International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Pathomorphological Study of Pasteurella Multocida Positive Bovine Lungs

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ABSTRACT

Aims: To describe the gross and histopathological lesions in lungs of bovines infected with Pasteurella multocida, and to document the associated pathomorphological changes in cattle and buffaloes in Jabalpur, Madhya Pradesh, India.

Study Design: Cross-sectional observational pathomorphological study.

Place and Duration of Study: Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India.

Methodology: Pneumonic lungs from 68 bovines (cattle and buffaloes) were collected from slaughterhouses and postmortem examinations. Gross pathological lesions were systematically recorded. Lung tissue sections were fixed in 10% neutral buffered formalin, processed by standard histopathological techniques and stained with Haematoxylin and Eosin (H&E) and Masson's Trichrome. Species-specific PCR targeting virulence genes (ompH and omp87) was used to confirm Pasteurella multocida infection.

Results: Gross examination revealed congestion and haemorrhages in 61.76% of cases, consolidation in 44.12%, fibrinous exudation in 32.35% and interlobular septal thickening in 26.47%. Three lungs (4.41%) were confirmed positive for P. multocida. Histopathology of confirmed cases showed severe vascular changes including alveolar flooding with fibrinopurulent exudate, necrosis, marked bronchiolar epithelial degeneration and desquamation, perivascular and peribronchiolar cuffing, and fibrinous pleuritis. Masson's Trichrome staining confirmed mild to moderate fibrosis in chronic cases.

Conclusion: The gross and histopathological findings in this study are consistent with fibrinous bronchopneumonia caused by P. multocida in bovines. The results highlight the progressive nature of pasteurellosis, ranging from acute to chronic forms and provide valuable baseline pathological data for the region.

Keywords: Pasteurella multocida, bovine lungs, gross pathology, histopathology, bronchopneumonia.

1. INTRODUCTION

India has the largest bovine population in the world and livestock plays a pivotal role in the agricultural economy. Pasteurellosis is the number one killer among cattle and buffaloes in tropical Asia and Africa. In India, the disease remains endemic and, despite extensive control efforts, accounts for approximately 50–60% of bovine mortality. The disease mainly develops under stressful conditions during hot and humid weather, though it may occur at any time throughout the year (Bahr et al., 2021 and Clemmons et al., 2021). Pasteurellosis is an acute, fatal, septicaemic disease of cattle and buffalo caused by the bacterium Pasteurella multocida, which commonly resides in the upper respiratory tract and oropharynx of animals

as part of their normal flora and is generally considered an opportunistic pathogen. Predisposing factors such as age, immune status, stress, poor management, dietary changes, environment, transportation and co-infection are also responsible for disease onset (Freeman et al., 2022).

Pasteurella multocida (P. multocida) is a gram-negative bacterium associated with a variety of diseases in animals, including haemorrhagic septicaemia in cattle and buffaloes, fowl cholera in poultry, atrophic rhinitis in pigs, snuffles in rabbits, and pneumonic and septicaemic pasteurellosis in sheep, goats, wild animals and humans (Mostaan et al., 2020). P. multocida isolates are classified based on capsular antigens into five serogroups (A, B, D, E and F) (Townsend et al., 2001), each showing host and disease-producing specificity. In addition, virulence factors such as capsules, outer membrane proteins, fimbriae and adhesins play important roles in the immunopathological changes of diseases caused by P. multocida (Bostan et al., 2016). Clinical signs such as serous to purulent nasal discharge, elevated body temperature, dyspnoea, open-mouth breathing with extended neck, respiratory failure and death may occur within hours of the first symptom appearance (Peek et al., 2018).

The diagnosis of pasteurellosis in bovines by various tools including history, clinical signs, necropsy and histopathological changes is valuable in establishing a presumptive disease diagnosis. Traditional bacteriological techniques are considered time-consuming and lack reliability with certain limitations (Hashem et al., 2022).

Although extensive published research is available on P. multocida, scarce information is documented concerning its pathology in Jabalpur, Madhya Pradesh. In order to address this gap, the current study was planned to describe the structural changes in lung tissues of cattle and buffaloes infected with P. multocida.

2. MATERIAL AND METHODS

2.1 Place and Area of Work

The present work was conducted in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India.

2.2 Sample Collection

Most affected parts of lung tissues were collected in 10% buffered formalin for histopathological studies. Samples were obtained from slaughtered buffaloes and during postmortem examinations of cattle and buffaloes submitted to the Department of Veterinary Pathology.

2.3 Pathomorphological Study

2.3.1 Gross Pathology

Carcasses of bovines were examined systematically for the presence of various gross pathological lesions. The lungs were specifically examined for gross lesions of pneumonia, with emphasis on location, distribution, colour, size and consistency on palpation, along with detailed examination of the cut surface of the lesions, as described by Thompson (1983).

2.3.2 Histopathology

Lung tissue samples were collected and fixed in 10% neutral buffered formalin, then processed for histopathological examination following standard procedures as described by Gridley (1960).

Processing of Tissue:

Thin slices of lung tissue were dehydrated through three changes of acetone for 30, 30 and 45 minutes respectively, followed by clearing in three changes of benzene for 30, 30 and 45 minutes. Subsequent

impregnation in four changes of molten paraffin wax (each for one hour) was performed. The tissues were then embedded in paraffin wax using L-moulds.

Section Cutting:

Paraffin-embedded tissue blocks were sectioned at a thickness of 4–5 micrometres (µm) using a rotary microtome. The sections were floated on a warm water bath and mounted on clean glass slides smeared with egg albumin as an adhesive.

