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BACTERIOLOGICAL AND PATHOLOGICAL INVESTIGATION OF GOAT LUNGS IN MYMENSINGH AND DETERMINATION OF ANTIBIOTIC SENSITIVITY

M. M. Rashid, M. J. Ferdoush, M. Dipti, P. Roy, M. M. Rahman, M. I. Hossain and M. M. Hossain*

Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202

ABSTRACT

The foremost important goal of the present study was to investigate the bacteriological and pathological conditions in lungs of goats slaughtered in four different slaughter houses/places of Mymensingh Sadar, Mymensingh and in addition to it, antibiotic sensitivity test of commonly used antibiotics in Veterinary practices were performed on isolated bacteria. A total of 75 lungs of slaughtered goats were examined individually and out of which 20 affected lungs were collected for histopathology and bacterial isolation respectively from Mymensingh Sadar, Mymensingh in Bangladesh, during the period from January to May 2013.The lung lesions was grossly recorded 40% in goats (30 out of 75 lungs examined). Grossly, the lung lesions were categorized into (a) hemorrhages 35% (b) congestion 25% (c) hemorrhage and congestion 15% (d) emphysematous lung 15% and (e) hepatization in lung10%. In histopathology, lung lesions were categorized into (a) bronchopneumonia 30%, (b) pneumonia 25% (c) hemorrhagic pneumonia 20% (d) emphysema 15%, (e) purulent pneumonia 10%. *Pasteurella sp.* (15%) was isolated from the lung lesions of hemorrhagic pneumonia, *E. coli.* (25%) from bronchopneumonia and *Staphylococcus sp.* (40%) from purulent pneumonia, focal pneumonia and emphysema, and mixed infection (*Staphylococcus sp.* and *E. coli*) 20%. Finally antibiotics sensitivity test was performed on isolated bacteria to which ciprofloxacin was more powerful than others (penicillin, amoxicillin, streptomycin, nalidixic acid and kanamycin) tested and the second one was oxytetracyclin.

Key words: Bacteria, pathology, goat lung, antibiotic sensitivity

INTRODUCTION

Lung, the important organ of respiratory system, is vulnerable to many infectious and non-infectious agents causing various pathological conditions in farm animals. Among the inflammatory and non-inflammatory disease conditions, pneumonia either acute or chronic causes debility and death leading to great economic loss to the farmers and distress (Alam *et al.*, 2001; Ferdausi *et al.*, 2008). Women rear only few goats that are grazed usually on free pastures. These pastures are usually found to be contaminated with various infectious agents that can get access through inhalation and thus they cause pneumonia. A large number of goats are being brought to the Veterinary Clinics in different parts of Bangladesh for their treatment. Among them, a considerable number is of respiratory problem, especially pneumonia. A variety of causes are responsible for development of pneumonia (Rahman *et al.*, 1976). The knowledge of pathogenesis, gross, microscopic changes of lung and antibiotics sensitivity test on isolated bacteria will help the veterinarian for diagnosis and treatment of the diseases. Related works in the context of Bangladesh on goat lungs lesions are very rare. This paper describes the occurrence of lung lesions, gross and microscopic changes and isolation, identification of bacteria from the affected lungs and also to determine antibiotics sensitivity test on isolated bacteria.

MATERIALS AND METHODS

Collection of samples

The samples were collected from four slaughter houses/places of Mymensingh Sadar (Masua bazar, KR market, Bolashpur and Kewatkhali). Lungs were collected from goats immediately after slaughter. A total of 20 affected lungs out of 75 examined were collected for pathological studies and 20 swabs were collected from inner core of lungs by cotton bud aseptically and immediately placed in Falcon tube containing 10ml nutrient broth for isolation of bacteria. All the samples were transferred to the Histopathology and Bacteriology Laboratory, Department of Pathology, Bangladesh Agricultural University, Mymensingh for histopathology, isolation and identification of bacteria respectively.

