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A Necropsy based Investigation on Concurrent Infection of PPR and Emerging Opportunistic Bacterial Pathogens in Sheep and Goat

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ABSTRACT

Background: There is scanty literature available about concurrent infection of Pestes des Petits (PPR) and emerging opportunistic bacterial pathogens in field conditions. Thus, study was conducted to investigate the above based on necropsy.

Methods: Systematic necropsy examination was conducted on 50 small ruminant carcasses. Detailed study included virological, bacteriological, gross and microscopic investigation on tissues collected using standard techniques. PPR virus (PPRV) antigen was detected in various samples using sandwich Enzyme Linked Immunosorbent Assay (s-ELISA). Bacterial pathogens were isolated on blood agar and identified by VITEK® system.

Result: Eleven goats and nine sheep had concurrent infection. Highest positivity for PPRV antigen was observed in nasal and tracheal swab, lungs and lungs associated lymph nodes. *Pasteurella multocida* was the major bacteria found to cause fibrinious hemorrhagic broncho-alveolar pneumonia followed by *Klebsiella pneumoniae spp. pneumoniae* producing suppurative bronchoalveolar pneumonia in the presence of PPR infection. *Sphingomonas paucimobilis* and *Acinetobacter baumannii,* human pathogen were also isolated and found to produce severe hemorrhagic interstitial pneumonia and serofibrinous bronchopneumonia respectively in presence of PPR which is novel finding. Our study is the first report of necropsy based investigation on concurrent infection in sheep and goats.

Key word: Bacteria, ELISA, Goat, Lesions, PPR, Sheep.

INTRODUCTION

Small ruminants contribute significantly to the economy of most rural communities in developing countries. Therefore they are called as poor man's cow (Ngambi *et al.* 2013; Singh *et al.* 2014; Sunder *et al.* 2020). Though small ruminants contribute towards alleviation of poverty, their productivity is hampered by infectious diseases and poor husbandry practices. Compared with cattle, there are limited studies on small ruminants' health and the scientific literature available is highly fragmented and sometimes, incomplete (Singh *et al.* 2010). The tendency of these animals to huddle and group rearing practices further predispose them to infectious and contagious diseases. The condition becomes adverse when bacterial as well as viral infections are combined particularly under adverse weather conditions as very well documented in large ruminants (Hemlatha *et al.* 2020).

One area which is poorly documented is the magnitude of multiple infections, by different types of pathogens such as viruses, bacteria, and parasites that result in respiratory diseases in sheep and goats. Among this one, important respiratory disease affecting small ruminants is peste des petits ruminants, PPR. The disease is caused by morbillivirus formerly peste des petits ruminants virus (PPRV). PPR virus is known to cause severe immunosupression which further makes animal more susceptible to secondary bacterial infections such as Pasteurella, Klebsiella species and other pathogens (Bhaskar *et al.* 2009; Manimaram *et al.* 2017; Kumar *et al.* 2020). Concurrent infection in small ruminants is responsible for high morbidity and mortality. Necropsy examination is one of the important diagnostic methods used in the field for the diagnosis of diseases (Benavides *et al.* 2015). It is very helpful to study pathogenesis of a disease (Chauhan *et al.* 2011). Despite our best efforts with available literature search, we could not find reports on the concurrent infections caused by PPR and bacterial pathogens based upon necropsy. Therefore, the present study was conducted on the dead carcasses of sheep and goat suspected to be died of PPR and mixed infection to investigate aetiological agent and pathomorphological lesions in order to understand the pathogenesis of mixed infection.

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MATERIAL AND METHODS

Detailed necropsy examination was conducted on 50 carcasses of small ruminants received during November 2018 to April 2019 at Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. Out of fifty, twenty carcasses comprising of eleven goats and nine sheep were found to have severe involvement of respiratory lesions. These 20 carcasses were suspected to be died due to PPR and their data is included in the present investigation. Respiratory organs showing lesions were collected for bacteriological isolation, detection of PPRV antigen and pathomorphological study.

Bacteriological isolation and identification

Heart blood, nasal swabs, tracheal swabs, samples from lung and lung associated lymph nodes showing lesions were collected in sterile petridishes. Streaking was done on various agar such as Mac conkey agar, sheep blood agar and Eosin Methylene Blue (EMB) agar procured from Hi media. Plates were incubated at 37°C for 24-48 hrs and restreaking was done on Tryptic Soya Agar to get pure colonies for VITEK® analysis (Gokmen *et al.* 2019). The card for each Gram positive or negative group of bacteria was automatically filled by a vacuum device, sealed and inserted into the VITEK 2 reader and subjected to a kinetic colorimetric measurement.

