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Bacteriological and Histopathological Investigation of Pneumonia in Black Bengal Goat

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Abstract

The study was designed to evaluate the isolation and identification of causative bacteria from caprine pneumonic lungs, antibiotics sensitivity test with isolated bacteria, gross and histopathological changes to determine the types of pneumonia. A total of 210 Black Bengal goat carcasses were subjected to gross examination and infected with pneumonia were collected for bacteriological and histopathologica study. Pneumonia was detected in 40 carcasses. Bacteriological examinations revealed the presence of ovine pathogens, such as *Staphylococcus spp*. (35%), *Escherichia coli* (25%), Pasteurella spp. (20%), *Streptococcus spp.* (15%), and Klebsiella spp. (5%). The antibiogram study indicated that the most effective antimicrobial agents against all the tested isolates were ciprofloxacin; ceftriaxone; and oxytetracycline; could be the choice of antibiotics for the treatment of pneumonia in Black Bengal goats. The gross lesions were recorded as hemorrhage (25%), congestion (20%), hemorrhage and congestion (30%), emphysema (15%), and hepatization (10%) of lungs. Based on the histopathological study, 30% of affected carcasses were bronchopneumonia, 25% with fibrinous bronchopneumonia, 20% with hemorrhagic pneumonia, 15% with interstitial pneumonia, and 10% with purulent bronchopneumonia. Both gross and histopathological lesions of above diseases were enunciated. However, other etiology may also have a considerable effect on the occurrence of pneumonia in Black Bengal goat of Bangladesh.

Keywords: Antibiogram; Black Bengal goat; Histopathology; Isolates; pneumonia; Livestock; Etiology; Mortality; Morbidity; Shipping fever; Ciprofloxacin; Ceftriaxone; Oxytetracycline; Bronchopneumonia; Capsules; Bacteria; Virus; Parasites; Fungi

Introduction

Black Bengal goats called as a "poor man's cow" is the second important livestock in Bangladesh. Goat rearing is easy, less expensive, less laborious and highly profitable business [1], is seriously hampered due to various diseases. The diseases affecting the respiratory tract: Pesti Des Petits Ruminants (PPR), Contagious Caprine Pleuropneumonia (CCPP) and Pasteurellosis impose a substantial loss through high morbidity and mortality [2,3]. Furthermore, most of the infectious agents that because the respiratory disease is ubiquitous in nature and are normal inhabitants of the nasopharynx [2]. Unfortunately, a large number of goat and lamb population die due to pneumonia at the early stage of their lives including reduced growth rate, carcass condemnation and consequent substantial economic impact on animal husbandry because of the need of medicine and vaccination programs [3,4]. Pneumonia sometimes called goat "shipping fever" is one of the most common problem encountered today associated with expensive treatments. Therefore, early diagnosis and proper treatment are necessary for effective control of the diseases [5].

Pneumonia caused by the interaction of several pathogens (bacteria, virus, parasites, fungi), host defence, environmental factors [6], and stress [7,8]. Though viral, fungal and other causal agents are responsible for the goat pneumonia, the present study conducted to investigate pneumonia caused by bacterial pathogens. Some bacteria as like Pasteurella spp. and *Staphylococcus spp.* normally found in the respiratory tract of healthy goat, however, they sometimes cause pneumonia during the stress conditions or in association with other pathogens. Klebsiella spp. causing pneumonia, while it produces virulence factors such as smooth lipopolysaccharide, pili for adhesion to host cells, capsules (K antigen) that are antiphagocytic aid the bacterium in its competition with the host for iron uptake [9,10]. The respiratory problem in kids less than one year thought to be an association with Pasteurella spp. and Parainfluenza-3 [11].

For the prevention and control of goat pneumonia, isolation and characterization of causal agents and antibiogram study of isolates are essential. Thus, a present study was undertaken to isolate the pathogen responsible for pneumonia in Black Bengal goat with their antibiogram and histopathological study.

