

Original Research Article

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Pathology of *Salmonella choleraesuis* Related Respiratory Infection in Piglets, Its Isolation, Identification and Antibigram

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ABSTRACT

Keywords

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Ninety two piglet carcasses showing respiratory lesion presented to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur for necropsy were selected for detailed pathological and bacteriological studies. Five lung samples were found to be positive for *Salmonella choleraesuis* infection based on culture, morphology, biochemical tests and *fliC* gene based PCR. Gross lesions of *Salmonella* infected piglets revealed severe congestion, oedema, areas of consolidation, extensive haemorrhage and emphysema in lungs, frothy exudates in tracheal lumen and enlargement and congestion of the bronchial lymph nodes. Microscopically oedema fluid, infiltration of inflammatory cells in the interstitium and haemorrhages were the prominent changes in lungs characteristic of interstitial pneumonia were noticed. Bronchial lymph nodes showed lymphoid depletion and haemorrhages. This study established the prevalence, pathology, molecular detection *S. choleraesuis* in respiratory infection in the pig population in Thrissur.

Introduction

Salmonella is one of the most important enteric pathogen affecting intensively reared weaned piglets below five months of age. Porcine *Salmonella* serovars are mainly classified as host restricted serovars typified by *S. choleraesuis* and ubiquitous serovars typified by *S. typhimurium*. *S. derby* and *S. typhimurium* were considered as the most commonly isolated serovars from pigs. The main mode of transmission of *Salmonella* was considered as faeco-oral route but aerosol transmission was also suggested to be the important route (Fedorka Cray *et al.*, 1995).

Worldwide increase in antimicrobial resistance to non-typhoid *Salmonella* species has become a major problem for public health concern. Antibiotic resistance cause reduced effect on both conventional and newer antibiotic therapy, thereby aggravating the situation. In a study conducted by Jean *et al.*, (2006) human and swine isolates were shown to have the same pulsotype and they hypothesized that *Salmonella* transmission from swine to humans caused antibiotic resistance in humans due to the use of antibiotics in veterinary practice at sub

therapeutic level or as growth promoters in feed. The present study envisaged better understanding of isolation, identification and pathomorphology of *S. choleraesuis* in respiratory infections of piglets which will be helpful in adopting proper therapeutic modalities and initiating better control measures.

Materials and Methods

Lung samples of piglets showing respiratory lesions which were brought for postmortem examination at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Kerala during a period from January to September, 2016 were collected. Detailed postmortem examination of carcasses showing respiratory lesions was performed. Lung samples were collected in 10 per cent neutral buffered formalin and in sterile Petriplates for histopathology and bacteriological examinations respectively. *Salmonella* was isolated using MacConkey agar and subjected to biochemical tests using HiAssorted™ Biochemical test kit (HI-MEDIA). The identification of bacteria was done as per standard protocols prescribed by Bergey's manual (Quinn *et al.*, 1994).

Isolates positive in conventional methods were subjected to *fliC* based *S. choleraesuis* specific primer as per Chiu *et al.*, (2005). Forward primer 5'-AAG GAA AAG ATC ATG GCA CAA-3' and reverse primer 5'-GAA CCC ACC ATC AAT AAC TTT G-3' were used to get an amplicon size of 963bp. Amplification of PCR product was confirmed by one per cent agarose gel electrophoresis. The gel was visualized and documented in a gel documentation system (Bio-Rad laboratories, USA). All positive *Salmonella* isolates were analyzed for antimicrobial resistance by the disc-diffusion method (Cockerill *et al.*, 2013) using commercially available antibiotic discs (HiMedia

laboratories Limited, Mumbai). Representative lung tissues were processed and embedded in paraffin. Tissue sections were taken at 4-5 µm thickness and routine haematoxylin and eosin staining (Bancroft and Cook, 1995) was done and examined under light microscope.

Results and Discussion

Salmonella choleraesuis is a host restricted serovar producing systemic infection in weaned piglets less than five months of age and characterized by various clinical signs like fever, lethargy, reduced appetite, shallow expiratory dyspnea, huddling behavior and severe scouring. In the present study, five out of 92 samples were positive for *S. choleraesuis* (5.4 per cent). This result was similar to the results of Bahadur *et al.*, (2016), Foley *et al.*, (2008). Bose (2015) reported 60 per cent *S. choleraesuis* infection in lung samples of piglets. This difference in results could be due to different factors like sample size, environmental condition, housing and management of animals, geographical area and virulence of organism.

