

# Pathomorphological Investigation on Membranous Tracheitis in an Adult Buffalo

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## ABSTRACT

Membranous tracheitis is an important pathological condition affecting the trachea in animals, usually associated with immunosuppression. This report describes the pathological findings associated with diphtheric tracheitis in an immunocompromised adult buffalo. A carcass of an adult buffalo, with a history of dyspnea, and a large amount of reddish foam flowing from the mouth and nose, presented for necropsy. Detailed postmortem examination was conducted and representative tissue samples were collected for bacteriological isolation and histopathological examination. Macroscopic examination revealed whitish, pale or red coloured blood mixed heavy froth in lumen of trachea with a tightly adhered greenish colour diphtheric membrane to mucosa. Lungs showed, circular to irregular shaped blackish suffusions, consolidation, along with thin layer of fibrin covering all the lobes. Microscopic examination revealed fibrinous tracheitis and serofibrinous pneumonia in lungs. Mediastinal lymphnode showed severe lymphoid depletion. Bacteriological isolation from tracheal swab and lungs confirmed the presence of *Escherichia coli* using VITEK 2 system indicating its role in pathogenesis of diphtheric membrane formation in presence of immunosuppression.

**Key words:** Buffalo, Diphtheric membrane, *E. coli*, Necropsy, Trachea.

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## INTRODUCTION

Livestock sector is an important subset of Indian agriculture and it plays a crucial role in Indian economy. According to 20<sup>th</sup> livestock census 2019 (DAHD, 2019), population of cattle and buffaloes in India contributes around 37.28 % (192.49 million) and 21.23 % (109.85 million) of the total livestock population, respectively. Haryana shares 1.9 million cattle and 4.3 million buffalo in total population. Diseases in cattle and buffaloes lead to losses in milk yield thus lowering the return on the investments. Bovine Respiratory Disease (BRD) is a general term for respiratory diseases in bovines caused by a range of factors, singly or in combination. A major cause of economic losses, BRD affects the lower respiratory tract/lungs (pneumonia) or upper respiratory tract (rhinitis, tracheitis, bronchitis). BRD in bovines is a multifactorial disease complex including infection with a wide range of conditionally or obligatory pathogenic viruses, bacteria and exposure to stressors (Duff and Galyean, 2007). In presence of different stressors including weaning, changes of feed, variation in ambient temperature, humidity and weather opportunistic pathogens take upper hand and proliferate leading to formation of lesions in respiratory tract. A number of bacterial disease conditions like colibacillosis, pasteurellosis, mycoplasmosis, *Mannheimia haemolytica* infection, *Histophilus somnus* infection, salmonellosis, tuberculosis and paratuberculosis (Griffin *et al.*, 2010) affects respiratory system of bovines resulting in the heavy mortality and decline in overall production. However membranous tracheitis is rarely reported in

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buffalo. Therefore the present study describes a case of membranous tracheitis in an adult buffalo.

## MATERIALS AND METHODS

An adult buffalo suspected to be died due to respiratory tract infection was brought to the Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar for post-mortem examination. Clinical history was recorded from the postmortem requisition form stating reddish froth from nostrils dyspnoea and sudden death.

Detailed post-mortem examination was conducted as soon as the carcass was brought to the post-mortem hall to avoid further autolysis and putrefactive changes under aseptic conditions. The carcass was examined for any injuries, markings etc. and observed for any gross pathological lesions in the respiratory system (nasal turbinate, nasopharynx, trachea, lungs, mediastinal lymph nodes). All target organs were observed for any change in color, texture, size, exudate or any other abnormal change, if present.

**Cytopathology:** Touch impression smears were taken from lesions of lungs. Impression smears were stained with field stain, Leishman stain, Giemsa stain and Gram's stain to demonstrate the pathological changes and aetiological agent.

**Histopathology:** During post-mortem examination, representative tissue samples from respiratory system such as nasal turbinate, nasopharynx, trachea, lung and mediastinal lymph node were collected in 10% neutral buffered formalin for histopathological examination.

