



RESEARCH ARTICLE

**Prevalence and pathological studies on ovine pneumonic pasteurellosis in Kashmir valley, India**

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**Özet**

**Dar LM, Darzi MM, Mir MS, Rashid A, Abdullah S, Hussain SA.** Hindistan'ın Kashmir vadisi koyun pasteurellozisi üzerine prevalans ve patolojik araştırmalar. *Eurasian J Vet Sci*, 2012, 28, 4, 199-203

**Amaç:** Araştırmanın amacı Hindistan'ın Kashmir vadisinde kesime tabi tutulan koyunlarda pasteurellozisin prevalans ve patolojisini belirlemektir.

**Gereç ve Yöntem:** Farklı mezbahalardan elde edilen 2155 koyun akciğeri incelendi. Büyük pneumonik lezyon bulunduran akciğerler toplandı. Bakteriyolojik kültür için farklı akciğer loplardan örnekler toplandı. Bakteriyolojik incelemeye göre dokuların histopatolojik incelemeler yapıldı. Örneklerde bağ doku Masson's Trichrome boyası, elastin Hart's metodu, neutral ile asit mukopolisakaritler Combined Alcian Blue PAS tekniği ve mast hücreler Toluidine Blue boyası ile boyandı.

**Bulgular:** İncelenen 956 lezyonlu akciğerin 398 adetinden *Pasteurella* spp. izole edildi. İncelenen genel populasyon dahil edildiğinde genel prevalans %18.46 (398/2155) olarak belirlendi. Kış (%29.76) ile ilkbahar (%21.03) mevsiminde gözlenen prevalansın yaz (%9.4) ve sonbahar (%12.35) mevsiminden istatistiki olarak önemli ( $p < 0.01$ ) oranda yüksek belirlendi. Makro ve histopatolojik incelemeler fibröz pneumonisi ile uyumlu belirlendi.

**Öneri:** Kashmir vadisinde yüksek oranda koyun pasteurellozisi bulunmaktadır. Bu bulgular hastalığın neden olduğu ekonomik kayıpları önlemek için hastalığa karşı geliştirilecek kontrol işlemlerine yardımcı olacaktır.

**Abstract**

**Dar LM, Darzi MM, Mir MS, Rashid A, Abdullah S, Hussain SA.** Prevalence and pathological studies on ovine pneumonic pasteurellosis in Kashmir valley, India. *Eurasian J Vet Sci*, 2012, 28, 4, 199-203

**Aim:** To describe prevalence and pathology of pneumonic pasteurellosis in sheep slaughtered in Kashmir Valley, India.

**Material and Methods:** 2155 ovine lungs from various abattoirs were examined. Lungs with gross pneumonic lesions were collected. Specimens taken from the different lobes were collected for bacteriological culture. Based on the bacteriology, tissues were processed for histopathological examination. Duplicate sections were stained for connective tissue by Masson's Trichrome Stain, elastin by Hart's method, neutral and acid mucopolysaccharide by Combined Alcian Blue PAS technique, and mast cells by Toluidine Blue Stain.

**Results:** Out of 956 grossly pneumonic lung samples, *Pasteurella* spp. was isolated from 398 affected lung samples giving an overall prevalence of 18.46% (398/2155) in the population studied. The prevalence was significantly ( $p \leq 0.01$ ) higher in winter (29.76%) and spring (21.03%) as compared to autumn (12.35%) and summer (9.4%). Gross and histopathological examination revealed features typical of fibrinous pneumonia.

**Conclusion:** Ovine pasteurellosis is highly prevalent in Kashmir Valley. These findings will help in developing better control measures against the disease to prevent the ensuing economic losses

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Anahtar kelimeler: Koyun, pasteurellozis, histopatoloji

Keywords: Sheep, pasteurellosis, histopathology

## ► Introduction

Livestock sector plays a multifaceted role in socio-economic development of rural households in a developing country, like India. Among various sectors of animal husbandry, sheep husbandry contributes substantially by providing mutton, wool, manure and hides. Economic losses associated with various diseases in sheep and other domestic animals, are often the result of a complex interaction between infection, poor management and environmental condition. Among the various health related problems, pneumonic pasteurellosis is the major cause of deaths in the lambs and decreased productivity in the older animals (Carter 1973).

Pneumonic pasteurellosis, also known as respiratory manheimiosis, is the most common example with a wide prevalence in ruminant animals. The disease, in its typical clinical form, is highly infectious and, often fatal. Pneumonic pasteurellosis is one of the oldest diseases affecting domestic animals. Nonetheless, it still remains a major problem (Tizard 1992). Like many potential pathogenic microorganisms, *Pasteurella multocida* and *Mannheimia haemolytica* are often detected in upper respiratory tract and the upper part of the digestive tract of apparently healthy animals and does not seem to cause any disease until suppression of immunity of the host occurs such as due to shipment and exposure to persistent cold climatic conditions (Timoney et al 1988, Tizard 1992, Rados-tits et al 2000).

