Pathological Studies on Lung Abscesses in Sheep Slaughtered in Kashmir Valley, India

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(Recieved 11 April 2012/ Accepted 22 Jun 2012)

Abstract

The present study was conducted in Kashmir valley of India to investigate the prevalence and pathology of lung abscesses in sheep, slaughtered in different organized abattoirs. These abattoirs were visited between January 2010 to February 2011 and a total of 1455 lungs were examined. Out of these 18.9% lungs had abscesses, with higher incidence in young sheep (60%) than in adult ones (40%). Grossly, abscesses were observed in one or more lung lobes and were either single or multiple. In majority of lungs, abscess sizes varied from pea to walnut size, but in some cases large abscesses were also observed. Histopathologically, abscesses were characterized by a central caseo-necrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells and few mononuclear cells and macrophages. Most of the abscesses revealed presence of Gram positive bacterial infection where as chronic abscesses indicated both Gram positive and Gram negative bacterial infection. Fibrous tissue proliferation around the pyogenic membrane of the chronic abscesses was noticed. Disruption and disorientation of elastin fibres was also a prominent feature. Increased concentration of both acid and neutral mucopolysaccharides was observed in and around the lesion. Purulent material of abscesses revealed marked metachromasia. The study revealed that lung abscesses in domestic sheep are highly prevalent in Kashmir valley. Thus, there is a need to introduce appropriate control measures of diseases affecting the lungs to minimize the incidence of lung affections and hence reduce the ensuing economic losses.

Keywords: Abscess; sheep; lung; Kashmir; histopathology; Prevalence

Introduction

In a developing agriculture based country, like India, the importance of animal husbandry in the economic progress of the people cannot be underestimated. Livestock sector plays a multifaceted role in socio-economic development of rural households. Among various sectors of animal husbandry, sheep husbandry contributes substantially by providing mutton, wool, manure and hides, particularly under temperate, semiarid and arid climates. 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, respiratory ailments are the major cause of deaths in the lambs and decreased productivity in the older animals. The respiratory system constitutes the most extensive surface that is exposed directly to the ambient environment. Although, the respiratory system possesses a local innate defense mechanism, yet it is highly vulnerable to the various environmental insults. Any sudden change in the environment interferes with the local defense rendering the system more vulnerable (Bruere et al., 2002). Abscesses have been reported in several internal organs in sheep especially in lungs, livers, mediastinal and bronchial lymph nodes (Batey, 1986). Lung abscesses can have considerable consequences. This form of disease can cause major economic losses because the infected animals have to be culled from breeding flocks due to poor phys-

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ical condition or decreased fertility and further, whole carcasses or lungs are condemned at abattoirs (Izgur *et al.*, 2010).

Kashmir valley is located in the northern Himalayas approximately at an altitude of 1730 m above the mean sea level and falls between 32° 17' to 36° 58' North latitude and 73° 26' to 80° 30' East longitude. The temperature ranges between -4 to 32°C during various months of the year and average annual precipitation is around 70 millimeters. Spring is the wettest season and autumn is the driest. Sheep rearing is practiced extensively in Kashmir and contributes to 60% of the total meat consumption in the region. However, the base line epidemiological data describing the prevalence and pathology of various ovine lung affections have not been published in the peer-reviewed journals until now. The goal of the present study was to describe the prevalence and pathology of lung abscesses in sheep slaughtered in Kashmir Valley, India.

Materials and methods

Study material

A total number of 1455 sheep lungs were examined in different organized abattoirs from January 2010 to February 2011. Sheep were categorized as young (day 0 to 12 months of age) or adult (animals over 12 months of age). 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 of bronchial tree for the presence of lung abscesses.

Impression smears from various lung abscesses were taken after giving an incision. Also, smears were prepared from bronchial froth/exudate and stained with Wrights-Giemsa method. Representative tissue specimens from grossly affected lungs were collected in 10% neutral buffered formalin for histopathological examination.

