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Original article

Isolation and identification of bacteria from upper respiratory tract of Black Bengal goat in Bangladesh and investigation of some epidemiological parameters related to pneumonia

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ABSTRACT

Goat pneumonia (commonly known as goat shipping fever) is a common disease in Bangladesh affecting mostly at early age. The present study was aimed to isolation and identification of the associated bacteria and their antibiogram studies, and to investigate some epidemiological parameters relating to respiratory diseases especially pneumonia in Black Bengal goat. A total of 50 respiratory samples (nasopharyngeal swab) were collected. The associated bacteria were isolated and identified through commonly used bacteriological and biochemical methods. Besides, some epidemiological parameters related to goat pneumonia were investigated by direct observation and interviews of the owners. On the basis of morphology, staining, cultural and biochemical characteristics, the isolated organisms were identified as *Escherichia coli* (23%), *Staphylococcus* spp. (15%) and *Bacillus* spp. (8%). No bacteria could be isolated from 5(10%) samples. The isolated bacteria were found pathogenic in day-old-mice. The *Staphylococcus* spp. was highly sensitive to Erythromycin and resistant to Trimethoprim and Metronidazole. On the other hand, Nalidixic acid was found to be highly sensitive against *Bacillus* spp. Similarly, *E. coli* was highly sensitive to Ciprofloxacin, Norfloxacillin and Enrofloxacin. The pneumonia was found severe in the cases of mixed bacterial infection. Pneumonia was found prevalent mostly in young, female,

poorly nourished goats reared in unhygienic and high humid conditions. Rainy season and inappropriate management practices (e.g., rearing of mixed age group together, insufficient balanced diet, negligence to vaccination, wet shed or floor, negligence to give treatment etc.) also influenced the occurrence of the disease. The findings of this study would help in formulating appropriate strategies for the prevention and control of the disease.

1. Introduction

In the world, there are about 867 million goats, which are one of the major sources of meat and milk in many areas (Haenlein, 2004). Bangladesh, an Asian country, has 34.6 million goats and sheep (Rahman et al., 2003). Black Bengal goat is mostly (>90%) reared in Bangladesh (Amin et al., 2001). The goats are well-reputed in terms of fertility, adaptability to hot humid conditions, quality meat and high quality skin (Husain, 1999; Amin et al., 2001). In Bangladesh, semi-intensive farming system in goat rearing is increasing. However, the goats suffer from several diseases that reduce profitability (Berge et al., 2006). Respiratory bacteria are ubiquitous in nature and are normal inhabitants of the nasopharynx of Black Bengal goat. Thus, interpretation on the associated bacteria is sometimes very difficult. Goat respiratory infections exhibit various clinical syndromes exerting stressful condition to the goats (Islam et al., 2006).

Pneumonia is considered as one of the major diseases of goat. The disease is mostly caused by bacteria. However, the associated bacterial agents are not well-studied. In recent years, many attentions have been paid on studying contagious caprine pleuropneumonia (OIE, 2008). In Bangladesh, large number of goat population die each year due to pneumonia at the early stage of their lives. Early diagnosis and proper treatment are essential for effective treatment and prevention and control of the diseases (Islam et al., 2006). Epidemiological parameters could be studied which will help in taking appropriate strategies for the prevention and control of the diseases. However, there is paucity of literature that describes systematic observation of different diseases, kid mortality relating to epidemiological factors in intensively or semi-intensively reared Black Bengal goats. Therefore, the research work was aimed at understanding the prevalence of bacterial agents causing pneumonia in Black Bengal goat. Also, host and some environmental factors relating to goat pneumonia are assessed, and appropriate antibiotics against the bacteria have been suggested.

2. Materials and methods

2.1. Study area and sample collection

Nasopharyngeal swab samples (n=50) were collected directly from the respiratory tract of Black Bengal goat from Mymensingh area (Fig. 1) in Bangladesh with a history of suffering from acute respiratory problem especially showing typical signs of pneumonia. Following standard bacteriological specimen transportation procedure, the collected samples were brought to the Bacteriology Laboratory at the Department of Microbiology and Hygiene under Faculty of Veterinary Science, Bangladesh Agricultural University (BAU) for bacteriological analysis.

2.2. Investigation of epidemiological parameters relating to goat pneumonia

The data relating to the occurrence of goat pneumonia were collected by direct interview with the goat-owner and direct inspection of the animals. A pre-tested questionnaire was used for this purpose. The data were collected based on the parameters such as age, sex, environment, temperature, humidity, season, health status, and hygiene and management practices mentioned above.

