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Isolation and Identification of Etiological Agents from Diarrhoeic Goats

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Abstract: The present research was undertaken to isolate and identify the etiological agents responsible for diarrhoea in goats. For this purpose, faecal samples were cultured and subcultured in basic medium named Nutrient agar medium and Blood agar medium to obtain pure cultures of separate genera. From pure cultures, bacterial colonies were picked up and further cultured in different selective medium viz. MacConkey agar medium, Bacto fluid thioglycollate broth, Cooked meat broth, Brain-heart infusion broth, Eosin methylene blue agar medium and Salmonella-Shigella agar medium to observe the cultural characteristics of the isolates. In different selective medium, bacterial genera were identified on the basis of their cultural morphology (in case of solid media) and change of color and gas production (in case of liquid media). By Gram staining, short, single or paired gram positive and gram negative bacteria were observed under the electric microscope in the smears prepared from the pure colonies from basic and selective medium. Finally Biochemical tests viz., Catalase, Voges-Proskauer, oxidase, citrate utilization, Coagulase, Oxidation-fermentation, Carbohydrate fermentation test were performed to confirm the specific bacterial genera. Of the 20 experimented faecal samples, *Salmonella* sp. (5.0%), *Staphylococcus* sp. (10.0%), *Escherichia coli* (25.0%), *Bacillus* sp. (85.0%) and *Clostridium* sp. (65.0%) were identified as single or mixed infection. 5.0% diarrhoea were found non-bacterial infection. Therefore the present study provides opportunities for the characterization of such bacteria as well as for the prevention of diarrhoea and thus help in goat farming.

Key words: Isolation, identification, etiological agents, diarrhoea, goat

Introduction

Diarrhoeal disease seems to be one of the major community health hazards both for men and animals in most countries of the world. It is resulted from the enteritis, which is the inflammation of the intestinal mucosa, characterized by abdominal pain, loose faeces, increase in stool mass, stool frequency, vomiting tendency, or stool fluidity (dehydration) that contain 70-95% water. More than 14 L of fluid may be lost per day in severe cases of diarrhoea. The chronic form of diarrhoea may last for days or week and may culminate in death (Radostits *et al*., 1995).

Caprine diarrhoea occurs world wide in goats of any age. In Bangladesh, Diarrhoeal disease remains the most often reported clinical problem in goats. Goat diarrhoea is responsible for poor growth in kids and a significant loss of production both through morbidity and mortality. Some enteropathogens like bacteria, viruses, protozoa and helminthes have been recognized to be associated with diarrhoea (Radostits *et al*., 2000). This disease has been reported from UK, USA, Canada, Argentina, France, Australia, New Zealand, Papua New Guinea, South Africa, Switzerland, Iran, India,

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Algeria, Pakistan, China and Japan. Reports on diarrhoea associated with enteropathogens are very much limited from Bangladesh. Here, it may be mentioned that, research work on diarrhea in goat has not been undertaken earlier in Bangladesh. Therefore, an attempt was made to isolate and identify the enterobacteria from goat diarrhea.

Since 1992, goat rearing has become seriously impaired due to high mortality with diarrhoea like symptoms. The marginal and land-less farmers most easily live on rearing of goats in Bangladesh. So, goat is called the poor man's cow that is the second important livestock in Bangladesh which plays an important role in the rural economy and we can earn substantial amount of foreign currency by exporting skin and other by-products. The estimated goat population in Bangladesh in 1997-98 was 33.50 millions, which was 33.331 millions in 1996-97 and growth rate of goat is about 8.2% yearly whereas cattle growth rate is about 0.80%. The numbers of goat farm at the private sector were 5584 in 1997-98 (Amin, 1998). So, above information indicates the goat population in our country is increasing day by day.

At present, Asian and other under developing countries, agriculture means crop and cereal production and relatively less importance is given to livestock health and production. As a result, deficiencies of proteins become an unsolved problem. Therefore, enhancement on small ruminants production especially goat in the framework of small holder agriculture assume the great significance. So, present study will be helpful toward goat rearing and enhance dynamism of goat farming which not only alleviate the poverty but also boost up the national economy. Considering all above-mentioned points, the present work was designed with the following objectives:

- Isolation and identification of the bacterial agents through different selective culture media and biochemical tests.
- To find out the actual bacterial agents responsible for goat/kid diarrhea so that appropriate regulatory actions are taken.

Materials and Methods

The research work was carried out at Bacteriology Laboratory of Animal Health Research Division (AHRD), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh during October, 2005 to January, 2006. Diarrhoeic goats were collected from Bangladesh Livestock Research Institute's goat farm.