Histopathological Examination

Tissue sections were processed by routine paraffin embedding technique. Briefly, the fixed tissue specimens were cut into pieces of 2-3mm thickness and washed thoroughly with water for several hours before putting in ascending grades of alcohol for dehydration, followed by clearence in benzene and embedded in paraffin. Sections of 4-5 micron thickness were cut and stained with Harri's Haematoxylin and eosin method (H&E) (Luna, 1968). Duplicate sections were stained using Brown-Brenn method for demonstration of Gram positive and Gram negative bacteria. The sections were also selected based on preliminary histopathological examination and stained for connective tissue by Masson's Trichome Stain, elastin by Hart's method (Luna, 1968), neutral and acid mucopolysaccharide by Combined Alcian Blue PAS technique (Bancroft and Gamble, 2002) and mast cells by Toluidine Blue Stain (Humason, 1979) respectively.

Results

Prevalence and pathology

Out of total 1455 sheep lungs, 275 (18.9%) exhibited abscesses with higher incidence in young sheep 60% (165/275) than in adult ones 40% (110/275). Grossly, abscesses were observed in one or more lobes of lung and were either single or multiple. In most of the cases, abscesses were pea to walnut size, but in some cases large abscesses were also observed. In a few cases abscesses occurred in areas which were grossly consolidated. On dissection dirty grey to greenish cheesy pus (Fig. 1A) exuded from the abscesses. Mediastinal lymph nodes were grossly enlarged and elongated having adhesions with the margins of diaphragmatic lobes of either side in certain lungs. Cut sections of these lymph nodes often revealed thick, greenish cheesy pus (Fig. 1B).



Fig. 1A. Cut section of lung showing dirty grey to greenish pus in the abscess.



Fig.1B. Cut section of mediastinal lymph node revealing thick, greenish cheesy pus.

Histopathologically, focal areas of suppuration were observed in lung parenchyma. The abscesses were characterized by a central caseo-necrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells and few mononuclear cells and macrophages(Figs. 2A & B). The pleura was either thickened showing infiltration of polymorphonuclear cells or showed presence of micro-abscesses (Figs. 2C & D). Chronic abscesses were characterized by the zone of leukocytic infiltration which was in turn surrounded by a connective tissue capsule associated with infiltration of large number of mononuclear cells. Some abscesses revealed extensive connective tissue proliferation around the periphery as well as involving the pleura which was thickened with marked mononuclear cell infiltration. In a few cases associated with chronic bronchiolitis, fibroplasia was marked involving perivascular and peribronchiolar region. Purulent material was present in bronchi and bronchioles along with infiltration of polymorphonuclear cells (Fig. 2E). In general, severe congestion was observed in lung parenchyma along with the abscess (Fig. 2F). Focal areas of fibroplasias and atelectasis were evident.

In most of the sections from abscesses, Gram positive bacteria were demonstrated (Fig. 3A); however, most of the chronic abscesses revealed mixed infection with both Gram positive and Gram negative bacteria.

Impression smears from the bronchial exudates and abscess wall revealed presence of polymophonuclear leukocytes, desquamated epithelial cells and cells containing hypersegmented nuclei. Collagen deposition was noted around the pyogenic membrane of the chronic abscesses (Fig. 3B). Disruption and disorientation of elastin fibres was prominent around the abscess walls (Fig. 3C). In most of the abscesses, pyogenic membrane and infiltrating cells around the abcesses as well as within the purulent material were positive for acid mucopolysaccharides where as formed pus was positive for neutral mucopolysaccharides (Fig. 3D); however, in some chronic abcesses the connective tissue fibres around the abscess were positive for neutral mucopolysaccharides. Purulent material of abscesses revealed marked metachromasia (Fig



Fig. 2A. Section of lung revealing central caseonecrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells. H & E X40.



Fig. 2B. Higher magnification of Fig. 2A, revealing central caseo-necrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells and fibroblasts peripheral to the membrane. H & E. X100.



Fig. 2C. Section of lung revealing thickening of pleura with infiltration of polymorphonuclear cells. H & E. X100.



Fig. 2F. Section of lung revealing congestion in lung parenchyma adjacent to the abscess. H & E. X400.