Gross pathology

A total of 20 samples were examined for gross abnormalities of lungs. Gross tissue changes were observed and recorded carefully, and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathological studies at least for 3-7 days.

*Corresponding e-mail address: mmhossain04@yahoo.com.au

Histopathology

The formalin-fixed 20 lung tissues containing lesion were trimmed, processed, sectioned and stained as per standard procedure (Luna, 1968).

Isolation and identification of bacteria

Swabs from each of 20 lung samples were incubated for 24 hours at 37°C. From the nutrient broth, subcultures were made in nutrient agar, blood agar, and EMB agar and incubated at 37°C for overnight. The identification of the organisms was performed by the tests as described by Alam *et al.* (2001), Ferdausi *et al.* (2008) and Tonu *et al.* (2011). On the basis of colony and staining characters the bacteria were grouped into four types: *Pasteurella sp., E. coli, Staphylococcus sp*., and mixed colonies of *Staphylococcus sp.* and *E. coli*. . Two representative samples of *Pasteurella sp*. and three of *E. coli* were tested for biochemical test using sugars.

Polymerase chain reaction (PCR) for the detection of *E. coli*

PCR is a powerful technique used for DNA amplification. PCR was carried out in the present experiment in a final reaction volume of 25 - 200µl capacity thin walled PCR tubes in a programmable thermocycler using Promega PCR Master Mix Kit. The primers (Tonu *et al.*, 2011) used for the detection of the *E. coli* were shown in Table 1.

Table 1. Prmers used for the detection of *E. coli*

Primer	Primer sequence (5'-3')	Amplification products (bp)
ECO-f	GACCTCGGTTTAGTTCACAGA	585
ECO-r	CACACGCTGACGCTGACCA	

Table 2.Composition of reaction mixture for PCR per reaction

Component	Volume/ reaction
PCR Master Mix	12.5 µl
Primer Forward (100 pmol/µl)	0.5 µl
Primer Reverse (100 pmol/ µl)	0.5 µl
Nuclease free water	6.5 µl
Total (Master mix)	20 µl

Five microliter water was added instead of DNA to the control tube. The tube was then centrifuged shortly in mini centrifuge machine.

Thermal profile

Specific primers ECO-f and ECO-r targeting 16S rDNA were used in amplification reaction with a thermal profile :initial denaturation at 94^{o}C for 3 min, followed by 35 cycles for 94^{o}C for 30 seconds, annealing at 60^{o}C for 1 min, and extension at 68^{o}C for 2 min, with a final extension at 68^{o}C for 7 minutes and reaction was hold at 4^{o} C (Tonu *et al.*, 2011).

Electrophoresis of the amplified products and documentation

Agarose gel electrophoresis was conducted in 1X TAE buffer at 90 V for 30 minutes. One molecular weight marker 100bp DNA ladder size was electrophoresed alongside the PCR products. DNA bands were observed under UV light on a transilluminator and photographed by image documentation system.

Antibiotics sensitivity test

After preparation of nutrient agar plate, 20 µl of nutrient broth containing culture of bacteria was poured on agar plate and it was spread by L-shaped ladder.

Then prepared filter paper disc and commercial antibiotics disc (penicillin, amoxicillin, streptomycin, nalidixic acid and kanamycin) were placed on the agar plate by same distance according to centre by sterile forceps. Then it was incubated at 37ºC for over night. No growth of bacteria around the particular disc was considered as sensitive to that antibiotic, growth of bacteria around the disc was considered as resistance to that particular antibiotic and partial growth was considered as partially resistant to that particular antibiotic (Dipti, 2013).