Detection of PPR Virus antigen

For detection of PPR virus antigen, samples such as lung, lung associated lymph nodes, nasal swabs and tracheal swabs were collected and stored at -20°C till further analysis. PPR sandwich-ELISA kit for PPRV antigen detection was obtained from Rinderpest Laboratory, Division of Virology, Indian Veterinary Research Institute, Mukteswar. The test was performed strictly as per the protocol outlined in the user manual supplied with the kit and analyzed as per protocol.

Pathological examination

During necropsy examination, touch impression smears were taken from the affected organs, stained with Field stain and examined for the detection of cytopathological changes. Appropriate tissues from respiratory organs showing lesions were collected in 10% buffered formalin for histopathological examination, routinely processed and stained with Haematoxylin and eosin (H and E) for histological examination using light microscope.

RESULTS AND DISCUSSION

Bacteriological isolation and identification

Pasteurella multocida was isolated from the pneumonic lungs, lung associated lymphnodes, tracheal swab and heart blood of four goats and three sheep respectively. *Pasteurella multocida* produced typical dew drop like white glistening colonies (Asaduzzaman *et al.* 2013) on the blood agar (Fig 1A). Culturally, *Klebsiella pneumonia ssp pneumoniae* (*K. pneumoniae*) was confirmed as grey white mucoid colonies on the blood agar (Fig 1B) and lactose fermenting pink coloured colonies on the Mac conkey agar which was isolated from lungs, lungs associated lymph node and heart blood of two goats and two sheep. *Acinetobacter baumanii (A. baumanii)* was isolated from the lungs of goat which produced typical 'fish eye' colonies on the EMB agar (Fig 1C). *Sphingomonas paucimobilis* (*S. paucimobilis)* was isolated from lungs and lung associated lymph nodes of two goats (Cenzig *et al.* 2015). It produced white to yellowish colonies with turbid zone of hemolysis on the blood agar (Fig 1D). Lung tissue showed highest number of cultured colonies followed by the lung associated lymph nodes. Nowadays VITEK® based identification of Bacterial is very important as it gives accurate results and biochemical characteristics of bacteria. Similar studies were conducted in experimental infection in goat and sheep (Benavides *et al.* 2015; Cid *et al.* 2019) where *Pasteurella multocida* was isolated from lungs.

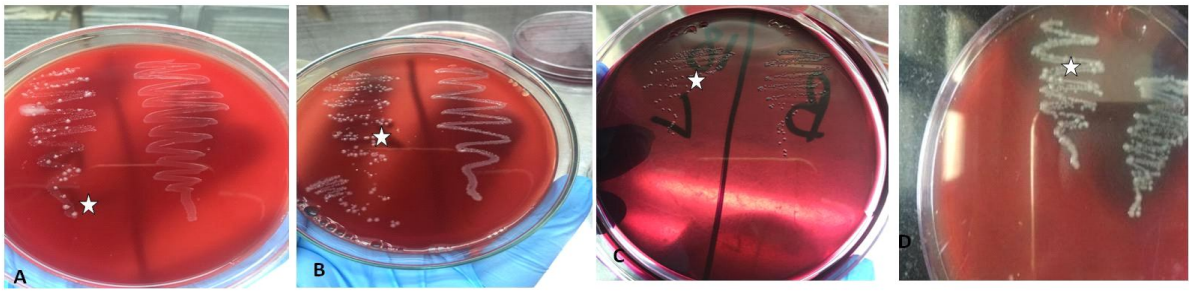

Fig 1: Cultural colony characteristics of bacteria isolated from various tissues of sheep and goat. A-Glistening dew drop like colonies of *Pasteurella multocida* on blood agar (star) B –White to grey small mucoid colonies of *Klebsiella pneumoniae ssp pneumoniae* on blood agar (star) C- Fish eye colonies of *Acinetobacter baumannii* on EMB agar (star); D-Hemolytic white to yellowish small colonies of *Sphingomonas paucimobilis* on blood agar (star).

Detection of PPRV antigen

Out of 200 samples of nasal swab, tracheal swab, lungs and lung associated lymphnodes collected from 50 carcasses; twenty carcasses were found to be affected by PPR virus. PPRV antigen was detected equally *i.e* (100%) in nasal swab, tracheal swab, lung associated lymph nodes and lungs of eleven goats and nine sheep respectively (Pandey *et al.* 2020). Similar study was conducted on different clinical samples of small ruminants (Mahajan *et al.* 2012) for detection of PPR antigen which revealed lung and nasal swabs are more appropriate for detection of PPR antigen.

