Pathological and bacteriological study of lobe distribution of lesions in buffalo (Bubalus bubalis)

Sayyari, M.[1]*; Sharma, R.[2]

[1]Department of Pathology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran. [2]Department of Pathology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

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Correspondence
Sayyari, M.,
Department of Pathology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran.
Tel: +98(711)2286950
Fax +098(711)2286940
Email: Sayyari@Shirazu.ac.ir

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Abstract

The correlation between the bacteriological agent of buffalo pneumonia and its pathologic characters were investigated. In the present study, 333 samples of buffalo lungs in Iran were studied for pneumonial lesions and evidence of bacterial and viral infection. The type of pneumonia was classified as interstitial, fibrinous or purulent bronchopneumonia and the anatomical location of lesions was also recorded. In 201 samples with interstitial pneumonia, the lungs were found to be noticeably elastic, edematous and pale. Microscopically, thickening of the alveoli walls, hyperplasia and increased numbers of monocytes was seen. Lesions were mostly found in the right and left diaphragmatic lobes. The 55 samples with fibrinous bronchopneumonia were macroscopically bright and marbled and firm in texture. Thickening of the alveoli walls and large numbers of neutrophils were evident at the microscopic level, and the majority of lesions were located in the diaphragmatic lobes. Microscopically, a large number of neutrophils but few macrophages were seen. Of the 24 samples with purulent bronchopneumonia, most lesions were found in the left diaphragmatic lobe. The main bacteria that were isolated were: *Pasturella spp, Staphylococcus aureus, Pseudomonas cuosis, Acinetobacter spp, Arcanobacterium pyogenes, Escherichia coli and Proteus spp*. Given the importance of buffalo in milk and meat production for Khuzestan province, this study could be considered as a basis for future attempts to reduce buffalo mortality due to respiratory diseases.

Introduction

In Khuzestan province (south west of Iran), buffalo mortalities due to pneumonia, particularly pasteurellosis is a common event and regardless of annual vaccination, the acute form of the disease emerges every year. The objectives of this study were to describe the nature and distribution of macroscopic and microscopic lung lesions in buffalo. The adult water buffalo appears to have a high degree of resistance to diseases. But they are more susceptible to pneumonia than cattle. Husbandry weather and immunity affect morbidity. In endemic areas, from 10 to 50 percent of the buffalo population acquired soid immunity through exposure or subclinical infection. Close herding and wetness predispose to an increased morbidity (Zaman *et al.,* 2007). In domestic animals, pneumonia can be classified into four morphologically distinct types i.e., Bronchopneumonia, Interstitial pneumonia, embolic pneumonia and granulomatous pneumonia according to the lesions' texture, distribution, appearance and exudation. Also, based on the inflammatory lesions' distribution in the lungs, the pneumonia can be categorized to the cranioventral (most cases of bronchopneumonia), multifocal (embolic pneumonia), diffuse (interstitial pneumonia) and local extensive (granulomatous pneumonia) pneumonia.The most common agents of suppurative bronchopneumonia, particularly in buffalo, are *Pasteurella multocida, Bordetella spp ,_Actinomyces pyogenes, Streptococcus spp., Escherichia coli* (*E. coli)* and various *Mycoplasma* species (Mandal *et al.,* 1995; McGavin *et al.,* 2007). Bronchopneumonia caused by *Pasteurella multocida* or *Mannheimia haemolytica* has a cranioventral lung distribution and affects ruminants of all ages worldwide. Both organisms are found in the nostrils and the upper respiratory tract and can become pathogenic under certain stress conditions, such as transportation and weaning (Ribble *et al.,* 1995; Davies *et al.,* 2004). Most of these organisms are secondary pathogens that require impairment of the pulmonary defense system before they can colonize the lung and establish infection.In fibrinous bronchopneumonia, the inflammatory process occurs in several adjacent lobules and therefore the exudate can spread rapidly

throughout the entire lobe. This type of bronchopneumonia is known as lobar pneumonia and is defined as the partial or entire involvement of a lobe in which the lesions are distributed. It can occur after shipping fever has been exacerbated by transportation, congestion, stress or infections, particularly by *Pasturella spp.* (Davies *et al.,* 2006; Prado *et al.,* 2007), and can lead to collapse of the affected part of the lung if the main airway is obstructed (Jubb *et al*., 2007). In buffalo, fibrinous pneumonia is mostly manifest as the lobar type (Mandal *et al.,* 1995; Ayse, 2000).

The pathogenesis of interstitial pneumonia is complex and involves interaction between external factors, including toxins, biological and environmental factors, with alveolar epithelium and type I and II pneumocytes. It can also arise from hematogenous injury to the alveolar capillary endothelium or alveolar basement membrane. In interstitial pneumonia, lesions cause the lung tissue to become enlarged, elastic, pale, edematous and emphysematous (Autio *et al.,* 2007). It is characterized by formation of a hyaline membrane, epithelialization and moderate forms of adenomatosis (Thorn *et al*., 2000; Welsh *et al.,* 2004).In Iran no systemic works have been performed on buffalo respiratory system .This study could be considered as a basis for future attempts to reduce the buffalo mortalities due to respiratory diseases.

