase and produce indole and ferment carbohydrates with slight gas production [13–15].

4. Genotypic Chracterization of *Pasteurella multocida*

Since the initial development of the PCR in 1985, the basic principle of *in vitro* nucleic acid amplification through repetitive cycling has had extensive application in all aspects of fundamental and applied clinical sciences [16]. The application of PCR technology for *Pasteurella multocida* identification was first reported in 1994 when primers constructed from the sequence of the toxA gene (encoding the dermonecrotic toxin implicated in progressive atrophic rhinitis) were used to detect toxigenic *Pasteurella multocida* strains. PCR techniques play a critical role in the clinical laboratory diagnosis as rapid and specific detection of microorganism. It has provided remarkable advances in the diagnosis of infectious agents, particularly in cases where the presence of organism is having significance. Lichtensteiger *et al.* [17] investigated the feasibility of PCR for accurate, rapid detection of toxigenic *Pasteurella multocida* from swabs. They developed a PCR protocol which resulted into amplification of an 846-nucleotide segment of the toxA gene. They developed a concordance of PCR results with (i) detection of toxA gene with colony blot hybridization, (ii) detection of toxA protein with colony immunoblot analysis, and (iii) lethal toxicity of sonicate in mice in a test set of 40 swine diagnostic isolates. Results of an enzyme-linked immunosorbent assay for toxA agreed with the other

assays except for a negative reaction in one of the 19 isolates that the other assays identified as toxigenic. They suggested that PCR detection of toxigenic *Pasteurella multocida* directly from clinical swab specimens should be feasible.

5. Gross pathological lesions

On post-mortem examination of dead pigs from natural outbreaks, the gross pathological lesions may be marked by congestion and petechial haemorrhages on all over the serous membranes. Widespread petechial haemorrhages in the wall of thoracic cavity is the hallmark of the disease. Hydrothorax with presence of straw-coloured fluid in thoracic cavity can be seen (**Figure 1**). All the visceral organs may exhibit petechial to ecchymotic haemorrhages on the serosal surfaces. In some animals, hydrothorax, pleurisy and hydropericardium can also be prominent [18, 19].

The lungs usually show congestions with varying degrees of consolidation and with a marked thickening of the interlobular septa, pleura and rubbery consistency of lungs. There may be petechiae over the lungs (**Figure 2**). In acute cases the lungs may be severely consolidated with liver-like consistency. Whereas, subacute to chronic infection manifest grossly by marbled appearance of lungs (**Figure 3**), rubbery consistency and thickening of pleura (**Figure 4**), and emphysematous changes in lungs [20–22]. Heart may be severely congested and there may be presence of petechial as well as haemorrhagic streaks and necrotic foci which can be visible upon removal of pericardium (**Figure 5**). Rounding of heart and haemorrhages were also observed by Kapoor *et al.* [23]. The liver is one of the severely affected organs in this disease. The lesions may be characterized by congestion, petechiae and multiple necrotic foci on the surface of liver [21, 22]. Splenomegaly is a constant lesion seen in all the cases. There may be haemorrhagic enteritis in pigs died of swine pasteurellosis.

