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Study on pathological changes in broncho pneumonia associated with *Mannheimia haemolytica* in Nellore brown sheep

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Abstract
Sheep that died due to respiratory illness were subjected to postmortem examination(n=61) and lung specimens were collected for isolation, PCR work and histopathology A total of 21 lung samples which were found positive for *Manneheimia haemolytica* through PCR were also subjected to study of gross pathology and histopatholgy. Observed necropsy lesions were *viz*., consolidation of cranial apical lobe, pleural adhesions, sero sanguinous fluid in thoracic cavity, fibrinous pneumonia, cut section showing muco- purulent exudates and froth in the trachea with congestion.
Lung histopathology revealed edema, haemorrhages, congestion and infiltration of PMN cells in alveoli. Also observed deposition of fibrin strands and haemosiderin pigment in interstitial tissue.

Keywords: sheep broncho pneumonia, *Mannheimia haemolytica*, gross lesions, histopathology

Introduction
Livestock sector holds a substantial share in fulfilment of human food demand and this share is expected to further get doubled by 2030. Small ruminants are valuable assets for developing countries like India with the potential for providing meat, milk, and wool. These animals are highly susceptible to respiratory diseases, which account for almost 50 % mortality amongst them. It was estimated that, irrespective of the etiology, the infectious respiratory diseases of sheep and goats contribute to 5.6 percent of the total diseases of small ruminants.
Respiratory tract diseases are considered to be most common entity of livestock health and pneumonia is considered as single greatest cause of death in sheep as they seems to have a very low resistance to respiratory tract infections.
Minor collateral ventilation between alveoli and extensive interlobular septa make the ruminants particularly sheep more vulnerable to frequent pneumonic episodes because this anatomical variation in lung alveoli limits the expulsion of alveolar exudate [1].
Though infectious pneumonia in sheep is multifactorial in etiology (Bacteria, Virus, Parasite), *Mannheimia haemolytica* is one of the most important respiratory pathogens of small ruminants causing serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs. *M. haemolytica* and mycoplasmas are the important/primary causes of respiratory infections and the pathogenicity enhances during the concurrent infection of the two organisms [2].
As *M. haemolytica* is reported to be a part of normal microbiota of upper respiratory tract, it is necessary to evaluate the lung lesions by histopathological examination as an important diagnostic tool. Because histopathology coupled with demonstration of pathogen from the lesion is far more superior to mere demonstration of the organisms from the nasal swabs [3].
Hence the present study was undertaken with an objective of histopathological studies besides cultural examination and, molecular confirmation with PCR to establish the pathology associated with *M. haemolytica.*

Materials and Methods
Pneumonic lungs were collected from the sheep died (n=61) during the post- mortem at Department of Veterinary Pathology, CVSc, Proddatur, and Animal Disease Diagnostic Laboratory, Kadapa.
Post-mortem examination was performed on sheep died due to respiratory infection in order to study the gross pathology and tissue specimens were collected in 10 % neutral buffered formalin for histopathological examination.

Tissue samples collected for histopathology were processed by routine paraffin embedding technique and sections of 4-5 µm thickness were cut and stained by routine Harri's Haematoxylin and Eosin method [4].

Results and Discussion

Gross post-mortem lesions

The lung samples collected during post-mortem examination were showing consolidation of cranial apical lobe (n=14), right lateral lobe (n=5), diaphragmatic lobe (n= 2), pleural adhesions (n=6), pleural fluid in thoracic cavity (n=6), froth in trachea (n=18) and congested trachea (n=17). Cut section revealed pale and dark areas with muco-purulent exudates in the airways (Table. 1 and Fig.1).

Similar necropsy findings in mannhaemic pasturellosis have been reported by many researchers [5, 6, 7, 8].

Table 1: Frequency of necropsy findings observed in sheep respiratory infections

Necropsy findings	Number Observed in Number of post-mortems
Froth in trachea	18 (24%)
Congested trachea	17 (17%)
Consoldiation of cranial apical lobe	14(14%)
Grey hepatization	10 (10%)
Pleural fluid in thoracic cavity	9 (9%)
Pleural adhesions	6 (6%)
Consolidation of right lateral lobe	5 (5%)