Materials and Methods

Of the 1,622 lungs inspected at the Ahwaz slaughterhouse, 333 lungs were diagnosed with pneumonia. Selection criteria included observable lesions such as hyperemia, bleeding, edema, discoloration, solidness or hardness. After anatomical locations of lesions in various lobes were recorded, samples were transported to the bacteriology laboratory in ice bags. Both aerobic and anaerobic bacteria were identified according to procedures described by Quinn *et al*. (1994) were cultured using standard biochemical tests. Macintosh (Germmany-hannover, lower Saxony) anaerobic glass jars with vents were used to culture obligate anaerobic bacteria in a H_2CO_2 atmosphere. Plates of Brewer's Agar inoculated with the lung swab were placed inside the anaerobic jar, which was then sealed and incubated at 37°C for 7 days. To avoid death of the obligate anaerobic bacteria on oxygen exposure, culture was carried out on reducing media. For preliminary confirmation, all anaerobes isolated on anaerobic Brewer's Agar were cultured aerobically, and organisms which failed to grow aerobically were considered true anaerobes. The cultures were then cultured using specific media containing either mannitol, indole or sorbitol. The samples were then placed into formalin (10%) to be fixed and prepared for pathological study. Finally, 3-5 mm pieces were taken for preparing sections 5-7 µm in diameter using the paraffin method, followed by hematoxylin and eosin staining. Each sample was examined twice to determine the type of pneumonia and identify the different isolated bacteria.

Results

Gross and microscopic lesions of the lung were categorized into three groups: interstitial pneumonia, fibrinous bronchopneumonia and suppurative bronchopneumonia (Table 1). A- Distribution of interstitial pneumonia: Out of all 201 (61%) samples, 12 (5.97%) were located in the left apical lobe, 15 (7.46%) in the right apical lobe, 14 (6.96%) in the left and accessory apical lobes (a), 69 (34.32%) in the right diaphragmatic lobe, 75 (37.31%) in the left diaphragmatic lobe, 8 (3.98%) in the accessory lobe and 8 (3.98%) in the medial lobe. The lungs affected by interstitial pneumonia are distinguishable macroscopically beholden to their pale color and rubber consistency and enlargement, thereby in some cases the rib tracks are found on the lung surface. Edema in the alveolar septa and inside the alveoli, hyperplasia of cells type II (epithelization), infiltration of lymphocytes and other mononuclear were observed microscopically, as well. From all of the samples affected by interstitial pneumonia, 201 samples were cultured, of which 33 (16.41%) and 168 (83.5%) samples were found to be positive and negative respectively. *Pasturella spp* from 13 samples (39. 4%), *Pasturella spp* together with other bacteria like *Arcanobacterium pyogenes*, *E-coli* and *Acinetobacter*

Table 1: Distribution of lesions in different lobes of lung.

No.	Lobe	Interstitial pneumonia		Fibrinous pneumonia		Supprative pneumonia	
		Total cases	Percentage	Total cases	Percentage	Total cases	Percentage
1	Left apical	12	5.9	-	-	-	-
2	Right apical	15	7.5	7	12.2	2	8.2
3	Left and accessory apical	14	6.9	6	10.9	-	-
4	Right diaphragmatic	68	34	19	34.5	9	3.7
5	Left diaphragmatic	75	37	18	32.7	11	45
6	Accessory	8	3.9	3	5.5	1	4
7	Middle	8	39	2	36	1	4
8	Total	201	100%	55	100%	24	100%

spp from 6 samples (18.18%), other bacteria such as *pseudomonas cuosis* from 2 samples (6.06%) in the left apical lobe, *staphylococcus epidermidis* from 1 sample (3.03%) in the right diaphragmatic lobe and from 2 samples (6.06%) in the left diaphragmatic lobe, *Staphylococcus non-haemolytic* and *Arcanobacterium pyogenes* from 9 samples (27.27%) were isolated.

Distribution of fibrinous broncho pneumonia: Out of the entire 55 (61.51%) samples affected by this type of pneumonia, 19 (34.54%) were located in the right diaphragmatic lobe, 6 (10.9%) in the auxiliary apical lobe (a), 7 (12.72%) in the right apical lobe, 18 (32.72%) in the right diaphragmatic lobe, 2 (3.63%) in the medial lobe and 3 (5.45%) in the auxiliary lobe. Macroscopically, the lungs affected by fibrinous pneumonia were found congested, heavy, and edematous with marbled lobules. At the microscopic level the dilatation of the alveoli walls due to edema and the abundance of pink liquid fibrin and the accumulation of neutrophils in alveoli spaces were seen. Out of the 55 (16.51%) samples , cultures were prepared from 24 samples, of which 16 samples were found positive as follows: in the right and left diaphragmatic lobes, 6 (37.5%) *pasteurella spp,* in the left diaphragmatic, 4 (25%) *pasteurella spp* and *Arcanobacterim pyogenes* and *E.coli*, in the right diaphragmatic lobe alone, 1 (6.25%) *Pasturella spp* and *E.coli*, in the right and left apical lobe 3 (18.75%), *pasturella spp* and *staphylococcus spp* and in the medial lobe 2(12.5%) *pasturrella Spp*.

