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# Pathomorphological studies of lung lesions in buffalo (*Bubalus bubalis*) in southern region of Rajasthan

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#### Abstract

The present study was carried out from January 2021 to November 2021 on the lungs of buffalo. During this period a total number of 98 samples of buffalo's lung, irrespective of their sex, age groups, and breeds were examined. Out of these, 76 representative samples of lungs were showed gross lesions, which were further subjected to histopathological examinations. An overall incidence of various pathological conditions was observed as 77.55%. The various forms of lungs affections were identified *viz.* emphysema 10.52%, atelectasis 2.63%, congestion and haemorrhages 6.57%, edema 2.63%, pneumonia 27.63% and hydatidosis 50%.

Keywords: Buffalo, histopathological examination, incidence, bronchopneumonia and hydatid cyst

# Introduction

The domestic water buffalo contributes a significant share of global milk production and is the major milk producing animal in several countries. Buffaloes have emerged as the prospective livestock species in India due to the triple purpose utility of milk, meat and draught. It is benefitting the farmers as well as the country's economy and food security (G.C. Banerjee, 1998) <sup>[5]</sup>. Lung affections are one of the most fatal and frequent health issues affecting the buffaloes. The thick connective tissue septa help in localization of infection and modified to thickened and oedematous, this may cause obstruction of airways while exhaling which leads to imbalance between inspiratory and expiratory volume (Argade *et al.*, 2019) <sup>[4]</sup>. The disease of lungs which may be either acute or chronic can cause debility and death resulting in great economic losses.

# **Material and Methods**

# Sample collection

A total of 98 buffalo's lung were examined out of which 76 animals showed various gross lesions of pathological significance in the lung used as material for the present study. The samples were collected from carcasses of buffaloes subjected to post-mortem examination to various veterinary clinics and various slaughter houses located in Udaipur, Dungarpur, Chittorgarh and Rajsamand districts of southern Rajasthan during the period from January 2021 to November 2021.

# Histopathology examination

Formalin-fixed tissues were processed by routine acetone-xylene technique, impregnated and embedded in paraffin wax. Sections were cut at 4-5  $\mu$ m thickness with the help of semi-automatic rotary microtome (Lillie, 1965)<sup>[12]</sup>. The sections were stained with haematoxylin and eosin (H&E) stain following conventional procedure (Luna, 1968)<sup>[14]</sup>.

# **Results and Discussion**

Total 76 tissue samples out of 98 samples were revealed definite lesions on gross and histopathological examination of lung in southern region of Rajasthan. In the present investigation, 77.55% incidences of pathomorphological lesions in lungs of buffaloes were noted. Tahssin and Alsamad (2020) <sup>[19]</sup>, Renu *et al.* (2021) <sup>[17]</sup> and Akalu *et al.* (2021) <sup>[1]</sup> reported an incidence of 23.21%, 47.48% and 91.76%. The lesions were grouped into abnormalities of inflation 10 (13.15%), circulatory disturbances 7 (9.21%), types of pneumonia 21 (27.63%) and parasitic condition 38 (50%).

This variability in the incidence might be attributed to different geographical areas and different methods of management system. The higher incidence of pathomorphological lesions in lung confirms that lung is the organ which is comparatively more susceptible to exposure to physical, chemical, and biological injuries because of its anatomical and histological characteristics.

 
 Table 1: Details of various pathological conditions in lungs of buffaloes

S. No.	Types of conditions	Number	Percentage
Ι	Abnormalities of inflation	10	13.15%
	i. Pulmonary emphysema	8	10.52%
	ii. Atelectasis	2	2.63%
Π	Circulatory disturbances	7	9.21%
	i. Congestion and haemorrhages	5	6.57%
	ii. Edema	2	2.63%
III	Types of pneumonias	21	27.63%
	i. Bronchopneumonia	15	19.73%
	a. Suppurative bronchopneumonia	12	15.78%
	b. Fibrinous bronchopneumonia	3	3.94%
	ii. Interstitial pneumonia	4	5.26%
	iii. Haemorrhagic pneumonia	2	2.63%
IV	Parasitic conditions	38	50%
	i. Hydatidosis		

# Abnormality of inflation

Abnormalities of inflation were noticed in 10 cases with the incidence of 13.15%. emphysema and atelectasis were included in abnormalities of inflation with the incidence of 10.52% and 2.63% respectively. Interstitial and alveolar emphysema was observed. [Fig. 1] Grossly, the emphysematous lungs were voluminous and greyish pale in color. A crepitating sound was heard on pressing and on cutting the affected portion. Emphysematous areas did not sink in the water. Microscopically, the alveoli were distended irregularly and due to rupture of inter alveolar walls, formation of air bubbles of variable sizes were seen in the lungs. The intact alveolar septa were thin and stretched with no blood. While the ruptured septa appeared as stumps projecting into the lumen. Pulmonary emphysema was reported earlier by Machhaliya et al. (2015)<sup>[15]</sup> and Renu et al. (2021) [17] with an incidence of 10.63% and 8.15% respectively. Decreased collateral ventilation makes them susceptible to interstitial emphysema. Agonal expiration in slaughtered animal makes their lungs prone to agonal emphysema. In pulmonary atelactasis, atelectatic lungs were dark and depressed. Microscopic examination, they showed collapse of the alveolar lumen with thickened interalveolar septa. Zeryhun and Alemu (2017)<sup>[24]</sup> and Tahssin and Alsamad (2020)<sup>[19]</sup> noted the higher prevalence of atelectasis with 6.4% and 5.83% respectively which might be the result of poor prevention of disease in that area and exposure of animals to stress factors like dust and overcrowding.