All isolates were found to be aerobic, gram negative, short rods giving non lactose fermenting colonies of 1-2 mm in MacConkey Agar and smooth, low convex pink colonies surrounded by pink hue in Brilliant green Agar (Figure 1). The primary genus identification and secondary serovar identification was done using different biochemical (Figure 2) and sugar fermentation tests and the results are presented in Table 1. This was in accordance with Irimie *et al.*, (2010), Sakano *et al.*, (2011) and Bose (2015).

Five bacterial isolates that were positive in conventional methods were subjected to PCR using a primer, specific for *S. choleraesuis fliC* gene and all the five samples got

amplified at 963bp (Figure 9). This was in accordance with Chiu *et al.*, (2005) and Bose (2015). Bose (2015) reported extra intestinal isolation of *Salmonella* from lungs, heart blood and bronchial lymph node of pigs based on conventional and PCR method. Nikbath and Sani (2016) reported that the combination of pre-culture, culture and PCR method can be used for the precise and accurate confirmation of *Salmonella*. In the present study, an accurate identification of *Salmonella choleraesuis* was done using conventional and molecular methods.

For identifying the antibiotic sensitivity of *S. choleraesuis*, all five positive isolates were

subjected to antibiogram (Figure 4). All the isolates were uniformly sensitive to cefixime, gentamicin, ciprofloxacin, amoxycylav, levofloxacin, ceftriaxone, chloramphenicol, cephalixin, nitrofurantoin, cefotaxime, ciprofloxin and resistance to ampicillin, clindamycin, nalidixic acid, penicillin G and erythromycin. In a study conducted by Chiu *et al.*, (2002) found only the third generation cephalosporines had the reliable activity against *S. choleraesuis*. Resistance is mainly due to the improper use of antibiotics in animal practice, mutation and also due to interspecies dissemination of resistant plasmids from other organism (Jean *et al.*, 2006).

Table.1 Biochemical and sugar fermentation test of isolates

SI No.	Test	Result
1	ONPG	-
2	Lysine utilization	+
3	Ornithine utilization	+
4	Urease	-
5	Phenyl alanine deamination test	-
6	Nitrate reduction	+
7	H ₂ S production	-
8	Citrate utilization	+
9	Voges Proskauer's test	-
10	Methyl red	+
11	Indole	-
12	Malonate utilization	-
13	Esculin hydrolysis	+
14	Oxidase	-
15	Arabinose	+
16	Xylose	+
17	Adonitol	-
18	Rahmnose	+
19	Cellobiose	-
20	Melibiose	+
21	Saccharose	-
22	Raffinose	-
23	Trehalose	+
24	Glucose	+
25	Lactose	-



Fig.1 Pink *Salmonella* colonies in Brilliant green agar



Fig.2 Indole (-), Methyl red (+), Voges Proskauer (-) & Citrate (+)



Fig.3 Lung-Unilateral consolidation of lungs



Fig.4 Antibiogram of *Salmonella* Choleraesuis

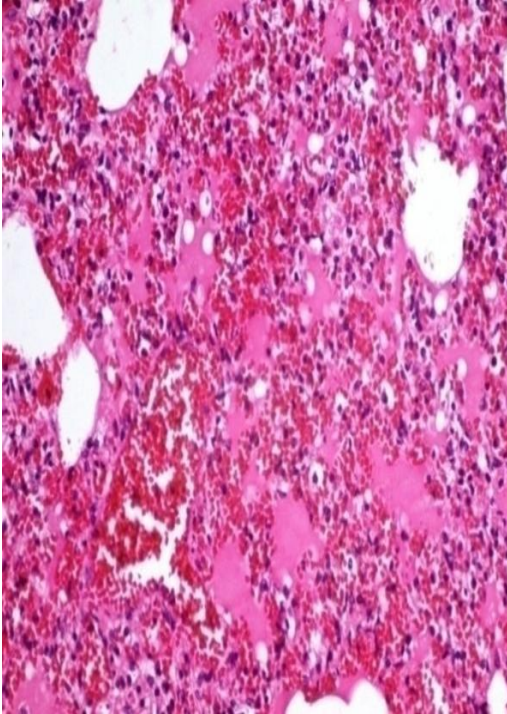


Fig.5 Lung-Pulmonary edema (H&E x400)