Staining of Tissues:

- **Haematoxylin and Eosin (H&E):** Tissue sections were stained with haematoxylin and eosin following the method described by Gridley (1960). After staining, sections were mounted using distyrene plasticizer xylene (DPX) and covered with coverslips for microscopic examination.
- **Masson's Trichrome Stain:** Tissue sections were mordanted by immersion in Bouin's fluid overnight, followed by cooling and washing under running tap water until the yellow colour disappeared. Sections were then stained with Weigert's iron haematoxylin for 10 minutes, followed by Biebrich scarlet–acid fuchsin solution for 15 minutes. Differentiation was carried out using phosphomolybdic acid–phosphotungstic acid solution for 10–15 minutes. Counterstaining was performed with aniline blue for 5–10 minutes, followed by dehydration, clearing and mounting.

3. RESULTS AND DISCUSSION

3.1 Gross Pathology of Pneumonic Bovine Lungs

Detailed gross pathological evaluation of pneumonic lungs was performed in 68 bovines, comprising lung samples collected from slaughtered buffaloes and during postmortem examinations of cattle and buffaloes. In several lungs, more than one type of pneumonic lesion was observed simultaneously, indicating overlapping pathological processes within the same lung tissue. Pneumonic lesions were consistently observed in all affected carcasses, with bilateral involvement of both right and left lungs.

Gross pathological examination revealed characteristic pneumonic changes. Congestion and haemorrhages were the most common lesions, recorded in 42 cases (61.76%), followed by consolidation in 30 cases (44.12%) and fibrinous exudation in 22 cases (32.35%). Thickening of the interlobular septa was observed in 18 cases (26.47%), while pulmonary oedema and hydatid cysts were each noted in 12 cases (17.65%). Emphysematous areas were seen in 10 lungs (14.70%), nodules in 3 cases (4.41%) and pleural adhesion in a single case (1.47%). Verminous pneumonia was seen in 4 cases (05.88%). The distribution of all gross lesions is summarised in Table 1.

Table 1. Percentage of gross pathological lesions associated with pneumonia in bovines

S.No.	Lung Lesions	No. of cases (%) (n=68)
1.	Congestion and Haemorrhages	42 (61.76)
2.	Consolidation	30 (44.12)
3.	Fibrinous exudates	22 (32.35)
4.	Thickened interlobular septa	18 (26.47)
5.	Pulmonary oedema	12 (17.65)
6.	Hydatid cyst	12 (17.65)

7.	Emphysema	10 (14.70)
8.	Verminous pneumonia	04 (05.88)
9.	Nodule	03 (04.41)
10.	Pleural adhesion	01 (01.47)

The high prevalence of congestion and haemorrhage observed in this study (61.76%) is notably higher than that reported by Akbor et al. (2007), who observed haemorrhages and congestion in only 16.25% of abnormal buffalo lungs. Similarly, Belkhiri et al. (2009) reported pulmonary congestion in 7.89% of cases in a large-scale survey of 870 bovine lungs, suggesting that the high frequency of these lesions in the present study reflects an acute to subacute disease process in a considerable proportion of animals.

Consolidation, observed in 44.12% of cases, is a hallmark of bronchopneumonia and typically indicates active bacterial infection. This aligns with the observations of Akbor et al. (2007), who described various stages of pneumonia (congested, fibrinous, purulent) in buffaloes, although their overall prevalence of specific pneumonic stages was lower. The high rate of fibrinous exudation (32.35%) and interlobular septal thickening (26.47%) in the present study strongly suggests the involvement of pathogens like Pasteurella multocida, which are classically associated with fibrinous pleuropneumonia. This finding is supported by Praveena et al. (2014), who described severe fibrinous bronchopneumonia with thickened septa in calves experimentally infected with P. multocida serotype A:1.

Emphysema was recorded in 14.70% of cases, remarkably similar to the 14.35% prevalence reported by Belkhiri et al. (2009). This consistency across studies suggests that emphysema is a common secondary lesion in bovine lungs, often resulting from forced respiration associated with chronic pneumonia. Hydatid cysts were found in 17.65% of cases, which is significantly lower than the 42.64% reported by Belkhiri et al. (2009), perhaps reflecting regional differences in parasite burden. Pleural adhesions in 1.47% of cases are comparable to the 2.5% thickened pleura reported by Akbor et al. (2007), representing chronic or resolving pleuritis.

3.2 Pathomorphological Study of Pasteurella multocida Infected Bovine Lungs

Out of 68 bovine pneumonic lungs examined, 03 (4.41%) lungs were found to be positive for species-specific P. multocida along with its associated virulence genes (ompH and omp87). Gross assessment of P. multocida-infected pneumonic lung lesions was carried out based on anatomical location, variations in colour, consistency and the nature of exudates by careful visual examination and palpation.

In general, pneumonic lesions in all P. multocida infected lungs showed bilateral involvement of different lobes, in variable severity, with deep red to purplish-red discolouration, heavy and voluminous appearance, reflecting the combined effects of natural infection dynamics, co-infections and environmental stressors under field conditions. Similar findings were also noted by earlier reports emphasising the cranioventral distribution of pasteurellosis associated pneumonia (Singh et al., 2007; Behera et al., 2023 and Rasheed et al., 2024).

