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Caprine lung diseases and causal bacteria

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Abstract

Pathological conditions in lungs of slaughtered goats were studied. Sixty lungs were examined and tissue samples and swabs obtained for histopathology and bacterial isolation, respectively. The prevalence of lung diseases was 58.3% (n=35). Gross lesions were categorized into: (a) haemorrhage and congestion 25% (b) emphysema 21.7% (c) hepatization 3.3% and (d) granulomatous nodules about 1 mm diameter 8.3%. On histopathological examination, 10 types of lesions were found: (a) bronchitis 6.7%, (b) small cell anaplastic carcinoma 3.3%, (c) pneumonia 6.7%, (d) interstitial pneumonia 15%, (e) emphysema 6.7%, (f) bronchopneumonia 3.3%, (g) purulent pneumonia 5%, (h) haemorrhagic pneumonia 3.3%, (i) pulmonary adenomatosis 1.7% and (j) no lesions 6.7%. *Pasteurella* sp. (11.7%), *Escherichia coli* (*E. coli*; 6.7%), *Staphylococcus* sp. (36.7%) and *Bacillus* sp. (3.3%) were isolated from the lungs. *Pasteurella* sp. was found in haemorrhagic pneumonia, interstitial pneumonia, small cell anaplastic carcinoma and bronchitis, followed by *Bacillus* sp. in haemorrhagic pneumonia, *E. coli* in interstitial pneumonia and pulmonary adenomatosis and *Staphylococcus* sp. from emphysema, bronchopneumonia, pneumonia, bronchitis and purulent pneumonia. (*Bangl. vet.* 2007. Vol. 25, No. 1, 9-16)

Introduction

The goat is an important species of livestock in third world countries. Bangladesh, a tropical agro-based developing country, possesses the third largest population in Asia of over 34 million goats (FAO, 2007). This represents over 57% of the total livestock population. Over 90% of the goats belong to the Black Bengal breed.

Lungs are vulnerable to many infectious agents. Pneumonia causes debility and death leading to great economic loss to farmers. Women commonly rear a few goats, grazed usually on free pastures. These pastures are usually contaminated with infectious agents that can get access through inhalation and cause pneumonia. A large number of goats are brought to the Veterinary Clinic or Hospital for treatment of respiratory problems, especially pneumonia. A variety of causes are responsible for pneumonia in goats (Rahman *et al.,* 1976). This investigation was undertaken to determine the status of lung diseases in Black Bengal goats.

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Materials and Methods

Samples

Lung samples were collected immediately after slaughter from four abattoirs in Mymensingh sadar upazila (sub-district) from May to September 2007. Out of 60 lungs examined 35 were affected, and were collected for pathological studies: 35 swabs were collected aseptically from inner core of lungs, and immediately placed in nutrient broth for bacteriology. All samples were transferred to the laboratory for histopathology and bacteriology.

Pathological studies

Gross : Sixty lungs were examined for gross abnormalities, which were recorded, and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathology.

Histopathology : The fixed tissues were processed as described by Luna (1968).

Photomicrography

Photomicrography was done using an Olympus PM-C 35 camera.

Isolation and identification of bacteria

Test tubes containing samples were incubated for 24 hours at 37˚C. From the nutrient broth, subcultures were done on blood agar, MacConkey agar, EMB agar, TSI agar and nutrient agar, and incubated at 37˚C overnight. The identification of the organisms was done as described by Freeman (1985) and Cheesbrough (2000). The organisms were isolated and identified on the basis of colony and staining characteristics and biochemical tests.

Biochemical tests

Catalase activity : Organisms were grown on a slope of nutrient agar. One ml 3% hydrogen peroxide was run down the slope and examined after 5 min for evolution of gases.

Coagulase test : Undiluted plasma (0.5 ml) was mixed with an equal volume of old broth culture for 18-24 hour at 37˚C for 4 hours and examined after 1 and 4 hours for a coagulum. Negative tubes were left at room temperature overnight and then re-examined.

Indole test : Peptone water (2 ml) was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added, shaken well and examined after one minute. Formation of a red layer was the indication of indole positive.

Methyl- red test : A colony of the test organism was inoculated in 0.5 ml of sterile glucose phosphate broth (as used in the V-P test). After overnight incubation at 35-37°C, a drop of methyl red solution was added. A positive reaction was shown by

a bright red colour, indicating acidity. A yellow or orange colour was indicated as negative.