**Bacteriological Isolation:** Tracheal swab and small tissue samples of lung showing lesions were collected in sterile petridishes with the help of scissors and forceps under sterile conditions. The surface of each tissue sample was cauterized with a red hot scalpel blade for decontamination. A deep incision was made in each sample using sterile scalpel blade, a sterile swab was dipped into the incised area and streaked onto various agar plates. Plates were incubated at 37°C for 24 h. Various agar such as sheep blood agar, MacKonkey agar, EMB agar and nutrient agar, were used initially and restreaking was done on tryptic soya agar to get pure colonies for VITEK® analysis. (Griffin *et al.*, 2010.)

**Identification by the VITEK 2 Compact:** 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 – incubator module (incubation temperature 35.5°C) and subjected to a kinetic colorimetric

measurement every 15 min. Data were analyzed using VITEK 2 database version 4.01.

## RESULTS AND DISCUSSION

As per history, the buffalo was having signs of respiratory distress, dyspnoea and weakness and was treated with dextrose, normal saline solution and antibiotics. Upon necropsy examination, nasal turbinates and nasopharynx were showing dark reddish discoloration and extensive haemorrhages. Trachea revealed whitish, pale or red coloured blood mixed heavy froth in lumen and greenish coloured diphtheritic membrane which was tightly adhered to the mucosa (Fig. 1). Beneath the membrane petechial to ecchymotic haemorrhages were present (Fig. 2). Lungs showed circular to irregular shaped blackish coloured suffusion type haemorrhages, consolidation of all the lobes and presence of whitish yellow layer of fibrin covering whole lung (Fig. 3, 4) cytopathological examination of trachea and lung impression smears revealed presence of bacteria, neutrophils and macrophages with toxic changes in nuclei and cytoplasm (Fig. 5) and fibrin strands in background suggesting fibrinous inflammation. Similar pathological findings were observed by Singh *et al.* (2009), Khin *et al.* (2010) and Sharma (2019) in lungs. Diphtheritic membrane formation over trachea is rarely reported.

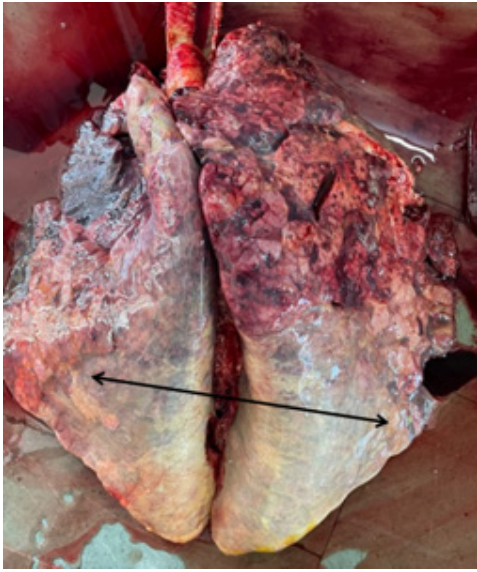
Histopathological findings in nasal turbinate showed necrosis of chondrocytes in cartilage, moderate to diffuse infiltration of leucocytes and distortion of epithelial cells. Nasopharynx showed loss of cilia, distortion and loss of epithelium, break in the continuity of epithelium and infiltration of leucocytes. Trachea showed necrosis of glands in mucosa and submucosa, loss of ciliated epithelium and presence of fibrinous exudate over the mucosal epithelium (Fig. 5). Lungs showed interstitial thickening due to presence of serofibrinous exudate (Fig. 6), congestion of blood



**Fig. 1:** Trachea showing formation of tightly adhered greenish coloured diphtheritic membrane (double arrow) on mucosa.



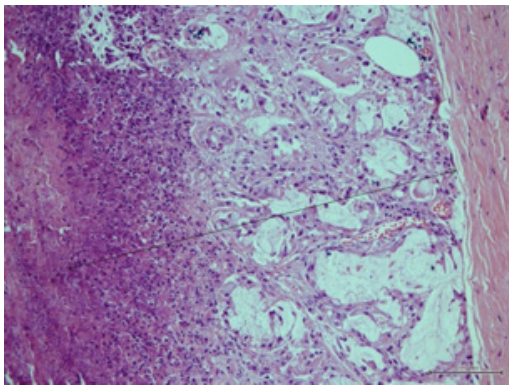
**Fig. 2:** Trachea showing presence of diffuse petechial to ecchymotic haemorrhages (arrow) on the mucosa just below the diphtheritic membrane.