# **Circulatory disturbances**

The incidence of circulatory disturbances was observed as 9.21% in the present study held at southern region of Rajasthan. Pulmonary congestion and haemorrhages were seen in 5 cases with the incidence of 6.57%. Grossly, diffuse areas of congestion and haemorrhages were noticed in all lobes of lungs. [Fig. 2] Microscopically, alveolar capillaries and blood vessels were filled with blood in case of congestion and haemorrhages were noticed in the lumen of alveoli and bronchioles. [Fig. 3] The same consistency with the present

findings were also noted by Belkhiri et al. (2009) [6] and Benhathat and Aggad (2017)<sup>[7]</sup> with the incidence of 7.89% and 5.35% respectively. The higher incidence of congestion and haemorrhages was observed by Gebrehiwot et al. (2015) <sup>[9]</sup> and Rana *et al.* (2020) <sup>[16]</sup>. Congestion is the result of decreased outflow of venous bloodwhich means it is a passive process. Haemorrhages might be attributed to trauma, pulmonary thromboembolism, septicaemia and disseminated intravascular coagulation. Pulmonary edema was observed in 2 cases (2.63%). Grossly, the lungs were enlarged and firm. When the surface was cut, edematous fluid was oozed out. Prominent alveolar septa were seen. Microscopically, a pink stained homogenous material was seen in alveoli and bronchi. This result shows coherence with the findings of Akbor et al. (2007)<sup>[2]</sup>, Benhathat and Aggad (2017)<sup>[7]</sup> and Syaghuswa and Vyambwera (2020) <sup>[18]</sup> with the incidence of 3.75%, 0.98%, 0.91% respectively. Higher incidence of circulatory disturbance was noticed by Rana et al. (2020) [16].

# Pneumonia

Pneumonia of different types was noticed during the study. A total of 21 cases showed pneumonia with the incidence of 27.63%. Yalew *et al.* (2018) <sup>[23]</sup> and Rana *et al.* (2020) <sup>[16]</sup> had the strong affirmation with this finding with the incidence of 33.33%. Emran *et al.* (2013) <sup>[8]</sup> observed the 18.5% incidence of pneumonia in lungs of buffalo. This wide variation in these findings might be due to different location, management practices, age of the animals and different stress factors.

Bronchopneumonia was noticed in 15 cases with the incidence of 19.73%, further noticed in two forms, suppurative bronchopneumonia with the incidence of 15.78% and fibrinous bronchopneumonia with the incidence of 13.94%. The incidence of interstitial pneumonia was 5.26% and the haemorrhagic pneumonia was 2.63%. In suppurative bronchopneumonia, grossly, the lungs were enlarged, firm and consolidated cranioventrally. The affected lobe had mosaic appearance due to patchy to diffuse areas of consolidation with intermingling normal. The pleura over the affected portion were grey or gravish vellow. Cut section revealed pale and dark areas with purulent and mucopurulent exudates in the bronchi and bronchioles. Microscopic examination revealed the localised lesions in lungs. The alveolar capillaries were congested, dilated and haemorrhages were also noticed. The lumen of alveoli, bronchi and bronchioles was filled with edematous fluid, inflammatory cellular exudates consisting of abundant neutrophils, few macrophages and cellular debris. [Fig. 11]. In chronic suppurative bronchopneumonia lobular pattern of consolidation was noticed in apical and diaphragmatic lobes. There were small purulent foci which were multiple in number. The airways were filled with purulent exudates. Microscopically, there were infiltration of polymorphs and mononuclear cells in the lumen of bronchi, bronchioles and alveoli. There was fibrous thickening of alveolar septa with cellular exudate in and around the wall of alveoli. Neutrophils were the predominant cells in the areas of suppuration. Pulmonary fibrosis, abscess formation, bronchiectasis and hyperplasia of lymphoid tissue were noticed in few cases. [Fig. 12]. Emran et al. (2013)<sup>[8]</sup> found the same cranioventral consolidation and Benhathat and Aggad (2017)<sup>[7]</sup> and Yadav et al. (2015)<sup>[22]</sup> also noticed similar gross changes in the pneumonic lung. In case of fibrinous bronchopneumonia, gross examination revealed, lungs were heavy, enlarged, grey to dark red in color and firm in consistency. Lung had tendency to sink on immersing in water. Interlobular septa were prominent. When the section was cut, there were oozing of serous exudate with fibrin from the bronchi. [Fig. 4]. The pleura was thickened and whitish grey in color. Fibrinous adhesions with the thoracic wall were also seen in some cases. Microscopically, there were the predominance of fibrinous exudates, intermingling with inflammatory cells in the alveolar septa. Alveolar capillaries were congested and alveoli were dilated. Alveoli were filled with fibrinous exudates, erythrocytes, few neutrophils and a large number of mononuclear cells. The interlobular septa were dilated, edematous and filled with fibrin. [Fig. 10]. Yalew *et al.* (2018) <sup>[23]</sup>, Yadav *et al.* (2015) <sup>[21]</sup> and Tahssin and Methaq (2020) <sup>[19]</sup> noticed the similar changes as described in the present study.