Maintenance of stock culture

To preserve organisms, they were inoculated at 37°C for 24 hours. After growth of the organisms the tubes were sealed with paraffin wax and kept at 4°C.

Results and Discussion

The prevalence of gross lung lesions was 58.3%, categorized into haemorrhages and congestion 25%, emphysema 21.7%, hepatization 3.3% (Fig. 1) and granulomatous nodules lesions about 1 mm diameter 8.3%.

Histologically, lesions were categorized into 10 types: bronchitis 6.7%, small cell anaplastic carcinoma 3.3%, pneumonia 6.7%, interstitial pneumonia 15%, emphysema 6.7%, bronchopneumonia 3.3%, purulent pneumonia 5%, haemorrhagic pneumonia 3.3%, pulmonary adenomatosis 1.7% and no apparent lesions 6.7% (Table 2).

Bronchitis : No specific gross lesions were found but the consistency of lung was hard on palpation. Microscopically, there was exudate in most of the lumen of the bronchus. Infiltration of neutrophils was found in the lumen and wall of the bronchus (Fig. 3).

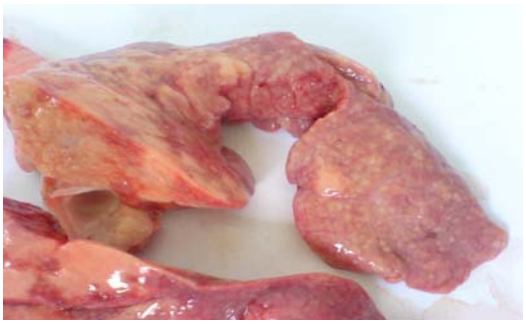

Fig. 1. Caprine lungs showing grey hepatization

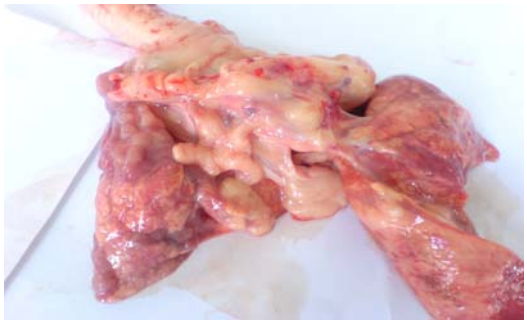

Fig. 2. Caprine lungs showing small nodule

Small cell anaplastic carcinoma : Grossly, the affected portion of lung showed consolidation. Microscopically, the epithelium was proliferated from the bronchial wall causing hypercellularity and loss of normal structure. The neoplastic cells consisted of single layer of cuboidal or low columnar epithelial cells with uniform shape and size.

Pneumonia : Grossly, there was haemorrhage and in some cases congestion. Excessive haemorrhages within the alveoli and inter-alveolar septa associated with leukocytic infiltration was found histologically. Similar infiltrations were noticed in

the bronchus. The wall of the bronchus showed inflammatory changes and mild necrosis in the mucosal layer.

Table 1. Gross pathology of goat lungs

Lungs lesions	No. of affected animal	Disorder (%)
a. Haemorrhage and congestion in lung	15	25
b. Emphysematous lung	13	21.7
c. Hepatization in lung	2	3.3
d. Granulomatous nodule like lesions in lung	5	8.3
Total	35	58.3

Table 2. Histopathology of goat's lung (n= 60)

Lungs lesions	No. of animal affected	Disorder (%)
a. Bronchitis	4	6.7
b. Small cell anaplastic carcinoma	2	3.3
c. Pneumonia	4	6.7
d. Interstitial pneumonia	9	15
e. Emphysema	4	6.7
f. Bronchopneumonia	2	3.3
g. Purulent pneumonia.	3	5.0
h. Haemorrhagic pneumonia	2	3.3
i. Pulmonary adenomatosis	1	1.7
j. No lesions	4	6.7

Interstitial pneumonia : Grossly, the lungs were reddish with small dark-red spots, which were hard on palpation. Microscopically, the alveolar septa were thickened due to accumulation of macrophages and lymphocytes with proliferation of fibrous connective tissue (Fig. 4).

Emphysema of lungs : The emphysematous area was pale in colour with elevated areas that were easily detected by compression with a finger. Many alveoli were distended with wide openings due to rupture of alveolar walls. The alveolar wall was thin and atrophic under the microscope.