RESULTS

A total of 75 goat lungs were examined and 30 were found to be apparently abnormal in naked eye (Table 3). Out of 30 abnormal lungs, 20 were collected for pathological and bacteriological investigation. The occurrence of affected goats with lung disorders at 4 slaughter houses/places was about 40%. Grossly the lung lesions were categorized into following types: (a) Hemorrhages 35% (b) Congestions 25% (Fig. 3) (c) hepatization (red and gray) of lungs 10% (Fig. 1 and 2), (d) hemorrhage and congestions 15% (e) Emphysema 15% (Table 4).
In histopathology, the lung lesions were categorized into: (a) pneumonia 25% (Fig. 3), (b) bronchopneumonia 30% (Fig. 4 and 5), (c) emphysema 15% (Fig. 5), (d) purulent pneumonia 10%, and (e) hemorrhagic pneumonia 20% (Fig. 5, Table 5).

The prevalence of *Pasteurella* sp. was 15% (Fig. 7), *E. coli* 25%, *Staphylococcus* sp. 40% and mixed infection (*Staphylococcus* sp. and *E. coli*) 20%. *Pasteurella* sp. was isolated from pneumonia and hemorrhagic pneumonia. *E. coli* was isolated from hemorrhagic pneumonia, pneumonia (severe) and bronchopneumonia. *Staphylococcus sp*. isolated from emphysema, bronchopneumonia and purulent focal pneumonia (Fig. 6).

a) Pneumonia

The prevalence of pneumonia was recorded 25% (5 out of 20 lungs). There was presence of hemorrhages and in some cases congestion in lung. Microscopically, there was excessive hemorrhages within the alveoli and inter alveolar septa associated with leukocytic infiltration. Haemorrhage and leukocytic infiltration were found in the bronchus. The wall of the bronchus also showed the inflammatory changes and mild necrosis in the mucosal layer. The lesions of 5 lungs were variable.

b) Broncho- pneumonia

The prevalence of broncho-pneumonia was recorded 30% (6 out of 20 lungs). The affected portion of lung was congested. In addition, the lung was consolidated on palpation. Microscopically, all 6 lungs showed exudates and infiltration of inflammatory cells mainly neutrophils in the bronchiolar wall as well as in the lumen of bronchiole. There was also deposition of exudates within the bronchi and alveoli.

c) Purulent pneumonia

The prevalence of purulent pneumonia was recorded 10% (2 out of 20 lungs). All 2 lungs showed hepatization (1 red and 1 gray). Microscopic lesions were characterized by the presence of inflammatory cells consisted of mainly neutrophils within the alveoli and in the lumen of the bronchioles in lung with gray hepatization while other one showed same lesions but with increased level of erythocytes in alveoli of lung which was the characteristics of red hepatization. In addition to this, adjacent to the bronchioles, there were focal infiltrations of large number of neutrophils and few lymphocytes .

d) Hemorrhagic pneumonia

The prevalence of hemorrhagic pneumonia was recorded 20% (4 out of 20 lungs). The lungs showed severe hemorrhage and congestion in all 4 lungs. Microscopically, there was excessive hemorrhages within the alveoli and inter alveolar septa associated with leukocytic infiltration. Hemorrhages and leukocytic infiltration were also found in the bronchus. The wall of the bronchus also showed the inflammatory changes. All 4 lungs did not show the similar level of intensity of hemorrhage.

(e) Emphysema of lung

The prevalence emphysema of lung was recorded 15% (3 out of 20 lungs). Emphysematous areas of all 3 lungs showed pale colour with elevated areas and that was easily diagnosed by compression of finger. Microscopically, many alveoli were distended and many had wide opening into each other or into a common space due to rupture of alveolar walls. The alveolar wall was thin and atrophic.

B. Bacterial isolation and identification

Out of 20 lungs 3 were positive to *Pasteurella* sp. (15%), 5 were positive to *E. coli* (25%), 8 were positive to *Staphylococcus sp.* (40%) and 4 others were found to be mixed infection (20%) (Table 6).