On the gross examination of lung in case of Interstitial pneumonia, lungs were pale, heavy and failed to collapse. Cut revealed а slightly meaty appearance. section Microscopically, thickening of inter-alveolar septa due to septal cell proliferation and infiltration of mononuclear cell was observed. The alveoli were distorted in shape. Hyperplasia of bronchial and bronchiolar epithelium into the lumen was observed. [Fig. 9]. In chronic interstitial pneumonia, deposition of fibrous tissue was noticed in interlobular septa and in or around the bronchioles. These findings showed coherence with the findings of Tahssin and Methaq (2020)<sup>[19]</sup> and Zhang *et al.* (2020)<sup>[25]</sup>. Haemorrhagic pneumonia, grossly, intensely red to brownish black patchy areas involving both apical and diaphragmatic lobes were observed. On incision of section affected, oozing of blood from the affected zone. Microscopically, the alveoli were partially or completely filled with erythrocytes and some amount of serous fluid admixed with some leukocytes. Some of the alveoli and bronchioles revealed a homogenous eosinophilic mass. [Fig. 8]. Renu et al. (2021) [17] also noted the engorgement of alveoli with erythrocytes and edematous fluid. These lesions were similar as observed by Valles et al. (2015)<sup>[20]</sup> and Renu et al. (2021)<sup>[17]</sup>.

# Hydatiodosis

Hydatidosis was observed in 38 cases out of total 76 pulmonary findings with the incidence of 50%. On gross examination, lung showed, multiple cysts of different sizes, located either superficially or deeply in the lung parenchyma. The cysts were filled with clear watery or serous fluid and were soft or fluctuating. On cut section, cysts were unilocular, most cases, watery fluid was escaped when the cysts were punctured. [Fig. 5 & 6] Few cysts were fertile with multiple cream coloured brood capsules were recorded. On the microscopic examination, hydatid cyst revealed, from within outward a germinal layer, a thick laminated elastic layer of hyaline membrane which was concentric. [Fig. 7] The hyaline layer was surrounded by connective tissue with infiltration of lymphocytes, macrophages and in some cases neutrophils and eosinophils were also noticed. In some cases, the hydatid cyst was surrounded by a zone of necrosis which was eosinophilic in nature. Variability in intensity of cellular infiltration was noticed. The neighbouring parenchyma was showing the congestion, atelectasis, compensatory emphysema, interstitial and purulent bronchopneumonia. Machhaliya et al. (2015)<sup>[15]</sup> noted the hydatidosis as the principal factor for causing damage to the lung parenchyma in his study. along with Zeryehun and Alemu (2017) [24] and Benhathat and Aggad (2017)<sup>[7]</sup>. Kamdi et al. (2018)<sup>[10]</sup> noted same microscopic

lesions with the presence of interstitial and bronchopneumonia. These gross & microscopic lesions showing the strong affirmation with the findings of Lat-lat *et al.* (2006) <sup>[11]</sup> and Belkhiri *et al.* (2009) <sup>[6]</sup>.



Fig 1: Interstitial emphysema- bubbles of in the interstitial septa



Fig 2: Haemorrhage-subpleural trapped air foci with variability in sizes.



Fig 3: Haemorrhage-microphotograph showing erythrocytes in lumen of alveoli and bronchioles with alveolar septal fibrosis. H&E, 400X.



Fig 4: fibrinous bronchopneumonia - oozing of fibrinous exudate.



Fig 5: Gross picture showing the hydatid cyst in the lung parenchyma.



Fig 6: Cut section showing the Yellow and white translucent membrane attached to cyst wall



Fig 7: Microphotograph having concentric layers of hyaline in cyst wall. H&E, 40X.



Fig 8: Haemorrhagic pneumonia- microphotograph showing infiltration of erythrocytes, fibrin and inflammatory cells in alveoli and interstitium. H&E, 100X



Fig 9: Interstitial pneumonia- microphotograph of lung showing the thickening of alveolar partitions, presence of edema, and free alveolar cavities. H&E, 100X.



**Fig 10:** Fibrinous bronchopneumonia- microphotograph of lung showing the fibrinous exudate. H&E, 400X.



**Fig 11:** Acute suppurative bronchopneumonia-microphotograph showing the infiltration of cellular exudate and filling of bronchiole with edema, desquamation of bronchiolar epithelium and surrounded by Shaves tissue close with longe size mass. U.S.E. 400X





Fig 12: Chronic suppurative bronchopneumonia- microphotograph showing the inflammatory exudate in bronchial lumen, sero-purulent exudate in alveoli, infiltration of leucocytes and necrosis at some places. H&E, 100X.

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