CLINICOPATHOLOGICAL ATTRIBUTES OF SWINE PASTEURELLOSIS

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Received: February 6, 2015; Accepted: March 20, 2015

Abstract: Swine pasteurellosis was observed in descript as well as non-descript pigs of Chhattisgarh, India. Affected animals exhibited pyrexia, dullness, staggering gait, anorexia, serous nasal discharge and dyspnoea. Case fatality rate was 95% in adult animals and 100% in piglets. Typical lesions of oedematous swellings noted in the pharyngeal region, these swellings spread to the ventral cervical region and brisket of affected pigs. Gross lesions included severe pneumonia and haemorrhages in lungs, petechial haemorrhages on serous membranes and other visceral organs. Lymph nodes were enlarged, oedematous and haemorrhagic. The blood smears from heart blood and tissue impression smears revealed bipolar organisms indicating the presence of Pasteurella spp. The cultures obtained were subjected to Gram’s staining for checking the purity, bipolar morphology and biochemical characterization of the organisms. Molecular characterization revealed Pasteurella multocida, capsular type B. On histopathological examination, lungs showed typical fibrinous bronchopneumonia, multifocal suppuration. Pleura was severely thickened. Heart of some pigs showed presence of thrombi, haemorrhages and necrosis of myocardium.

Key words: Swine Pasteurellosis, Pasteurella multocida, Haemorrhagic septicaemia

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, the present study describes the clinical and pathological alterations following natural infection in pigs. Yet, Pasteurellae have been shown to be a common microflora of the upper respiratory tract in normal animals [4]. 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 characterised by rapid course of high fever, respiratory distress, dullness, depression and followed by death generally within a day of infection [5].
Pathogenesis of \textit{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 [6]. The key virulence factors identified in \textit{Pasteurella multocida} include capsule, lipopolysaccharides, surface adhesions, iron regulated and iron acquisition proteins [7].

**MATERIALS AND METHODS**

Outbreaks and sporadic cases suspected for pasteurellosis in pigs were attended in Raipur, Durg and Rajnandgaon districts of Chhattisgarh during the period May 2011 to October 2012. A total of 13075 samples were collected during the study period. Out of 13075 samples, 854 samples from both natural outbreaks and sporadic cases were processed for making tentative diagnosis of swine pasteurellosis. Case fatality rate was 95% in adult animals and 100% in piglets.

The affected animals were off fed and had high fever (41-42°C). About 95% mortality in affected pigs occurred. The atmospheric temperature and humidity were recorded between 24.5°C – 29.5°C and 76% – 93% respectively. The infected pigs were examined clinically. Detailed necropsy was conducted and gross lesions were recorded. The blood smears were examined for the presence of teaming number of bipolar organisms indicating the presence of \textit{Pasteurella spp}. The organisms were isolated from morbid as well as clinical blood/nasal samples of the pigs. Clinical samples, on sheep blood agar plates yielded tiny transparent, non-hemotytic colonies after an incubation of 24 hours at 37°C. The Gram’s negative cocci bacillary \textit{Pasteurella multocida} organisms were confirmed by biochemical characterization. The isolates of \textit{Pasteurella multocida} tested by PM-PCR were found to give an amplified product of 460 bp size using primer KMT1SP6 and KMT1T7 (Fig. 4). Capsular PCR analysis resulted in the amplification of a single band representing serogroup-specific regions of the biosynthetic loci in all of the isolates. The strains included in this study were confirmed as \textit{Pasteurella multocida} based on a positive PCR with primers specific for the capsular biosynthesis genes. All the isolates in present study were associated with pneumonia, were of capsular type B and yielded an amplification of 590 bp size. On amplification using ompH-specific primer, a PCR product of the expected size of 1.2 kb was obtained.

**RESULTS**

**Ante-mortem and post-mortem lesions:** Clinical examination of the ailing adult pigs revealed pyrexia (41-42°C), staggering gait, dullness, serous nasal discharge and dyspnoea leading to death after a clinical course of 4-6 days. Infected piglets showed high fever and serous nasal discharge and died within 24 hours of onset of fever. Oedematous swellings noted in the pharyngeal region, these swellings spread to the ventral cervical region and brisket of affected animals (Fig. 1). On post-mortem examination, petechiae and frothy exudate were present in trachea and bronchi. Petechiae were seen all over the serous membranes and peritoneum. There was hydrothorax with accumulation of straw coloured fluid. Endocarditis was observed in the heart, lungs showed severe consolidation along with the presence of multiple focal haemorrhages (Fig. 2). Spleen from most of the dead pigs were very much enlarged. Liver and kidneys were swollen and contained petechial haemorrhages. There was presence of haemorrhagic enteritis (Fig. 3). Lymph nodes were enlarged, oedematous and haemorrhagic.