Materials and Methods

Sample Collection and Processing

A total of 210 lung samples was obtained from different slaughterhouses of Bangladesh subjected to gross examination for the presence of pneumonic lesions. No details of sex breed or husbandry conditions of goat were available. Lungs with microscopic pneumonic lesions were attained by excluding parasitic lesions. Following the gross inspection, all the samples of apparently affect lungs transferred to the Microbiology and Pathology laboratory for bacteriological and histopathological examination. Samples of affected areas aseptically collected and placed in sterile plates. The outer surface of the pneumonic lungs first seared with a heated spatula before cutting the inner surface of lungs. The lung swabs aseptically collected from the inner core of lungs by cotton bud and immediately placed into the Falcon tube containing 10ml nutrient broth for isolation of causative bacteria.

Isolation and Identification of Causative Bacteria

Primary culture was performed in both nutrient agar and broth media. For sub-culturing, suspected bacteria were inoculated separately into different bacteriological agar media under the aseptic condition and subsequently incubated at 370C for 24 hours. Pure culture was made as per the procedures described by Cheesbrough, Buxton & Fraser [12,13]. In order to identify the isolated bacteria, cultural, morphological, and biochemical characteristics were performed. The cultural characteristics or colonial morphology of the bacteria grown on the nutrient and blood agar media was recorded. Gram staining method was performed to study the morphology and staining characteristics of bacteria according to the technique described by Merchant and Packer. Motility test was executed to differentiate motile bacteria from the non-motile one. Hemolytic activity of the bacteria has done according to the procedure mentioned by Carter [14]. Biochemical tests, such as- sugar fermentation, catalase, methyl red, indole production, the Voges-Proskauer, citrate utilization, TSI (Triple sugar iron) agar slant reaction, lysine iron agar, oxidation fermentation was performed according to the standard methods [13]. The classification and specification of the organisms were done by following the scheme presented in Animal Microbiology [12]. The stock culture was maintained based on the procedures of Carter et al. [15].

Antimicrobial Sensitivity Test

A disc diffusion method [16,17] was used to determine the susceptibility of the bacterial isolates against antibiotic agents. For this, commercially available 11 different antibacterial discs (Oxoid Ltd., Baring-stoke Hampshire, England) were used. The names and concentrations of the antibacterial discs were mentioned in Table 3. The interpretation on antibiotic susceptibility was recorded according to the guidelines of Clinical and Laboratory Standard Institute.

Pathological study of pneumonic lungs

All the positive (infected) lung samples carefully examined for gross abnormalities. The gross tissue lesions were observed and recorded carefully, and representative part of tissue samples was fixed in Bouin's fluid for further histopathological studies. Afterward, the preserved samples were dehydrated in alcohol, cleared in xylene, impregnated and embedded in paraffin wax, sectioned at seven (7)µm and finally stained with hematoxylin and eosin (H&E) for histopathological examination as described by Luna [18].

Results

Isolation and Identification of Causative Bacteria

The prevalence of Klebsiella spp. 5%, *Streptococcus spp*. 15%, Pasteurella spp. 20%, *Escherichia coli* 25%, *Staphylococcus spp.* 35%. Klebsiella spp. was isolated from bronchopneumonia and interstitial pneumonia. *Streptococcus spp*. and *Staphylococcus spp.* Isolated from bronchopneumonia, hemorrhagic pneumonia, and purulent bronchopneumonia. Pasteurella spp. and *Escherichia coli* were isolated from hemorrhagic pneumonia, bronchopneumonia and fibrinous bronchopneumonia (Table 1).

Table 1: Prevalence of isolated bacteria in affected pneumonic lungs (n=40).