To the authors' knowledge, the base line epidemiological data describing the prevalence and pathology of ovine pneumonic pasteurellosis in Kashmir valley have not been published in the peer-reviewed journals until now. Hence, the present study was undertaken to investigate the prevalence and pathology of pneumonic pasteurellosis in the sheep destined for slaughter in Kashmir valley.

## ► Material and Methods

2155 sheep lungs were examined in different organized abattoirs. Following slaughter, lungs were first examined in situ and any lesions observed were noted. Then whole lungs from all the animals were collected and thoroughly screened by visual examination, palpation and dissection. 956 of 2155 lung samples with gross lesions in favour of pneumonia were subjected to microbial culture.

A portion of the specimens taken from the different lobes of the affected lungs were collected. These samples were placed into sterile plastic bags. Sections measuring one to two inches were aseptically removed, immersed in the 70% alcohol, opened and minced with sterile forceps and inoculated in 10 ml trypticase soy broth (TSB). The TSB was then incubated for 2 hours at 37 °C and then streaked into blood agar plate (BAP, 5% blood in blood agar base). The

BAP was incubated at 37 °C for 24 hours. Based on the colony characteristics and appearance, the suspected colonies were then isolated. The colonies in both culture media were studied for their shape, size, color, and haemolysis capacities. Further, the isolated pure cultures were identified according to the procedure described by Cowan (1974).

Based on the isolation of *Pasteurella* spp., formalin fixed tissue specimens were processed by routine paraffin embedding technique. Briefly, the samples were cut into pieces of 2-3 mm thickness and washed thoroughly with water for several hours before putting in ascending grades of alcohol for dehydration, followed by clearance in benzene and embedded in paraffin. Sections of 4-5 micron thickness were cut and stained with Harri's Haematoxylin and eosin method.

Specific staining techniques were also carried on paraffin embedded sections. The sections were selected on the basis of histopathology and were stained for connective tissue by Masson's Trichome Stain, elastin by Hart's method, neutral and acid mucopolysaccharide by Combined Alcian Blue PAS technique and mast cells by Toluidine Blue Stain.

The results were analyzed statistically using chi-square with confidence level 95%.

## ► Results

### • Prevalence

Out of 956 grossly pneumonic lung samples, *Pasteurella* spp. was isolated from 398 pneumonic lung samples giving an overall prevalence of 18.46% (398/2155) in the population studied. The frequency of isolation was significantly ( $P \leq 0.01$ ) higher in winter (29.76%) and spring (21.03%) as compared to autumn (12.35%) and summer (9.4%), (Table 1).

Table 1. Seasonal prevalence of pneumonic pasteurellosis in sheep ( $p \leq 0.01$ ).

Season	Total lungs examined	Total No. positive	Percentage
Spring	523	110	21.03
Summer	542	51	9.40
Autumn	502	62	12.35
Winter	588	175	29.76
Total	2155	398	18.46

### • Pathology of lungs with pneumonic pasteurellosis

Grossly, the lungs were characterized by patchy to diffuse areas of consolidation covered by a layer of fibrin, mostly in the apical, cardiac and anteroventral portions of diaphragmatic lobes (Figure 1). Cut surfaces of the consolidated lung tissues revealed well circumscribed hemorrhagic areas with red tinged mucopurulent discharge oozing out from bronchi and bronchioles. Pleuritis was also evident grossly.



Figure 1. Lung showing bilateral consolidation in apical, cardiac and cranioventral lobes covered by thin layer of fibrin.

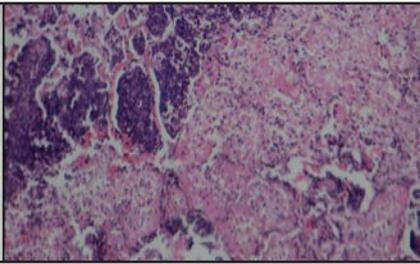


Figure 2. Section of lung showing fibrinocellular exudates in the alveoli (H&E).  $\times 400$ .

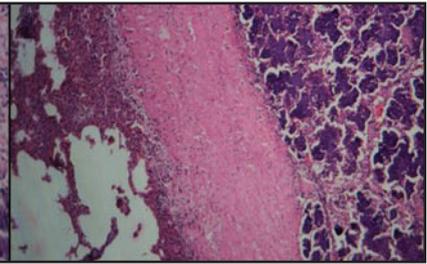


Figure 3. Section of lung showing thickening of interlobular septae with infiltration of fibrinocellular exudates (H&E).  $\times 400$ .