Table 3. Investigation of goat after slaughtering

Name of the slaughter houses/places	No of goat examined	No of goat affected	% Affected
Masua bazar, Sadar	35	15	42.86
Kewatkhali	20	10	50
KR market	12	2	16.67
Bolashpur	8	3	37.5
Total	75	30	40

Table 4. Gross pathology of lung (N = 20)

Lung lesions	No. of lung affected	%Affected
Hemorrhage	7	35
Congestion	5	25
Hemorrhage and congestion	3	15
Emphysema of lung	3	15
Hepatization of lung	2	10

Table 5. Histopathology of lung (N = 20)

Lung lesions	No. of lung affected	% Affected
Pneumonia	5	25
Bronchopneumonia	6	30
Hemorrhagic pneumonia	4	20
Emphysema	3	15
Purulent pneumonia (focal)	2	10

Table 6. Prevalence of bacteria in lung (N = 20)

Findings	Types of lung lesions	No. of lungs affected	% Bacterial isolation
Staphylococcus sp.	Bronchopneumonia, purulent and focal pneumonia	8	40
E. coli	Bronchopneumonia, hemorrhagic pneumonia and pneumonia (severe)	5	25
Pasteurella sp.	Pneumonia and hemorrhagic pneumonia	3	15
Mixed infection (*Staphylococcus* sp. and *E. coli*)	Bronchopneumonia hemorrhagic pneumonia and purulent pneumonia	4	20

The prevalence of *Staphylococcus* sp. was recorded 40% (8 out of 20 lungs). They grew well on nutrient agar media and showed smooth circular, opaque often yellow-pigmented colony about 1 mm in diameter after overnight of incubation at 37°C. In nutrient broth, they grew well and showed dense turbidity with a powdery deposit. Smears from pure cultures revealed gram positive and round shaped bacteria occurring in cluster form.
The prevalence of *Pasteurella* sp. was recorded 15% (3 out of 20 lungs). They grew well on nutrient agar media and showed smooth, circular, grayish colony about 1 mm in diameter after overnight incubation at 37°C. In nutrient broth, they grey well and showed diffuse turbidity. Small grayish hemolytic zones were evident in blood agar media. Smear from pure culture revealed gram negative, bipolar, small rod shaped bacteria. In Leishman's stain bipolar characteristics of the organisms were diagnosed which took blue color. *Pasteurella* sp. fermented the sucrose, dextrose, maltose with production of acid but did not ferment lactose. So, isolated organisms were *Pasteurella* sp.

The prevalence of *E. coli* in lung swabs was 25% (5 out of 20 lungs). The organism produced smooth circular colonies with dark centers and metalic sheen on EMB agar. *E. coli* was a short rod, varying from coccoid bipolar shapes to long filamentous forms. It occurred singly or short chains and they were gram negative. Motility test was performed by hanging drop slide as mass movement was found (Merchant and packer, 1967). *E. coil* fermented dextrose, lactose, maltose, mannitol and sucrose. Two isolates of each group of *E. coli* were tested by ECO-f and ECO-r primer and showed 585-bp products after 1% agarose gel electrophoresis and found clear band formation (Fig. 8).

Antibiotics sensitivity test

Staphylococcus sp. was slightly sensitive to penicillin while *Pasteurella* sp. and *E. coli* were resistant to penicillin. *Staphylococcus* sp., *Pasteurella* sp. and *E. coli* were highly sensitive to oxytetracycline, streptomycin, kanamycin and ciprofloxacin. *Staphylococcus* sp., *Pasteurella* sp. and *E. coli* were partially resistant to amoxicillin. *Staphylococcus* sp. was sensitive to nalidixic acid while *Pasteurella* sp. and *E. coli* were completely resistant to nalidixic acid (Table 5 and Fig. 9).