No.	Isolated Bacteria	Types of Pneumonia observed in Pneumonic Lungs	No. of Lungs affected	% of Bacteria Isolation
1	*Streptococcus spp.*	Bronchopneumonia, hemorrhagic pneumonia and purulent bronchopneumonia	6	15
2	*Staphylococcus spp.*	Bronchopneumonia, interstitial pneumonia and purulent bronchopneumonia	14	35
3	*Escherichia coli*	Bronchopneumonia, hemorrhagic pneumonia and fibrinous bronchopneumonia	10	25
4	*Klebsiella spp.*	Bronchopneumonia and interstitial pneumonia	2	5
5	*Pasteurella spp.*	Bronchopneumonia, hemorrhagic pneumonia and fibrinous bronchopneumonia	8	20

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Biochemical Tests

Biochemical tests were conducted with all the isolates of causative bacteria by using five sugar media such as dextrose, maltose, lactose, sucrose, and mannitol. The detailed characterization of all the bacterial isolates is tabulated in Table 2.

Table 2: Results of biochemical characteristics of isolated bacteria from lung samples of Black Bengal goat.

Parameters	Characteristics of Bacterial Isolates				
Gram's reaction	+ve	+ve	-ve	-ve	-ve
Motility	-	-	+	-	-
Catalase test	-	+	+	+	+
Citrate test	-	-	-	+	-
Indole test	-	-	+	-	+
MR test	+	+	+	-	+
VP test	+	+	-	+	-
Growth on TSI	N/A	N/A	Slant-Red,	Slant-Red,	N/A
			Butt-Yellow	Butt-Yellow	
Growth on LIA	N/A	N/A	Slant and	N/A	N/A
			Butt-Red		
Sugar fermentation					
Dextrose	A	A	A & G	A & G	A
Maltose	A	A	A	A	-
Lactose	A	A	A	A & G	-
Sucrose	A	A	A	A	A
Mannitol	A	A	A	A	A
Most probable	Streptococcus	Staphylococcus	Escherichia	Klebsiella	Pasteurella
bacteria	spp.	spp.	coli	pneumoniae	spp.

N/A: Not applicable; A: Acid production only but no gas production; (+): Positive reaction; A & G: Acid & gas production; (-): Negative reaction.

Antibiogram Study

Antibiotic sensitivity pattern of different isolates was performed against eleven commonly used antibacterial agents. The antibiogram study was revealed that the isolates are highly sensitive to ciprofloxacin, oxytetracycline, and ceftriaxone, moderately sensitive to streptomycin, cefalexin, nalidixic acid, erythromycin, and resistant to gentamicin, bacitracin, penicillin, amoxicillin. The summary of antibiotic sensitivity pattern is shown in Table 3.

Table 3: Antibacterial sensitivity pattern of isolated bacteria.

Antibacterial Agents	Disc Concentration (µg / disc)	*Streptococcus spp.*	*Staphylococcus spp.*	*E. coli*	*Klebsiella pneumoniae*	*Pasteurella spp.*
Penicillin	10 unit / disc	++	++	-	-	-
Bacitracin	10 unit / disc	++	++	-	-	-
Streptomycin	25	+++	+++	++	-	++
Amoxicillin	30	++	++	-	-	-
Gentamycin	10	++	++	-	-	-
Erythromycin	15	+++	+++	++	++	++
Cefalexine	30	+++	+++	++	++	++
Ceftriaxone	30	+++	+++	+++	+++	+++
Nalidixic acid	30	+++	+++	-	-	-
Ciprofloxacin	30	+++	+++	+++	+++	+++
Oxytetracycline	25	+++	+++	+++	+++	+++

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Histopathology of Pneumonic Lungs

Grossly the lung lesions (Table 4) were categorized into following types: (a) hemorrhage 25% (b) Congestion 20% (c) hemorrhage and congestion 30% (d) emphysema 15% (e) hepatisation of lungs 10% (Figures 1 & 2). From histopathological study of pneumonic lung tissues of Black Bengal goat, five types of pneumonia were identified according to their histopathological changes (a) bronchopneumonia (30%), (b) fibrinous bronchopneumonia (25%), (c) hemorrhagic pneumonia (20%), (d) interstitial pneumonia (15%), and (e) purulent bronchopneumonia 10% (Table 5).

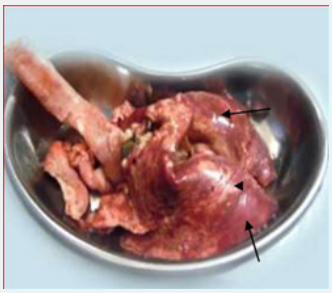

Figure 1: Hemorrhage and congestion of lungs.