Collection of Samples

The isolates of bacteria used in the present study belonged to 20 goats (Bengal goats, Jamunapari) aged between one month to three years which were noticed to have diarrhoea. Faecal samples were selected as experimental sample. Samples were collected randomly from those goats that were noticed Diarrhoeal sign. Table 1 represents collected samples and their place and date of collection.

For the purpose of sampling, sampling kit consisting of several sterile plastic bags, a marking pen, spatula and alcohol were taken. For isolation and identification, faecal samples were collected from the diseased goats directly from the rectum by using sterile plastic gloves. About 20 g of faeces were collected, kept in a polythene bag and tagged. At each time of collection hands were sterilized with alcohol (95%) and the bags were partially tilled with material. Then the bags were properly tied and labeled. Special care was always taken to avoid contamination as far as practicable.

Preparation of Culture Media

Commercially available media were used during this study. Different media were prepared according to the direction of the manufacturers for the culture and subculture of the organisms for their proper isolation and identification.

Table 1: Collected faecal samples and their place, sex, age, size and date of collection

Sample No.	Place	Sex	Age	Size	Date of collection
1627	BLRI Goat farm	Male	1 year 10 month	14 kg	17.10.2005
1254	BLRI Goat farm	Female	2 month 14 days	1.5 kg	17.10.2005
1379	BLRI Goat farm	Male	7 month	7 kg	20.10.2005
2012	BLRI Goat farm	Male	3 month	2 kg	27.10.2005
2082	BLRI Goat farm	Male	1 month 4 days	0.9 kg	02.11.2005
1954	BLRI Goat farm	Female	1 month 20 days	0.9 kg	10.11.2005
1445	BLRI Goat farm	Female	2 month 21 days	1.7 kg	17.11.2005
1390	BLRI Goat farm	Male	1 year 3 month	10 kg	20.11.2005
1623	BLRI Goat farm	Male	1 year 7 month	13 kg	22.11.2005
74	BLRI Goat farm	Female	2 month	1.1 kg	29.11.2005
311	BLRI Goat farm	Male	1 yr 10 month	14 kg	08.12.2005
2812	BLRI Goat farm	Female	2 month 14 days	1.5 kg	12.12.2005
510	BLRI Goat farm	Male	7 month	7 kg	18.12.2005
1860	BLRI Goat farm	Male	3 month	2 kg	22.12.2005
1170	BLRI Goat farm	Male	1 month 4 days	0.9 kg	27.12.2005
1410	BLRI Goat farm	Female	1 month 20 days	0.9 kg	29.12.2005
1960	BLRI Goat farm	Female	2 month 21 days	1.7 kg	03.01.2005
612	BLRI Goat farm	Male	1 year 3 month	10 kg	04.01.2005
1112	BLRI Goat farm	Male	1 year 7 month	13 kg	08.01.2006
1560	BLRI Goat farm	Female	2 month	1.1 kg	10.01.2006

Parasitological Examination of the Collected Samples by Direct Microscopy

Each of the faecal samples were examined on conventional direct smear method and followed by sedimentation methods to detect the parasitic eggs which were identified by their morphological features as described by Samad (2001).

Observation of Bacterial Growth in Different Culture Media

All the collected diarrhoeic faecal samples were examined for isolation and identification of bacteria. Samples were streaked on different agar medium, incubated at 37°C in aerobic incubator and in anaerobic incubator (containing 5% CO_2) for 24-48 h or sometimes 72 h.

Isolation of Colonies

From each petri dish plate, culture was subcultured in Nutrient agar and Blood agar medium and kept in aerobic and anaerobic condition respectively. Subcultures were repeated on both agar plate by streak plate method (Cheesbrough, 1985) until the colonies were considered the pure separately by visually and also by microscopically.

Final Selection of Isolates

The colonies were selected according to their size, shape, color, height, smoothness, margin and other main properties. Regarding to the above properties, colonies that possessing the highest positive signs were selected for further tests.

Purification of the Selected Isolates

For purification, the selected colonies were picked up by platinum loop and plating on agar plate (Nutrient agar and Blood agar) by streaking method.

Morphological and Cultural Studies for Identification of the Selected Colonies

The faecal samples were cultured in NA medium in aerobic incubator at 37°C for 24-48 h. Samples were also cultured in BA medium (5% Sheep blood) in anaerobic incubator (5% CO_2) at 37° for 48 h. The isolates were also cultured on different agar medium and inoculated in different broth. After incubation, the colonies showed different characteristics. For observing cultural characteristics, discrete colonies on the agar surfaces were selected to study their shape, size, consistency and color.

Preparation for Microscopic Examination

For microscopic examination, many methods are available to prepare the slides and here fixed smear method was applied.