Gross examination of the first case of pneumonic lungs revealed marked enlargement with bilaterally distributed lesions. The lungs appeared dark red in colour, indicating severe congestion, and were voluminous, firm, consolidated and non-collapsible. On incision, the cut surface of the lung parenchyma revealed oozing of frothy exudates and extensive hyperaemia. The gross lesions in this case are characteristic of acute pneumonic pasteurellosis caused by Pasteurella multocida. These changes reflect

severe vascular damage and increased capillary permeability induced by bacterial endotoxins, resulting in congestion, haemorrhage and exudation within the lung parenchyma (Tadesse et al., 2017). Similar gross findings have been consistently reported in both experimental and natural infections. Gourlay et al. (1989) described firm pneumonic consolidation following intratracheal infection in calves, while Singh et al. (2007) reported severe pulmonary congestion, haemorrhages and fibrinous exudates during outbreaks of pasteurellosis. The present findings correspond well with earlier observations of alveolar flooding and bronchial involvement in acute cases (Dagleish et al., 2010 and Praveena et al., 2014).

In the second case, the pneumonic lungs failed to collapse upon opening of the thoracic cavity and exhibited red to grey discolouration, with consolidated and firm, liver-like (hepatized) consistency on palpation. On cut section, mild to moderate oedema and hyperaemia were also evident in the lung parenchyma. Comparable gross features have been documented in subacute and chronic stages of P. multocida associated pneumonia. Dagleish et al. (2010) and Praveena et al. (2014) described progression from congestive and fibrinous lesions to firm, grey consolidated lungs with loss of normal elasticity in advanced cases. Persistent haemorrhagic foci within consolidated parenchyma have also been reported in natural outbreaks of pasteurellosis, reflecting ongoing vascular injury and incomplete resolution of inflammation (Tuzcu et al., 2020 and Elbatawy et al., 2022). The hard consistency suggests organisation of exudates and early fibrotic changes, which are commonly encountered in chronic bronchopneumonia due to P. multocida (Behera et al., 2023 and Mostafa et al., 2024).

In the third case, the pleural surface appeared dull and rough, with patchy to diffuse pleural thickening suggestive of fibrinous pleuritis. Some areas exhibited irregular multifocal necrotic foci and multifocal to coalescing areas of consolidation, with varying degrees of interlobular emphysema and rubbery consistency on palpation. Hepatization of lung tissue is widely reported as a classical gross feature of fibrinous bronchopneumonia caused by P. multocida. Consolidated and airless areas in lungs have been described in calves and buffaloes experimentally and naturally infected with P. multocida, where alveoli become packed with fibrin, inflammatory cells and erythrocytes, resulting in loss of spongy architecture (Gourlay et al., 1989; Dagleish et al., 2010; Praveena et al., 2014 and Tuzcu et al., 2020). Fibrinous pleuritis with pleural thickening and adhesions consistent with P. multocida infection has been consistently documented by Singh et al. (2007); Dagleish et al. (2010); Elbatawy et al. (2022) and Kumar et al. (2024).

Figure 01: Carcass of cattle for postmortem examination

Figure 02: Carcass of buffalo calf and buffalo for postmortem examination

Figure 03: Congestion and haemorrhages in bovine lung

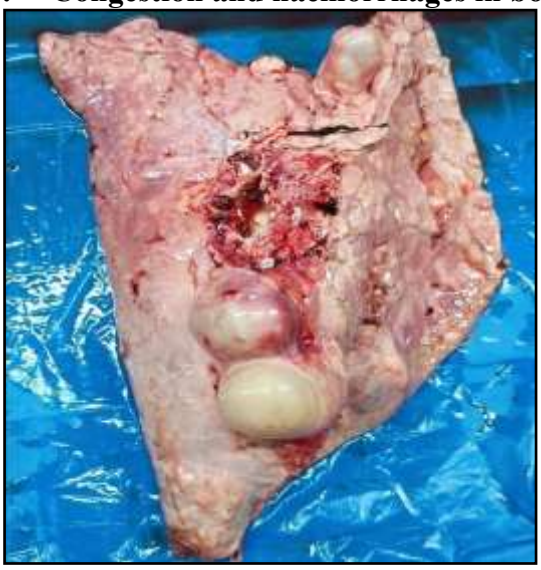

Figure 04: Hydatid cyst in bovine lung

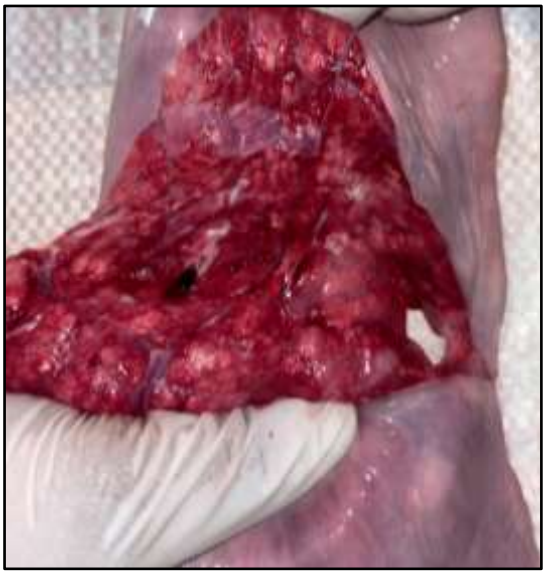

Figure 05: Cut section of bovine lung showing nodules (arrow)

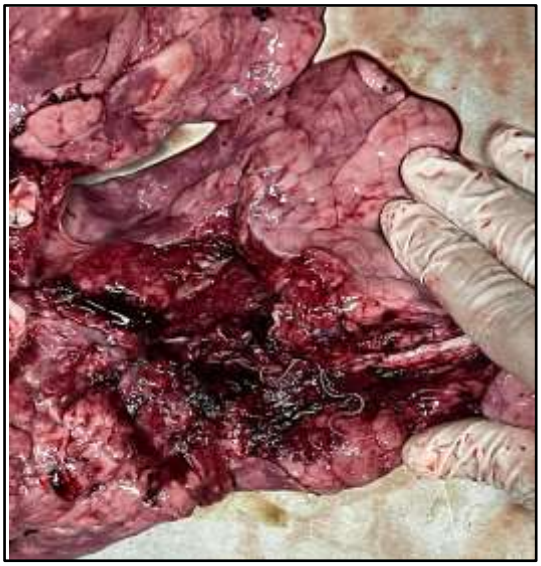

Figure 06: Verminous pneumonia in bovine lung (arrow)

