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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (24): 2021-2025, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.2021.2025

Isolation and Antibiotic Sensitivity of *Streptococcus pneumoniae* Infections with Involvement of Multiple Organs in Lambs

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Abstract: Respiratory diseases particularly lamb pneumonia is a multifactorial disease involving the interaction between host, etiological agent and environment. The present study was carried out to determine the causative agent of an outbreak of pneumonia in a sheep flock and to establish its pathogenicity and public health importance. The incidence occurred in sheep unit at Madhurikund farm of University (DUVASU), Mathura, Uttar Pradesh, India. At the time of incidence, the population of sheep at the farm was 90. Affected animals were clinically examined and nasal swabs and blood samples were collected from live animals, while morbid materials were collected from dead animals after postmortem examination. The etiological agent was isolated and characterized with conventional microbiological and biochemical methods. *Streptococcus pneumoniae* was the bacteria isolated from blood, different organs and cerebrospinal fluid. The antibiotic sensitivity revealed resistant to multiple drugs viz., penicillin, tetracycline, erythromycin, chloramphenicol, enrofloxacin and ciprofloxacin. Pathological examination revealed multiple involvements of organs with different degrees of inflammation and haemorrhages of the lower respiratory tract, lungs, liver, heart and kidney. Further, its pathogenicity was established by histopathological examination. In conclusion, presence of multi drug resistant *Streptococcus pneumoniae* in weaning lambs with the involvement of multiple organs appears to be an emerging zoonotic threat to human particularly in shepherds. This seems to be the first report of isolation of multi drug resistant *Streptococcus pneumoniae* from outbreak in lambs with multiple organ involvement in India.

Key words: *Streptococcus pneumoniae*, antibiotic sensitivity, lamb, histopathology

INTRODUCTION

Respiratory diseases especially lamb pneumonia is caused by interaction of various factors like host (immunological and physiological), etiological agent (virus, bacteria, mycoplasma) and environment factors (Brogden *et al*., 1998; Kumar *et al*., 2011, 2012). Out of many etiological agents *Streptococcus pneumoniae*, a commensal bacteria of the nasopharynx of animals, has been associated with a majority of cases of morbidity and mortality in young lambs due to pneumonia. They cause reduced growth, mortality in lambs and significant economic impact in terms of cost of treatment and condemnations in abattoirs (Jones *et al*., 1982; Goodwin *et al*., 2004). The present study revealed *S. Pneumoniae* as an etiological agent responsible for high morbidity and mortality rate in weaning lamb in an outbreak in Mathura district, Uttar Pradesh, India in 2010. The isolation of highly pathogenic *S. pneumoniae* is also of public health concern as sheep rearing community in India rear them within their premises.

MATERIALS AND METHODS

Study area, animal and management: The incidence occurred in sheep unit at Madhurikund farm of University (DUVASU), Mathura, Uttar Pradesh, India. At the time of incidence, the population of sheep at the farm was 90. The age of most of the affected animals was below one year. The animals were grazed during the day time on natural pasture and had free access to hay at night in properly sheltered pens. The flocks were kept in groups ranging from 20-35. Lambs suffering from respiratory disease were supplemented with concentrate feed in separate pen.

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They had *ad lib* supply of water. Sheep were drenched against internal parasites with albendazole in June and December. All were vaccinated against pasteurellosis with *Pasteurella multocida* vaccine (Biological Products Section, Badshabagh, Lucknow, India) and against Foot-and-Mouth Disease (Indian Immunologicals, Hyderabad, India).

History, clinical examination and data collection: An outbreak with respiratory and nervous signs occurred in the month of March, 2010. Infected and suspected animals were thoroughly examined by animal health researchers of University (DUVASU), Mathura for pyrexia (rectal temperature more than 105°F), abnormal respiration (sternal and abdominal respiration, polypnoea, dyspnoea), coughing and nasal discharge. Other signs like circling, staggering and paresis before death were also recorded. The health and basic record books of the flock, compiled by veterinary and animal care staff, were examined and analyzed for occurrence, history of disease, morbidity, mortality and other related epidemiological data. Clinical, serological, gross pathological and bacteriological investigations were carried out from infected animals, while post-mortem and histopathological examinations were conducted on dead animals.

Sample collection: Nasal swabs, blood samples, faeces and cerebrospinal fluids were collected from sick lambs whereas tissues such as liver, lung, kidney, lymph nodes and hearts were taken from dead lambs, during post-mortem examination.