Broncho-pneumonia : Grossly, the affected portion of lung was congested, consolidated and hard on palpation. Microscopically, there was exudation with infiltration of neutrophils in the bronchiolar wall and in the lumen of bronchioles. There was deposition of exudates within the peribronchial alveoli.

Purulent pneumonia : Grossly, the lung was consolidated and hard. Microscopic lesions were characterized by inflammatory cells, mainly neutrophils, within the alveoli and in the lumen of the bronchioles. Adjacent to the bronchioles, there were focal infiltrations of a large number of neutrophils and a few lymphocytes.

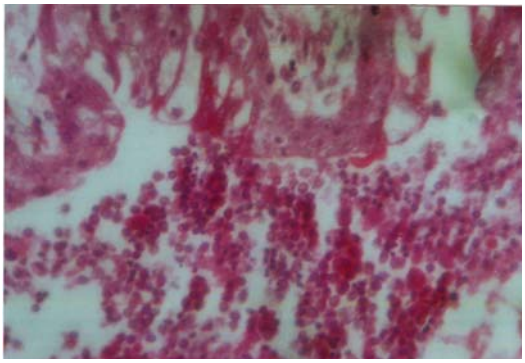

Fig. 3. Section of caprine lung showing bronchitis: Note exudate in most of lumen of the bronchus and infiltration of inflammatory cells mainly neutrophils in and around the bronchial wall (H&E, x 333)

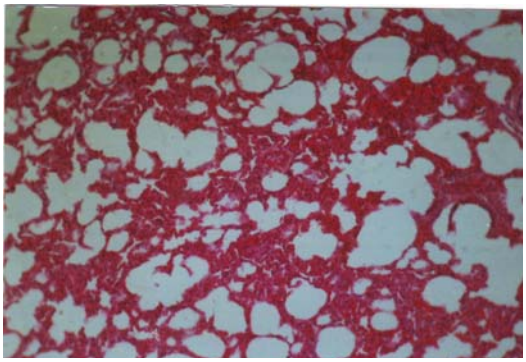

Fig. 4. Section of caprine lung showing interstitial pneumonia: Note the alveolar septa is thickened due to accumulation of macrophages and lymphocytes (H&E, x 830)

Haemorrhagic pneumonia: The lungs showed severe congestion. There were many microscopic haemorrhages within the alveoli and inter-alveolar septa, associated with leukocytic infiltration. Haemorrhages and leukocytic infiltration were found in the bronchus. The wall of the bronchus showed inflammatory change.

Pulmonary adenomatosis: No specific gross lesions were found but the consistency of lung was firmer on palpation. Glandular pattern of growth due to pneumocyte Type-II cells proliferation was found histologically.

Pus and cyst in lungs were recorded by Jubb *et al.* (1993); Radostits *et al.* (2002), but no such lesions were recorded in the present investigation.

The highest occurrence of microscopic lung lesions was interstitial pneumonia (15%) where *Pasteurella* sp. and *E. coli* were present. The role of these bacteria in the production of the lesion was not clear. However, the interstitial pneumonic lesions were characterized by thickening of inter-alveolar septa with macrophages, lymphocytes with proliferation of fibrous connective tissue. The histopathological lesions of interstitial pneumonia correspond with the findings of Jones *et al.* (1997); Zamri-Saad (2006). Histopathology of broncho-pneumonia, haemorrhagic pneumonia and pulmonary adenomatosis described in the present investigation were similar to those of Akbar (2007); Jones *et al.* (1997).

Bacterial isolation and identification

Among the 60 lungs, seven were positive for *Pasteurella* sp., four for *E. coli*, 22 for *Staphylococcus* sp. and two for *Bacillus* sp. (Table 3). *Pasteurella* sp. was isolated from haemorrhagic pneumonia, interstitial pneumonia, small cell anaplastic carcinoma and bronchitis. *Bacillus* sp. was isolated from haemorrhagic pneumonia. *E. coli* from interstitial pneumonia and pulmonary adenomatosis was isolated. *Staphylococcus* sp. from emphysema, bronchopneumonia, pneumonia, bronchitis and purulent pneumonia was found. The prevalence of *Pasteurella* sp. was 11.7%, *E. coli* 6.7%, *Staphylococcus* sp. 36.7% and *Bacillus* sp. 3.3% (Table 3).

Table 3. Detection of bacteria in the lung (n=60)

Finding	Total No. sample examined	No. of affected animal	Bacteria isolated (%)
Pasteurella sp.	60	7	11.7
E. coli		4	6.7
Staphylococcus sp.		22	36.7
Bacillus sp		2	3.3
Total	60	35	59.4

Isolation and identification of Pasteurella sp.