2.3. Isolation and identification of associated bacteria

Primary culture was performed in Nutrient agar and Nutrient broth media. For sub-culturing, suspected bacteria were inoculated separately onto different bacteriological agar media under aseptic condition and incubated at 37^0C for 24 hours. Pure culture was made as per the procedures described by OIE (2008) and Cowan (1985). In order to identify the isolated bacteria, cultural, morphological and biochemical characteristics were studied. The cultural characteristics or colonial morphology of the bacteria grown on the nutrient and blood agar media were recorded. Gram staining method was performed to study the morphology and staining characteristics of bacteria according to the technique described by Cheesbrough (1985). Motility test was performed (Cowan (1985) to differentiate motile bacteria from the non-motile one. Hemolytic activity of the bacteria was performed according the procedure mentioned by Carter (1986). Biochemical tests, such as- sugar fermentation, coagulase, catalase, Methyl Red, indole production, the Voges-proskauer, citrate utilization, TSI agar slant reaction, oxidation fermentation were performed according to the standard methods (Cheesbrough, 1985). The classification and specification of organisms was based on the scheme presented in Bergey's Manual of Systematic Bacteriology (Bergey et al., 1984). The stock culture was maintained following the procedures of Chowdhury et al. (1987).

Fig. 1. Area of sample collection in Bangladesh. The black arrow indicated the area of this study.

2.4. Pathogenesis test in mice

The pathogenesis test was performed according to the procedure described by Roy et al. (2012). In brief, day-old mice of both sexes were used for experimental production of pneumonia. The mice were divided into five groups consisting of ten mice in each. Three groups were inoculated with individual species of bacteria and one group was infected with mixed organisms. One group was kept as control. An amount of 0.5 ml inoculum ($5x10^6$

CFU) was administered orally and the mice were observed for up to 7 days. Re-isolation and confirmation of the organisms from the experimentally infected mice was made.

2.5. Antibiotic sensitivity test

A previously described disc diffusion method (Bauer et al., 1966; Khan et al., 2005; Roy et al., 2012; Masud et al., 2012) was used to determine the susceptibility of the bacterial isolates against antibiotic agents. For this, commercially available 14 different antibiotic discs (Oxoid Ltd., Baring-stoke, Hampshire, England) were used. The names and concentrations of the antibiotic discs are mentioned in Table 1. The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007; formerly known as NCCLS).

3. Results and discussion

3.1. Epidemiological investigations

The data obtained from the epidemiological study are presented in Table 2. Goat pneumonia was observed mostly in young (1-12 months), female, poorly nourished animals reared in unhygienic and high-humid conditions. Rainy season and inappropriate management practices (mentioned in the materials and methods) enhanced the occurrence of the disease. The study is in support of several other findings (Pinheiro et al., 2000; Lancelot et al., 2002; Kumar et al., 2004a; Kumar et al., 2004b; Islam et al., 2006) where more than one predisposing factors such as environmental and management factors (housing, climate etc.), imbalance nutrition, immune status of the goat were involved in the onset of goat pneumonia.

Table 1
Antibiotics used in antibiogram studies.

Antibiotic disc	Concentration (µg/disc)
Penicillin	6
Gentamicin	10
Ciprofloxacin	5
Amoxicillin	10
Erythromycin	5
Ampicillin	30
Trimethoprim	25
Nalidixic acid	30
Azithromycin	15
Furazolidone	15
Enrofloxacin	5
Norfloxacin	10
Pefloxacin	5
Metronidazole	5

3.2. Isolation and identification bacteria in nasopharyngeal swab of Black Bengal goat

On the basis of morphology, staining, cultural and biochemical characteristics, three different bacteria were isolated from the samples (Table 3 and Table 4). Out of 50 samples, 22 (44%), 15 (30%) and 8 (16%) samples were found positive for *E. coli*, *Staphylococcus* spp. and *Bacillus* spp., respectively. No bacteria could be isolated from 5 (10%) samples supporting the findings of Yimer and Asseged (2007) and Elsheikh and Hassan (2012) who found 16.7% and 49% samples were negative for any bacteria, respectively. The incidence of different bacteria isolated from goat correlated with the findings of Yimer and Asseged (2007), Obasi et al. (2001), Ajuwape et al. (2004), Adler et al. (2007) and Shafarin et al. (2007) with slight variation. However, a prevalence of only 2.94% of *Staphylococcus* spp. was observed in pneumonic lesions (Elsheikh and Hassan, 2012) indicating that Black Bengal goats are in high risk for the occurrence of bacterial infection including pneumonia. Besides the bacteria isolated from this study, several other organisms are reported for the occurrence of pneumonia in goat such as-

