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Research Article

PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON LUNGS OF BOVINE WITH RESPIRATORY AFFECTIONS

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ABSTRACT

Respiratory affections are important causes of mortality in bovines. *Pasteurella multocida* is one of the major bacterial pathogens isolated from bovines affected with respiratory diseases. The present investigation was carried out to study the pathomorphological changes and detection of *Pasteurella multocida* by immunohistochemistry in lungs collected from 18 bovine carcasses with history of respiratory illness. Grossly, lungs revealed congestion, haemorrhages, oedema, thickened interstitial septa and nodular lesions. The histopathological lesions were categorized as congestion, haemorrhages, oedema, emphysema and pneumonia. Histopathologically, pneumonia was noticed in 6 out of 18 cases. It was further classified as serous (1/18), sero-fibrinous (1/18), interstitial (1/18) and granulomatous type (3/18). In none of these cases, *P. multocida* antigen was detected by immunohistochemistry.

Keywords: Bovines, Immunohistochemistry, Lungs, Pathomorphological, *Pasteurella multocida*

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In bovine species, affections of respiratory system are one of the major causes of mortality (Patrick, 2009). Respiratory disease complex is a multi-factorial syndrome and develops as a result of complex interaction between host, environmental factors and pathogen (Duff and Galyean, 2007). Primarily, a virus modulates the host defence mechanism and thereby allows the colonisation of secondary invader bacteria in respiratory tract. *Pasteurella multocida* (*P. multocida*) is one of the primary bacterial pathogens involved in such clinical syndromes (Welsh *et al.*, 2004). It causes widespread damage in the body, particularly in respiratory system. Besides, routine bacterial culture methods, molecular techniques such as polymerase chain reaction (PCR) and other important ancillary techniques such as immunohistochemistry (IHC) play an important role in diagnosis of bacterial infections. The IHC not only detects the antigen, but also unfurls the site of bacterial localization. It can also be used as a potential tool to study pathogenesis of a disease. Keeping above in view, the present study was undertaken to describe the pathological lesions and detection of *P. multocida antigen* in the lungs of dead bovines with history of respiratory illness.

MATERIALS AND METHODS

The present study was conducted on eighteen (18) bovine carcasses that were presented to the Department of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar for post-mortem examination during the period from August, 2018 to February, 2019. The animals from the field cases with respiratory signs or suspected for pasteurellosis were included in this study. The lungs were thoroughly examined and gross lesions were recorded. The tissue samples of the lungs were collected and fixed in 10% neutral buffered formalin for further histopathological and immunohistochemical studies. The tissues were routinely processed and embedded in the paraffin. Thereafter, paraffin embedded tissues were cut into sections of 4 µm thickness and stained with haematoxylin and eosin (H & E) as per the conventional method (Luna, 1968).

The immunohistochemical staining was also done in formalin fixed paraffin embedded tissue sections. The IHC was carried out with standard procedure as described earlier by Bhat *et al.* (2016). For IHC, 4 µm sections were mounted on 3-Aminopropyl-triethoxy-silane coated slides. Briefly, the sections were deparaffinized in the xylene and then rehydrated through descending grades of ethanol. The antigen retrieval was carried out by heating the sections dipped in 0.01 M citrate buffer (pH: 6.0) in the microwave oven at 800 W for 30 minutes (15 cycles of 2 minutes each). Endogenous peroxidase was blocked by 3% hydrogen peroxide prepared in the absolute methanol for 60 minutes. The sections were incubated with mouse raised anti- *P. multocida* B:2 hyperimmune sera used in 1:2500 dilution and kept at 4 °C overnight. Thereafter, sections were incubated with secondary antibody and extravidin peroxidase (1:20 dilutions) (Sigma Aldrich) for 30 minutes each at 37 °C. The sections were washed thrice for 5 minutes each with the phosphate buffered saline (pH: 7.2-7.4) in between consecutive steps from antigen

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retrieval till colour development throughout the entire process. Colour reaction developed with 3-Amino-9-ethyl-carbazole (Sigma Aldrich) as per the manufacturer's instructions. Then, sections were counter stained lightly with Mayer's haematoxylin. The brick red or reddish brown colour reaction was considered as positive reaction. The positive and negative controls were also used during the process. For positive control, the lung sections of rabbits experimentally infected with the *P. multocida* B:2 were used. However, in the negative control, sections were incubated with 1% bovine serum albumin (diluent used for antibodies and extravidin) instead of the mouse raised anti-*P. multocida* B:2 hyper immune sera.