Pasteurella sp. grew well on nutrient agar and showed smooth, circular, greyish colony about 1 mm in diameter after 24 hour at 37°C. In nutrient broth, they grew well and showed diffuse turbidity. Small greyish haemolytic zones were evident in blood agar. In McConkey agar, they showed small pink colonies about 1-2 mm in diameter. Smear from pure culture revealed Gram-negative, bipolar, small rod-shaped bacteria. In Leishman's stain bipolar characteristics of the organisms were detected with a blue colour (Fig. 5). *Pasteurella* sp. was catalase-positive and indole-negative. *Pasteurella* sp. fermented sucrose, dextrose and maltose with the production of acid but did not ferment lactose.

Isolation and identification of Staphylococcus sp.

Staphylococcus sp. grew well on nutrient agar and showed smooth circular, opaque often yellow-pigmented colonies about 1 mm in diameter after 24 hour at 37°C. In nutrient broth, they grew well and showed dense turbidity with a powdery deposit. Smears from pure cultures revealed Gram-positive, round bacteria in cluster form.

Isolation and identification of Escherichia coli

The organism produced smooth circular colonies with dark centres and metallic sheen on EMB agar. *E. coli* produced pink colonies on MacConkey's agar. *E. coli* was a short rod, varying from coccoid bipolar shapes to long filamentous forms. It was occurred singly or in short Gram-negative chains. *E. coil* fermented dextrose, lactose,

maltose, mannitol, sucrose and dulcitol. The organisms were MR positive, VP negative and produced indole.

Isolation and identification of Bacillus sp.

Bacillus sp. grew readily on blood agar. On agar they produced haemolytic zone. The bacilli were Gram-negative, rod-shaped cells in chain form (Fig. 6).

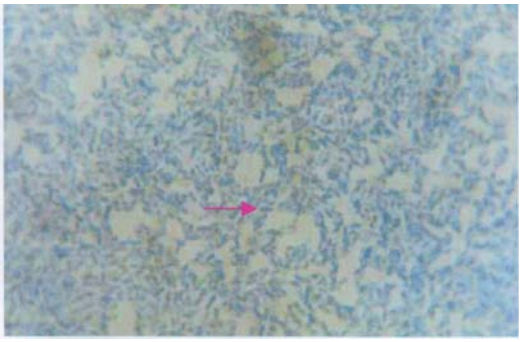

Fig. 5. Smear from pure culture of *Pasteurella sp.* stained with Leishman's stain : Note the Gram-negative, rod-shaped blue bacteria (x 830)

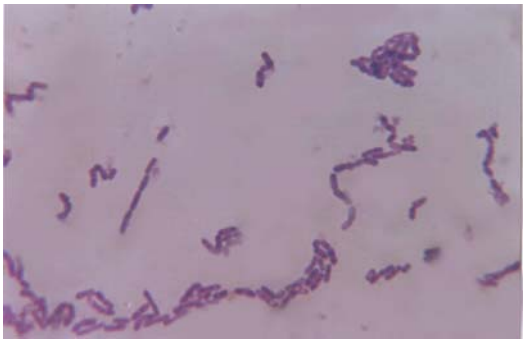

Fig. 6. Smear from pure culture of Bacillus sp. is stained with Gram's stain: Note Gram-negative, rods in chain form (x 830)

Pneumonia (chronic interstitial pneumonia, broncho-pneumonia, congested stage of pneumonia, fibrinous pneumonia, purulent pneumonia) are caused by *Pasteurella multocida* (Shafarin *et al*., 2007; Zamri-saad *et al*., 2006); *Pasteurella haemolytica* (Brogden *et al*., 1998); *Corynebacterium* sp. (Sharma and Dwivedi, 1977); *Staphylococcus* sp. and *Streptococcus* sp. (Almeida *et al*., 1986; Ugochukwu, 1986) and *Escherichia coli* (Kapur *et al*., 1976). However, *Pasteurella* sp. (11.7%), *Escherichia coli* (6.7%), *Staphylococcus* sp. (36.7%) and *Bacillus* sp. (3.3%) were identified.

It cannot be clearly explained the role of bacterial agents in the development of lung lesions. Neoplasms (Archer *et al*., 2007; De Las Heras *et al*., 2006) have also been reported. However, no attempt was made to characterize other aetiological agents except bacteria that warrant further study.

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