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CHARACTERIZATION OF BACTERIA ASSOCIATED WITH PNEUMONIA IN BLACK BENGAL GOATS

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ABSTRACT

The present research work was undertaken for the characterization of the bacterial pathogens responsible for pneumonia in black Bengal goats. Nasal swab samples (n = 50) were collected from the pneumonic black Bengal goats in Mymensingh and Sirajgonj districts. Samples were inoculated onto nutrient agar, eosin methylene blue (EMB) agar, MacConkey agar, and blood agar media for isolation of bacteria. Identification of bacteria was performed by the Gram's staining method, cultural properties and biochemical tests. Antibiotic sensitivity of bacterial isolates was performed against 11 antimicrobial agents. *Pasteurella spp* were isolated from 25 cases, and *Staphylococcus spp* from 13 cases. Mixed infection caused by the *Pasteurella spp* and *Staphylococcus spp*. were recorded in 12 cases. *Pasteurella spp* produced whitish, opaque circular and translucent colonies on nutrient agar, smooth, convex, glistening colonies on EMB agar and no hemolysis on blood agar. *Staphylococcus spp* have shown gray white or golden yellowish colonies on nutrient agar. Golden yellow colonies without hemolysis or whitish colonies with hemolysis were also produced by *Staphylococcus spp*. on the blood agar media. *Pasteurella spp* were indole positive, MR-VP negative and ferment dextrose, sucrose and mannitol with the production of acid. The *Staphylococcus spp* were positive to MR-VP, coagulase and catalase reactions, negative to indole test and fermented five basic sugars with acid production. Results of cultural and biochemical tests supported that these two isolates belonged to *P. multocida* and *S. aureus*. *P. multocida* were highly sensitive to ciprofloxacin and resistant to penicillin. *S. aureus* found to be highly sensitive to erythromycin, tetracycline, enrofloxacin, and norfloxacin and less sensitive to amoxicillin.

Key words: Goats, pneumonia, bacteria, antimicrobial agents

INTRODUCTION

Goat is the second important livestock in Bangladesh. It plays an important role in the rural economy and earns substantial amount of foreign currency through exporting skin and other by products (Alam, 1993). The estimated goat population in Bangladesh in 2002 was 36.9 million and in 2001 was 34.4 million (FAO, 2003), most of which belong to black Bengal breed (Rahman *et al.,* 1976). It is mainly reared for meat, milk and leather production (Faruk *et al.,* 2006). Rearing of goat is easy, less expensive, less laborious and highly profitable. In Bangladesh goat farming is substantially hampered due to outbreaks of diseases.

Pneumonia in goat is an infection of the lungs characterized by fever (40-41 ºC), anorexia, painful coughing, dyspnea, mucopurulent nasal discharge and depression. It is one of the most common respiratory illnesses in goats throughout the world (Ackermann and Brogden, 2000). Although pneumonia is more frequently occur in kids but it also infects adult goats. Both infectious and non infectious agents are responsible for lung affection. Among the infectious agents *Pasteurella maltocida* and *Pasteurella haemolytica* are more frequently associated with the outbreak of acute pneumonia and death of goats in all age (Falade, 2002). These bacteria are commonly found in the upper respiratory tract of healthy goats. Poor managemental condition, transportation stress, overcrowding pens, sudden environmental changes, poor housing conditions, viral infection (e.g. parainfluenza-3 virus), lung parasites and other stressful conditions increase goats' susceptibility to pneumonias. Pneumonia caused by *P. multocida* and *P. haemolytica* can lead to wide spread financial losses because of death, reduced live weight, delayed marketing, treatment cost and unthriftiness among survivors (Davies *et al.,* 1997; Daniel *et al.,* 2006).

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Pneumonias were reported in the black Bengal goats in Bangladesh (Islam *et al.* 2006). To the best of our knowledge there is no report concerning the characterization of bacterial pathogens causing pneumonia in the black Bengal goats in Bangladesh. Identification of bacteria is essential for undertaking effective prevention and control measures against pneumonia in black Bengal goats. This study was therefore designed to characterize the bacterial pathogens associated with pneumonia and selection of appropriate antibiotic for treatment of this disease in goats.

