MOLECULAR DETECTION AND CARDIOPULMONARY PATHOLOGICAL STUDIES OF PORCINE PASTEURELLOSIS IN PIG POPULATION OF CHHATTISGARH

TIGGA, M.,* GHOSH, R. C., MALIK, P.,1 CHOUDHARY, B. K. AND MISHRA, C. S.

Department of Veterinary Pathology, College of Veterinary Science and A.H., Kamdhenu Vishwavidyalaya, Durg, Anjora, Chhattisgarh; 1CCS, National Insitute of Animal Health, Baghpat (U.P.). E. mail: chiyamum@gmail.com, Cell: 07389245959

Received: June 5, 2015; Revised: June 26, 2015; Accepted: June 30, 2015

Abstract: Outbreaks of swine pasteurellosis were observed in descript as well as non-descript pigs of Chhattisgarh, India. It is an economically important infectious disease of pigs, has brought great economic losses to the pig farmers by high rates of morbidity and mortality. Affected animals exhibited pyrexia, dullness, staggering gait, anorexia, serous nasal discharge and dyspnoea. Affected pigs revealed 66% mortality and 100% case fatality rates in pre-weaned piglets. The article describes the molecular characterization and cardiopulmonary pathological studies on swine pasterurellosis. Diagnosis of disease was confirmed by PCR on Pasteurella multocida isolated from clinical samples. Lungs, the predominantly affected organ were severely congested with varying degrees of consolidation and with a marked thickening of the interalveolar septa, pleura and rubbery consistency of lungs. Heart was severely congested and there were presence of petechial as well as haemorrhagic streaks and necrotic foci visible upon removal of pericardium. Histopathological examination showed haemorrhages and necrosis of myocardium, acute fibrinous pneumonia characterized by serofibrinous exudation and infiltration with polymorphonuclear cells, macrophages and erythrocytes.

Key words: Swine pasteurellosis, Pneumonia, Pasteurella multocida

INTRODUCTION

Pasteurella multocida is an important primary animal pathogen for over a century and is becoming crucial as human pathogen [1] leading to a disease process termed pasteurellosis. Pasteurella multocida which causes haemorrhagic septicaemia (HS) of ruminants is believed to enter the host via respiratory and oral routes. It 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.

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 [2]. Pathogenesis of P. multocida is a complex interaction between host specific factors and specific bacterial virulence factors and depends on the bacterial strain, the animal model and their interactions [3]. The key virulence factors identified in Pasteurella multocida include capsule, lipopolysaccharides, surface adhesions, iron regulated and iron acquisition proteins [4]. The protein toxin from Pasteurella...
*multocida* cause necrotic changes in vascular endothelium and induce all of the major symptoms in infected animals [5]. In Chhattisgarh state outbreaks of HS occur every year in different districts and cause high economic losses but many outbreaks remain unrecorded because of the lack of proper investigation and monitoring system. Therefore, there is a need of investigation of the organism at genotype level, pathology caused by the disease and also to ascertain the relative efficacy of available vaccines in conferring protection in vaccinated herd. Surveillance for HS in the Indian subcontinent is very important for understanding the disease pattern and for providing better control measures for the disease, and in turn reduces the losses to livestock owners. Unfortunately, little is known about the prevalent strain of *Pasteurella multocida* strain being circulated in the field and host response to vaccination procedures and even less about the transmission of the disease or why outbreaks continue to occur despite the intensive vaccination programme.

**MATERIAL AND METHODS**

Field outbreaks of swine pasteurellosis in descript and non-descript pigs were attended in Raipur, Durg and Rajnandgaon district of Chhattisgarh state. Clinically infected and dead animals were observed thoroughly. A total of 13075 samples were collected and processed during the study period from June 2011 to October 2013. Detailed necropsy of carcasses was conducted and gross lesions were recorded. Heart blood and representative tissue samples from heart, lungs, liver, spleen, kidneys and Lymph nodes were collected for bacteriological isolation and the tissue samples were preserved in 10% Formal saline solution for histopathological studies. The clinical samples were processed for bacteriological isolation and molecular characterization. Morbid tissue samples then processed by routine histopathological techniques and stained with haematoxylin and eosin stains [6].

The extraction of genomic DNA was done by using Xpedition™ Fungal/Bacterial DNA MiniPrep from Zymo Research Corporation USA. DNA extraction method was followed as per the protocol supplied along with the kit. PCR was carried out in thermal cycler (Master cycle, Eppendorff, Germany) using *Pasteurella multocida* specific primer [7] to amplify the KMT1 gene fragment, capsular gene fragment and outer membrane protein H (ompH) gene fragment. The PCR products along with 100 plus bp DNA ladder electrophorised in 1 per cent agarose gel containing ethidium bromide (5ìl /100ml). PCR products were visualized as a single fluorescent band under UV light in Gel documentation system.