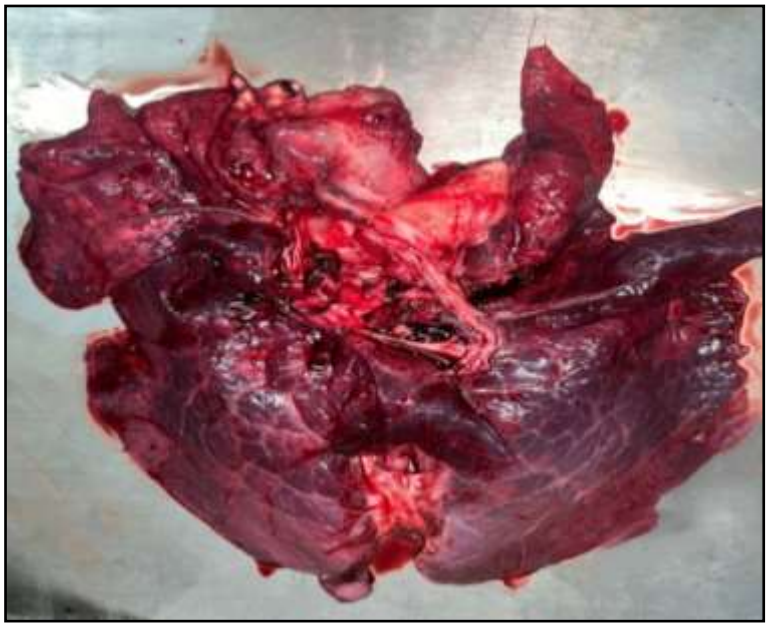

Figure 07a: *P. multocida* positive bovine lung showing bilaterally distributed pneumonic lesions

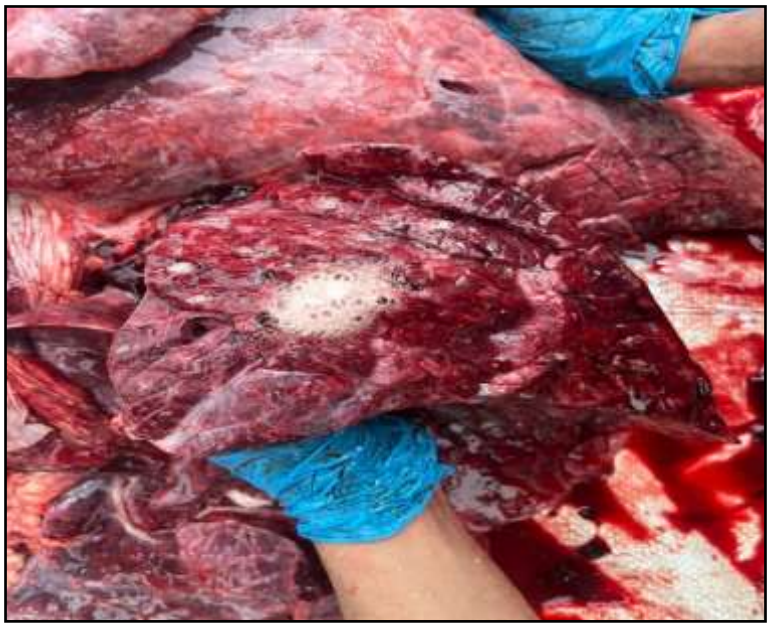

Figure 07b: Cut section of *P. multocida* positive bovine lung showing frothy exudate and extensive hemorrhage

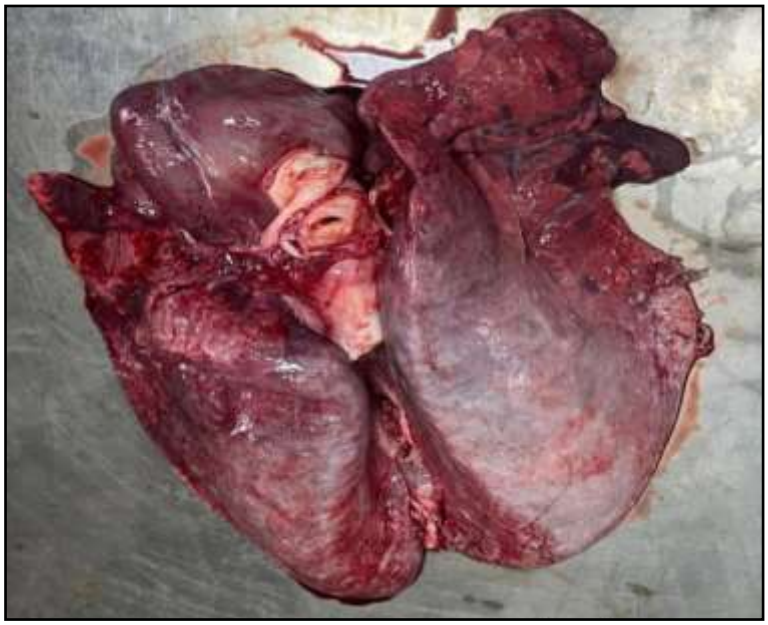

Figure 08a*: P. multocida* positive bovine lung showing congestion (arrow) and consolidation (arrowhead)

