

Pneumonia in slaughtered sheep in south-western Iran: pathological characteristics and aerobic bacterial aetiology

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Keywords

Actinomyces pyogenes,
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Histopathology,
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Pneumonia,
Sheep,
Staphylococcus aureus.

Summary

In this study, the lungs of 1,000 sheep carcasses were subjected to gross examination and those suspected to be infected with pneumonia were studied at histopathological level as well as examined for presence of bacteria. Pneumonia was detected in 42 (4.2%) carcasses. Based on histopathological lesions, 45.24% were affected with suppurative bronchopneumonia, 20.93% with interstitial pneumonia, 11.9% bronchointerstitial pneumonia, 7.14% with fibrinous bronchopneumonia and 2.38% with embolic pneumonia. In addition, 11.9% of the lungs showed lung abscesses and 2.33% were affected with pleuritis without involving pulmonary parenchyma. Bacteriological examination revealed presence of ovine pathogens, such as *Pasteurella multocida* (24.53%), *Staphylococcus aureus* (20.75%), *Klebsiella pneumoniae* (15.09%), *Corynebacterium pseudotuberculosis* (7.55%) and *Actinomyces pyogenes* (1.89%). The most common form of pneumonia was suppurative bronchopneumonia with moderate amounts of fibrin deposits on the pleural surface and inside the bronchioles and alveoli.

Casi di polmonite in pecore macellate nel sud ovest dell'Iran: caratteristiche patologiche ed eziologia batterica aerobica

Parole chiave

Actinomyces pyogenes,
Corynebacterium pseudotuberculosis,
Iran,
Istopatologia,
Klebsiella pneumoniae,
Pasteurella multocida,
Polmonite,
Pecore,
Staphylococcus aureus.

Riassunto

Lo studio riporta i risultati dell'esame macroscopico di 1.000 carcasse di pecore e di esami istopatologici e di isolamento di batteri contaminanti effettuati in casi di sospetta polmonite. Casi di polmonite sono stati riscontrati in 42 carcasse (4,2%). L'esame istopatologico delle lesioni ha evidenziato broncopolmonite suppurativa (45,24%), polmonite interstiziale (20,93%), polmonite broncointerstiziale (11,9%), broncopolmonite fibrinosa (7,14%) e polmonite embolica (2,38%). Inoltre, sono stati riscontrati ascessi nell'11,9% e pleurite senza interessamento del parenchima polmonare nel 2,33% dei polmoni esaminati. L'esame batteriologico ha evidenziato la presenza di alcuni patogeni ovini: *Pasteurella multocida* (24,53%), *Staphylococcus aureus* (20,75%), *Klebsiella pneumoniae* (15,09%), *Corynebacterium pseudotuberculosis* (7,55%) e *Actinomyces pyogenes* (1,89%). I casi di broncopolmonite suppurativa con moderate quantità di deposito di fibrina sulla superficie della pleura e all'interno di bronchioli ed alveoli rappresentano il reperto più comune.

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Introduction

Respiratory diseases are common in all species of domestic animals. Pathogenesis is multifactorial, and the diseases appear due to the interaction of infectious micro-organisms (bacteria, mycoplasma, viruses and fungi), host defence, environmental factors (22) and stress (33, 38). Respiratory diseases in all major sheep-producing countries result in lamb mortality, reduced growth rate, carcass condemnation and consequent substantial economic impact on animal husbandry because of the need to activate chemotherapeutic and vaccination programmes (15, 21).

Age, geographic location, nutrition and climate are determining factors on the type of microorganism causing pneumonia. In addition, rearing systems, stress factors, climatic changes, unhygienic conditions, sudden changes in feed and a low level of herd health status are stated as predisposing factors to bacteria and viruses. A wide variety of bacteria are found in the upper respiratory tract. Airways and lung parenchyma remove the infectious agents that are deposited, so that the deeper structures are less often attacked. Most of the infectious agents are normal inhabitants of the nasopharynx. These bacteria, after growing in the nose and throat, extend downwards and produce multiple bacterial infections. Pneumonia is a common disease of sheep. Lamb losses are generally connected with bacterial infections leading to pneumonia, diarrhoea, and subsequent sepsis, which is a potential complication of pneumonia (5).