MATERIALS AND METHODS

Collection of specimens

Nasal swab samples (n = 50) were aseptically collected from the pneumonic Black Bengal goats from Ullaparasadar upazilla, Sirajgang; Bangladesh Agricultural University (BAU) goat farm and BAU veterinary clinic. Goats were manifested the clinical signs of fever (40-41 ºC), anorexia, mucopurulent nasal discharge, coughing, anorexia and depression.

Isolation of bacteria

Nasal swab samples were inoculated separately onto the nutrient agar and blood agar, eosin methylene blue (EMB) agar and MacConkey agar media and incubated at 37 °C for 24 hours. The colonies on primary cultures were repeatedly sub-cultured by steak plate method until the pure cultures with homogenous colonies were obtained (Cheesbrough, 1985).

Characterization of bacteria

In order to identify and differentiate bacterial pathogens isolated from pneumonic goats different characteristics of bacteria such as: cultural characteristics, cellular characteristics and biochemical characteristics were studied.

Cultural characteristics or colonial morphology (e.g. size, margin, elevation and colour) of bacteria grown on the nutrient and blood agar media were recorded. Gram's staining method was performed to study the cellular morphology and staining characteristics of bacteria according to the technique described by Merchant and Packer (1967). Biochemical tests such as: sugar fermentation, coagulase, catalase, M-R, V-P and indole tests were performed according to the standard methods (Cheesbrough, 1985).

Antibiotic sensitivity assay

Disc diffusion assay was used to determined antimicrobial susceptibility of isolates against 11 different antimicrobial agents such as: amoxicillin, ampicillin, penicillin, tetracycline, erythromycin, azithromycin, enrofloxacin, norfloxacin, furazolidone, gentamicin and ciprofloxacin following the standard methods (NCCLS, 2003).

RESULTS AND DISCUSSION

Isolation of bacteria

Fifty nasal swabs were collected from 50 goats. *Pasteurella* spp were recovered from 25 cases and *Staphylococcus spp* were isolated from 13 cases. Mixed infection caused by *Pasteurella spp* and *Staphylococcus spp* were recorded in 12 cases. The distribution of bacteria in the nasal swab samples of pneumonic black Bengal goats is shown in Table1**.**

Table 1. Distribution of the bacteria in the nasal swabs samples of pneumonic black Bengal goats

No. of goats examined	Name of bacteria	No. of culture positive samples (%)
50	*Pasteurella* spp	25 (50)
	Staphylococcus spp	13 (26)
	Pasteurella spp + *Staphylococcus* spp	12 (24)

Berge *et al.,* (2006) isolated *P. multocida* and *P. hemolytica* from the respiratory tract of sheep and goats. Yimer and Asseged (2007*)* also reported *Staphylococcus* spp, *E. coli*, *Corynebacter* spp, *Klebsiella* spp and *Bacillus* spp in the respiratory tract of sheep. Data of bacterial isolation in our study are in agreement with the findings of Oros *et al.,* (1997), Ozbey and Muz (2004) and Shafarin *et al.,* (2007).

Cultural, morphological and staining characteristics

Staphylococcus spp and *Pasteurella* spp were grown in the nutrient agar and blood agar media. No growths of *Staphylococcus* spp were noticed on the EMB and MacConkey agar media. Both hemolytic and non-hemolytic *Staphylococcus* spp were recovered from the nasal swab samples. *Pasteurella* spp did not produce hemolysis on blood agar. *Pasteurella* spp were grown on EMB agar but did not grow on MacConkey agar. *Staphylococcus* spp were round shaped arranged in clustered and showed Gram positive reaction. *Pasteurella* spp were Gram negative, coccobacillary shaped arranged in single or pair form. These results are in agreement with Heddleston and Wessman (1975) and Mork *et al.,* (2010). The summary of cultural, morphological and staining characteristics of bacteria recovered from nasal swabs is given in Table 2.