Table 5. Antibiotics (available in veterinary practice) sensitivity test on isolated bacteria

Antibiotics	Name of isolated bacteria		
	Staphylococcus sp.	*Pasteurella* sp.	*E. coli*
Penicillin	±	-	-
Oxytetracycline	+	+	+
Amoxycillin	±*	±*	±
Streptomycin	+	+	+
Ciprofloxacin	+	+	+
Kanamycin	+	+	+
Nalidixic acid	+	-	-

N.B: Partially sensitive = ±, Partially resistant = ± *, Sensitive =+ and Resistant= -

DISCUSSION

The present investigation was conducted on slaughtered goats in Mymensingh Sadar to categorize the different lung lesions, isolation of bacteria, and finally antibiotics sensitivity test (commonly used antibiotics in Veterinary practices) was performed on isolated bacteria. In the present study, the prevalence of gross lung lesions was recorded 40% in Black Bengal (it was not mentioned in M&M and result sections) goat at Mymensingh in Bangladesh. Ferdausi *et al.* (2008) and Alam *et al.* (2001) recorded the gross lung lesions were 58.33% and 6.66%, respectively. This variation compared to present investigation might be due to some calculating factor and frequency of diseases. Ugochukwu (1985) reported gross lung lesions of goat with an incidence of 75%. But Almeida *et al.* (1986), Kaya and Erganis (1991) reported an incidence of 35.5% and 55.8%, respectively. On the basis of gross findings, this variation of incidence might be due to variation of geographical location or virulence of the causal agent. However, the findings of present investigation were almost similar to other authors (Almeida *et al.*, 1986, 35.5% and Kaya and Erganis, 1991, 55.8%). Though geographical location and breeds of goats were different, the prevalence of lung lesion in present investigation was less than that of findings of Ugochukwu (1985) who reported 75% incidence.

In this investigation, the highest prevalence of histopathological lung lesions was bronchopneumonia (30%) and the isolated bacteria were *Pasteurella sp*. and *E. coli* from this lesion. The second highest prevalence of lungs lesions recorded 25% were pneumonia then hemorrhagic pneumonia (20%) and emphysema (15%). The less commonly occurring lungs lesions were purulent pneumonia (10%) and the isolated bacteria was *Staphylococcus sp*. Ferdausi *et al.* (2008) reported that histopathologically the lung lesions were pneumonia 6.67%, bronchopneumonia 3.33%, purulent pneumonia 5%, hemorrhagic pneumonia 3.33% etc. This variation might be due to some calculating factor as authors calculated out of total examined goat but this investigation was conducted within only affected lungs of goats. Histopathology of bronchopneurnomia and hemorrhagic pneumonia described in this present investigation corresponded to the lesions of other investigators (Jones *et al.*, 1997; Alam *et al.*, 2001; Akbor *et al.*, 2007).

Figure 1. Red hepatization of lung

Figure 2. Gray hepatization of lung

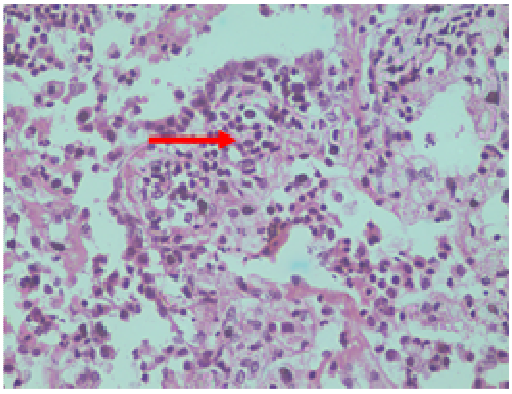

Figure 3. Section of goat lung showing pneumonia (severe) with *E. coli* (H & E; 40X)

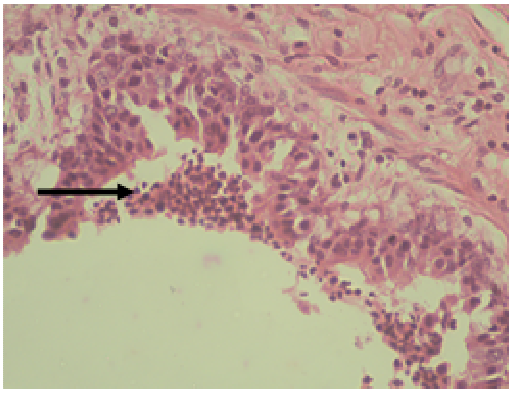

Figure 4. Section of goat lung showing bronchopneumonia with *E. coli* infection with (H & E; 40X)