**RESULTS**

The affected animals were anorexic and had high fever (41-42°C). About 95% mortality in affected pig herd was recorded. Typical cases of oedematous swellings noted in the pharyngeal region, ventral cervical region and brisket of affected adult pigs. On post-mortem examination, Petechiae and frothy exudates 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. All the visceral organs exhibited petechial to ecchymotic haemorrhages on the serosal surfaces. In some animals, hydrothorax, pleurisy and hydropericardium were also prominent.

Grossly, the lungs were severely congested with varying degrees of pneumatic changes. There were petechiae over the lungs (Fig.1). In acute cases the lungs were severely consolidated with liver-like consistency. Whereas, subacute to chronic infection was manifested grossly by marbled appearance of lungs, rubbery consistency and thickening of pleura, sub-pleural haemorrhages (Fig.2) and emphysematous changes in lungs. Heart was severely congested and there were presence of petechial as well as haemorrhagic streaks and necrotic foci visible upon removal of pericardium (Fig.3).

Lungs, the primarily affected organ, microscopically showed a variety of lesions. There was presence of oedema in lungs. The pneumatic lesions...
Tigga et al,
microscopically revealed as fibrinous pneumonia, necrotizing fibrinohaemorrhagic pneumonia, interstitial pneumonia and purulent bronchopneumonia (Fig.4). The acute fibrinous pneumonia characterized by serofibrinous exudation and infiltration with polymorphonuclear cells, macrophages and erythrocytes was observed. The bronchial and alveolar lumen was filled with infiltrated erythrocytes, polymorphonuclear cells and macrophages. The alveoli showed variable changes from congestion to severe haemorrhages (Fig.5). There were thickening of pleura and sub-pleural hemorrhages. Pleura and alveolar septae were thickened with fibrin, oedema and infiltration of polymorphonuclear cells. The pathological modifications viz. generalized lymphadenopathy, submandibular and brisket edema, acute fibrinous pneumonia, proctitis, acute colitis, hemorrhagic typhilitis, chronic bronchopneumonia with abscessation and pleuritis have been found to be common lesions of pneumonic pasteurellosis in pigs.

Haemorrhages and necrosis were evident in heart. There was sub-pericardial haemorrhage. Presence of erythrocytes in between the myocardial fibers was remarkably noted during the study. There were severe necrotic changes in myocardium. Necrosis of myocardium and haemorrhage were seen as predominant lesion (Fig.6). Loss of striations of muscle fibers was noticed during the present study. There was presence of thrombi in the blood vessels.

**Fig.7:** Detection of Pasteurella multocida by species specific PCR (PM-PCR). Lane M: 100 plus DNA ladder Lane 1: Positive control, Lane 2-7: Test Isolates

**Fig.8:** Detection of Capsular type of Pasteurella multocida by PCR. Lane M1 & M2: 100+ bp DNA ladder, Lane 1: Positive control, Lane 8: Negative control, Lane 2 to Lane 7 test isolates.

**PCR detection of Pasteurella multocida:** The isolates of Pasteurella multocida tested by Pasteurella multocida species specific PCR (PM-PCR) were found to give an amplified product of 460 bp size using primer KMT1SP6 and KMT1T7 (Fig.7). Three pairs of primer sets (CAPA, CAPB and CAPD) were used in the capsular PCR analysis of different isolates of Pasteurella multocida. However, only primer pair of CAPB resulted in the amplification of a single band of 590 bp representing the capsular type B of Pasteurella multocida (Fig.8). All the isolates in present study were associated with pneumonia were of capsular type B and yielded same amplicon size of 590 bp. On amplification using ompH-specific primer, a PCR product of the expected size of 1.2 kb was obtained.

**DISCUSSION**

Pasteurella multocida type B is responsible for HS in dairy cattle and buffalo and many outbreaks are reported every year from all over India [8,9,10]. Literature revealed that pigs that recover from the disease act as a reservoir of Pasteurella multocida type B not only for pigs, but also for nearby dairy herds. Two outbreaks of swine pasteurellosis in Punjab were recorded and found that Pasteurella multocida Serotype B was associated with both the outbreaks [11]. 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. Chronic bronchopneumonia with abscessation and pleuritis have been found to be common lesions of pneumonic pasteurellosis in pigs. Post-mortem findings and pathological changes suggestive of swine pasteurellosis have also been reported by other workers [11,12]. Thus, due to highly contagious nature of disease and conducive environmental stress, the disease has played a major role in huge economic loss in affected pig population.

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.

REFERENCES