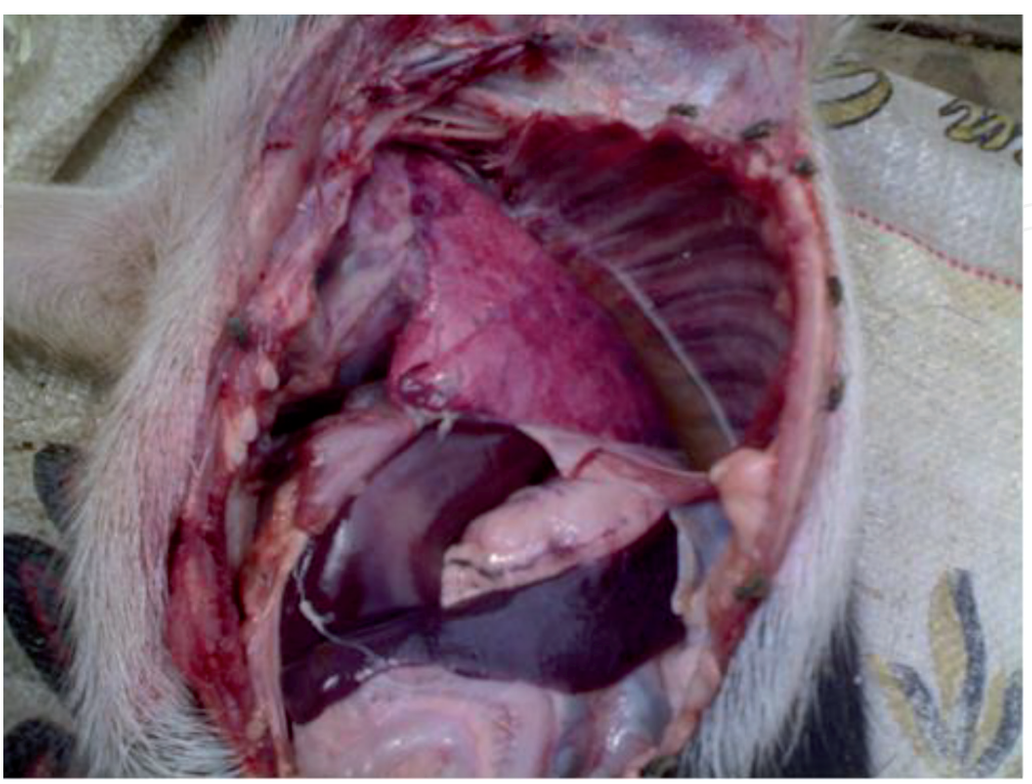

Figure 1.
Consolidation of lungs, hydrothorax, congested liver and splenomegaly in a pig died of swine pasteurellosis.

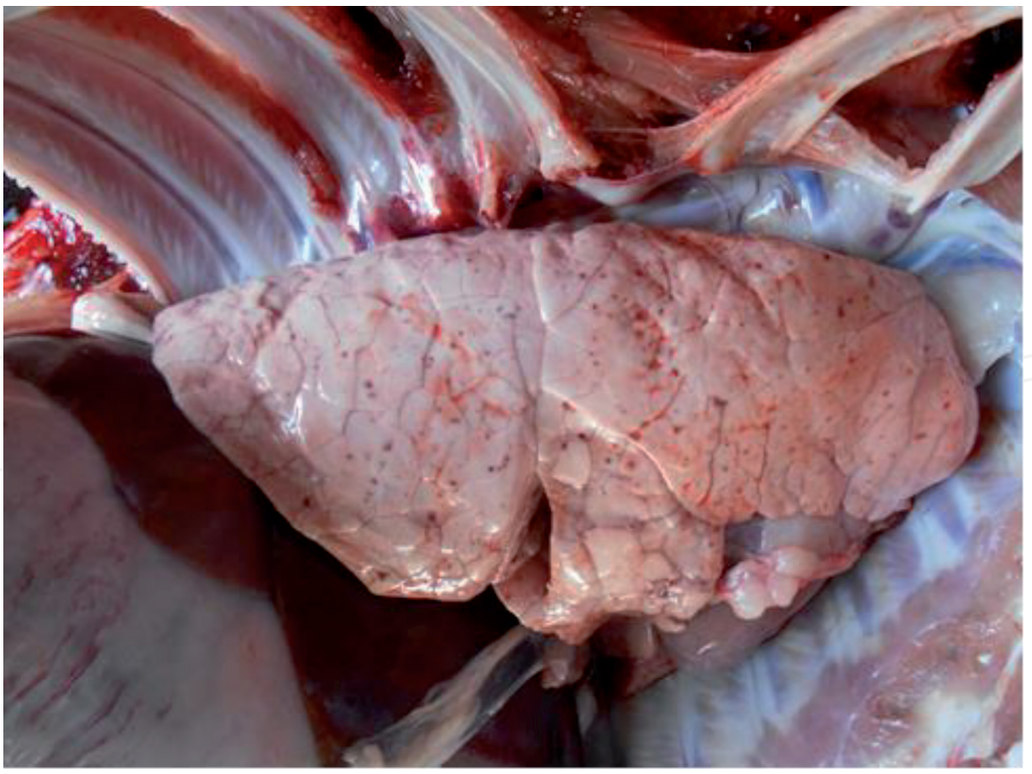

Figure 2.
Petechiae over lungs of a pig died of swine pasteurellosis.