Histopathological examination of the affected lungs revealed features typical of fibrinous pneumonia and pleuritis. The alveoli were filled with fibrin and interlobular septae were thickened with infiltration of fibrino-cellular exudate comprising predominantly of mononuclear cells including neutrophils (Figures 2 and 3). Bronchi and bronchioles revealed epithelial desquamation and were plugged by the inflamma-

tion of neutrophils, some of which were disintegrated. The area surrounding necrotic foci revealed vascular engorgement and the sero-fibrinous inflammatory exudates with abundant polymorphs and mononuclear cells (Figure 6). In addition emphysema and oedema were associated findings in most of the affected lungs. Special staining revealed deposition of collagen fibers indicative of connective tissue proliferation only in 12

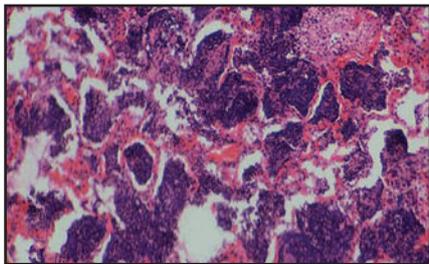


Figure 4. Section of lung revealing presence of oat shaped cells in the alveoli (H&E).  $\times 400$ .

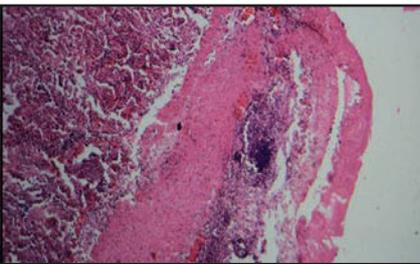


Figure 5. Section of lung showing thickening of pleura due to fibrin deposition with infiltration of neutrophils (H&E).  $\times 400$ .

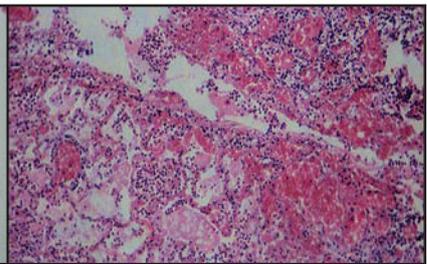


Figure 6. Section of lung revealing vascular engorgement and serofibrinous inflammatory exudates (H&E).  $\times 400$ .

tory exudates and cellular debris. The alveoli revealed presence of large number of leukocytes in the lumen, especially macrophages in the form of basophilic spindle shaped cells or oat shaped cells often arranged in a streaming pattern, pathognomonic for fibrinous pneumonia (Figure 4). These oat shaped leukocytes formed whorl like structures in and around the alveoli. Pleurae were often thickened with fibrinous exudates associated with neutrophilic infiltration (Figure 5). Affected lungs were often associated with focal or wide irregular areas of necrosis which stained pink with eosin. There was homogenous infiltration

of 398 affected lungs (Figure 7). Staining of tissues for elastin revealed varying degrees of elastic tissue disruption which was prominent in the emphysematous and atelectatic areas (Figure 8). Staining of affected tissues by Combined Alcian Blue-PAS technique revealed varying degrees of acid and neutral mucopolysaccharide reaction in different types of lesions. Bronchial epithelium and oat cells were positive for acid mucopolysaccharides; while, fibrin within alveolar lumen and interlobular septae was positive for neutral mucopolysaccharides (Figure 9). No mast cell reaction was evident around the lesions.

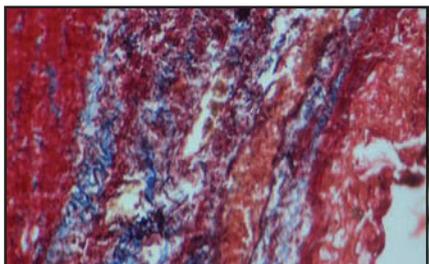


Figure 7. Section of lung revealing collagen deposition in the pleura (Masson's Trichrome Method).  $\times 400$ .

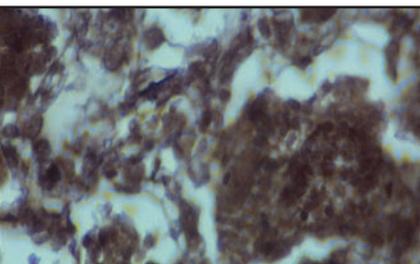


Figure 8. Section showing disruption and disorientation of elastic tissue in emphysematous and atelectatic areas (Harts Method).  $\times 400$ .