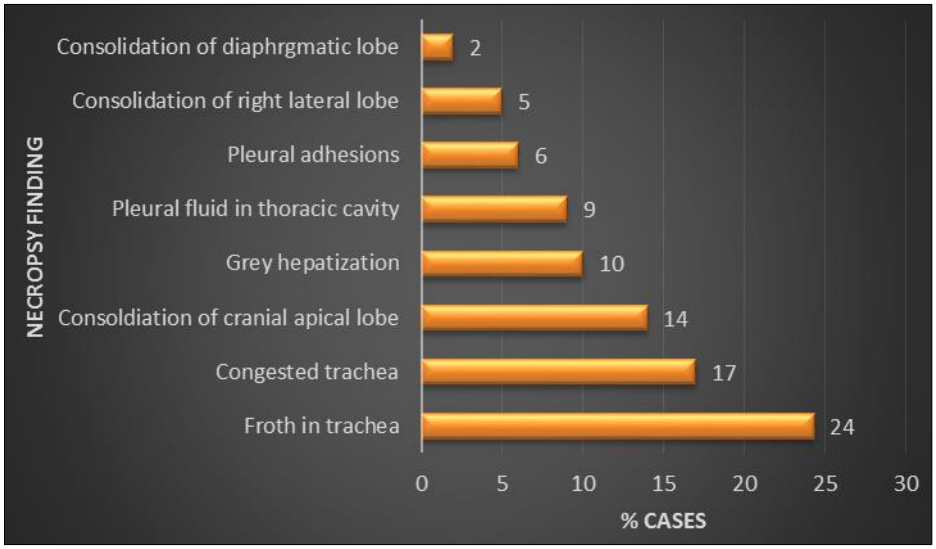

Fig 1: Necropsy findings observed in sheep respiratory infections

During mannhaemic pasturellosis alveolar macrophages, neutrophils and mast cells release maximum amount of proinflammatory cytokinins (IL-1, IL- 8 and TNF-α) and simultaneous release of free radicles and leucocytes contributes to injury and necrosis of bronchiolar and alveolar cells.

Initial stages of fibrinous broncho pneumonia, there is massive vascular changes due to leucotoxins which increase the permeability of blood air barrier that leads to leakage of fibrin and protein rich fluid in to alveolar lumen. These exudation of plasma proteins, inflammatory cells, fibrin and edema fluid results in obliteration of alveoli [8].

Predominance of the lesions in cranio-ventral portions of the pneumonic lungs is attributed to shortness and abrupt branching of airways, greater deposition of infectious organisms, reduced vascular perfusion, gravitational sedimentation of exudates and regional differences in ventilation. Consolidation of pneumonic lungs is due to loss of airspaces because of exudation and atelectasis [8].

Histopathology

Histopathological findings observed in the current study includes, fibrinous pneumonia, interstitial pneumonia, infiltration of polymacro nuclear cells in alveoli, edema, hyperaemia, haemorrhages and bronchial atelectasis and enlarged peri-bronchial capillaries.

Edema in lungs is characterized by exudation of protein rich fluid in to alveoli and interstitial tissue that appeared as homogenous eosinphilic material upon H&E staining (Fig. 52, 53).

Similar findings like deposition of fibrin and PMN cells infiltration and edema of alveoli were reported by earlier workers [5, 6, 8].

These histopathological findings observed in the present study might be due to the damage caused by *M. haemolytica* through persistent generation of virulence factors such as LPS, leucotoxin that causes severe intravascular thrombosis in capillaries, pulmonary lymphatics and veins leading to ischaemic necrosis in lungs [7].

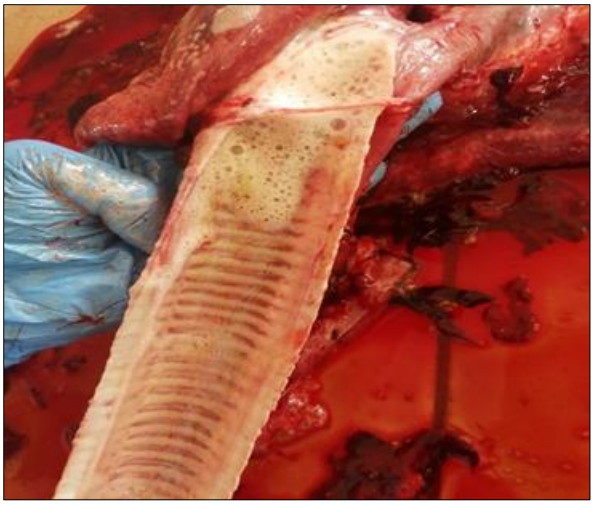

Fig 2: Trachea: Froth and congestion of trachea

Fig 3: Lungs: Consolidation of apical lobe

Fig 4: Sheep with broncho pneumonia showing moderate muco-purulent nasal discharges

Fig 5: Sheep with broncho pneumonia showing unilateral muco-purulent nasal discharges

Fig 6: Heart: Epicardial petichal haemorrhages

Fig 7: Lungs: Severe congestion with diffuse areas of hemorrhages involving all the lobes.