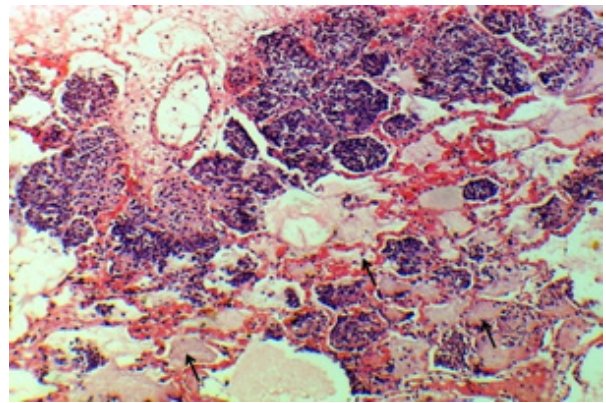
**Fig. 3:** Lungs showing presence of whitish yellow layer of fibrin covering whole lung (double arrow) and diffuse petechial to ecchymotic haemorrhages.



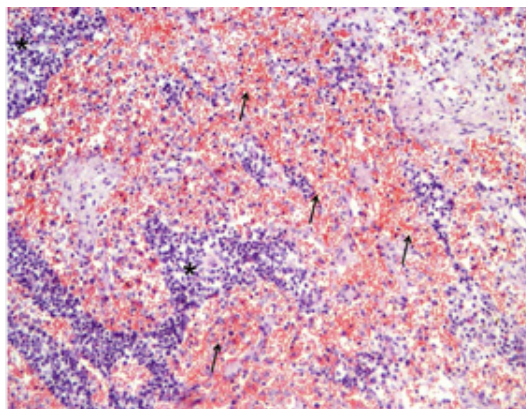
**Fig. 4:** Lungs showing consolidation, firm consistency and diffuse petechial to ecchymotic haemorrhages.



**Fig. 5:** Trachea showing presence of necrotic tissue debris, neutrophilic infiltration and necrosis of glands in mucosa and submucosa (double arrow) (H&E stain X100)



**Fig. 6:** Lungs showing presence of dirty pink coloured fibrin strands in lumen of alveoli along with infiltration of leucocytes (arrow), blocking of alveolar lumen by plug of leucocytes (asterisk) and thickening of interstitial space due to capillary haemorrhages -Fibrinous bronchoalveolar pneumonia (H&E stain X100)



**Fig. 7:** Mediastinal lymph node showing extensive haemorrhages (arrow) and lymphoid depletion in corticomedullary area (asterisk) (H&E stain X200)

vessels and areas of haemorrhages along with moderate infiltration of leucocytes. Jackson *et al.* (1987) also found more or less similar common lesion in cases of fibrinous bronchopneumonia. Ayroud *et al.* (2000) and Loneragan *et al.* (2001) had reported the similar findings of interstitial pneumonia in cattle. Mediastinal lymph node showed extensive haemorrhages, congestion, haemosiderin deposition, and lymphoid depletion in cortical areas (Fig. 7). More or less similar lesions were also observed by Akbor *et al.* (2007) in trachea and lungs of buffaloes.

Bacteriological isolation from tracheal swab and lungs showed formation of metallic sheen like cultural colonies on MLA. Pure culture was identified as *Escherichia coli* using VITEK-2 system with excellent identification. *E. coli* is found to cause fibrinous tracheitis in cattle calf (Griffin *et al.*, 2010). However there are limited reports. Therefore present investigation on membranous tracheitis due to *E. coli* is significantly contributing in pathomorphological studies due to bacterial infection. In presence of immunosuppression as evidenced by severe lymphoid depletion, toxins of *E. coli* produce their toxic effects and turned to pathogenic. Further study is needed on toxins and their role in pathogenesis of respiratory lesions.

On the basis of the present findings, it is reasonable to conclude that *E. coli* have potential to produce significant pathomorphological changes in presence of immunosuppression that can lead to death. Further studies are needed on serotypes of *E. coli* and their molecular characterisation with special reference to bovine respiratory diseases.

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