Gross and histopathological examination: Post-mortem examinations of five dead lambs were conducted immediately after death. During necropsy, special emphasis was given to respiratory organs. Portion of the internal organs of dead lambs (lungs, spleen, liver, kidney, lymph nodes, and heart) were collected for bacteriological and histopathological examination.

Bacteriological culture and identification: Standard aseptic procedures were followed for bacterial isolation. Briefly, the surface of the organs were cleaned with denatured alcohol and flamed immediately following the procedures of Carter *et al.* (1995). It was followed by searing with heated spatula before the inner tissues were chopped and collected in PBS (pH 7.4) and further inoculated into sterile screw capped test tubes containing 5 mL of Tryptose soya, PPLO and SDA broth. Similar, procedures were followed for grossly non-pathologic specimens of the organs. Blood samples collected aseptically were also inoculated in the similar pattern with the help of sterilized bacteriological loop. Bacterial isolation was performed from nasal swabs, blood samples and cerebrospinal fluids of sick sheep and internal organs of dead lambs.

Samples and a loop full culture after 24 h of incubation were inoculated onto Nutrient agar, MacConkey Lactose agar (MLA), Sabouraud's Dextrose agar (SDA) and 5% sheep blood agar plates and incubated aerobically/microaerobically at 37°C for 24 h (Quinn *et al*., 2002). Swab and lung specimens were also inoculated in PPLO broth and incubated at 37°C under 5% CO_2 for 48 h (Rosengarten *et al*., 1994). Samples from PBS (7.4) were triturated, filter sterilized (0.22 micron syringe filter) and inoculated on MDBK cell lines and incubated at 37°C under 5% CO_2 for 48 h in CO_2 incubator.

After incubation, the plates were observed for presence of bacterial colony. On the blood agar, morphological characteristics and size of the colony, presence or absence of haemolysis, type of haemolysis and pigment production were observed and noted. Thereafter single isolated colonies were aerobically sub cultured on Tryptose soya agar slant and preserved at 4°C for downstream biochemical and fermentative identification tests. Colony characteristics on blood agar, catalase and oxidase tests, hydrogen sulfide (H_2S) production, indole, Methyl Red (MR), Voges-Proskauer (VP), citrate utilization, urease production, motility and different carbohydrate fermentation tests and Gram staining were applied for bacterial identification according to Barrow and Feltham (1995).

Antibiotic sensitivity test: Bacterial isolates were tested for antibiotic sensitivity testing by the disk diffusion method following the NCCLS (2002) guidelines. The antibiotics used in the study were Amikacin (30 μg), Amoxycillin-clavulanic acid (20/10 μg), Ampicloxacillin (10 μg), Ciprofloxacin (30 μg), Chloramphenicol (30 μg), Enrofolxacin (10 μg), Erythyromycin (15 μg), Levofloxacin (5 μg), Streptomycin (10 μg), Tetracycline (30 μg), Penicillin (10 IU), Amoxicillin (30 μg), Cefotaxime (30 μg), Ceftriaxone (30 μg) and Kanamycin (30 μg).

RESULTS AND DISCUSSION

The outbreak occurred at the end of winter season i.e., March 2010. Lambs were showing the symptoms of pneumonia and most of the mortality occurred in lambs. On clinical examination, recurring pneumopathies were observed with signs of dyspnoea, polypnoea, pyrexia, dry coughing and progressive emaciation in almost all the cases examined. Few lambs had abundant mucoid to purulent nasal discharge, infrequent lacrimation, scouring

or conjuctival congestion and symptoms of pneumonia along with nervous disorders as circling, inco-ordination and inability to rise and move ultimately leading to death. Necropsy showed the presence of greenish-red frothy exudates in the trachea and bronchi. The lungs of affected animals were enlarged and congested. The mucous membrane of trachea, bronchi and bronchioles were highly congested. There was moderate to high degree of enlargement of mediastinal and prebronchial lymphnode. Interstitial multifocal pyogranulomatous pneumonia was present in a few cases. Pleuritis was found in cases with pyothorax present in one of these cases. Pyogranulomatous meningoencephalitis was found in four cases, two of them showing necrotizing lesions (Fig. 1). In one case, necrosuppurative peritonitis was observed. The most common histopathological changes observed were a diffuse mixed infiltration of neutrophils and histiocytes, with lesser extent of lymphocytes, thickening of the inter-alveolar septa and multifocal bacterial colonies with cocoid forms. Pathological results were strongly consistent with bacterial infections. All the lambs died had empty stomach. Pathological changes in liver, lungs, kidney, heart and lymph nodes were also observed and were suggestive of bacterial infection due to infiltration of neutrophils in tissues (Fig. 1a-e).