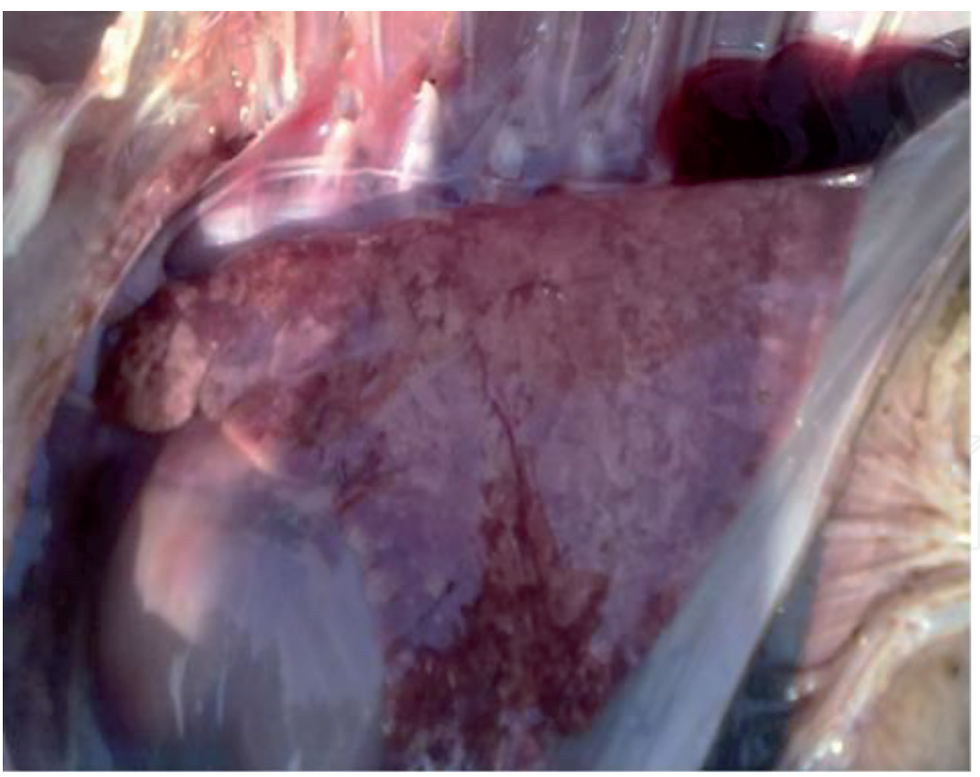

Figure 3.
Marbled appearance of lungs of a pig died of swine pasteurellosis.

6. Histopathological lesions

Lungs, the primarily affected organ, microscopically shows a variety of lesions from congestion of capillaries with thickened interlobular septa and atelectasis to severe lesions of perivascular and bronchial infiltration of inflammatory cells. There may be

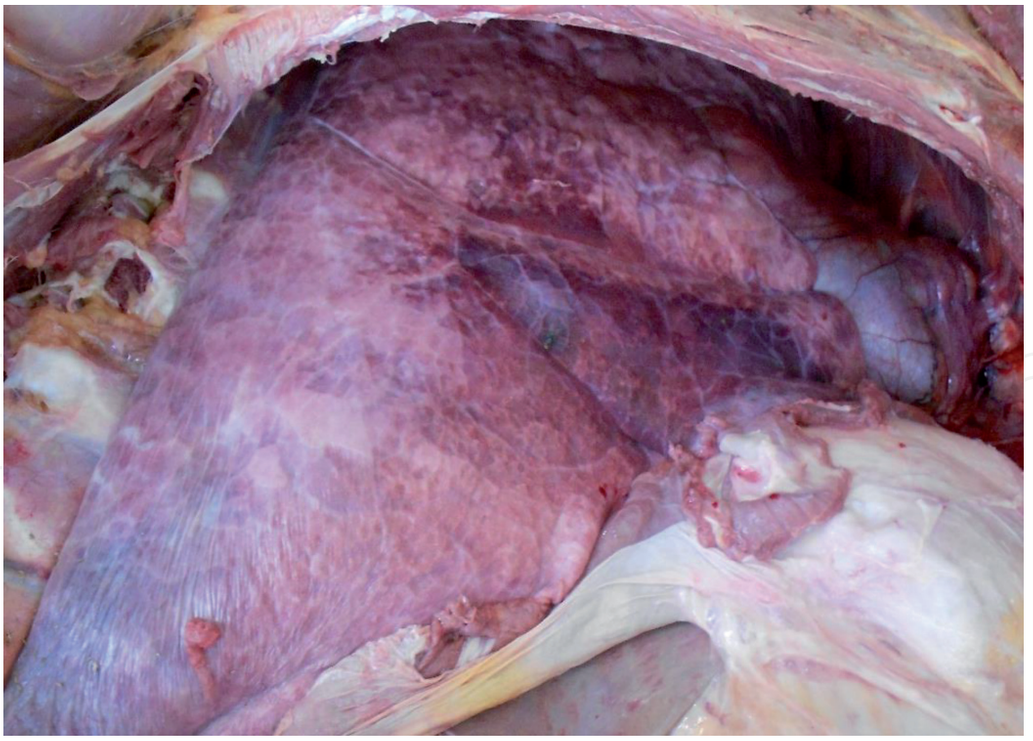

Figure 4.
Rubbery consistency of lungs and pleural thickening in a pig died of swine pasteurellosis.