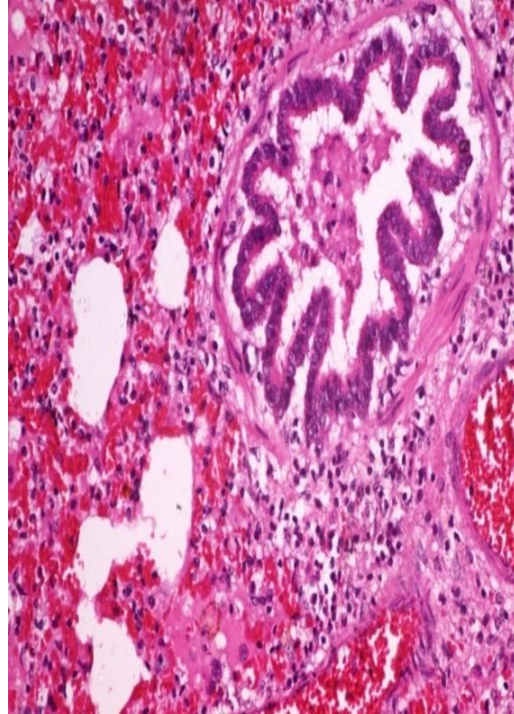


Fig.6 Lung-Broncho-interstitial pneumonia (H&E x400)

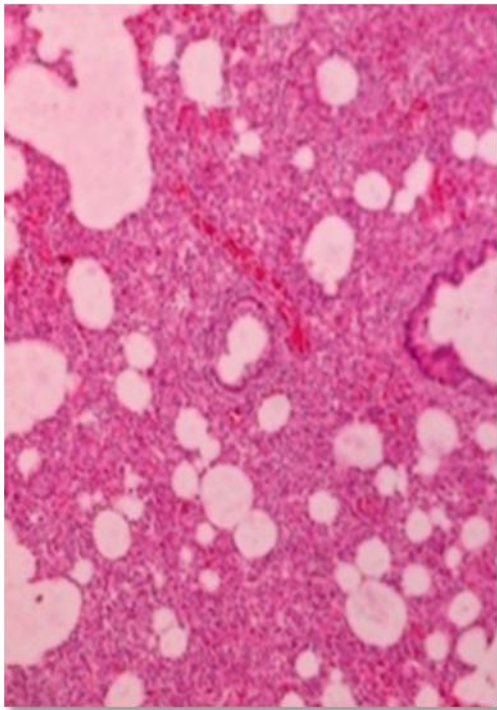


Fig.7 Lung-Interstitium haemorrhages (H&Ex100)