Sheep represent a great resource in Iran. The highlands of the Chaharmahal-Bakhtiary Province (south-west Iran) are an important part of the national economy for sheep production. Because of the important economic impact on the sheep industry, bacterial pneumonia has been extensively studied experimentally and in the field (10). This study was designed to isolate the aerobic bacteria from different types of ovine pneumonic lungs, to describe the histopathological findings, and to evaluate the frequency of different types of pneumonia in sheep. Therefore, the aim of this work is the acquisition of basic knowledge of the pathogenic bacteria associated with ovine respiratory diseases in Iran.

Materials and methods

Area

Chaharmahal-Bakhtiary province is located in south-west Iran. This province is 16,533 km² in size, within the central part of the Zagros mountains. The latitude in the southern zone is between 31° 4'

and 42° 4' north. Longitude is 49°39' west to 51° 21' east. Due to the natural situation of this province, it includes various climates. The origin of rainfall in the province is mostly Mediterranean and Sudanese atmospheric flows, which enter this region from the west and south and affect the region for a period of eight months (October to May). The average rainfall of the province is about 560 mm per year.

Sample collection

In this study, lungs from 1,000 native sheep slaughtered at Shahrekord slaughterhouse underwent gross examination for the presence of pneumonic lesions. No details of sex, breed or husbandry conditions of the sheep were available, and all the animals were submitted for routine slaughter. The lungs with macroscopic pneumonic lesions were obtained (organs showing parasitic lesions were excluded). Following gross inspection, the samples of apparently affected lungs were taken for pathologic and microbiologic investigations. In addition, 10 lungs appearing healthy at gross inspection were examined for isolation of bacteria as a control group.

Pathological investigation

Tissue samples 1 cm³ in thickness were fixed in 10% neutral-buffered formalin for histopathological examination. The samples were then dehydrated in graded ethanol and embedded in paraffin. Sections 5 µm in thickness were stained with haematoxylin and eosin and examined by an ordinary light microscope.

Bacterial isolation

Samples of the affected areas were aseptically collected and placed in sterile plates kept in an icebox and were submitted to the Bacteriology Department. The outer surface of the pneumonic lungs were first seared with a heated spatula before the cut inner surface of the lungs were cultured on blood agar by contact with addition of 5% sheep blood and McConkey agar. The plates were then aerobically incubated at 37° C for 24-48 h. Subcultures were made and pure cultures of each strain were obtained. Identification of the isolated bacteria was performed according to the standard procedures and included morphology of the colonies on blood agar plates, presence and type of haemolysis, Gram staining, cytochrome oxidase, catalase, indole production, urease production, sulfhydryl acid production (TSI), oxidation/fermentation, motility and growth ability under aerobic conditions (31). Biochemical characters of isolates were determined with commercial test kits.

Results

Pathological findings of pulmonary lesions

The lungs of 42 (4.2%) sheep were affected at gross level by various types of pneumonia and pulmonary abscesses. No bacterial isolation and lesions were observed in ten apparently normal lungs. Pneumonias were classified into 5 subgroups according to their macroscopic appearances (texture, exudation and distribution of lesions) and microscopic findings (26). The morphologic diagnosis and bacteriological results in the 42 cases of ovine pulmonary lesions examined as well as normal lungs at gross examination are given in Tables I and II.

Macroscopic and histopathological characteristics of the diagnosed pneumonia

Suppurative bronchopneumonia

The most commonly encountered pneumonia was suppurative bronchopneumonia (45.24%, $n = 19/42$). The gross appearance showed irregular consolidation with lobular pattern. The cranial, middle and accessory lobes were the main affected areas. Consolidation involved more right lung than left. The pulmonary parenchyma was firm in texture. Depending on the age and nature of the process, the consolidated lungs varied from dark red in acute to grey-pink and grey in chronic form (Figure 1). In the acute phase, the cut surface of the consolidated lobules was moist and purulent

exudate leaked from small airways. There were numerous small, greenish-yellow purulent foci scattered throughout the affected cranioventral lobes. In the chronic phase, abscesses of different sizes, with fibrous capsules surrounding them, were observed in the affected lobes.

Histopathologically, neutrophil-rich exudates were present in the alveolar spaces and lumens of the bronchioles and bronchi, and in some occasions an admixture of various amounts of cell debris, mucus, fibrin, neutrophils and macrophages were observed in these areas. The inflammatory process confined to the individual lobules and normal alveoli were seen adjacent to the alveoli filled with neutrophilic exudates. In severe cases, purulent exudates completely filled the entire lumen of alveoli and bronchioles. Due to complete or partial obstruction of the airways some of the lobules showed atelectasis and/or emphysema. The bronchiolar epithelium showed necrosis and mixed populations of neutrophils and lymphocytes infiltrated the lamina propria and formed typical peribronchiolitis. Multiple areas of necrosis and occasionally sequestration foci were consistently observed within the pneumonic portions.