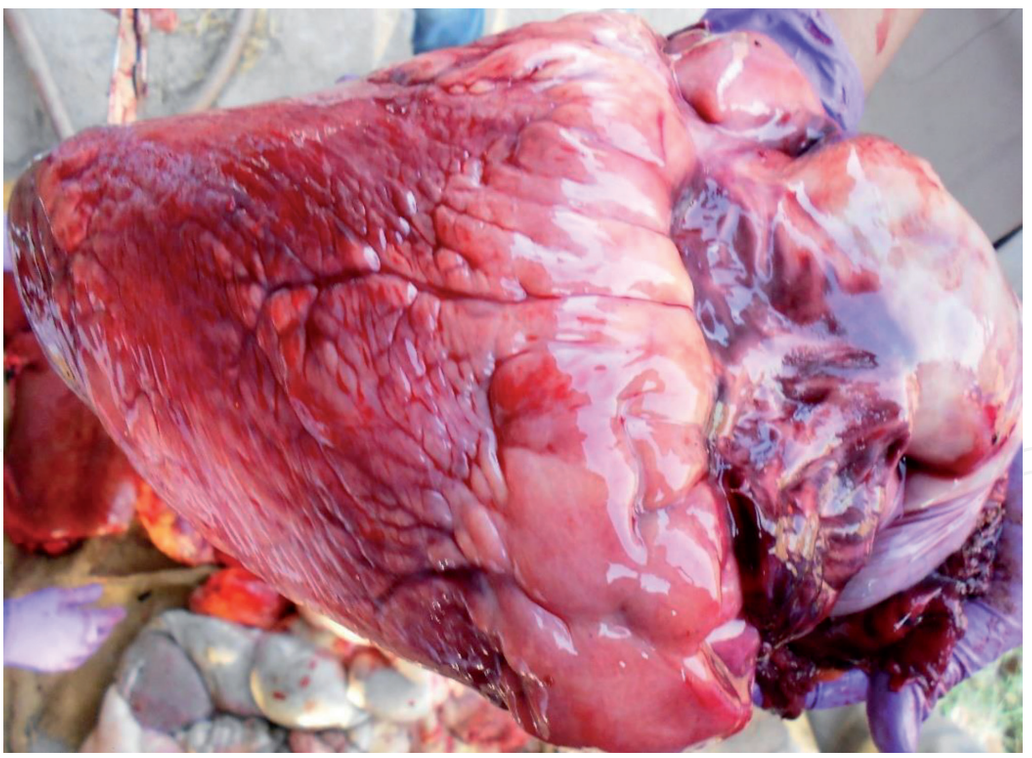

Figure 5.
Severe congestion and presence of haemorrhagic streaks in heart of a pig.

presence of oedema in lungs. The pneumonic lesions microscopically characterized by fibrinous pneumonia (**Figure 6**), necrotizing fibrinohaemorrhagic pneumonia, (**Figure 7**), interstitial pneumonia (**Figure 8**) and purulent bronchopneumonia (**Figure 9**). The acute fibrinous pneumonia characterized by serofibrinous exudation and infiltration with polymorphonuclear cells, macrophages and erythrocytes may

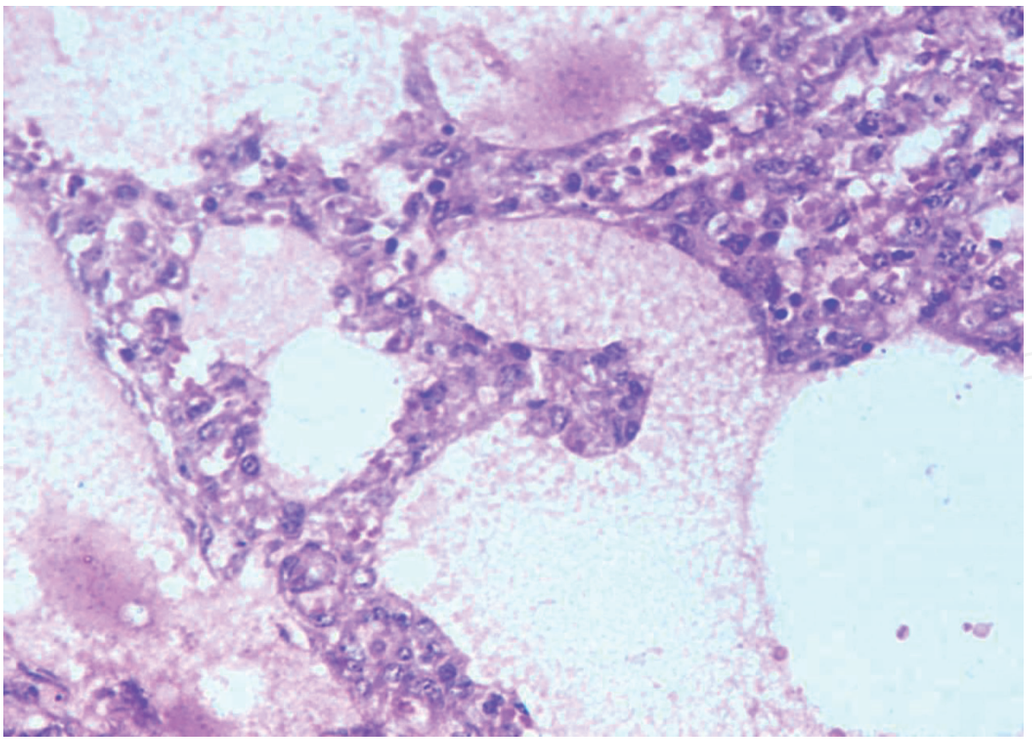

Figure 6.
Photomicrograph showing Fibrinous exudate in alveoli (H&EX400).