RESULTS AND DISCUSSION

The details of species, age, sex, gross and histopathological lesions and results of IHC for the detection of *P. multocida* B:2 antigen in bovine lungs are presented in table 1. Grossly, the lungs revealed thickened pleura, congestion, haemorrhages, oedema, consolidation, emphysema and thickened inter-alveolar septa. Congestion was either focal or diffused and it was either restricted to apical lobes or diffused throughout the diaphragmatic lobes (Fig. 1). This condition might have been due to terminal supply of trachea into cranial lobes which can easily transmit the pathogen from upper respiratory tract at this site and results in production of pathological lesions. Belkhiri *et al.* (2009) also reported congestion in bovine lungs (7.89%) collected from the Tiaret slaughter house. Haemorrhages were also noticed and these were categorized as petechial or ecchymotic type. The bovine lungs revealed emphysema and appeared pale and enlarged (Fig. 2). In some other cases, septae were more prominent (Fig. 3). Similarly, Belkhiri *et al.* (2009) also reported emphysema in 14.35% cases of bovine lungs. Mellau *et al.* (2010) reported emphysema in the lungs of cattle slaughtered in Arusha, Tanzania with the incidence rate 13.10%. In present study, lungs also revealed oedema characterized by oozing out of sero-sanguinous fluid from the cut surfaces. However, a lower incidence (0.98%) of oedema in cattle lungs was reported by Benhathat and Aggad (2017). In one case, fibrin deposits were noticed over pleura. In this study, consolidation of the lungs along with presence of cheesy exudate was observed in three cases. In two cases, the consistency of lungs was hard and a gritty sound was heard while cutting. Multiple white foci of small sizes with hard consistency were noticed in one case (Fig. 4).

The attempts were made for isolation of *P. multocida* on blood agar from lung samples. However, in none of the samples, *P. multocida* could be isolated and the scenario of non occurrence might probably be due to less number of cases or due to the possibility of antibacterial treatment given to the animals. Contrary to our study, Tegtmeier *et al.* (1999) isolated *P. multocida* from the lungs of 10 (13.89%) calves out of 72. Similarly, Fulton *et al.* (2009) also isolated *P. multocida* from 54 (24.50%) bovine lungs out of the 220 that died with clinical signs of bovine respiratory disease. Karimkhani *et al.* (2011) isolated *P. multocida* from 6 (2.5%) bovine lung samples out of 240, collected from Urmia's slaughter house. They suggested that animal, its breed, sex, age and season could be the possible factor for the occurrence of these positive cases.

On histopathological examination, lung sections revealed congestion, haemorrhages, oedema, emphysema and pneumonia. A total of 11 out of 18 cases revealed congestion. The haemorrhages were noticed in lung parenchyma as well as stroma. Pulmonary oedema was noticed in 6 cases and it varied from moderate to severe. It was characterized by accumulation of pinkish fluid in alveolar lumen. Similarly, pulmonary oedema was reported by Belkhiri *et al.* (2009) in bovine lung samples collected from the Tiaret and Batna's slaughter houses. In the present study, emphysema was noticed in 5 out of 18 cases. It was characterized by the enlargement of alveolar spaces along with destruction of alveolar septa and formation of giant alveoli (Fig. 5). Similarly, Argade *et al.* (2019) reported that the microscopic changes suggestive of emphysema were characterized by enlarged air spaces in the lungs of Murrah buffalo that died with a history of respiratory distress. Microscopically, pneumonia was diagnosed in 6 cases out of 18 in the present study and it was further classified as serous (1/18), sero-fibrinous (1/18), interstitial (1/18) and granulomatous type (3/18). Serous pneumonia was characterized by accumulation of fluid in alveolar lumen and presence of few mixed types of inflammatory cells (both polymorphonuclear and mononuclear). Sero-fibrinous pneumonia revealed pleuritis and presence of sero-fibrinous exudate in the lung parenchyma. The interstitial pneumonia was characterized by proliferation of interstitial septae and intra-alveolar fibroblasts along with infiltration of mononuclear cells mainly lymphocytes. Similar changes have been reported by earlier authors (Raji *et al.*, 2012; Sushma *et al.*, 2016; Benhathat and Aggad, 2017). Similar to present study, Raji *et al.* (2012) and Benhathat and Aggad (2017) also reported pneumonia in 8.79% and 10.84% cases of cattle lungs collected from slaughterhouse. In present study, granulomatous pneumonia was observed in 3 out of 18 cases. These cases were characterized by caseous necrosis with calcification in the centre, infiltration of lymphocytes, macrophages, epithelioid

Table 1

Details of species, age, sex, gross and histopathological lesions in bovine lungs and results of immunohistochemistry for detection of *P. multocida* B:2 antigen