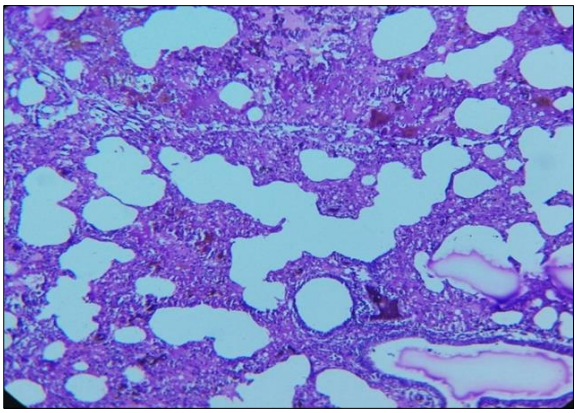

Fig 8: Sheep lung section showing edema and infiltration infiltration of PMN cells, H&E, X100

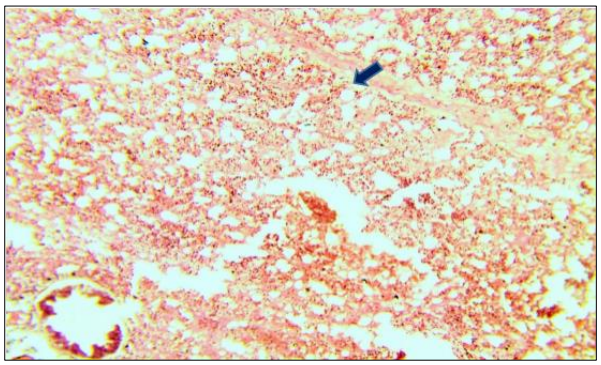

Fig 9: Sheep lung section showing Infiltration of PMN cells in interstitial space surrounding alveoli, Diffuse distribution of haemosiderin, Fibrin deposits (Blue arrow), H&E, x100

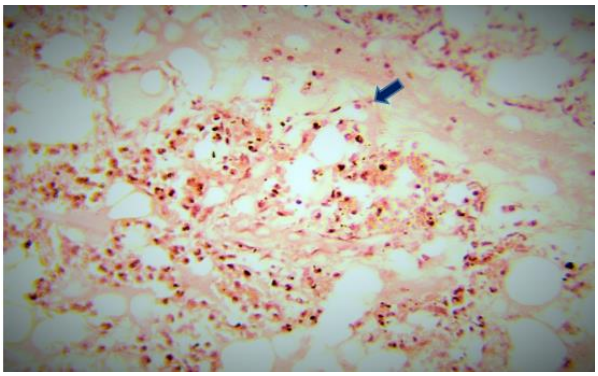

Fig 10: Lung section showing Infiltration of PMN cells in interstitial space surrounding alveoli, Diffuse distribution of haemosiderin, Fibrin deposits (Arrow), H&E, x400

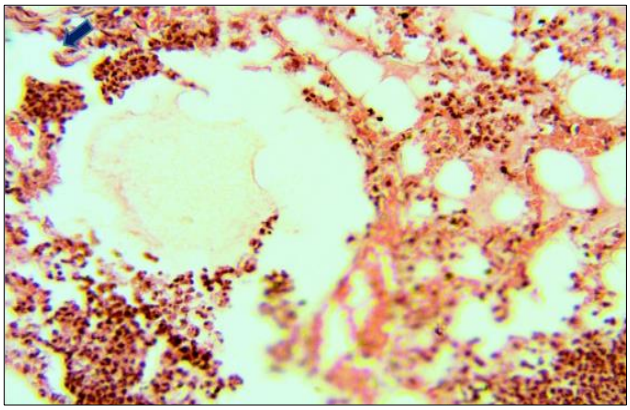

Fig 11: Sheep lung section showing Infiltration of PMN cells in interstitial space surrounding alveoli, Macrophages, PMN, Fibroblasts (blue arrow), H&E, x400 (Arrow).

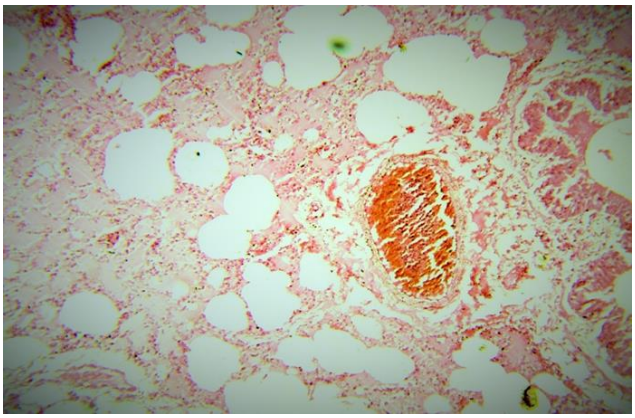

Fig 12: Lung section showing Inter alveolar edema, congestion, PMN infiltration, H&E, X100

Conclusion

In this study *Mannheimia haemolytica* was predominantly isolated from both nasal swabs and lung samples which was corroborated by post-mortem lesions and histopathology. Lungs showed characteristic macroscopic lesions associated with *Mannheimia haemolytica* like congestion, consolidation of apical lobe, sero sanguinous fluid in thoracic cavity, tracheal congestion, pleural adhesions. Histopathology revealed infiltration of PMN cells, fibrin, edema, congestion of lung alveoli. Gross pathological lesions clubbed with specific histopathological lesions can be used as best indicator of the bronchopneumonia associated with *M.haemolytica.*

Acknowledgements

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