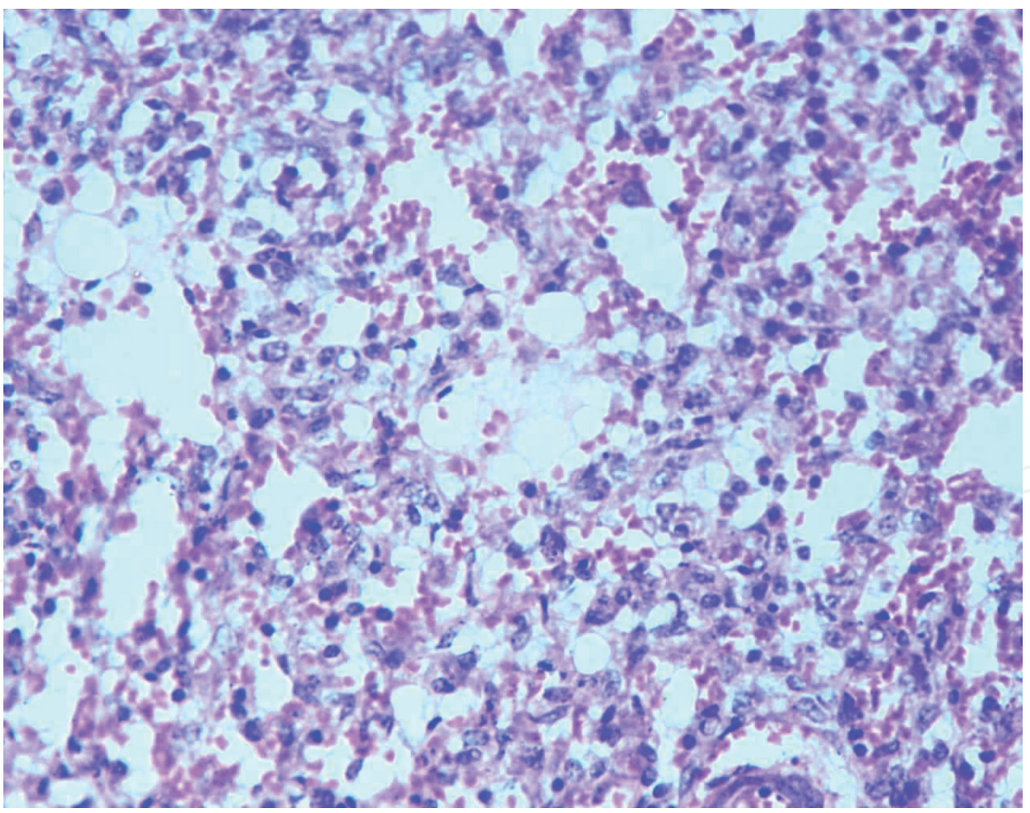

Figure 7.
Photomicrograph showing necrotizing fibrinohaemorrhagic pneumonia (H&E X400).

be observed. The bronchial and alveolar lumen usually filled with infiltrated erythrocytes, polymorphonuclear cells and macrophages (**Figure 10**). The alveoli showed variable changes from congestion to severe haemorrhages. Pleura and alveolar septa get thickened with fibrin, oedema and infiltration of polymorphonuclear cells. [23–26].

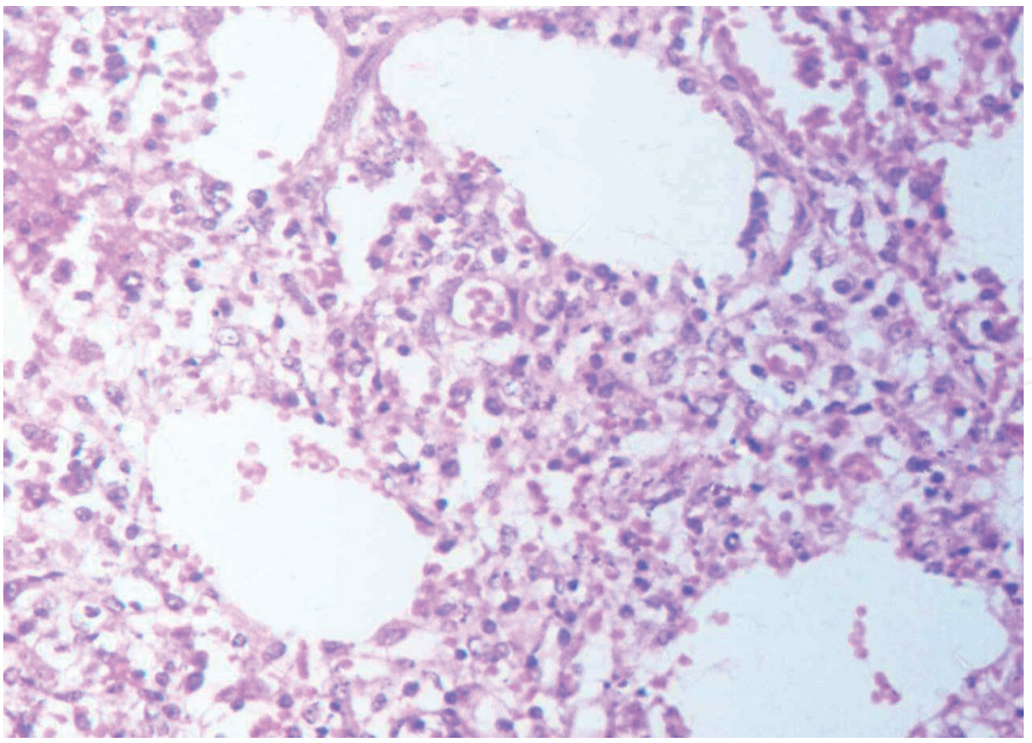

Figure 8.
Photomicrograph showing interstitial pneumonia (H&E X400).