In the chronic phase, thickening of alveolar walls, mainly by lymphocytes and macrophage infiltration, was evident. Cellular infiltration in the alveolar lumen, and occasionally walls, consisted of macrophages, lymphocytes and neutrophils. Goblet cells of the bronchiolar epithelium showed hyperplasia and changed the exudates to mucopurulent. Varying degrees of bronchiolar lymphatic tissue hyperplasia (BALT) was another common finding. In some cases, extensive peribronchiolar lymphoid accumulation narrowed the bronchiolar lumina. Occasionally,

Table I. The prevalence of pneumonia types, pulmonary lesions and associated bacteria in affected lungs compared with normal lungs.

Type of pneumonia	<i>Pasteurella multocida</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Corynebacterium pseudotuberculosis</i>	<i>Actinomyces pyogenes</i>	Total bacteria isolated
Purulent bronchopneumonia ($n = 19/42$) (45.24%)	6 (11.32%)	5 (9.43%)	4 (7.55%)	3 (5.66%)	–	18 (33.96%)
Fibrinous bronchopneumonia ($n = 3/42$) (7.14%)	2 (3.77%)	–	2 (3.77%)	–	–	4 (7.55%)
Interstitial pneumonia ($n = 9/42$) (21.43%)	1 (1.89%)	–	1 (1.89%)	–	–	2 (3.77%)
Embolic pneumonia ($n = 1/42$) (2.38%)	–	–	1 (1.89%)	–	–	1 (1.89%)
Bronchointerstitial pneumonia ($n = 5/42$) (11.9%)	1 (1.89%)	2 (3.77%)	1 (1.89%)	–	–	4 (7.55%)
Pulmonary abscesses ($n = 5/42$) (11.9%)	3 (5.66%)	1 (1.89%)	2 (3.77%)	1 (1.89%)	1 (1.89%)	8 (15.09%)
Total bacteria	13 (24.53%)	8 (15.09%)	11 (20.75%)	4 (7.55%)	1 (1.89%)	37 (69.81%)
Grossly normal lungs	–	–	–	–	–	–

Table II. Details of morphological diagnosis and bacteriological results in 42 cases of ovine pneumonia and pulmonary lesions as well as apparently normal lungs.

Number of cases (n = 42)	Morphological diagnosis	Bacteria isolated
19	Purulent bronchopneumonia	<i>Klebsiella pneumoniae</i> (n = 2)
		<i>K. pneumoniae</i> + <i>Staphylococcus aureus</i> (n = 1)
		<i>K. pneumoniae</i> + <i>Corynebacterium pseudotuberculosis</i> (n = 2)
		<i>Pasteurella multocida</i> (n = 5)
		<i>Staphylococcus aureus</i> (n = 3)
		<i>P. multocida</i> + <i>C. pseudotuberculosis</i> (n = 1)
		No bacteria isolated (n = 5)
3	Fibrinous bronchopneumoni	<i>P. multocida</i> + <i>S. aureus</i> (n = 2)
		No bacteria isolated (n = 1)
9	Interstitial pneumonia	<i>S. aureus</i> (n = 1)
		<i>P. multocida</i> (n = 1)
		No bacteria isolated (n = 7)
1	Embolic pneumonia	<i>S. aureus</i> (n=1)
5	Bronchointerstitial pneumonia	<i>K. pneumoniae</i> (n = 1)
		<i>K. pneumoniae</i> + <i>S. aureus</i> (n = 1)
		<i>P. multocida</i> (n = 1)
		No bacteria isolated (n = 2)
5	Pulmonary abscesses	<i>K. pneumoniae</i> (n = 1)
		<i>P. multocida</i> + <i>S. aureus</i> (n = 2)
		<i>P. multocida</i> + <i>C. pseudotuberculosis</i> (n = 1)
		<i>Actinomyces pyogenes</i> (n = 1)
10	Normal lungs	No bacteria isolated

severe chronic suppurative pleuropneumonia resulted in abscess formation. As shown in Tables I and II, the organism most commonly isolated from the suppurative bronchopneumonia was *Pasteurella multocida*. Other bacteria isolated included *Klebsiella pneumoniae*, *Corynebacterium pseudotuberculosis* and *Staphylococcus aureus*.