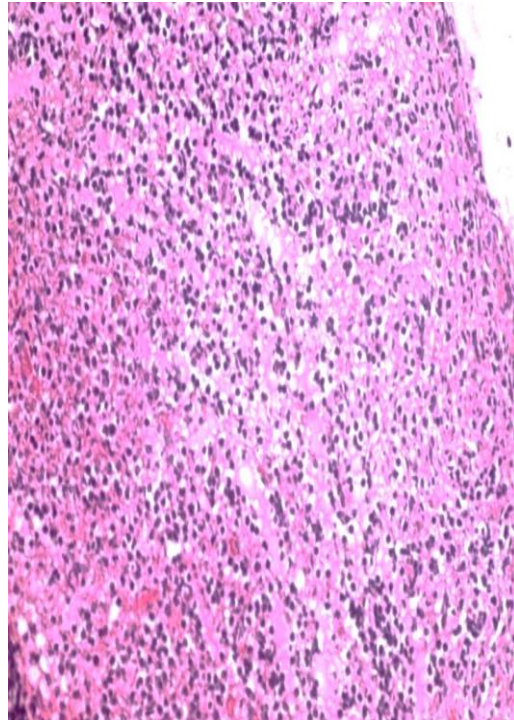
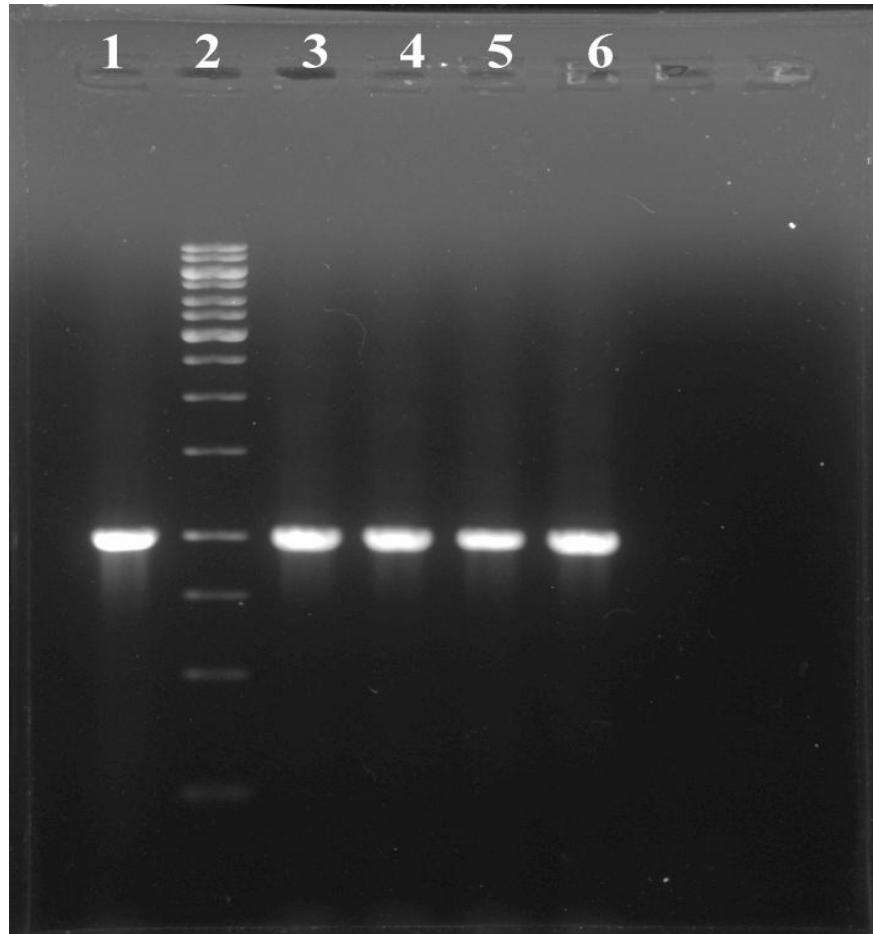


Fig.8 Bronchial lymph node-Severe lymphoid depletion (H&E x 400)

Fig.9 *fliC* gene based *Salmonella choleraesuis* specific PCR



Lane 1, 3, 4, 5, 6 - Samples
Lane 2- 1kbp DNA ladder

On postmortem examination, piglets revealed cyanosis of ear, snout, feet and ventral abdomen. Lungs were voluminous and characterized by the presence of severe oedema, congestion, cranio-ventral consolidation and marked areas of emphysema (Figure 3). Bronchial lymph nodes were congested and enlarged. The presence of froth in tracheal lumen was also observed. These findings were in accordance with those reported by Baskerville and Dow (1973), Carlson *et al.*, (2012), Karanja *et al.*, (2013) and Bose (2015).

Microscopically, presence of oedema in the alveolar lumen and interlobular septum was

observed (Figure 5). Interstitial pneumonia characterized by infiltration of mononuclear inflammatory cells, oedema and haemorrhages were noticed in interstitium (Figure 7). Mild to moderate thickening of interlobular septa with proliferation of fibroblast, oedema and infiltration of inflammatory cells were evident. Necrotic debris with eosinophilic exudates was observed in the bronchial and bronchiolar lumen (Figure 6). Bronchial lymph nodes showed moderate to severe lymphoid depletion (Figure 8). This was in accordance with the findings of Bakerville and Dow (1973), Carlson (2012), Karanja *et al.*, (2013) and Bose (2015). LPS component of

Salmonella species is responsible for the vascular damage and thrombosis which in turn led to the increased permeability of vascular endothelium leading to osmotic imbalance and oedema (Carlson, 2012). Lopez (2012) stated that the oedema is an integral part of early inflammation due to the action of leukotrienes, cytokines and other vasoactive amine released by the inflammatory cells and epithelial cells. Bose (2015) stated that emphysematous changes could be a compensatory mechanism of lung alveoli in occlusions. In the present study also various lesions as observed in the lung could be attributed to the toxic and inflammatory effects of infection.

Thus the present study clearly shows lung is an important organ for the colonization of *S. choleraesuis* infection which may play an important role in pathogenesis. Molecular technique such as PCR could provide rapid and sensitive detection of *S. choleraesuis* and offer advantage over culture methods which would help in timely identification.

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