S. No.	Animal details (Species, Age, Sex)	Gross lesions	Histopathological lesions/ Diagnosis	IHC results
1.	Buffalo, 6 y, F	Diffused congestion	Congestion and mild haemorrhages	Negative
2.	Cow, 5y, F	Congested apical lobes	Mild pleuritis, thickened interstitial walls andserous pneumonia	Negative
3.	Buffalo, 4y, F	Marked emphysema	Congestion, haemorrhages, emphysema and thickened inter-alveolar septa	Negative
4.	Buffalo calf, 7m, M	Congested apical lobes and haemorrhages	Marked congestion and haemorrhages, thickened interalveolar septa, focal areas of oedema and emphysema	Negative
5.	Buffalo, 4y, F	Haemothorax	Congestion, haemorrhages and moderate oedema	Negative
6.	Buffalo, 8y, F	Consolidated lobes, presence of fibrin, yellowish fluid in thoracic cavity	Granulomatous pneumonia	Negative
7.	Buffalo, Adult, M	Consolidated lobes, presence of tubercles and caseative necrosis	Granulomatous pneumonia	Negative
8.	Cow calf, 4m, F	Focal consolidated areas, congestion and prominent septa	Granulomatous pneumonia (Positive for *Mycobacterium tuberculosis* by PCR)	Negative
9.	Buffalo calf, 1m, F	Focal congestion and haemorrhages	Sero-fibrinous pneumonia, severe haemorrhages with infiltration of fibrin and inflammatory cells	Negative
10.	Cow calf, 7 days, M	Congested apical lobes	Congestion, haemorrhages and mild thickening of inter-alveolar septa	Negative
11.	Buffalo calf, 1m, M	Severe congestion and haemorrhages	Pulmonary oedema, emphysema, infiltration of inflammatory cells	Negative
12.	Buffalo calf, 4m, F	Slightly congested	Serous pneumonia, mild serous exudate, severe congestion and haemorrhages	Negative
13.	Buffalo calf, 5m, M	Severely congested	Pulmonary oedema, severe congestion and haemorrhages	Negative
14.	Buffalo, 3y, F	Consolidated lobes,congestion and haemorrhages	Interstitial pneumonia, thickening and infiltration in the interalveolar septa	Negative
15.	Buffalo, Adult, F	Leathery consistency, Emphysema	Emphysema and congestion, multifocal areas of infiltration of inflammatory cells mainly lymphocytes	Negative
16.	Buffalo, 14y, F	Severe congestion and oedema	Pulmonary oedema and congestion	Negative
17.	Buffalo, 6y, F	Emphysema and mild congestion	Emphysema and mild congestion along with oedema	Negative
18.	Cow calf, 6m, M	Congestion	Congestion, pulmonary oedema, haemorrhages and mild infiltration of leucocytes in alveoli	Negative

y: year, m: month, M: male, F: female

cells and Langhan's type giant cells and surrounded by fibrous capsule (Figs. 6 & 7). In one of these cases, the DNA of *Mycobacterium tuberculosis* was detected by PCR as confirmed by the Disease Investigation Laboratory, Department of Veterinary Public Health and epidemiology, LUVAS, Hisar. Similar to present findings, a number of such cases have been reported from different parts of India (Thakur *et al.*, 2012; Kaur *et al.*, 2019) and other parts of the world (Shitaye *et al.*, 2006; Carvalho *et al.*, 2015). *P. multocida* antigen was not detected in any of the lung sections by IHC also. However, positive control lung tissue revealed the presence of *P. multocida* antigen (Fig. 8). Earlier studies by Yaman *et al.* (2018) stated that the antigen of *Pasteurella* spp. was detected in the cytoplasm