Fibrinous bronchopneumonia

Fibrinous bronchopneumonia was detected in 7.14% (n = 3/42) of the affected animals (Table I). Macroscopic lesions were similar to suppurative bronchopneumonia except that fibrin was dominant and the lesions were lobar in nature. The distribution of the lesions was almost anteroventral. The apical and cardiac lobes were the most affected parts, but in severe cases infection was more extensive, involving substantial portions of the diaphragmatic lobe. The diseased portions of lung became remarkably consolidated, dark red in colour and firm in consistency due to pulmonary congestion, oedema and fibrin accumulation. A thin layer of fibrin usually covered the pleural surface of the affected lobules. Microscopically,

the affected lungs were dominated by diffuse capillary congestion. Presence of multifocal areas of necrosis and variable amounts of fibrinous exudate in the lumen of the alveoli and bronchioles were the most conspicuous and predominant features. The interlobular septa and pleura were thickened by fibrin, neutrophils and oedema. Some necrotic areas were surrounded by a rim of elongated cells, often referred to as 'oat cells', which were severely degenerated neutrophils mixed with alveolar macrophages. Fibrinous pleurisy with or without adhesion and extensive and widespread vascular thrombosis was evident in these animals. The thrombi were usually limited to small blood vessels, capillaries and lymphatics of the pneumonic lungs (Figure 2). In the bacterial examination, *P. multocida* and *S. aureus* were isolated from these cases (Table II).

Interstitial pneumonia

Nine animals (n = 9/42) (21.43%) showed interstitial pneumonia. The gross lesions were distributed throughout the lungs, often with more severe involvement of dorsocaudal regions. The affected



Figure 1. Macroscopic appearance of bronchopneumonia. Dark red consolidation of cranioventral lobes (arrows). *Pasteurella multocida*, *Klebsiella pneumoniae*, *Corynebacterium pseudotuberculosis* and *Staphylococcus aureus* were isolated from this type of pneumonia.

lungs were enlarged and were diffuse red to pale in appearance. Rib impressions were seen on the costal surfaces of the diaphragmatic lobes. The affected lung failed to collapse if pressed (Figure 3). No evidence of exudates could be detected in cut surfaces or air passages. The texture of the lungs was rubbery. The histopathological features of the affected lungs showed a marked increase in mononuclear cells and occasionally mild fibrosis in the interalveolar septa and presence of varying numbers of macrophages within the alveolar lumina. In some cases, hyperplasia of pneumocyte type II was seen. Microscopically, there was no obvious exudate in the alveolar spaces and airways. Interstitial pneumonia was associated with *P. multocida* and *S. aureus* in two lung samples. No bacteria were isolated from the other seven affected cases (Tables I and II).

Bronchointerstitial pneumonia

Bronchointerstitial pneumonia was detected in five animals (11.9%, $n = 5/42$). The affected lungs were diffuse red, wet, and failed to collapse. In contrast to interstitial pneumonia, the anterior lobes of the affected lungs showed red consolidation. Histopathologic findings revealed mixed characteristic features of suppurative bronchopneumonia and interstitial pneumonia.

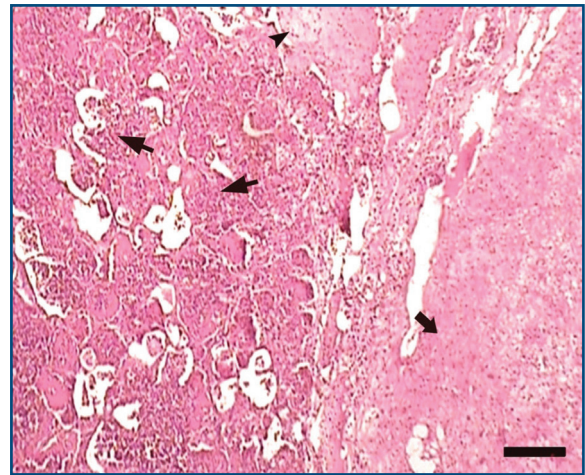


Figure 2. Histopathological characteristics of fibrinous bronchopneumonia. Deposits of fibrinopurulent exudate in alveoli (thin arrows) and interalveolar septa (thick arrow), presence of thrombi in capillary (arrow head). *Pasteurella multocida* and *Staphylococcus aureus* were isolated from this type of pneumonia, (H&E staining), (Scale bar: 250 μ m).