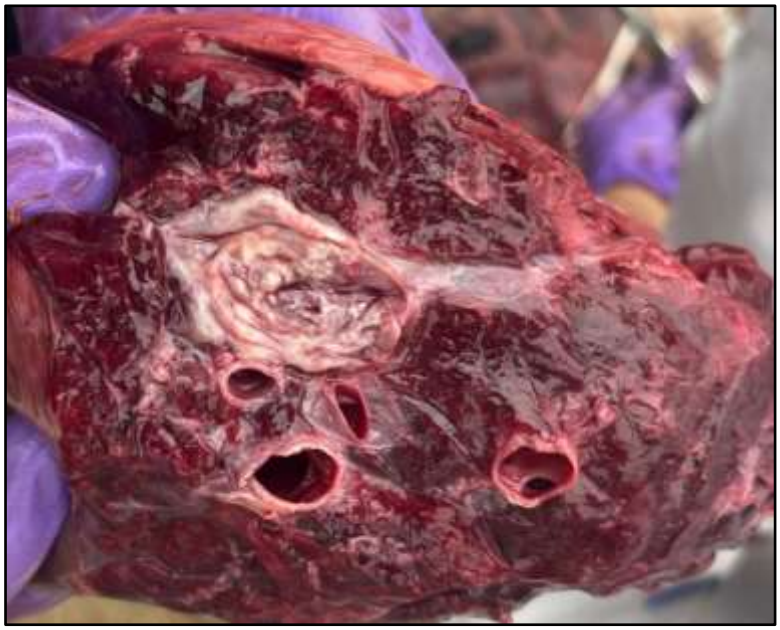

Figure 08b: Cut section of *P. multocida* positive bovine lung showing consolidation

3.3 Histopathology

3.3.1 Alveoli

Emphysematous changes with alveolar overdistension and septal rupture were observed, while in some areas the alveoli were filled with homogeneous eosinophilic material along with congestion of blood vessels. The alveolar spaces were variably distended and filled with abundant inflammatory exudate consisting predominantly of erythrocytes, neutrophils, macrophages, fibrin strands and necrotic cellular debris. In several areas, alveoli showed complete obliteration due to dense fibrinopurulent exudation, resulting in marked consolidation, and foci of coagulative necrosis with loss of normal alveolar architecture were evident. These findings align with the characteristic pathogenicity of P. multocida

described in bovine respiratory disease complexes and are in close agreement with Annas et al. (2014), who reported generalised severe congestion and multifocal haemorrhages in the lungs of infected buffaloes. Similarly, Chung et al. (2015) and Hedau et al. (2017) described marked haemorrhage and congestion as primary histological features. The presence of homogeneous eosinophilic alveolar material is indicative of pulmonary oedema, corroborated by Hedau et al. (2017) and Praveena et al. (2014).

3.3.2 Interalveolar Septa

The interalveolar septa were markedly thickened due to congestion, oedema and infiltration by inflammatory cells, predominantly neutrophils and mononuclear cells. Capillaries within the septa were dilated and engorged with blood. In areas adjacent to necrotic foci, septal architecture was disrupted and fibrin deposition was evident. These observations are in agreement with Doyle-Baker et al. (2020), Mostafa et al. (2024) and Singh et al. (2007), who identified thickened alveolar septa with increased lymphocytes and macrophages as a common change.

3.3.3 Bronchi and Bronchioles

Bronchi and bronchioles exhibited marked inflammatory changes. Bronchiolar epithelial degeneration, desquamation and hyperplasia were evident. There was luminal accumulation of neutrophils, fibrin, mucus and cellular debris. Bronchial and bronchiolar walls showed infiltration of inflammatory cells particularly neutrophils and macrophages accompanied by congestion and oedema. Necrosis of the bronchial epithelium, indicating severe suppurative bronchitis and bronchiolitis with perivascular and peribronchiolar cuffing by inflammatory cells, was frequently observed. These findings are aligned with the report by Singh et al. (2007), which described degeneration of bronchiolar lining epithelium. The severe suppurative bronchitis and bronchiolitis mirror the results of Elbatawy et al. (2022), who observed bronchioles packed with mucopurulent exudate. Perivascular and peribronchiolar cuffing is further supported by Yaman et al. (2018), who detected peribronchial and peribronchiolar mononuclear cell infiltration.

3.3.4 Pleura

Fibrin deposition on the pleural surface with inflammatory cell infiltration was noted, consistent with fibrinous pleuritis and congestion. Mild to moderate fibrosis was evident, with collagen fibres appearing blue in lung sections stained with Masson's Trichrome stain. The fibrin deposition and inflammatory infiltration correspond with the findings of Praveena et al. (2014) and Elbatawy et al. (2022), who reported fibrinous pleuritis with adhesions and pleural thickening in severe cases. The mild to moderate fibrosis confirms chronicity of infection, supported by Mostafa et al. (2024), who described fibrosis as a hallmark of chronic interstitial pneumonia and chronic bronchopneumonia in P. multocida infections.