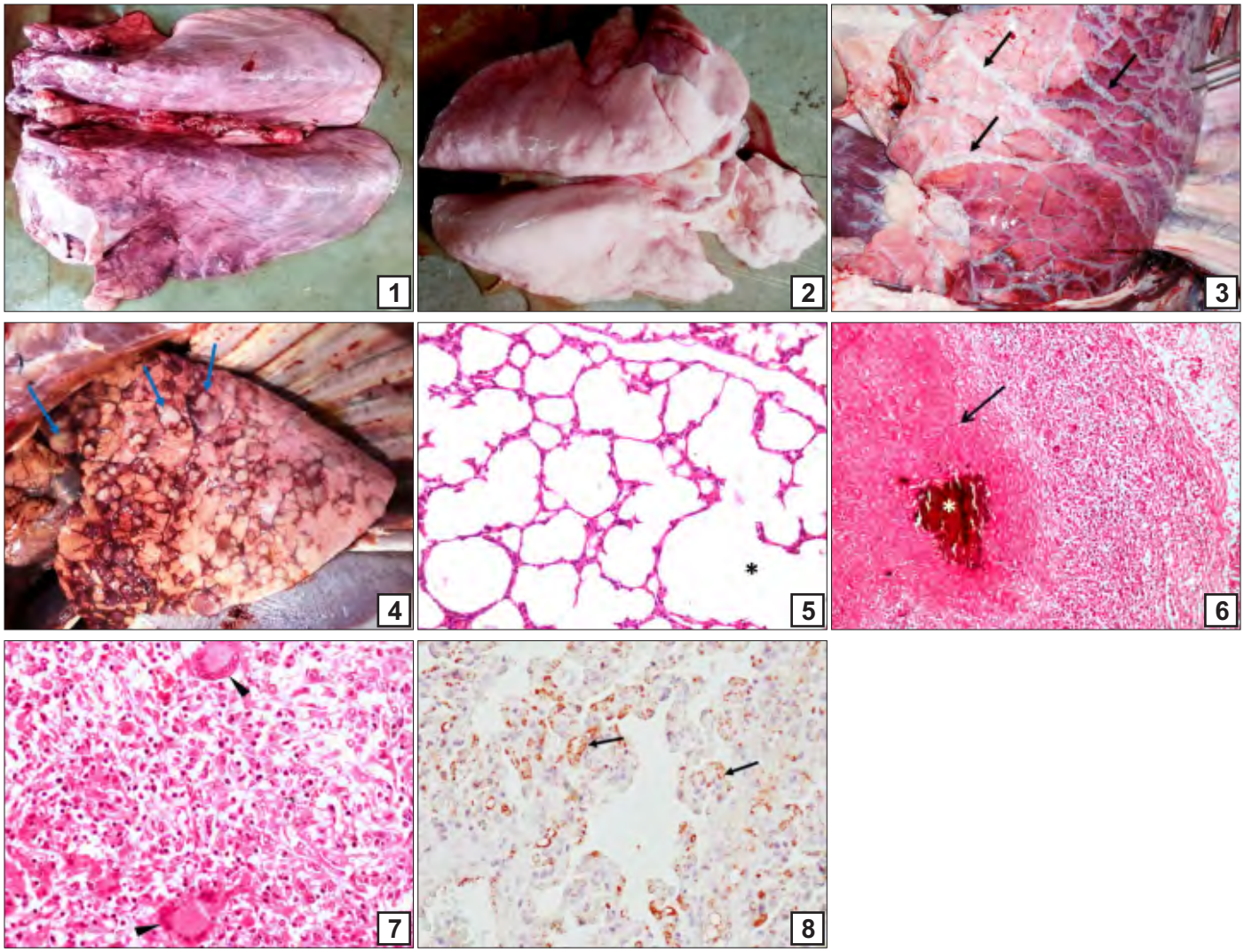

Fig. 1-8. (1) Bovine lungs with diffused congestion in diaphragmatic lobes; **(2)** Emphysematous bovine lungs showing pale and sponge-like appearance; **(3)** Bovine lungs showing congestion and distended and prominent septa (arrows); **(4)** Bovine lungs showing multifocal areas of nodular lesions (arrows); **(5)** Photomicrograph of lung showing emphysema characterized by the formation of giant alveoli (asterisk). H&E×400; **(6)** Photomicrograph of lung showing caseative necrosis (arrow) with calcification in the centre (asterisk) surrounded by fibrous tissue capsule. H&E×100; **(7)** Photomicrograph of lung showing multinucleated Langhan's type giant cells (arrow heads). H&E×400; **(8)** Photomicrograph of lung from *P. multocida* infected rabbit (positive control) showing reddish-brown immunopositive reaction depicting *P. multocida antigen* in parenchyma (arrows). IHC×200

of the epithelial cells of the bronchi and bronchioles in the lung sections which were collected from naturally-infected cattle of slaughter house in Turkey. The desquamated cells present in the lumen of bronchi and bronchioles also revealed the presence of *Pasteurella* antigen. Similarly, Bhat *et al.* (2016) also detected the *P. multocida* antigen in 16 swine lungs (22.5%) by IHC.

Although various pathological conditions were noticed in the bovine lungs in the present study, *P. multocida* was neither isolated nor its antigen was detected by IHC. Some bacteria other than *P. multocida* were isolated from 13 out of 18 cases as identified by Vitek 2. However, further characterization of these bacteria is under progress. So, it may be stated that the pathological lesions observed in the present study may be associated with these bacteria or some other etiological agents. Moreover, a large number of samples need to be screened by different diagnostic methods to look for the incidence and prevalence of pasteurellosis in disease outbreaks in Haryana.

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