Preparation of the Smear

A portion of bacterial culture was taken out by a sterilized loop and smeared on a slide and a very thin film was made which was allowed to dry in air. The smear was fixed by slightly heating the slide over a sprit lamp.

Staining

For the observation of the presence of the organisms (bacteria) and also to study their cellular morphology, selected isolates were stained. From the entire medium, isolates were collected and stained with Gram's staining method as per recommendation of Cowan (1985).

Biochemical Studies of the Selected Isolates

According to the procedure described by Buxton and Fraser (1977-78), the biochemical tests viz., Catalase, Voges-Proskauer, Oxidase, Citrate utilization, Oxidation-fermentation and Coagulase tests were performed. Carbohydrate fermentation test was performed with five basic sugars (Dextrose, Sucrose, Lactose, Maltose and Mannitol) and thereby production of acid or gas or both.

Results and Discussion

Microscopic Studies and Staining Properties of the Bacterial Isolates

Bacteria were identified on the basis of colony shape, arrangements and their ability to persisting safranine color in gram staining test (Table 2).

Cultural Characteristics

The cultural characteristics of bacterial isolates were observed on the basis of pigmentation, form, margin and elevation. Cultural colony characteristics of the bacteria isolated from the diarrhoeic goats are presented in Table 3.

Results of Parasitic Examination

Different microscopic slides were prepared by smearing method from the isolates. No parasitic egg was found. This may be due to the goats were treated with anthelminthic drugs just 14 days back.

Haemolytic Activities of the Isolated Organisms

In sheep BA medium, the haemolytic activities of five isolates of each colony of sample no. 1623, 74, 1390, 1627 and 379 from NA medium were tested for haemolysin production of which 6 (75.0%), 5 (62.5%), 7 (87.5%), 6 (75.0%) and 7 (87.5%) isolates were found positive respectively which were to able for haemolysin production, respectively (Table 4).

Table 2: Microscopic studies and staining properties of the bacterial isolates

Sample No.	Shape of colony	Characteristics arrangements	Gram staining
1623	Very short plump rods	Single	(-) ve
74	Small coccus	Cluster or pair	(+) ve
1390	Short plump rods	Single, pair or short chain	(-) ve
1627	Rod with square ends	Single, pair or long chain	(+) ve
379	Rod with blunt ends	Single, pair or group	(+) ve

Table 3: Cultural colony characteristics of the bacteria on different media

		Sample No.				
Cultural media	Colony appearance	1623	74	1390	1627	379
Nutrient broth		Uniform fine turbidity	Uniform fine turbidity	Flocculent	Uniform turbidity	Uniform fine turbidity
Nutrient agar media	Pigmentation	White	White	White to grayish	Grayish white	Grayish white
	Form	Round	Round	Circular	Circular	Circular
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth
	Elevation	Raised	Raised	Raised	Raised	Raised
Blood agar media						
Pigmentation	White	White	White	Creamy	Grayish	
	Form	Round	Round	Circular	Round	Circular
	Margin	Raised	Raised	Raised	Raised	Entire
	Elevation	Raised	Raised	Raised	Raised	Raised
	Haemolysis	Haemolysis	Haemolysis	Haemolysis	Haemolysis	Haemolysis
MacConkey agar media	Pigmentation	Pale, colorless	No growth	Rose pink	No growth	No growth
	Form	Circular		Circular		
	Margin	Entire		Entire		
	Elevation	Raised		Raised		
Eosin-Methylene Blue agar media	Pigmentation	No growth	No growth	Yellow green	No growth	No growth
	Form			Circular		
	Margin			Entire		
	Elevation			Raised		
Salmonella-Shigella agar media	Pigmentation	Colorless	Colorless	Pink	No growth	Colorless
	Form	Round	Round	Round		Round
	Margin	Smooth	Smooth	Smooth		Smooth
	Elevation	Raised	Raised	Raised		Raised
Cooked meat broth	Color change	No	No	No	Yes	Yes
	Turbidity	No	No	No	Yes	Yes
Brain-heart infusion broth	Color change	No	No	No	Yes	Yes
	Gas	No	No	No	Yes	Yes
Skimmed milk broth	Coagulation	No	Yes	No	No	Yes

Circular (Unbroken peripheral edge), Entire (sharply defined, even), Raised (Slightly elevated), Uniform fine turbidity (finely dispersed growth throughout the solution), Flocculent (flaky aggregates dispersed throughout the solution)

Table 4: Results of haemolytic activities of the isolates on BA medium

	Haemolytic activities	
Sample No.	Tested No.	Positive No. (%)
1623	6	75.0
74	5	62.5
1390	7	87.5
1627	6	75.0
379	7	87.5

Biochemical Tests

The biochemical characteristics of bacterial isolates were observed on the basis of broth color, or gas formation, or clot formation. The different isolates of the organisms showed identical results in different biochemical tests. The actual causes for which the manifestation of more or less identical result in biochemical tests by the five groups of known identified isolates were not clear. It is thought that all isolates in the present study possess some common genetic materials which are responsible for

the manifestation of similar type of biochemical reaction as reported by Pandey *et al.* (1979) and Hodna *et al.* (1982). Biochemical characteristics of the bacteria isolated from the diarrhoeic goats are presented in Table 5.