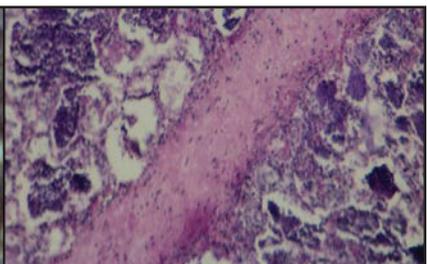


Figure 9. Section of lung showing interlobular septa positive for neutral mucopolysaccharides (Combined Alcian Blue PAS).  $\times 300$ .

## ► Discussion

In the present study, over all prevalence of ovine pneumonic pasteurilosis was found to be 18.46% in Kashmir valley, India. Earlier workers from other parts of the country have reported prevalence varying from 1.14% to 24.67% (Chattopadhyay et al 1986, Kamil 1989, Srinivasan et al 2003, Dhand et al 2004, Kumar 2005). The higher prevalence observed in winter (29.76%) and spring (21.03%) as compared to autumn (12.35%) and summer (9.4%) was in agreement with Pfeffer et al (1983). Further, it has been well established that development of pneumonic pasteurilosis is highly mediated by complex interactions between the naturally existing causative organism in the upper respiratory tract, the immunological status of the animal and the role of predisposing factors in the initiation of infection. The majority of *Pasteurella* spp. infections are mostly endogenous, caused by the normally resident bacteria on the upper respiratory tract, although exogenous infections can also occur by direct contact with sick animals or through infected aerosols. In either situation, the stress is an intrinsic condition that is consistently reported to increase the susceptibility to various types of infectious diseases in animals (Stephens 1980, Biondi and Zannino 1997). The role of stress in the natural incidence of pneumonic pasteurilosis is clearly evident by the fact that the disease onset is mainly associated with sudden exposure to stressful situations created by adverse physical, environmental or climatic conditions. The most common examples of these include extremely cold weather, overcrowding in a limited space, poor ventilation, bad management, rough handling and distant transport or shipping (Thomson et al 1975, Slocombe et al 1984, Radostits et al 2000).

The gross and histopathological observations were typical of fibrinous pneumonia and were in concordance with observations of earlier workers (Kamil 1989, Brogden et al 1998, Singh and Singh 1999, Tehrani et al 2004, Kumar 2005, Oruc 2006, Odugbo et al 2006). Presence of macrophages in the form of basophilic spindle shaped cells or oat shaped cells often arranged in a streaming pattern in and around the alveoli might be attributed to an imbalance with increased procoagulant activity and decreased fibrinolytic activity of the pulmonary leukocytes leading to increased fibrin deposition, and decreased fibrin removal from the alveolar tissue resulting into the "Oat shaped cell" formation (Jubb et al 1993). The focal and wide irregular areas of necrosis observed in affected lungs might be attributed to endotoxins and leukotoxins produced by the bacteria (Jubb et al 1993, Jones et al 1997, Oruc 2006, Ezzi et al 2007).

Most of the affected lungs revealed no connective tissue proliferation which might be attributed to acute nature of the condition (Dungworth 1993 and Lopez 2001). Varying degrees of elastic tissue disruption observed in the emphysematous and atelectatic areas

was in agreement with various studies reported earlier (Carnes 1968, Chrzanowski et al 1980, and Keller and Mandl 1972). Further, it has been suggested that emphysema is induced by the proteolytic destruction of elastin and elastolytic proteinases derived from leukocytes and macrophages (Dar et al 2012). Mucopolysaccharide reaction observed in different lesions might be attributed to their probable role in the inflammation (Darzi et al 2003, Shah 2008). Also, increased amounts of mucopolysaccharides in and around the lesions may be attributed to prolonged irritative action of different insults believed to determine hypersecretion of these substances (Lupu et al 1959). No mast cell reaction in and around the lesions could be demonstrated. Ramez-Romero et al (2000) also reported scarce mast cells in the areas with severe pneumonic lesions. It has been suggested that mast cells disappear due to degranulation during an acute inflammatory response depending on intensity of the lesion and time of exposure (Cheville 1994).

## ► Conclusions

The study showed that pneumonic pasteurilosis is highly prevalent in domestic sheep in Kashmir Valley, with higher rates in winter and spring than those in summer and autumn. Grossly, the affected lungs were characterized by patchy to diffuse areas of consolidation covered by a layer of fibrin, mostly in the apical, cardiac and anteroventral portions of diaphragmatic lobes. Histopathological examination of the affected lungs revealed alveoli filled with fibrin and thickened interlobular septae with infiltration of fibrinocellular exudate comprising predominantly of mononuclear cells and neutrophils. The results of this study will help in developing better control measures against the ovine pneumonic pasteurilosis to prevent the further economic losses.

## ► Acknowledgment

The bacteriological and histopathological examination of the affected lung tissues during the present study was conducted in the Department of Veterinary Microbiology and Department of Veterinary Pathology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Kashmir, India. The authors express their sincere gratitude to the personnel of both the departments who were present during this period.

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