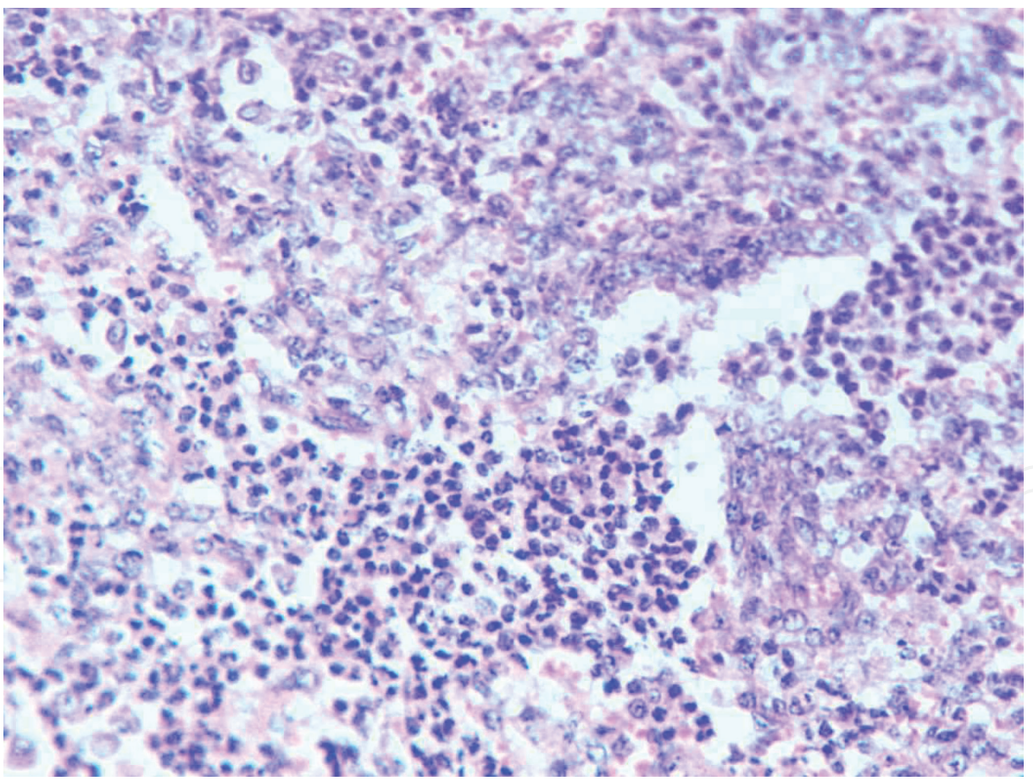

Figure 9.
Photomicrograph showing flooding of polymorphonuclear cells in alveoli (H&E X400).

Haemorrhages and necrosis may be evident in heart. Sub-pericardial haemorrhage and presence of erythrocytes in between the myocardial fibers may be remarkably noted. Myocardial necrosis can be marked as loss of striations of muscle fibers (**Figure 11**). There may be presence of thrombi in the blood vessels and fibrinous pericarditis in heart as a common finding. Liver is the consistently