Figure 3. Macroscopic appearance of interstitial pneumonia. Affected lung is pale, enlarged, uncollapsed and rib impressions are observed on diaphragmatic surface. *Pasteurella multocida* and *Staphylococcus aureus* were isolated from this type of pneumonia.

While the alveolar walls were thickened mainly by lymphocytes and macrophages, neutrophils and fibrin were accumulated in the alveolar lumens and the air passages. In some cases, multinucleated giant cells were seen in the alveoli. Pneumocyte type II showed mild hyperplasia. Alveolar collapse was also seen. There were spheroidal to ovoid concentrically

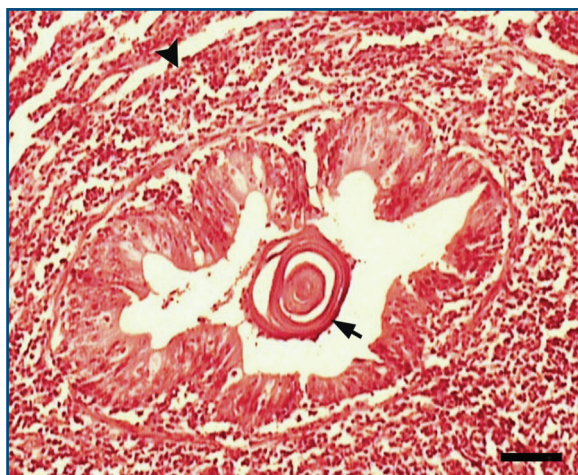


Figure 4. Lamellar corpora amylacea in the alveolar space of a lung affected by bronchointerstitial pneumonia (arrow) and thickening of alveolar space (arrow head), (H&E staining), (Scale bar: 100 μ m).

lamellar corpora amylacea in some of the alveoli (Figure 4). BALT hyperplasia and perivascular cuffing was another microscopic feature. *Pasteurella multocida*, *Klebsiella pneumoniae* and *S. aureus* were isolated from three cases of bronchointerstitial pneumonia. No bacteria were isolated from the other two animals (Table II).

Embolic pneumonia

Embolic pneumonia was detected in 2.38% ($n = 1/42$) of the affected sheep and gross characterization was by multifocal nodules of the same size distributed randomly throughout the pulmonary lobes. The gross lesions were white foci, small in size (4-6 mm), surrounded by a discrete, red, hemorrhagic halo (Figure 5). Microscopically, these nodules showed multifocal neutrophilic aggregations that were randomly scattered throughout the pulmonary lobes. The bacteria isolated from embolic pneumonia was *S. aureus*.

Pulmonary abscesses

In this study, the prevalence of pulmonary abscess ($n = 5/42$, 11.9%) was considered without concurrent bronchopneumonia. The abscesses ranged from 2 to 10 cm in diameter and occurred as single or sometimes multiple instances in one or more lobes. Some of them were very large and involved an entire pulmonary lobe. Lung abscesses containing viscous white-yellow odourless pus were found in the affected lung and mediastinal lymph nodes (Figure 6). Chronic abscesses were often surrounded by reactive fibrous walls. Bacteriological examinations from the centre of each abscess revealed growth of *S. aureus*, *P. multocida*, *K. pneumoniae*, *C. pseudotuberculosis* and *Actinomyces pyogenes* (Table II).

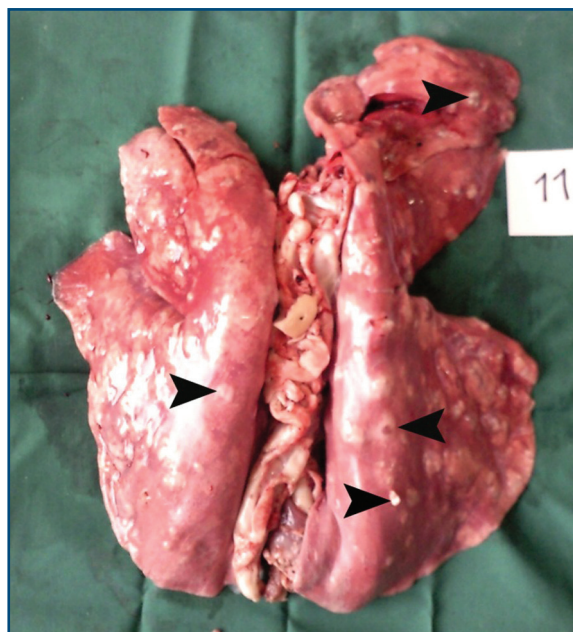


Figure 5. Macroscopic appearance of embolic pneumonia caused by *Staphylococcus aureus*. White, raised foci of 4-6 mm in size are observed throughout pulmonary lobes (arrow heads).