Fig. 2D. Section of lung showing micro pleural abscesses. H & E. X40.



Fig. 3A. Section of lung revealing presence of Gram positive cocci. Brown-Brenn method.X400.



Fig. 2E. Section of lung revealing presence of plug of purulent material in bronchus. H & E. X100.



Fig. 3B.: Section of lung showing collagen deposition around pyogenic membrane of abscess. Masson's Trichome method. X 400.



Fig. 3C. Section of lung showing disruption of elastic fibres at the periphery of abscess wall. Hart's method. X 300.



Fig. 3D. Section of lung revealing pyogenic membrane and infiltrating cells around the abscess positive for acid mucopolysaccharides, where as formed pus positive for neutral mucopolysaccharides. Combined Alcian Blue PAS. X 300.



Fig. 3E. Section of lung showing metachromasia in the center of purulent material. Toluidine Blue method. X 100.

Discussion

In the present study 18.9% lungs revealed abscesses in one or more lobes. Earlier workers have reported that ovine lung abscess prevalence ranged from 3.8% to 18% in different regions of country (Vyas et al., 1984; Chattopadhyay et al., 1986; Kamil, 1989; Mellau et al., 2010). The incidence of lung abscesses was found to be more in young sheep than in adults. Although lung abscesses have been reported in sheep of all ages, lambs appear to be the more susceptible due to progressive waning of passive immunity (Alley, 2002). Further, these observations in the present study were in accordance with observation made by earlier workers (Orr, 1995; Smits et al., 1998; Clark et al., 2001; Fairley et al., 2002) who reported higher prevalence of lung abscesses in lambs compared to the adult sheep.

The gross and histopathological features observed in the present study were generally similar to those described by earlier workers (Chattopadhyay *et al.*, 1986, Kamil, 1989, kumar, 2005). Most of the cases revealed presence of Gram positive organisms in histopathological sections and in some cases both Gram positive and Gram negative organisms were demonstrated. These findings were in concordance with the findings of Kamil (1989).

Chronic abscesses were having varying degrees of connective tissue proliferation in the adjoining areas. These observations were similiar as reported by earliers workers (Dungworth, 1993 and Lopez, 2001). Further this might be an attempt of the body to contain or circumscribe the active infectious zone. Also, the cytokines released by inflammatory cells might facilitate fibroplasia by paracrine action. Varying degree of disruption of elastic tissue around the abscesses observed was in agreement earlier reports (Carnes, with the 1968; Chrzanowski et al., 1980; Keller and Mandl, 1972, Lappalainen et al., 2005). It has been opined that emphysema is induced by the proteolytic destruction of elastin, by elastolytic proteinases derived from leukocytes and macrophages (Kuhn and Starcher, 1980; Tetley, 2002).

Both acid and neutral mucopolysaccharides were found to be increased in the affected areas. This may be attributed to their probable role in the inflammation (Darzi *et al.*, 2003; Shah, 2008). Also, increased amounts of mucopolsaccharides in and around the lesions may be attributed to prolonged irritative action of different insults, which are believed to determine hypersecretion of these substances (Lupu *et al.*, 1959, Izgur *et al.*, 2010).

Metachromasia could be demonstrated in the purulent material giving an indication of probable mast cell reaction. Mast cells have been found to play an important role in lung inflammation producing compounds and cytokines that induce acute phase response and induce neutrophil infiltration besides acting as antigen presenting cells (Abraham *et al.*, 1996; Abraham *et al.*, 1997). Further, it has been observed that mast cells contain proteases and growth factors that make them an important component in the chronic lung diseases (Malaviya *et al.*, 1996; Inoue *et al.*, 1996; Cairns and Walls, 1997; Abraham *et al.*, 1997; Metcalfe *et al.*, 1997).

The study showed that lung abcesses in domestic sheep are highly prevalent in Kashmir Valley. Thus, there is a need to introduce appropriate control measures of diseases affecting lungs to minimize the incidence of lung affections and hence reduce the ensuing economic losses.

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