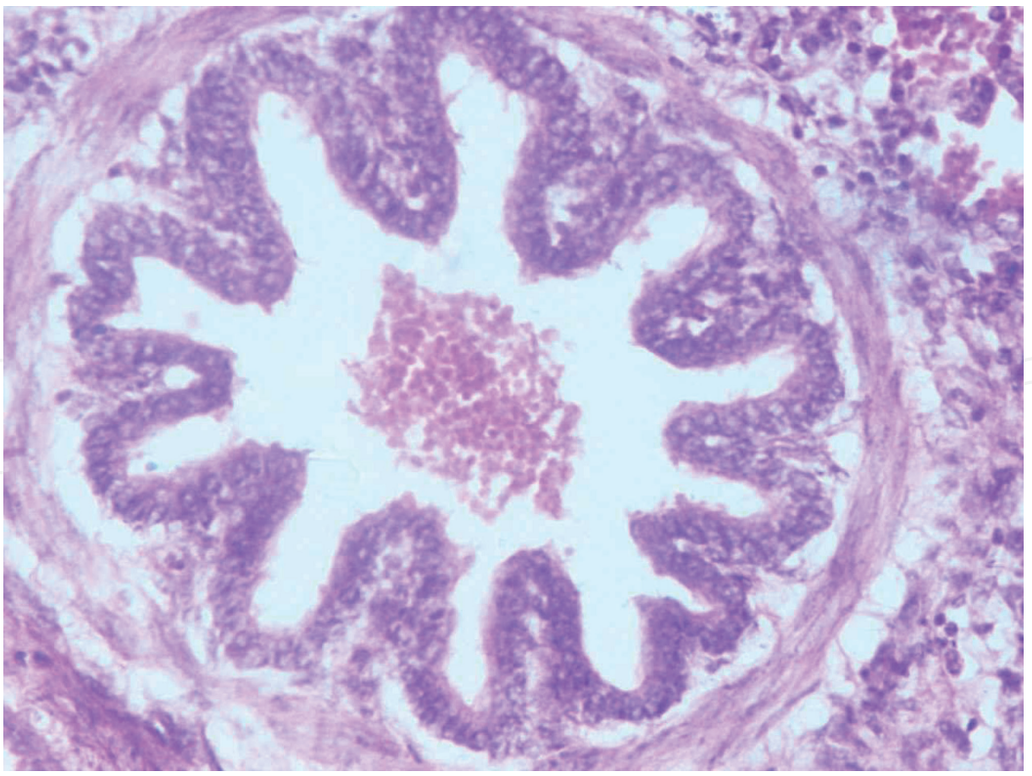

Figure 10.
Photomicrograph showing bronchiolar lumen containing exudate composed of erythrocytes (H&E X400).

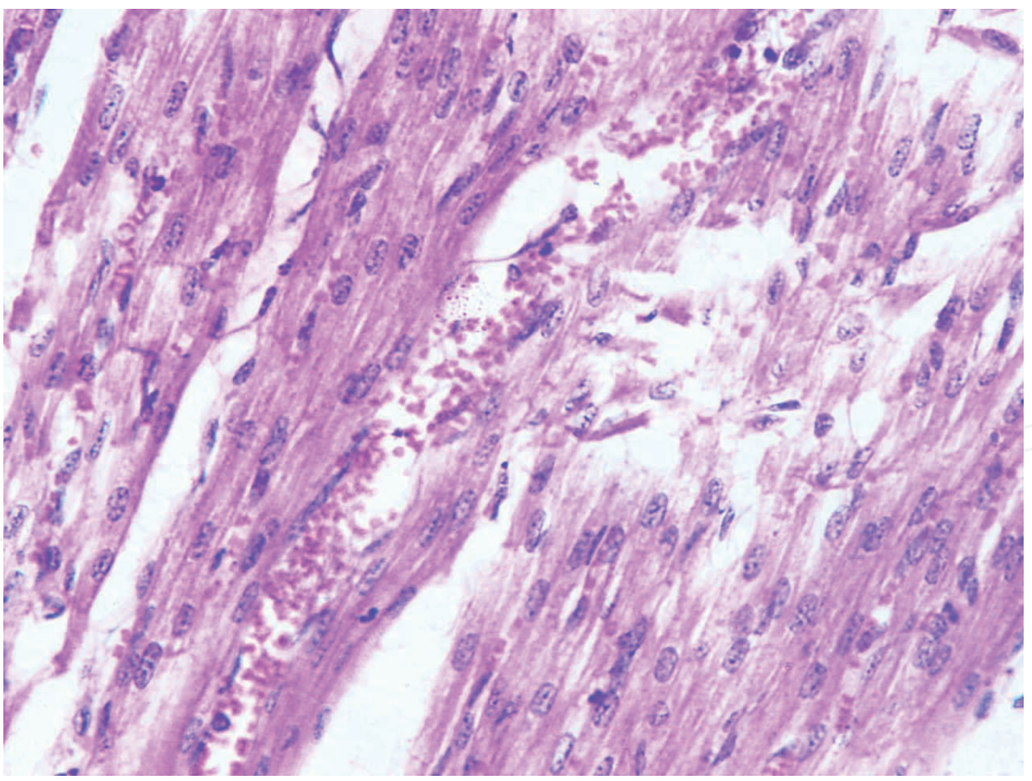

Figure 11.
Photomicrograph showing necrosis of myocardium and haemorrhages (H&E X400).

affected organ in this disease and the lesions reveal as invariably dilated and engorged blood vasculature and sinusoids. Focal areas of haemorrhages are usually seen. There will be hepatocytic swelling and increased activity of Kupffer cells in the parenchyma and focal areas of degenerative changes and hepatocytic necrosis.