Microbiological examination of nasal swab, blood and cerebrospinal fluid revealed no growth in PPLO broth, MDBK cell lines and SDA as there was no change in

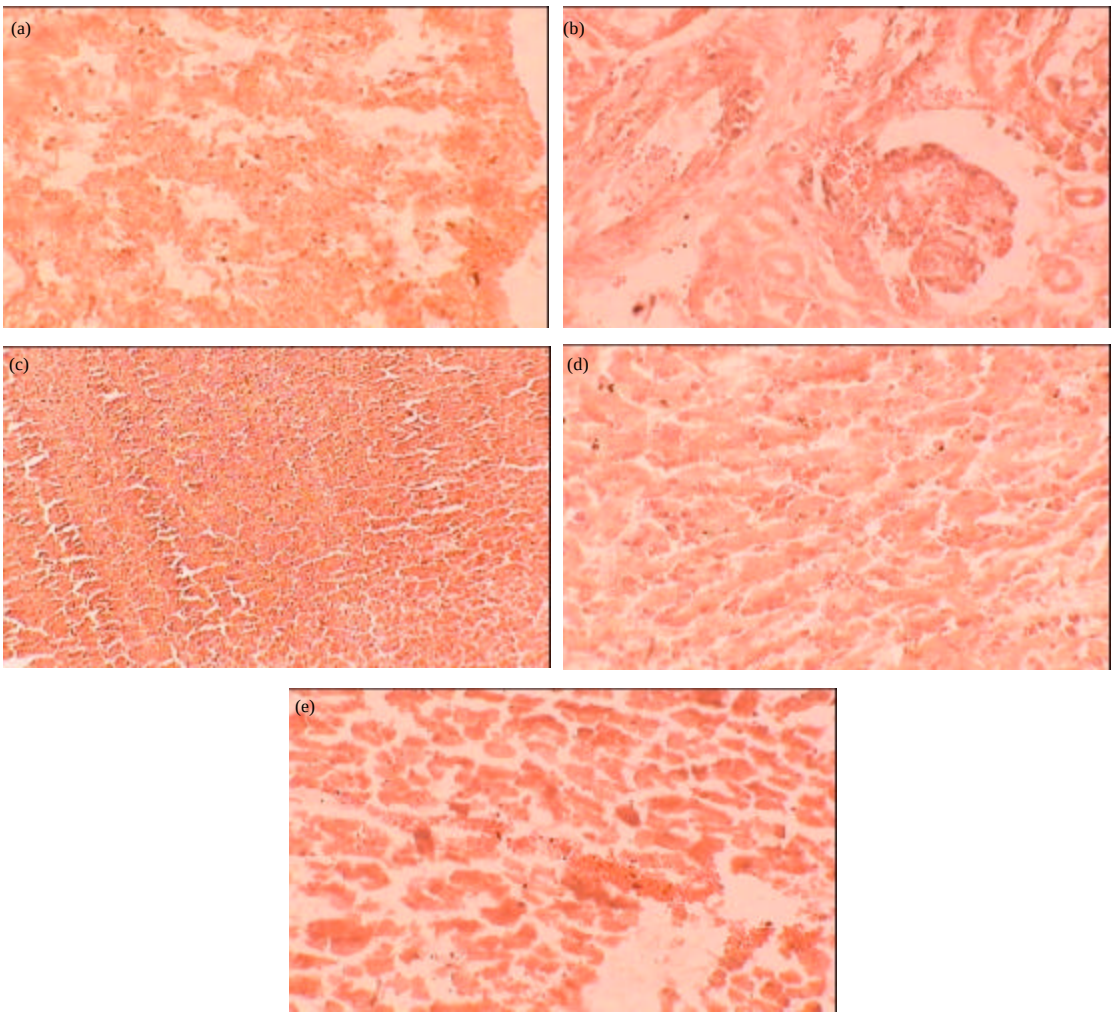

Fig. 1(a-e): (a) The interstitial multifocal pyogranulomatous pneumonia and congestion, (b) Hemorrhages and neutrophils infiltration in kidney, (c) Hemorrhages and neutrophils infiltration in lymph nodes, (d) Hemorrhages and neutrophils infiltration in liver and (e) Hemorrhages and neutrophils infiltration in heart