Figure 6. A large abscess containing viscous white-yellow odourless pus in the mediastinal lymph node. *Staphylococcus aureus*, *Pasteurella multocida*, *Klebsiella pneumoniae*, *Corynebacterium pseudotuberculosis* and *Actinomyces pyogenes* were isolated from pulmonary and mediastinal lymph node abscesses.

Microbiology findings

A total of 37 bacterial isolates were collected from the 43 ovine pulmonary lesions cultured. Of these isolates, 56.75% ($n = 21$) were Gram-positive and 43.24% ($n = 16$) were Gram-negative. The rate of pure and mixed culture was 39.53% and 23.26%, respectively. A summary of the bacteriological results is presented in Tables I and II.

Discussion

Respiratory diseases are common in various species of domestic animals particularly the herbivores. Because of its major economic impact on the sheep industry, through consequences such as death, retarded growth and reduced weight-gains in recovered animals, slaughterhouse wastage, drugs and labour costs, bacterial pneumonia has been extensively studied experimentally and in the field. Daniel *et al.* (9) evaluated the prevalence and onset of lung lesions and their impact on growth of lambs and showed that severe lung lesions could lead to greatly decreased growth performance of the animals.

The present study was designed to refine and correlate the histopathological pattern of ovine pulmonary lesions with their bacterial aetiologies. Of 1,000 lungs examined, 42 (4.2%) cases showed gross lesions of various types of pneumonia and pulmonary abscesses. Pneumonias were classified into 5 subgroups according to their macroscopic and microscopic appearances, including suppurative bronchopneumonia (45.24%), fibrinous bronchopneumonia (7.14%), interstitial (21.43%), bronchiointerstitial (11.9%) and embolic pneumonia (2.38%). Also, prevalence of pulmonary abscesses was obtained (11.9%). In bacteriological examinations, *Pasteurella multocida* (24.53%), *Staphylococcus aureus* (20.75%), *Klebsiella pneumoniae* (15.09%), *Corynebacterium pseudotuberculosis* (7.55%) and *Actinomyces pyogenes* (1.89%) were detected as important aerobic bacterial agents and the causative factors of various pulmonary lesions. Because of financial limitations, it was not possible to isolate anaerobic bacteria.

Pasteurella multocida and *S. aureus* were isolated from two of the seven lungs affected with interstitial pneumonia. It was possible these bacteria were secondary invaders and/or environmental contaminants because viruses are normally the etiologic agents of this type of pneumonia. In addition, lack of substantial exudate, thickening of alveolar septa and presence of multinucleated syncytial cells in alveoli affected with bronchiointerstitial pneumonia suggested viral agents as the primary aetiology of interstitial pneumonia, with further contamination of the infected lungs by bacteria. Concentric lamellar bodies known as corpora amylacea were present in the alveolar spaces in this form of pneumonia. Lin *et al.* (24) reported corpora amylacea in 36% of lambs affected by chronic nonprogressive pneumonia. They stated corpora amylacea may result from bronchiolar stenosis and stagnation of exudates. Bronchiointerstitial pneumonia had all characteristics of interstitial pneumonia, with neutrophils and cell debris additionally accumulating in some alveoli and

bronchioles. Ettorre *et al.* (13) studied pneumonia in lambs in Italy and reported lamellar concentrations of protein material known as corpora amylacea in 1.9% of affected cases.