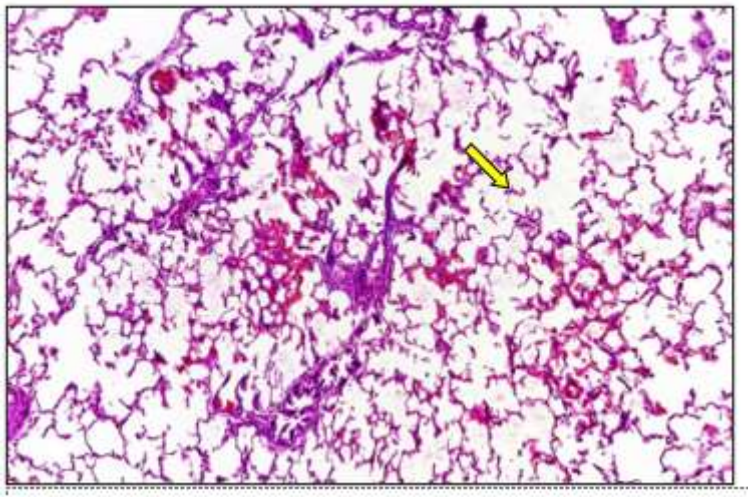

Figure 10*:* Microscopic section of *P. multocida* positive bovine lung showing over distension of alveoli with septal rupture (arrow), H&E X 100

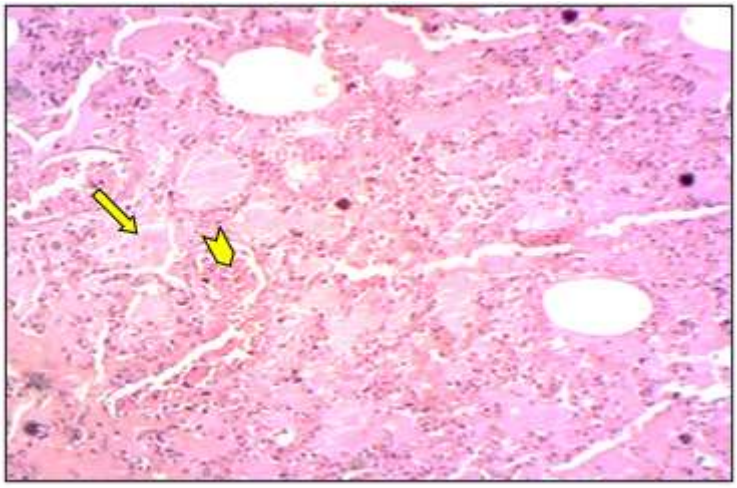

Figure 11: Microscopic section of *P. multocida* positive bovine lung showing homogenous eosinophilic material in alveoli (arrow) with congestion of blood vessels (arrowhead), H&E X 400

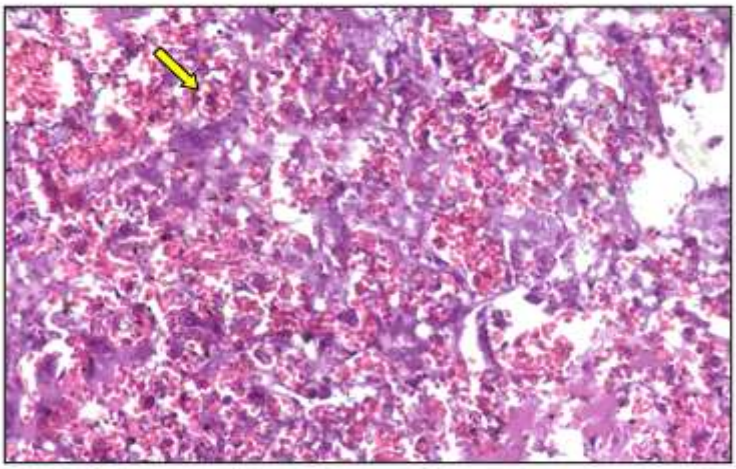

Figure 12: Microscopic section of *P. multocida* positive bovine lung showing infiltration of erythrocytes in alveoli (arrow), H&E X 400

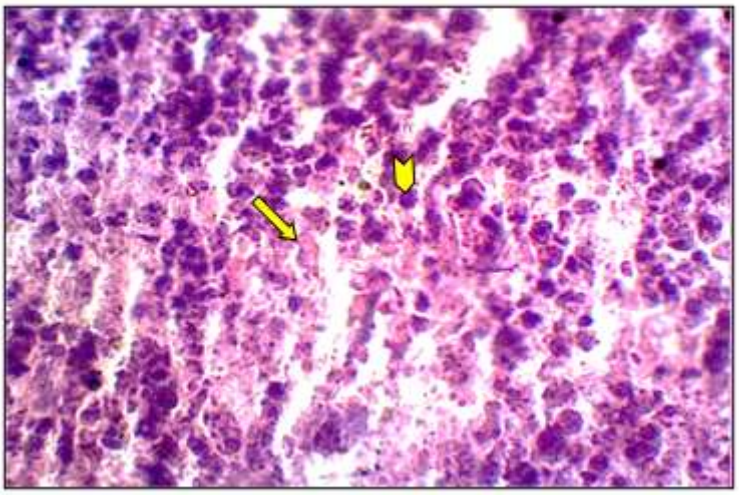

Figure 13: Microscopic section of *P. multocida* infected bovine lung showing necrosis (arrow) and infiltration of inflammatory cells (arrowhead), H&E X 400