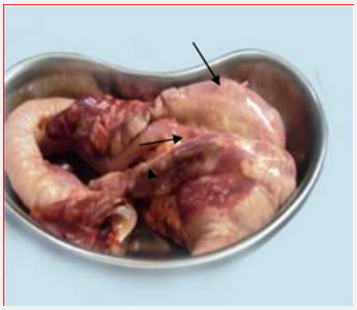

Figure 2: Emphysema and gray hepatisation of lungs.

Table 4: Gross pathological lesions of pneumonic lungs (n=40).

No.	Types of lung lesions	No. of lungs affected	% Affected
1	Hemorrhage	10	25
2	Congestion	8	20
3	Hemorrhage and congestion	12	30
4	Emphysema of lung	6	15
5	Hepatization of lung	4	10

Table 5: Different types of pneumonia observed in pneumonic lungs of Black Bengal goats (n=40).

No.	Types of pneumonia	No. of lungs affected	% Affected
1	Bronchopneumonia	12	30
2	Fibrinous bronchopneumonia	10	25
3	Hemorrhagic pneumonia	8	20
4	Interstitial pneumonia	6	15
5	Purulent bronchopneumonia	4	10

Lungs with bronchopneumonia were characterized by exudates in the alveoli, congestion of blood vessels, hemorrhages and sometimes hyperplasia of associated bronchial lymphoid tissues (Figure 3A). Lungs with fibrinous bronchopneumonia were characterized by inflammatory zone around the respiratory bronchiole with abundant fibrinous exudates and visible fibrin strand, interlobular septa, distended pleura and alveoli because of fibrinous exudation and neutrophilic exudation (Figure 3B). Hemorrhagic pneumonia was categorized by excessive haemorrhages within the bronchi, alveoli and interalveolar septa related to leukocytic infiltration (Figure 3C). While the lungs with interstitial pneumonia were considered by severe congestion of blood vessels, hemorrhage in the alveoli, inflammatory cells in the lumen of bronchus, reactive cells in and around the bronchial wall, and sometimes inter-alveolar septa were thickened due to the accumulation of mononuclear cells and proliferation of fibrous connective tissue (Figure 3D). Bronchopneumonia with endothelial damage marked the consolidation of the parenchyma with many neutrophils in the alveoli and severe desquamation of the bronchial epithelium with hyperplasia of the bronchial associated lymphoid tissues was categorized as purulent bronchopneumonia (Figure 3E).

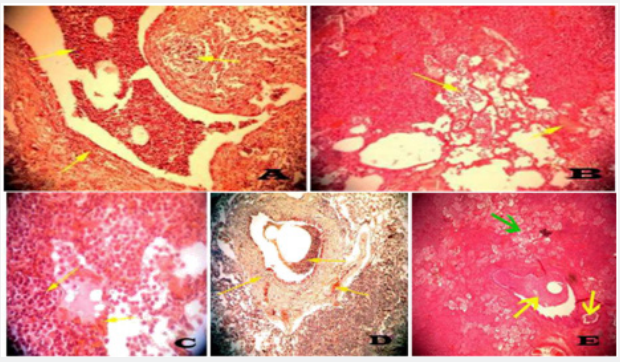

Figure 3: Microscopic appearance of different types of pneumonia.

Discussion

In our current study, five types of bacteria isolated and identified by biochemical and staining properties from pneumonic Black Bengal goats. These isolated organisms are common in pneumonic lung tissues as widely documented by several authors [9,19-24]. These bacteria are also common in pulmonary mixed infections since the respiratory pathways act as a reservoir for potentially pathogenic micro- organisms which develop into pneumonia following stress, decline of hygiene measures or climatic conditions [25], with obvious pneumonic lesions than when a single bacterium was incriminated [26]. *Staphylococcus spp.* was the major organism followed by *Escherichia coli* and Pasteurella spp. isolated from lung swabs [27,28]. *Staphylococcus spp.* resides in the upper respiratory tract and is involved in diseased processes only when stress conditions prevail [29]. Ferdausi et al. [30] reported the prevalence rate of *Staphylococcus spp.*, *Pasteurella spp.*, and *Bacillus spp.* were 36.67%, 11.67%, and 3.33%, respectively.