Numerous studies have been conducted on the pathology and bacteriology of ovine pulmonary lesions in various geographic situations. These have shown that the pattern of pneumonia and their bacterial agents were variable from region to region, depending on managing system, meteorological situations, sanitation, and age. Oruc (28) studied the correlation between the bacteriologic agents of lamb pneumonia with their corresponding pathological pattern and showed a prevalence rate of 35.41% pneumonia among 740 diseased or dead lambs. The author described the lung lesions histopathologically as acute-catarrhal (17.56%), muco-purulent (14.50%), purulent-necrotic (9.54%), fibrinous (26.72%), fibrino-necrotic (5.73%), interstitial (18.32%), and verminous pneumonia (7.63%). The bacteriological study described in (28), *Mannheimia haemolytica* (*Pasteurella haemolytica*) (56.14%), *E. coli* (24.56%), and *P. multocida* (10.52%) were detected as the main causative agents of various pneumonias in lambs. The findings reported in (28) are contrary to that of the present study, in which *M. haemolytica* and *E. coli* were not isolated from any of the pneumonic lungs. Hazirolu *et al.* (17) studied the pathology and microbiology of pneumonic lungs and reported a prevalence of 3.6% among 13,588 lambs. Macroscopic lesions were identified as atypical pneumonia in the cranial lobes. In the histology they reported proliferative pneumonia in most cases and stated that it was normally accompanied by exudative characteristics. *Mannheimia haemolytica* was isolated from 51.6%, *Mycoplasma ovipneumoniae* from 43.0% of pneumonic lungs and 26.2% of the cases showed mixed infection by both organisms. They described a close relationship between *M. haemolytica* and exudative inflammation ($p < 0.05$).

Abubakr *et al.* (1) studied pneumonia in 35 experimentally-infected sheep and 28 goats by intratracheal route with bacteria isolated from naturally-diseased sheep and goats in Sudan. According to their findings, bronchopneumonia was associated with *S. aureus* and *Streptococcus* spp, fibrinous pneumonia was associated with *Proteus mirabilis*, *Pseudomonas aeruginosa* or a combination of these two bacteria with *Mycoplasma arginini*, while chronic interstitial pneumonia was associated with a combination of *S. aureus*, *Streptococcus* spp. and *M. arginini*. Ikede (18) studied the respiratory lesions in 60 dead sheep in a livestock station at Ibadan in Nigeria over a 15-month period and showed that purulent pneumonia (48%), fibrinous pneumonia (12%), interstitial pneumonia (5%), giant cell pneumonia (3%), verminous pneumonia

(3%) and mycotic pneumonia (2%) were the main types of pneumonia in that farm. The organisms isolated from the pulmonary lesions of the above study included *M. haemolytica*, *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Mycoplasma arginini* and *Aspergillus fumigatus*. Similar to the above findings, the purulent bronchopneumonia was the most common form of the pneumonia.

Pasteurella multocida was the most common isolated microorganism from infected sheep, with a rate of 35.14% in the present study. *Pasteurella* spp. are normal bacterial flora of the upper respiratory tract and opportunistic pathogens that are normally responsible for respiratory infection in small ruminants. These bacteria cause septicemia in humans. Guillet et al. (16) reported *P. multocida* and meningitis in two-month-old twin infants after household exposure to a slaughtered sheep. This report emphasized that sheep could be a source of human contamination with *Pasteurella* species. Domestic celebrations involving religious sacrifices of these animals without appropriate hygiene may therefore be associated with infections of humans that are in close vicinity of the infected sheep. It has been postulated that the father of these twins who sacrificed the infected sheep was colonised with *P. multocida* and infected his two infants. Contact with pets or other possible sources of *Pasteurella* infection should be considered by using strict hygiene rules to minimize colonization of infection by contact. This organism is one of the main etiological agents of ovine pneumonic outbreaks, but compared to *M. haemolytica* (*P. haemolytica*) its significant contribution in ovine pneumonia has been largely ignored. No *M. haemolytica* was isolated from the affected lungs of the present study. Odugbo et al. (27) isolated *P. multocida* from pneumonic lungs of sheep and stated that *Pasteurellae* not belonging to *M. haemolytica* could not be neglected when considerations concerning ovine pasteurellosis are made. They explained that when the pneumonic pasteurellosis due to *M. haemolytica* became controlled in the field, the prevalence of *P. multocida* pneumonia increased significantly. Jaramillo-Argano et al. (20) isolated *Mannheimia* spp. and *P. multocida* strains from bovine pneumonic lungs in a slaughterhouse in Mexico and showed *P. multocida* (60.3%) and *Mannheimia* spp. (39.7%) as the main causative agents of bovine pneumonia in that area. However, in other studies, *M. haemolytica* was reported as the most frequent isolate of bovine pneumonic lungs (19, 37).