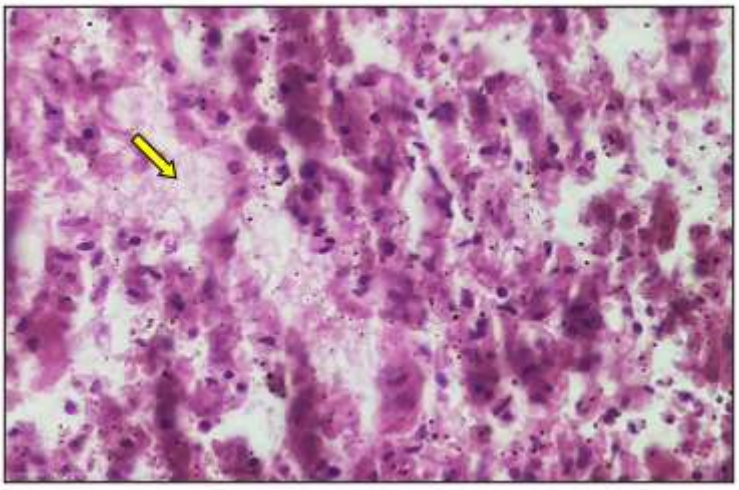

Figure 14: Microscopic section of *P. multocida* positive bovine lung showing with fibrinopurulent exudation in alveoli (arrow), H&E X 400

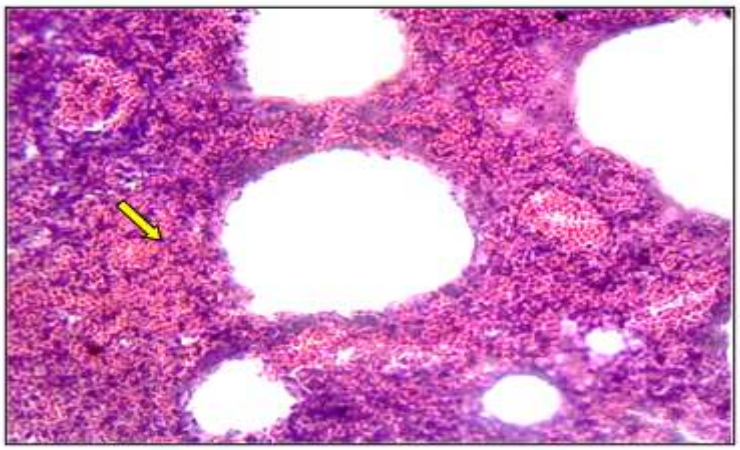

Figure 15: Microscopic section of *P. multocida* positive bovine lung tissue showing thickened interalveolar space with dilated and engorged blood vessels, (arrow), H&E X 200

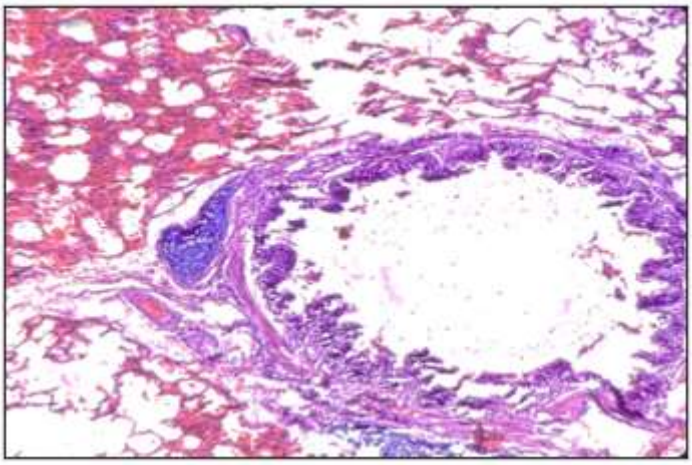

Figure 16: Microscopic section of *P. multocida* positive bovine lung with denudation of bronchiolar epithelium (arrow), emphysema (arrowhead) and thickened interalveolar septa, H&E X 200

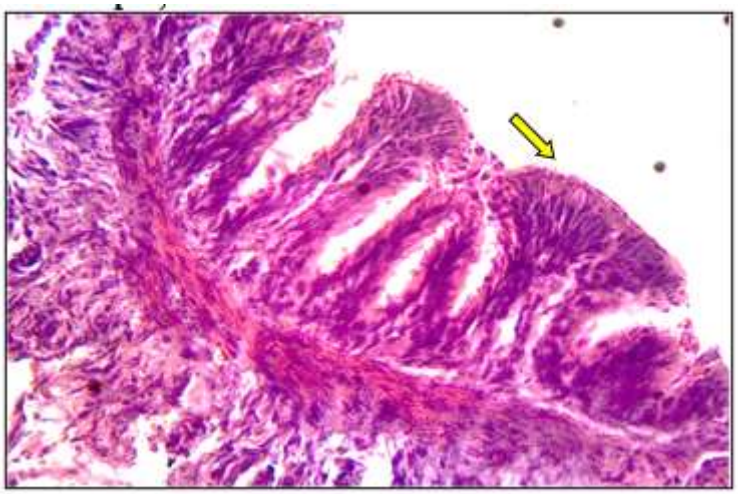

Figure 17: Microscopic section of *P. multocida* positive bovine lung showing bronchiolar epithelial hyperplasia (arrow), H&E X 400

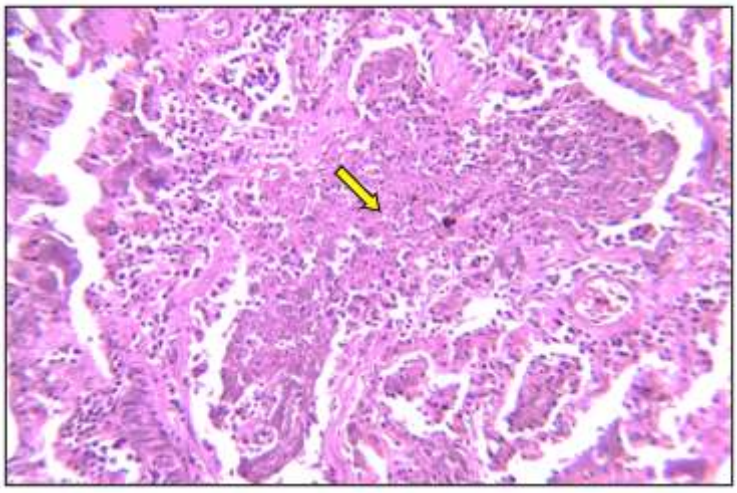

Figure 18: Microscopic section of *P. multocida* positive bovine lung with infiltration of inflammatory cell in the lumen of bronchiole (arrow), H&E X 400