colour, cytopathic effects and fungal colonies, respectively which were suggestive of no involvement of mycoplasma, virus and fungi. However, bacterial colonies were observed on tryptose soya agar under microaerophilic conditions. Presumptive identification of isolated bacteria was made by traditional physiological and biochemical methods. These include mucoid dew drop like colonies, Gram's positive diplococci, positive to catalase reaction, alpha hemolytic activity on 5% sheep blood agar, optochin (ethyl hydrocupreine) susceptibility, positive qualleng's reaction and bile solubility (Facklam and Carey, 1985). *S. pneumoniae* was the bacterium isolated from clinical specimens and from lungs, liver, CSF and heart blood of lambs died with signs of respiratory disease. On antibiotic sensitivity, *S. pneumoniae* isolates were resistant to penicillin, tetracycline, erythromycin, chloramphenicol, enrofloxacin, ciprofloxacin but found susceptible against other used antibiotics.

Among respiratory tract infections, bacterial diseases have drawn attention due to their varied clinical manifestations, disease severity and emergence of strains resistant to a number of chemotherapeutic agents (Woldemeskel *et al*., 2002). Respiratory disease is multifactorial in sheep (Lacasta *et al*., 2008) and a number of causative agents were responsible for the respiratory disease complex including streptococcal infections. The present study revealed *S. pneumoniae* as major and potent cause of mortality in the young lambs. Similarly, Bekele *et al.* (1992) also isolated *Streptococccus* spp. as major pathogen in association with other etiological agents from pneumonic sheep and Garedew *et al.* (2010) reported 35% cases with single etiological agents predominantly *Streptococcus* in pneumonic sheep. Various workers (Soni and Sharma, 1990; Ozkara, 1998; Salgam *et al*., 1998; Sasani *et al*., 1998; Raji *et al*., 1999; Kumar *et al*., 2000) have reported isolation of diverse bacterial species from sheep sick of respiratory diseases and also established the role of commensal organisms in pneumonia including *S. pneumoniae*. Progress in understanding the pathogenesis of pneumonia has been slow because of its complex etiology and varied epidemiology (Woldemeskel *et al*., 2002). In the present study, *S. pneumoniae* monoculture was isolated not only from respiratory tract but also from other vital organs and even in cerebrospinal fluid. Thus, it appears to involve multiple organs during outbreak particularly in young lambs. Adequate hygienic conditions were not maintained in the covered shed and the enclosures were over crowded. Moreover, clinically ill sheep were not separated from the apparently healthy animals and antibiotic treatment was given without sensitivity test. As the isolated *S. pneumoniae* was multi drug resistance, it persisted in the flock inspite of antibiotic treatment. For many years, Penicillin and Chloramphenicol has been the mainstay of treatment for pneumococcal disease in developing countries, as they are both inexpensive and effective. Unfortunately, the study revealed resistance against them and other commonly used antibiotics viz., Enrofloxacin, Ciprofloxacin, and Erythromycin. Thus, rapid increase in resistance to penicillin and other antimicrobial agents worldwide has made the choice of antimicrobial agent for *S. pneumoniae* infections more difficult and costly (Friedland and McCracken, 1994). To the best of author's knowledge, this seems to be the first report of isolation of *S. pneumoniae* from cerebrospinal fluid from the cases of infections in lambs and the first report of multi drug resistant *S. pneumoniae* infection outbreak in lambs with multiple organ involvement in India.

CONCLUSION

Presence of multi drug resistant *Streptococcus pneumoniae* in weaning lambs with the involvement of multiple organs appears to be an emerging zoonotic threat to human beings especially in shepherds. The possible reason behind that shepherds commonly belong to backward classes of community and mostly rely on common treatment of sick animals from nearby medical stores instead of nearby veterinary clinics and these shepherds may be the source of transmission of infection to society.

ACKNOWLEDGMENTS

The cooperation of staff of Madhurikund farm, Smt. Mamta, (Department of Veterinary Microbiology and Immunology), Sri Jai Prakash (Department of Veterinary Pathology) and Sri Pankaj Kumar (College of Biotechnology) are highly valued. The authors are also thankful to Head of Department of Veterinary Microbiology and Immunology; Department of Veterinary Epidemiology and Preventive Medicine; Department of Veterinary Pathology; Dean of College of Veterinary Sciences and Animal Husbandry and Hon'ble Vice chancellor, Uttar Pradesh Pandit Deen Dayal Uphadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evum Go-Anusandhan Sansthan (DUVASU), Mathura; for providing all the support and necessary facilities for conducting this study.

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