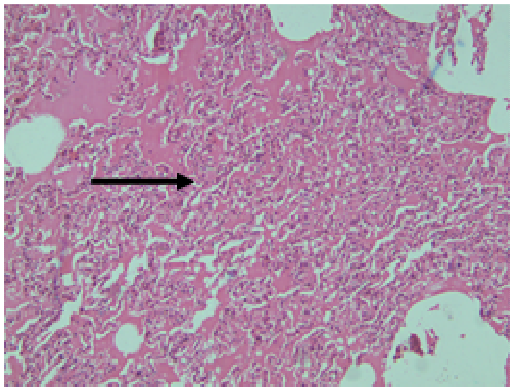

Figure 5. Section of goat lungs showing mixed infection with *E. coli* and Staphylococcus sp. by bronchopneumonia and hemorrhagic pneumonia (H & E; 10X)

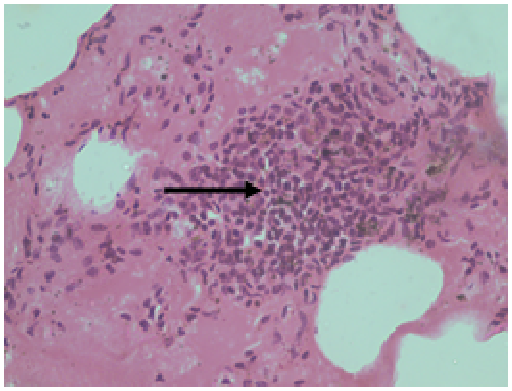

Figure 6. Section of goat lung showing focal purulent pneumonia with Staphylococcus sp. infection (H &E ; 40X)

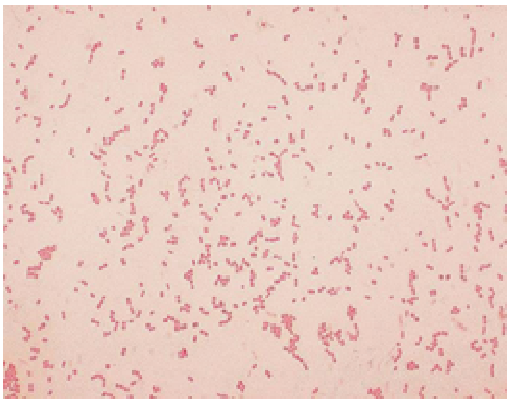

Figure 7. Smear from pure culture of *Pasteurella* sp. and stained with Gram's stain: Gram negative bipolar rod shaped bacteria

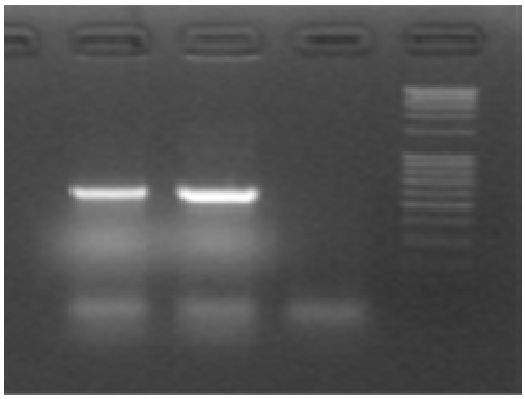

Figure 8. M= 100bp Marker, NC= Negative control, S1= sample 1 of E. coli isolate and S2=sample 2 of E. coli isolate