In our study, *K. pneumoniae* (15.09%) was isolated from the pulmonary abscesses, chronic bronchopneumonias and relatively fewer cases of bronchointerstitial pneumonia, whereas in most of the previous studies, *Klebsiella* has rarely been isolated from the pneumonic lungs of different

species of animals. Gameel et al. (14) identified *K. pneumoniae* subspecies ozaenae from small nodules on the chest wall and in the lungs of sheep slaughtered in Al-Ahsa abattoir, Saudi Arabia. Ludford and Stevens (25) detected *K. pneumoniae* from a case of canine pneumonia. Boguta et al. (8) isolated this bacterial flora from the nasal cavity of 80 foals with upper respiratory tract infection, as well as 20 healthy foals. They isolated *K. pneumoniae* and *E. coli* from the lung abscesses and purulent bronchopneumonia. In addition, no bacterial species with recognised pathogenicity was isolated from the healthy animals. In agreement with the current study, *K. pneumoniae* has been found to be a common cause of lung abscesses in human medicine. Most of the lungs abscesses occur in patients with a predisposition to aspiration and systemic or local immune-compromised status, such as chronic lung disease, malignancies, urinary tract infections, septicemia, soft tissue infections or diabetes mellitus (6, 7, 30, 36).

Al-Tarazi (4) studied bacterial aetiologies together with histopathological changes of pneumonia in 284 lungs of slaughtered camels in the northern parts of Jordan and reported the prevalence rate of 10.2% pneumonia in camels. Based on his findings, the pathological lesions of the pneumonic lungs comprised chronic proliferative bronchopneumonia (20.69%), chronic pleuropneumonia (6.9%), interstitial pneumonia (58.6%) and lung abscesses (10.34%). *M. haemolytica* and *Pseudomonas aeruginosa* were the most frequent isolated organisms from cases of chronic proliferative bronchopneumonia and chronic pleuropneumonia. *E. coli* and *Klebsiella* spp. were isolated from interstitial pneumonia. *S. aureus*, *A. pyogenes* and haemolytic streptococci were isolated from the lung abscesses. In their study, the most frequently isolated organisms were *E. coli* (26.66%), *Klebsiella* spp. (14.66%), *Pseudomonas aeruginosa* (12%), *S. aureus* (10.66%), and *M. haemolytica* (6.66%).

In our study, *S. aureus* (29.73%) was another potentially pathogenic bacterium that was isolated mainly from bronchopneumonia and pulmonary abscesses. This bacterium is the main inhabitant of the upper respiratory mucosa, playing a pathogenic role in immune-compromised hosts. It also has zoonotic implications, with opportunities for reciprocal transmission between man and domestic animals when natural barriers are compromised (3).

In the present investigation, *C. pseudotuberculosis* was isolated from the suppurative bronchopneumonia and pulmonary abscesses, and *A. pyogenes* was isolated from one case of pulmonary abscess. *A. pyogenes* is considered to be a common inhabitant of the upper respiratory and genital tracts of domestic animals (32). It is an

important opportunistic pathogen, responsible for suppurative infections in a variety of domestic animals. It has recently been reclassified as the genus *Actinomyces* on the basis of rRNA sequence (2, 12, 23, 35). *C. pseudotuberculosis* is a mycolic acid containing, facultative intracellular actinomycete associated with the development of abscesses in a variety of mammalian hosts (11, 29, 34).

This study revealed a correlation between the type of pneumonia and the isolated bacteria in sheep. The results of the present study showed that *P. multocida* was the most common bacteria, suggesting its importance in respiratory problems of sheep in this area. *P. multocida* was more likely to cause bronchopneumonia with moderate amounts of fibrin. Therefore, its role in small ruminants should receive more attention. No bacterium was isolated from those cases that did not show apparent gross lesions of pneumonia nor in some samples of pneumonic lungs. This could have been due to antibiotic therapy before slaughter, the etiological

agents disappearing and gross lesions recovering or possibly organisms other than bacteria were involved in inducing the pneumonia. There might have been some other predisposing factors such as parasites, viruses and *Mycoplasma* spp. Further studies are required to determine the significance of other pathogens such as *Verminous pneumonia*, and pneumonias due to *Mycoplasma* organisms, viruses and other aetiologies.

Conclusions

Pasteurella multocida was the most common bacterial agent isolated from the pneumonic lungs in this area. This coincides with some studies but differs from others that considered *M. haemolytica* the most pathogenic bacteria. *K. Pneumoniae*, reported as a rare aetiology of pneumonia in small ruminants, was also highly prevalent in this area. The prevalence of *K. pneumoniae* as a sheep pathogen in Iran has not been previously reported.

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