Suppurative bronchopneumonia: Out of a total of 24 (7.20%) lesions, 11(45.82%) in the left diaphragmatic lobe, 9 (37.5%) in the right diaphragmatic lobe, 2 (8.23%) in the right apical lobe, 1 (4.16%) in the medial lobe and 1 (4.16%) in the auxiliary lobe were seen.

Macroscopically the suppurative lungs were found red to grey, solid and pale showing dark and suppurative exudate in the bronchi and bronchioles and debris and secretions in the bronchus and bronchioles in which inflammatory cells of neutrophils and macrophages are predominant. Out of 22 (7.2%) suppurative pneumonia, cultures were prepared from16 samples and 13 samples (81.25%) were found positive as follows: 3 (23.07%) *Ecoli*, 4 (30.76%) *Arcanobacterim pyogenes* and 3 (23.07%) *Staphylococcus aureus* 3 (23.07%) *proteus spp.*

Discussion

The results of histopathological and bacteriological examinations in the present survey showed that 74% lung of buffaloes had interstitial pneumonia. It is believed that viruses and bacteria play primary and secondary roles in interstitial pneumonia, respectively In a previous study, parainfluenza type 3 virus was found in 73% of buffalo lungs affected by interstitial pneumonia. The affected lungs were pale and elastic, and most of the lesions were confined to the diaphragmatic lobes. Microscopically, lungs were found to have thicker alveolar walls, a hyaline membrane in the alveoli, an increase in alveolar epithelial cells, no polymorphs in the alveoli and a predominance of mononuclear cells (Ribble *et al.,* 1995; Tegtmeier *et al.,* 1999). In a retrospective study on respiratory diseases conducted in 121 farms hosting 21.5 million cattle, interstitial pneumonia was found to be the most prevalent form of pneumonia, and hyaline membrane formation was the most significant microscopic lesion (Loneragan *et al.,* 2002). Other lesions included thickening of the alveolar septa, which was seen in 46% of cattle, and accumulation of inflammatory mononuclear cells, which was seen in all lung of cattle that had suffered from interstitial pneumonia. Results from our study are similar to findings of other researchers. (Mandal *et al.,* 1995; Ayse, 2000). In fibrinous bronchopneumonia, the major findings were the accumulation of exudate and the thickening of alveolar walls due to the increase of fibrin and neutrophils. The accumulated fibrin that formed thick yellow plaques over the pleural surface is characteristic of this disease. Buffalo are very sensitive to pasturellosis, and mortalities have been reported worldwide (Welsh *et al.,* 2004). In Khuzestan province, the disease occurs even in vaccinated cattle in the form of a peracute respiratory disease showing fibrinous exudates .A fibrinous bronchopnemonia mortality rate of between 10 and 75% has been reported among calves that had been transported, and the main factor that exacerbated the disease was collection of the animals from different areas (Ribble *et al.,* 1995). Out of 8222 macroscopically examined lungs that had been taken from the abattoir , pneumonia was detected in 500 (6.1%) samples.Also the Bacteria including *Pasteurella multocida* (13.6%), S*taphylococcus aureus* (7.2%), *Corynebacterium spp. (*6%), *Streptococcus spp,* (3.8%)*, E. coli* (2.8%), *Bacillus spp.* (2.2%), *Pseudomonas spp.* (2%) and *Pasteurella* and mixed bacteria (25%) were isolated and identified from 311(82.2%) lungs (Ayse, 2000). In a study conducted on 40 cattle, *Pasteurella multicida* and Respiratory syncytial virus(RSV) were isolated from the respiratory tract of 34% and 21% of animals respectively.it agreed that fibrinous pneumonia was caused by *Pasteurella* spp. (Thorn *et al.,* 2000). The simultaneous Isolation of the virus, and *Pasteurella spp*. and other secondary agents such as *Corynebacterium* and *Staphylococcus* were reported too (Dyer *et al.,* 2004; Stepe *et al.,* 2005). In acute cases, cultures obtained from lung lesions or tracheal swabs or washes will be diagnostic. In chronic cases, bacterial cultures may be less rewarding; although *Pasteurella spp.* or *Mannheimia spp.* may have caused the initial infection, cultures taken later may reveal

Arcanobacterium pyogenes, a common cause of lung abscesses (Storz *et al.,* 2000). In the present study, purulent pneumonia was observed in the diaphragmatic, medial and apical lobes. Bacterial agents such as *Arcanobacterium pyogenes*, *E. coli*, *Pasturella spp* and *Staphylococcus aureus* were isolated. The high percentage of lesions due to interstitial and fibrinous bronchopneumonia observed in the buffalo warrant further study to elucidate the causative factors. Factors such as insufficient feed intake, hot and humid weather conditions and gas emissions from oil rigs might be influential.

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