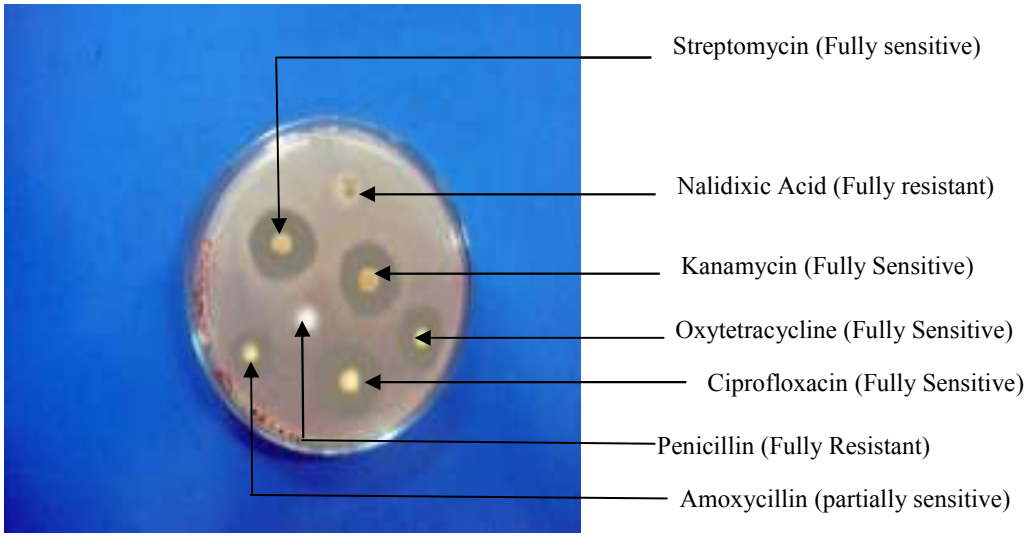

Figure 9. Antibiotics sensitivity test on isolates of *E. coli*

In this investigation three types of bacteria like *Pasteurella* sp. (15%), *Escherichia coli* (25%) and *Staphylococcus sp.* (40%) were isolated and identified by cultural character, staining properties and by PCR for *E. coli* only by using primer (ECO-r and ECO-f). Ferdausi *et al.* (2008) reported *Pasteurella* sp. (11.67%), *Staphylococcus* sp. (36.67%) and *Bacillus sp.* (3.33%). This investigation could not find out *Baillus* sp. but isolation of *Staphylococcus sp*. (40%) and *Pasteurella* sp. (15%) were almost similar to that of findings. *Pasteurella* sp. was isolated from pneumonia, hemorrhagic pneumonia and emphysematous lungs. *E. coli* was isolated from bronchopneumonia and severe pneumonia.

In bronchopneumonia the peribronchial tissue of the bronchus showed severe inflammatory lesions. So, on the basis of this finding, it might be speculated that the path of infection was in bronchogenous route. However, in recent theory whatever the route of infection, the severity of lesion depends on the virulence of causal agent (Jones *et al.*, 1997). The histopathological lesions of pneumonia and emphysema were similar with the description of other authors (Jones *et al.*, 1997; Alam *et al.*, 2001; Akbar *et al.*, 2007).

Staphylococcus sp. was isolated from focal pneumonia and purulent pneumonia. As this bacteria is known as pyogenic bacteria that's why it might be speculated that this purulent pneumonia was produced by the infection of Staphylococcus sp. The lesion of purulent pneumonia was scattered in the lung parenchyma. On the basis of the distribution of lung lesion, the path of infection might be due to hematogenous route.

Staphylococcus sp. was slightly sensitive to penicillin while *Pasteurella* sp. and *E. coli* were resistant to penicillin. *Staphylococcus* sp., *Pasteurella* sp. and *E. coli* were highly sensitive to oxytetracycline, streptomycin, kanamycin and ciprofloxacin. *Staphylococcus* sp., *Pasteurella* sp. and *E. coli* were partially resistant to amoxicillin. *Staphylococcus* sp. was sensitive to nalidixic acid while *Pasteurella* sp. and *E. coli* were completely resistant to nalidixic acid..

Finally, this study on lung lesions in goat has focused the different disease conditions and antibiotics sensitivity test on isolated bacteria. In maximum cases, the frequency of pathogenicity of causative agents was not studied from the lesions. In near future, the attempts should be taken to detect the pathogenicity of the causative agents.

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