Carbohydrate Tests

The carbohydrate characteristics of the bacteria isolated from the diarrhoeic goats are presented in Table 6.

Oxidation-fermentation Properties of Bacteria

All isolates of 1623, 74, 1390 and 1627 were found positive for fermentation whereas isolates of 379 showed both fermentative and oxidative properties (Table 7).

Provisional Identification of Selected Bacteria

Table 8 Contains the provisional identification of selected isolates obtained from diarrhoeic goat's faecal samples. The main etiological agents were *Salmonella* sp., *Staphylococcus* sp., *E. coli*, *Bacillus* sp. and *Clostridium* sp.

The present study includes isolation and identification of bacteria recovered from faecal samples of diarrhoeic goats of Bangladesh Livestock Research Institute's goat farm. Most of the clinical signs and bacterial isolates that are recorded in the present study are correlated with the study in calves by the authors like Chakrabarty *et al.* (1980), Chattopadhyay and Harbola (1988), Baldassi *et al.* (1995) and Uzal *et al.* (1996).

Of the 20 diarrhoeic goat faecal samples examined, 19 (98%) had enterobacterial infection, of which 1 sample (5.0%) were found positive for *Salmonella* spp., 2 samples (10.0%) for *Staphylococcus* sp., 5 samples (25.0%) for *E. coli*, 17 samples (85.0%) for *Bacillus* sp., 13 samples (65.0%) for *Clostridium* sp. and 1 sample (5.0%) were found negative for bacterial infection.

Table 5: Results of biochemical characteristics of the selected isolates

	Sample No.				
Name of experiment	1623	74	1390	1627	379
Catalase test (24 h incubation)	+	+	+	+	-
Voges-Proskauer test (48 h incubation)	-	-	-	-	-
Oxidase test (24 h incubation)	+	-	-	+	+
Citrate utilization (24 h incubation)	-	-	-	-	+
Coagulase test (24 h incubation)	-	+	-	-	+

(+) sign indicates positive reaction, (-) sign indicates negative reaction

Table 6: Results of Carbohydrate characteristics of the Isolates

		Sample No.				
Carbohydrate fermentation (24-48 h incubation)		1623	74	1390	1627	379
Dextrose	Color	Yellow	Yellow	Yellow	Yellow	Yellow
	Gas	+	+	+	+	+
	acid	+	+	+	+	-
Lactose	Color	Colorless	Yellow	Yellow	Yellow	Yellow
	Gas	-	+	+	+	+
	acid	-	+	+	+	-
Sucrose	Color	Colorless	Yellow	Yellow	Yellow	Yellow
	Gas	-	+	+	+	+
	acid	-	+	+	+	-
Maltose	Color	Yellow	Yellow	Yellow	Yellow	Yellow
	Gas	+	+	+	+	+
	acid	+	+	+	+	-
Mannitol	Color	Yellow	Yellow	Yellow	Yellow	Colorless
	Gas	+	+	+	+	+
	acid	+	+	+	+	-

(+) sign indicates positive reaction, (-) sign indicates negative reaction

Table 7: Oxidation-fermentation properties of bacteria isolated from diarrhoeic goats

	Peptone water containing glucose and bromothymol blue		
Sample No.	Without a layer of paraffin	With a layer of paraffin	Results
1623	+	+	Fermentative
74	+	+	Fermentative
1390	+	+	Fermentative
1627	+	+	Fermentative
379	+	+/-	Fermentative/Oxidative

(+) Sign indicates positive reaction, (-) Sign indicates negative reaction

Table 8: Provisional identification of selected isolates

Sample No.	Provisional identification name
1623	*Salmonella* sp.
74	*Staphylococcus* sp.
1390	*E. coli*
1627	*Bacillus* sp.
379	*Clostridium* sp.

Conclusion

Bengal goats and Jamunapari are potential and economic livestock of Bangladesh. A large number of goat populations are decreasing due to diarrhoea in every year. This study was undertaken to identify the main causal agents of diarrhoea and it was found that *Salmonella* sp., *Staphylococcus* sp., *E. coli*, *Bacillus* sp. and *Clostridium* sp. play a vital role for goat diarrhoea. Results of this study will help to develop an effective treatment method of goat diarrhoea against those bacteria.

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