Table 2. Summary of cultural and staining characteristics of bacteria isolated from nasal swabs of pneumonic black Bengal goats

Cultural characteristics			Staining characteristics			Identified organisms
Nutrient agar	Blood agar	Eosine-Methylene Blue agar	Shape	Arrangement	Gram's staining reaction	
Gray white or golden yellowish colony	Golden yellow colony without hemolysis or whitish colony with hemolysis	No growth	Cocci	Cluster	Gram positive	*Staphylococcus* spp
Whitish, opaque circular and translucent colony	Whitish, opaque circular colony with no hemolysis	Smooth, convex, glistening colony	Cocco-bacillary	Single or pair form	Gram negative	*Pasteurella* spp

Biochemical Characteristics

Staphylococcus spp fermented five basic sugars such as: dextrose, maltose, lactose, sucrose and mannitol. *Pasteurella* spp fermented only three basic sugars: dextrose, sucrose and mannitol. Only acid production was noticed following sugar fermentation reaction by these bacteria. *Staphylococcus* spp were positive to catalase, coagulase, MR and VP tests. Indole test was negative for *Staphylococcus* spp. *Pasteurella* spp were catalase, coagulase, MR and VP negative and indole positive. These results are in accordance with Tefera and Smola (2002) and OIE manual (2004). The summary of biochemical characteristics is shown in Table 3.

Table 3. Summary of biochemical characteristics of bacteria isolated from nasal swabs of pneumonic black Bengal goats

Fermentation reactions with carbohydrates					Catalase test	Coagulase test	MR test	VP test	Indole test	Identified organisms
D	ML	L	S	MN						
+	+	+	+	+	+	+	+	+	-	*Staphylococcus aureus*
+	-	-	+	+	+	-	-	-	+	*Pasteurella multocida*

D = Dextrose; ML = Maltose; L = Lactose; S = Sucrose; MN = Mannitol; + = Positive reaction; – = Negative reaction

Data of the biochemical and cultural characteristics indicated that isolated bacteria were *Staphylococcoccus aureus* and *Pasteurella multocida*. These results are in agreement with Hawari *et al.,* (2008) and Yimer and Asseged (2007*)*. Similar results were also reported by Amaechi and Ugbogu (2006) and Tefera and Smola (2002).

Antibiotic sensitivity assay

Staphylococcus aureus were highly sensitive to tetracycline, erythromycin, enrofloxacin and noflaxacin, moderately sensitive to furazolidone and gentamycin, less sensitive to amoxicillin and penicillin. On the other hand *Pasteurella multocida* were highly sensitive to ciprofloxacin, moderately sensitive to tetracycline, erythromycin, azithromycin and gentamycin, less sensitive to ampicillin, amoxicillin and resistant to penicillin. Summary of antibiotic sensitivity pattern of bacteria isolated from nasal swabs of pneumonic black Bengal goats is furnished in Table 4.

Table. 4. Summary of antibiotic sensitivity pattern of bacteria isolated from nasal swabs of pneumonic black Bengal goats

Bacterial isolates	Antibiotic sensitivity profiles										
	A	AP	P	TE	E	AZM	ENR	NOR	FR	GM	CIP
Staphylococcus aureus	+	ND	ND	+++	+++	ND	+++	+++	++	++	ND
Pasteurella multocida	+	+	-	++	++	++	ND	ND	ND	++	+++

E = Erythromycin; GM = Gentamicin; TE = Tetracycline; A = Amoxycillin; ENR = Enrofloxacin; NOR = Norfloxacin; FR = Furazolidone; AP = Ampicillin, CIP = Ciprofloxacin; AZM = Azithromicin; P = Penicillin; +++ = Highly sensitive; ++ = Moderately sensitive; + = Less sensitive and - = Resistant, ND = Not done

In clinical aspects antibiotic sensitive assay serves as a guide to choose the correct antibiotic to be used in the field (Coates and Hoops, 1980). The findings recorded in this study are in agreement with the result of Catry *et al.* (2002), Amaechi *et al.* (2006) and Berge *et al.* (2006). The results of antibiotic sensitivity assay recorded in this study are also in accordance with Kawamoto *et al.* (1990).

Data of this study indicated that the bacterial infections caused by *S. aureus* and *P. multocida* were responsible for pneumonia in black Bengal goat. Further study is required for molecular characterization of *S. aureus* and *P. multocida* isolated from pneumonic goats.

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