Actinomyces pyogenes, *Arcanobacterium pyogenes*, *Neisseria catarrhalis* and *Proteus vulgaris* (Obasi et al., 2001), *Pasteurella multocida* (Obasi et al., 2001; Hutt and Goossens, 2001; Ozbey and Muz, 2004; Shafarin et al., 2007), *Streptococcus pyogenes* (Obasi et al., 2001); *Mycoplasma capricolum* (Ostrowski et al., 2011). In addition to bacteria, other organisms (e.g., fungus, virus etc.) may be involved in goat pneumonia. However, our target was to find out the bacterial cause only.

3.3. Pathogenicity test in mice

On the observation, all the mice were found dead within 24 hours indicating that all the three different bacterial types were pathogenic. In contrast, mice of the control group were found alive. These are in support of the findings of Roy et al. (2012).

Table 2
Prevalence of goat pneumonia based on some epidemiological parameters.

Epidemiological patterns	Level of patterns	Goat examined (no.)	Prevalence (%)
Age	1-6 months	250	6 (2.4)
	7-12 months	500	7(1.4)
	13-18 months	300	3(1)
Sex	Male	300	2(0.6)
	Female	250	4(1.6)
Season	Rainy	250	5(2)
Hygiene status	Poor	250	7(2.8)
	Moderate	200	4(2)
	Good	200	2(1)
Health status	Poor	400	10(2.5)
	Moderate	250	3(1.2)
	Good	300	1(0.3)
Humidity	High	300	4(1.3)

Table 3
Frequency distribution of isolated Bacteria from respiratory system of Black Bengal goat.

Samples examined	Isolated Bacteria	Frequency (%)
n=50	*Staphylococcus* spp.	15 (30)
	Bacillus spp.	8 (16)
	Escherichia coli	22 (44)
	Absent of any bacteria	5 (10)

Table 4
Results of biochemical characteristics of Isolated Bacteria from respiratory system of Black Bengal goat.

Isolated Bacteria	Fermentation properties with Carbohydrate					Catalase test	Coagulase test	Indole test	Methyle-Red test	Voges-proskauer test	Citrate Utilization test
	D	ML	L	S	MN						
Staphylococcus spp.	+ A	+ A	+ A	+ A	+ A	+	+	_	_	_	_
Bacillus spp.	+ A	+ A	+ A	+ A	+ A	+	_	_	_	_	_
Escherichia coli	+ AG	+ AG	+ AG	+ AG	+ AG	+	_	+	+	_	_

D= Dextrose, ML= Maltose, L= Lactose, S= Sucrose, MN=Manitol, A= Production of Acid only, AG=Production of Acid and Gas, + = Positive reaction, - = Negative reaction.

3.4. Antibiotic sensitivity patterns of the isolated bacteria

The isolated *Staphylococcus* spp. was found to be sensitive to Erythromycin, moderately sensitive to Ampicillin and Amoxicillin, and resistant to trimethoprim and metronidazole. Nalidixic acid was found to be sensitive for *Bacillus* spp., whereas the isolate was moderately sensitive to Erythromycin and Azithromycin and resistant to Amoxicillin, Metronidazole, Penicillin, Trimethroprim and Ampicillin. *E. coli* was highly sensitive to Ciprofloxacin, Norfloxacillin and Enrofloxacin, and moderately sensitive to Amoxycillin, Peafloxacin, Furazolidone. The findings are in agreement with the results described by Catry et al. (2002), Nazir et al. (2005), and Amaechi and Ugbogu (2006). However, due to indiscriminate use of antibiotics in Bangladesh, slight variation was found.

4. Conclusion

To the best of our knowledge, this is the first report of bacteriological association with pneumonia in Black Bengal goat of Bangladesh. Three different bacteria were isolated from nasopharyngeal swabs. The bacteria were identified to be strains of- *Escherichia coli* (23%), *Staphylococcus* spp. (15%) and *Bacillus* spp. (8%). No bacteria could be isolated from 5 (10%) samples. However, further studies are needed to check whether any non-culturable bacteria are present or not. The isolated bacteria were found pathogenic in day-old-mice. Erythromycin, Nalidixic acid, Ciprofloxacin, Norfloxacillin and Enrofloxacin could be the choice of antibiotics for the treatment of pneumonia in Black Bengal goat caused by the isolated bacteria. The nutritional status and management practice for the young Black Bengal goat should be improved for conserving its health.

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