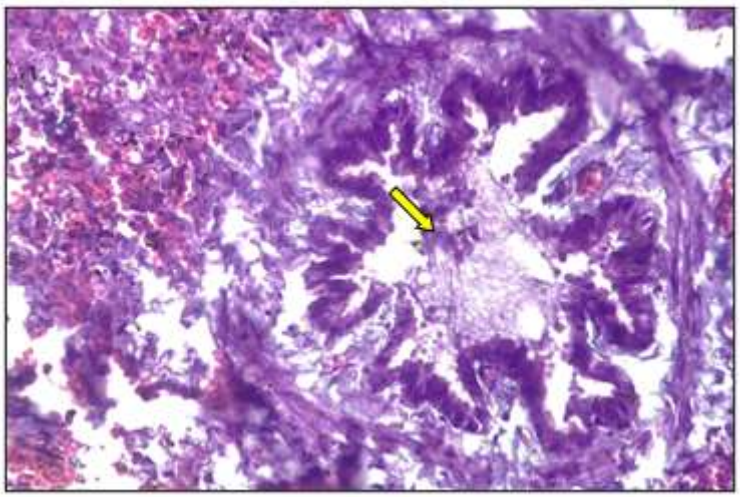

Figure 19: Microscopic section of *P. multocida* positive bovine lung showing degeneration of bronchiolar epithelium and increased fibrin (arrow), H&E X 200

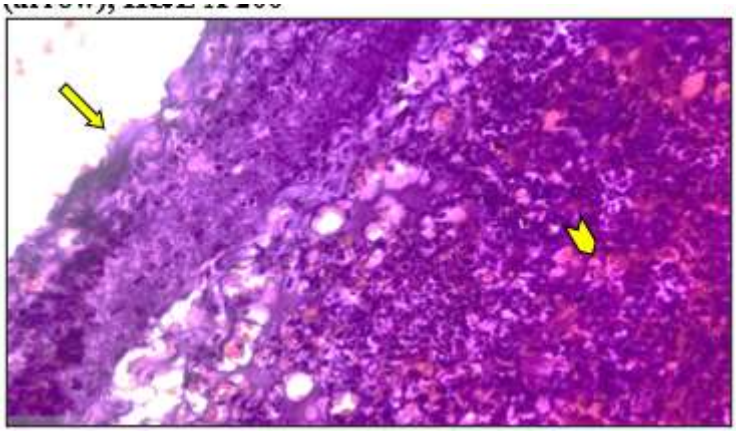

Figure 20: Microscopic section of *P. multocida* positive bovine lung with fibrinous pleuritis (arrow) and congestion (arrow head), H&E X 400

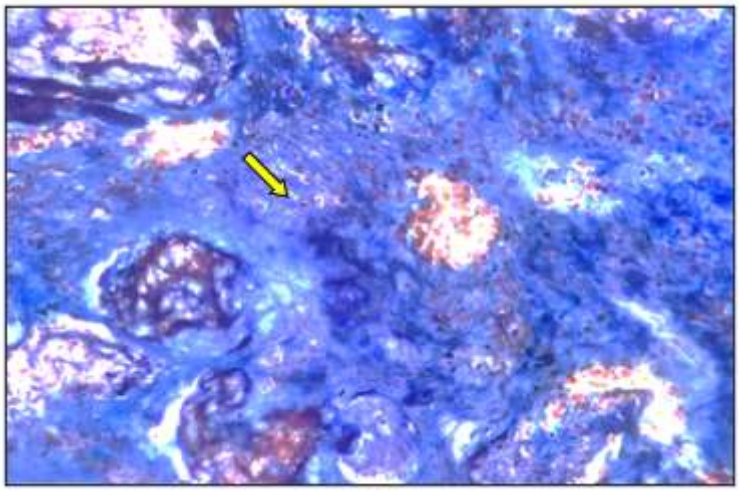

Figure 21: Microscopic section of *P. multocida* positive bovine lung with increased fibrin (arrow), Masson Trichome X 400

4. CONCLUSION

The present study documents comprehensive gross and histopathological findings in Pasteurella multocida-infected bovine lungs in Jabalpur, Madhya Pradesh. The gross lesions ranged from severe congestion, haemorrhage and fibrinous exudation in acute cases to hepatization, fibrosis and pleural adhesions in chronic cases. Histopathological examination revealed alveolar flooding with fibrinopurulent exudate, coagulative necrosis, bronchiolar degeneration, perivascular cuffing and fibrinous pleuritis. Masson's Trichrome staining confirmed collagen deposition indicative of chronicity. The findings are consistent with fibrinous bronchopneumonia caused by P. multocida and provide significant baseline pathological data for the region. This information may assist in improving diagnostic accuracy, disease management and control strategies for bovine pasteurellosis in central India.

ACKNOWLEDGEMENTS

The authors express sincere gratitude to the Dean, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, for providing necessary facilities. The authors are also thankful to all faculty and technical staff of the Department of Veterinary Pathology for their support during the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

All experiments have been examined and approved by the Institutional Animal Ethics Committee (IAEC) of Nanaji Deshmukh Veterinary Science University, Jabalpur.

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