Pathology

Carcasses, from which *Pasteurella multocida* and PPR virus was detected, showed fibrinous hemorrhagic brochoalveolar pneumonia characterized by presence of whitish fibrin layer over the surface of the lung along with diffuse massive hemorrhages (Fig 2A). Trachea was filled with severe whitish sticky froth. Touch impression smears from lungs showed strands of fibrin mixed with froth and toxic changes in the macrophages and neutrophils (Fig 2B). Tracheal epithelium was detached from the lamina propria and showed focal leucocytic infiltration microscopically. Lungs showed presence of fibrinous exudates in the alveoli along with marked congestion and multifocal areas of hemorrhages (Fig 2C), stage of grey hepatisation, formation of syncytia in the bronchioles and bronchi were characteristic of PPR (Fig 2D). Goats were comparatively, more affected than sheep and produced severe lesions in lungs. *Pasteurella multocida* was the most prominent micro-organism isolated from the respiratory tissues. It showed maximum association with PPR. It is known that *Pasteurella multocida* inhabits the upper respiratory tract (Cid *et al.* 2019; Kumar *et al.*

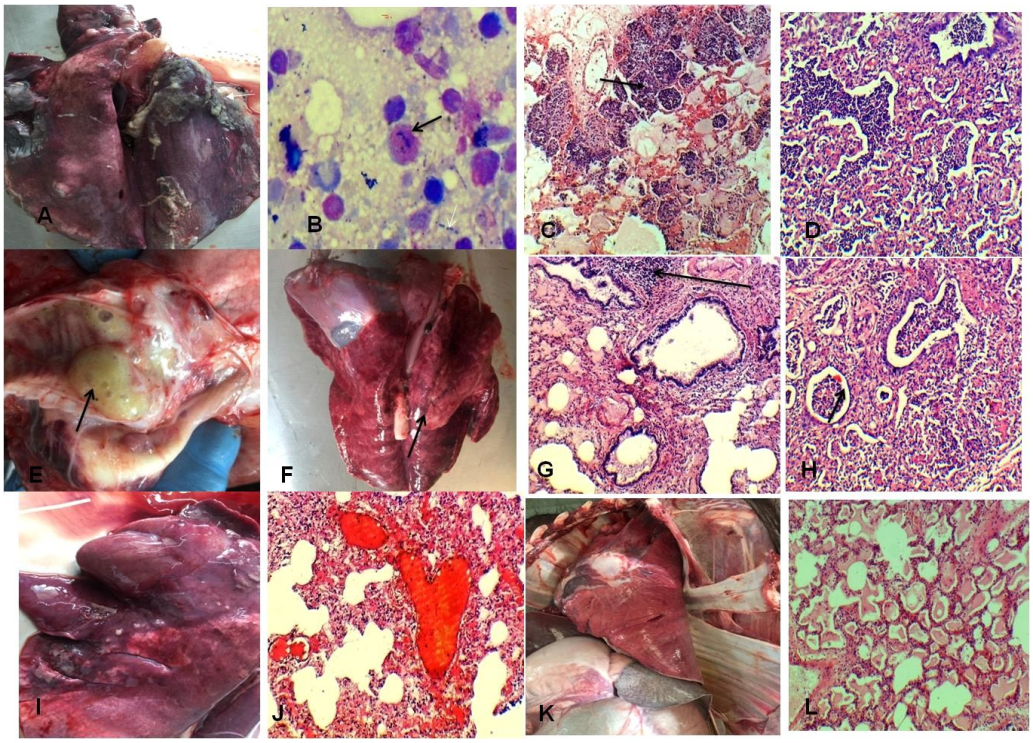

Fig 2: Pathomorphological lesions associated with concurrent infection of PPR and associated bacterial pathogen.
A- Lungs: cardiac and diaphragmatic lobe covered with thick whitish layer of fibrin B-Presence of bipolar organism (white arrow) in frothy exudate of lung and intracytoplasmic inclusion in epithelial cells (black arrow) Leishman stain 1000 X oil immersion; C- Lungs- blocking of alveolar lumen by plug of leucocytes (arrow head) and thickening of interstitial space due to capillary haemorrhages along with presence of fibrinous exudate H and E stain x100, D-Lungs: presence of heavy infiltration of leucocytes in alveoli with thickening of interstitial space H and E stain X100; E-Oozing of frothy thick yellow coloured suppurative exudate (arrow) from trachea, F-Lungs: severe dark red discoloration of lungs with multifocal areas of petechial to ecchymotic haemorrhages on diaphragmatic lobe of lungs and few raised whitish areas (arrows), G-Lungs: alveoli filled with necrotic debris and moderate infiltration of neutrophils surrounding bronchiole (arrow) H and E stain X 100, H- Lungs: bronchioles and alveoli filled with leucocytes and formation of syntitia with thickening of interstitial space (arrow) H and E stain X100; I-Severe diffuse hemorrhages, congestion, wet appearance of lungs and presence of white layer of fibrin at focal area (arrow) (*Sphingomonas paucimobilis* and PPR); J-Lung: thickening of interstitial space due to excess hemorrhages, congestion of blood vessels and mild infiltration of leucocytes in hemorrhagic pneumonia (*Sphingomonas paucimobilis* and PPR); K-Red discoloration and heavy and voluminous appearance of lungs (*A. baumanii* and PPR) , L- Lung capillary hemorrhages, infiltration of leucocytes in interstitial space and presence of serofibrinous exudate in lumen (*A. baumanii* and PPR)H and E stain X 100.