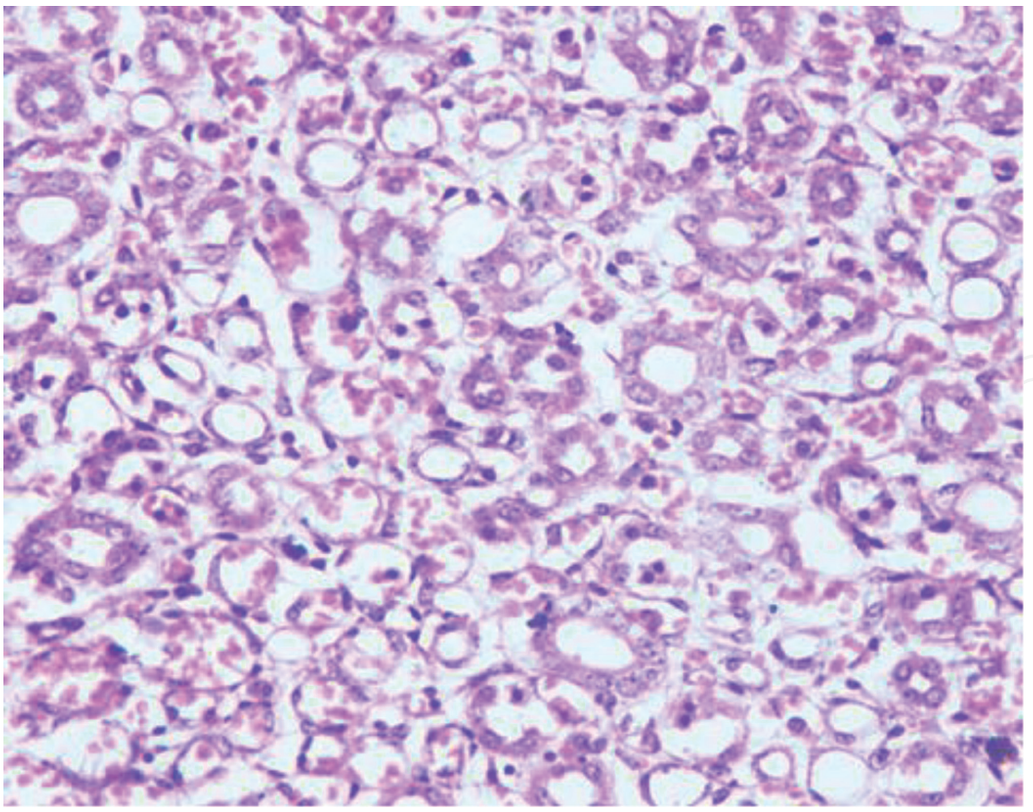

Figure 12.
Photomicrograph of kidney showing haemorrhages and sloughing of lining epithelium of renal tubules (H&E X400).

The hydropic degeneration of hepatocytes are characterized by cytoplasmic vacuolations and areas of hepatocytic necrosis with cellular infiltration. There may be dilatation of sinuses and disruption of hepatic cords seen in affected animals [20, 22, 27]. Lesions in Kidneys reveal as vascular congestion and focal areas of haemorrhages. Haemorrhages used to be intertubular (**Figure 12**). Cortical tubular epithelium may invariably swollen or degenerated with increased cytoplasmic granularity. The degenerative and necrotic changes of tubular epithelium will be diffuse in nature. Generalized degenerative and necrotic changes in the tubular epithelial cells may also be seen with variable severity from mild to high [20, 28].

There will be depletion of lymphocytes from germinal centre of the spleen and widespread necrosis can also be seen Spleen reveals as variably dilated and engorged vasculature, haemosiderosis, necrosis of lymphoid elements and infiltration of inflammatory cells mainly neutrophils [29, 30]. The microscopic lesions in the intestine are characterized by haemorrhages, necrosis of villi epithelium and desquamation of lining epithelium with focal infiltration of mononuclear cells in lamina propria and increase number of goblet cells [25].

7. Conclusions

Swine Pasteurellosis is an acute infection in swine caused by members of the *Pasteurella multocida*. Swine throughout the world are affected by pasteurellosis. *Pasteurella multocida* of swine can be isolated from natural field cases. Field isolate of *Pasteurella multocida* on sheep blood agar yield non-hemolytic, round, grayish, smooth or mucoid colonies. The isolates are Gram negative, cocco-bacilli in morphology and non-motile facultative anaerobe. Biochemically the isolates are positive for oxidase, catalase, indole production, reduction of nitrate, glucose and sorbitol

fermentation, but fail to ferment lactose, arabinose and adonitol. The isolate also found negative for citrate, lysine decarboxylase, urease, phenylalaline deamination and H_2S production. The genomic DNA of test isolates and organisms upon PCR using the primer pair KMT1SP6 and KMT1T7 produce an amplified product of approximately 460 bp size. On post-mortem examination of dead pigs, the gross pathological lesions observed in different organs marked by congestion, petechial to ecchymotic haemorrhages on serosal surfaces of all the visceral organs. The histopathological examination reveals as acute fibrinous pneumonia with variable degree of haemorrheges; mild to severe congestion and focal haemorrhages in heart, with loss of striation of heart muscle, thrombus formation in cardiac blood vessels, hydropic degeneration in liver, haemorrhages, hepatocytic necrosis and increased activity of Kupffer cells in the hepatic parenchyma and mild to severe necrosis in renal tubules with presence of focal haemorrhages.

Acknowledgements

Authors are thankful to the Dean, Veterinary College, Anjora, Durg, Chhattisgarh and Indian council of Agricultural Research for providing the necessary facilities to carry out the research.

Conflict of interest

The authors declare no conflict of interest.

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