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However, our current study could not find out any Bacillus spp. but the prevalence rate of *Staphylococcus spp.* and Pasteurella spp. Were almost similar with findings. Rashid et al. [31] also observed the similar data likewise *Staphylococcus spp.* (40%), *Escherichia coli* (25%), Pasteurella spp. (15%) and mixed infections (Escherichia coli and *Staphylococcus spp.* 20%) which were almost adjacent to the present findings, but mixed infections aren't considered in our present work. *Staphylococcus spp.* and *Streptococcus spp.* isolated from bronchopneumonia, interstitial pneumonia, and purulent bronchopneumonia. As this bacterium is known as pyogenic bacteria thus, it might speculate that this purulent bronchopneumonia produced by these microbes. Lesions of purulent pneumonia were scattered in the lung parenchyma. Based on the distribution of lung lesions, the path of infection might be due to the hematogenous route. The antibiogram study revealed that the *Streptococcus spp.* and *Staphylococcus spp.* Were moderately sensitive to penicillin, bacitracin, amoxicillin, and gentamycin while *Escherichia coli,* Klebsiella spp. and Pasteurella spp. were resistant. Furthermore, *Streptococcus spp.* And *Staphylococcus spp.* were highly sensitive to streptomycin, erythromycin, cephalexin, ceftriaxone, nalidixic acid, ciprofloxacin, and oxytetracycline while *Escherichia coli,* Klebsiellapneumonia and Pasteurella spp. were moderately sensitive to streptomycin, erythromycin, cephalexin. All isolated bacteria were highly sensitive to ciprofloxacin, oxytetracycline, and ceftriaxone. The findings recorded in this study are in agreement with the results of Ghanem et al [9,21,32-37].

In consideration of the most effective antimicrobial agents against all the tested isolates, ciprofloxacin, oxytetracycline and ceftriaxone [38] are the drugs of choice in the treatment of pneumonia in Black Bengal goats. The variation of antibiotic sensitivity pattern of the respiratory bacterial isolates might be due to the presence of resistance genes. Indiscriminate use of antibiotics for treating the infected animals might also be responsible for acquiring antibiotic resistance. In this investigation, grossly the lung lesions categorized into hemorrhage 25%, congestion 20%, hemorrhages and congestion 30%, emphysematous lungs 15%, hepatization in lungs 10%. Pus and cysts in lungs recorded by Jubb et al. [39]. However, no such lesions recorded in the present investigation. The highest prevalence recorded for bronchopneumonia followed by fibrinous bronchopneumonia, hemorrhagic pneumonia, interstitial pneumonia, and purulent bronchopneumonia. Histopathological study of pneumonic lungs by Ferdausi et al. [30] reported pneumonia 6.67%, bronchopneumonia 3.33%, purulent bronchopneumonia 5%, and hemorrhagic pneumonia 3.33%. This variation might be due to some calculating factor as authors calculated out of total examined goat, but this investigation conducted within only affected lungs of a goat. Histopathology of bronchopneumonia and hemorrhagic pneumonia described in this present investigation corresponded to the lesions of other investigators [40-47].

Conclusion

The results of a bacteriological and histopathological study of pneumonic lung tissues indicated that the microbial factors might play an important role in the development of pneumonia in Black Bengal goat. However, there are some effective antimicrobial agents like ciprofloxacin; ceftriaxone; and oxytetracycline for the treatment of pneumonia. Nevertheless, some bacterial organisms such as Pasteurella spp., *Streptococcus spp., Staphylococcus spp.,* Klebsiella spp [48-58]. And *Escherichia coli* responsible for the development of goat pneumonia. Further study for other etiology and molecular understanding of these bacteria required to characterize the causative agent of goat pneumonia.

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