2020). In the present study, it is postulated that PPR infected animals which are already immune compromised predisposed them to secondary *P. multocida* infection. Further, severity of lesions might be due to *Pasteurella multocida* toxin (PMT) which might have weakened the immune response by different toxin-related immune evasion strategies, facilitating the multiplication and survival of *P. multocida* in the host. Toxins produced by *Pasteurella multocida* alone or in combination with products of inflammatory cells induced necrosis of wall of blood vessels in lungs. (Kalorey *et al.* 2008). Similar findings were reported in experimental infection (Cid *et al.* 2019).

Sheep carcasses showed lesions associated with purulent hemorrhagic bronchopneumonia from which *Klebsiella pneumoniae ssp pneumoniae* and PPR virus detected. Nostrils showed frothy discharge whereas trachea and bronchi were filled with yellow colored purulent frothy material (Fig 2E). Grossly, small abscesses were present along with marked consolidation of lungs (Fig 2F). Impression smears of lungs showed toxic changes in neutrophils, presence of bacteria and syncytia formation. Histopathological examination showed presence of abscess with heavy infiltration of neutrophils surrounding bronchioles and stage of grey hepatisation (Fig 2G and 2H). *Klebsiella pneumoniae ssp pneumoniae* was the second most important bacteria isolated from the lungs and lung associated lymphnode from sheep and goat. However, sheep were found more susceptible to the concurrent infection of PPR and *Klebsiella*. The isolation of *K. pneumoniae* from lung abscesses and lymphnodes in presence of PPR virus is to the best of our knowledge, the first report of the concurrent infection. Purulent lesions in lungs were attributed to the capsular mucopolysaccharide of the Klebsiella (Jang *et al.* 2010).

Concurrent infection of *Sphingomonas paucimobilis* and PPR was observed in two goats. Patchy to diffuse areas of the haemorrhages, consolidation in the apical, cardiac and anterior parts of the diaphragmatic lobes of lungs were the main lesions (Fig 2I). Trachea showed presence of froth and reddish discoloration of mucosa. Microscopically, lungs showed thickened alveolar wall and marked congestion. The alveolar and bronchiolar lumen was filled with erythrocytes and pinkish serous fluid. Alveoli and bronchioles were disoreinted, presence of mononuclear cells and homogenous eosinophilic masses due to lysis of erythrocytes in the lumen producing hemorrhagic pneumonia (Fig 2J). This study is the first report of concurrent infection of *Sphingomonas paucimobilis* and PPR virus in goats. *Sphingomonas paucimobilis* was found responsible for hemorrhagic interstitial pneumonia when present along with PPR virus in natural infection. These findings are in accordance with earlier report (Cenzig *et al.* 2015) who stated that *Spingomonas* spp can produce respiratory infection in immunosupressed animals. Concurrent infection of *Acinetobacter baumannii,* human pathogen and PPR virus was observed in one goat. Gross lesions were characterized by presence of froth in tracheal lumen and moderate congestion of mucosa. Petechial to ecchymotic areas of hemorrhages on diphragmatic lobes of lung were also evident. (Fig 2K). Microscopic appearance of lungs showed presence of capillary hemorrhages, infiltration of leucocytes in the interstitial space and presence of sero-fibrinous exudate indicating serofibrinous bronchopneumonia (Fig 2L). Again, this is the first report of concurrent infection of PPR and *Acinetobacter baumannii. Acinetobacter baumanii* acts as a potential veterinary pathogen for dogs and cats (Francey *et al.,* 2000; Kolk *et al.* 2019; Nocera *et al.* 2020). In the present study, co-infection of PPR and *Acinetobacter baumanii* caused serofibrinous bronchopneumonia in the presence of down-regulated immune system in goat due to PPR virus.

CONCLUSION

Necropsy based diagnosis is found to be important tool as it has provided significant gross and microscopic lesions for identifying cause of death in the present study. Interaction of bacterial infection and PPR virus has produced severe degree of gross lesions and microscopic lesions typically, in the respiratory system. *Pasteurella multocida* and *Klebsiella pneumoniae* produced fibrinohemorrhagic and suppurative bronchoalveolar pneumonia in small ruminants, respectively. *Sphingomonas paucimobilis* was identified and isolated for the first time from the lungs of goat in presence of PPR infection. *Acinetobacter baumannii* rarely reported in domestic animals; was found to be pathogenic in concurrence with PPR. These bacteria are re-emerging as pathogenic organisms. However more research work is needed to throw light on the pathogenesis of concurrent infection considering large population of small ruminants.

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