**Bacteriological characterization:** The blood smears from heart blood and tissue impressions revealed teaming number of bipolar organisms indicating the presence of \textit{Pasteurella spp}. The organisms were isolated from morbid as well as clinical blood/nasal samples of the pigs. Clinical samples, on sheep blood agar plates yielded tiny transparent, non-hemotytic colonies after an incubation of 24 hours at 37°C. The Gram’s negative coccobacillary \textit{Pasteurella multocida} organisms were confirmed by biochemical characterization. The isolates of \textit{Pasteurella multocida} tested by PM-PCR were found to give an amplified product of 460 bp size using primer KMT1SP6 and KMT1T7 (Fig. 4). Capsular PCR analysis resulted in the amplification of a single band representing serogroup-specific regions of the biosynthetic loci in all of the isolates. The strains included in this study were confirmed as \textit{Pasteurella multocida} based on a positive PCR with primers specific for the capsular biosynthesis genes. All the isolates in present study were associated with pneumonia, were of capsular type B and yielded an amplification of 590 bp size. On amplification using ompH-specific primer, a PCR product of the expected size of 1.2 kb was obtained.

**Histopathological Lesions:** Microscopically, Lung showed typical fibrinous bronchopneumonia, multifocal suppuration, septae were thickened with fibrin, combined with cellular infiltration and edema (Fig. 5). Alveoli were filled with fibrinous exudate, erythrocytes and polymorphonuclear cells. Pleura was severely thickened. There were sub-pleural haemorrhages. Heart of some pigs showed presence of thrombi, haemorrhages and necrosis of
Fig. 1: Oedematous swelling in the pharyngeal, ventral cervical region and brisket of an adult Hampshire crossbred pig; Fig. 2: Photograph showing hydrothorax, consolidation of lungs, splenomegaly and petechiae on thoracic wall of 6 months old Yorkshire crossbred pig; Fig. 3: Photograph showing haemorrhagic enteritis in pig; Fig. 4: Detection of *Pasteurella multocida* by species specific PCR (PM-PCR). Lane M: 100 plus DNA ladder, Lane 1: Positive control, Lane 2: Isolate S5, Lane 3: Isolate S11b, Lane 4: Isolate S12R, Lane 5: Isolate S25, Lane 6: Isolate S31b, Lane 7: Isolate S42c, Lane 8: Negative control; Fig. 5: Microphotograph showing fibrinous exudation, flooding of polymorphonuclear cells and erythrocytes in alveoli (H&E X400); Fig. 6: Microphotograph showing necrosis of myocardium and haemorrhages (H&E X400); Fig. 7: Section of Liver showing extensive haemorrhages (H&E X400).
myocardium (Fig. 6). Glomeruli and kidney tubules showed haemorrhages and necrotic changes. Severe congestion and haemorrhages were observed both in cortex and medulla of lymph nodes. There were haemorrhages and necrosis in liver (Fig. 7).

DISCUSSION

*Pasteurella multocida* is considered as a commensal organism in the upper respiratory tract and tonsils and causes disease outbreaks in swine, cattle, buffalo, sheep and goats under extreme environmental conditions, or in animals immunosuppressed by viral infections [8]. This organism is the most common pathogen isolated from pigs housed under poor husbandry conditions, e.g. overcrowding and poor ventilation. The high mortalities observed in many outbreaks of swine pasteurellosis in India [9]. Similar outbreak of haemorrhagic septicaemia in pig herd has been reported earlier with variable degree of necrotic myocitis. The ulcerative skin lesions were absent during recent outbreak. Oedematous swellings were noted in the pharyngeal region of some of the infected pigs which is the predominant manifestation in cattle and bufaloes. *P. multocida* type B:2, is responsible for hemorrhagic septicemia in dairy cattle and buffalo and many outbreaks are reported every year from all over India suggesting that this serotype is transmitted between bovine species and swine [10]. The pathological modifications viz. generalized lymphadenopathy, submandibular and brisket edema, acute fibrinous pneumonia, proctitis, acute colitis and hemorrhagic typhilitis. The clinical manifestations characteristic of HS infections includes increased body temperature, respiratory rate, salivation, depression and anorexia [11]. Chronic bronchopneumonia with abscessation and pleuritis have been found to be common lesions of pneumonic pasteurellosis in pigs [12].

The outbreak of pasteurellosis is attributed to impairment of host defences mechanism, strain and virulence of causative organism and various other physiological and environmental stress factors. Incidence of swine pasteurellosis in present case appears to have occurred due to high relative humidity during rainy season which is one of the important environmental stress factors. Post-mortem find-ings and pathological changes suggestive of swine pasteurellosis have also been reported by other workers [10,12]. Thus, due to its highly contagious nature and high mortality rate, the disease has played a major role in huge economic loss in livestock population of rural Chhattisgarh.

ACKNOWLEDGEMENTS

Authors are thankful to the Dean, Veterinary College, Anjora, Durg, Chhattisgarh and Director, ICAR-National Research Centre on Equines, Hisar, Haryana for providing the necessary facilities to carry out the research.

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