

PREVALENCE OF *SALMONELLA* ASSOCIATED WITH GOATS IN BANGLADESH

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Abstract

Salmonellosis is one of the life threatening diseases of goats in Bangladesh. Therefore, the present study was designed to study the prevalence of Salmonellosis, and the isolation and characterizations of the *Salmonella* spp. from apparently healthy and diarrheic goats. A total of 47 faecal samples was collected from selected places and cultured onto different prescribed media to isolate the *Salmonella*. In this study, 12.76% (6/47) of the samples were found to be positive for *Salmonella* spp. During culture, all of the *Salmonella* isolates produced round, smooth, opaque, translucent, and black colored colonies on SS agar medium. All of the isolated *Salmonella* spp. fermented dextrose, maltose, and mannitol with production of acid and gas but did not ferment sucrose and lactose. However, these isolates had shown negative for the Indole and Voges-Proskauer tests and positive for the Methyl-Red test. All of these isolates were subjected to a rapid plate agglutination test with polyvalent “O” (Poly ‘O’) and polyvalent “H” (poly ‘H’) antisera where positive agglutinations were observed. They were highly sensitive to ciprofloxacin, spiramycin, and gentamycin; moderately sensitive to oxytetracycline, streptomycin, and amoxicillin; less sensitive to sulphamethoxazole and resistant to penicillin-G. Based on the present findings, it may be concluded that the investigated *Salmonella* spp. from goats might be *S. typhimurium*, *S. enteritidis*, *S. brandenburg*, *S. salford*, *S. newbrunswick*, *S. newport*, or *S. dublin*. It was a preliminary study; therefore, further characterization is required using other serological and molecular techniques.

Keywords: Bangladesh, goats, *Salmonella*, salmonellosis, prevalence, sensitivity

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Introduction

Salmonellosis is a disease or a group of diseases caused by a wide variety of *Salmonella* serovars in various hosts including goats (OIE, 2006) which remain as a serious problem with public health significance throughout the world (Tabaraie *et al.*, 1994). Substantial economic loss results from mortality and poor growth of animals, birds, and human beings as well as the hazards of transmitting food poisoning with gastroenteritis to humans, representing a serious problem for the food industry (Cooke and Todd, 1990). The goat population of Bangladesh is suffering from various diseases and disease conditions e.g. brucellosis, salmonellosis, enterotoxaemia, *Peste des petits ruminants*, goat pox, and contagious ecthyma. From the reports of personnel in the field from various corners of Bangladesh, it is assumed that salmonellosis is one of the most important diseases in the country. *Salmonellae* are Gram negative, small, rod shaped, non-spore forming, non-capsulated, aerobic, and facultatively anaerobic organisms classified under the family Enterobacteriaceae (Burrows and Freeman, 1985; Gene, 2002; OIE, 2006). *Salmonellosis* assumes one of the following forms: peracute septicemia, acute enteritis, chronic enteritis, or a subclinical carrier state. Common clinical signs are septicemia, fever, enteritis, arthritis, and also abortion. But the clinical signs may vary from species to species (Blood *et al.*, 2003). *Salmonella* infection in goat occurs in all ages, all seasons, both in males and females, and is responsible for a considerable loss of kids and even may cause abortion in adults (Arruda *et al.*, 2004). This infection may be a problem in chevon raising and the goat rearing industry and in areas where it will surely impair the fattiness and sound health of goats. The poor quality and quantity of chevon leads to poor market value (Arruda *et al.*, 2004). A mortality rate of 14.52% in kids was reported in India due to Salmonellosis (Ghosh *et al.*, 1987).

Salmonella organisms were isolated from poultry (Begum, 1992), cattle (Islam, 2007), sheep (Kobayashi *et al.*, 2007), and

ruminants (Rahman, 2007). However, the epidemiological investigation of Salmonellosis in goats has not been studied in Bangladesh. Goat farmers also use antibiotics to treat infections indiscriminately without confirmatory diagnosis. As a result, drug resistant *Salmonella* organisms are emerging. In view of the above consideration, the present study was undertaken a) to study the prevalence of Salmonellosis in goats in some selected areas of Bangladesh considering age, sex, breed, seasons, and health status; b) to isolate and identify the *Salmonella* spp. from clinical specimens of goats; c) to characterize the *Salmonella* of goat isolates using cultural, biochemical, and serological techniques; and d) to develop remedial measures against the isolated *Salmonella* spp.

Materials and Methods

Collection of Animal Faecal Samples

Samples were collected from 4 sites: Bangladesh Agricultural University (BAU) veterinary clinic, the BAU goat farm, Babul goat farm in Aziz coloni, Boira, Mymensingh, and Savar goat farm. A total number of 47 faecal samples were collected from apparently healthy and diarrheic goats and then transferred.

Isolation and Cultivation of *Salmonella* spp.

According to Cheesebrough (1984), we selected the culture system and media as follows: nutrient broth was used to grow the *Salmonella* spp. and perform the hanging drop test, biochemical test, and antibiotic sensitivity test. Nutrient agar medium was used to grow the *Salmonella* spp. from the collected samples. *Salmonella*-shigella (SS) agar medium was used as a selective medium for *Salmonella* spp. which causes enhancement of the growth of *Salmonella* while inhibiting the growth of other contaminating organisms and shows typical colony characteristics. Brilliant green (BG) agar medium was used as a selected medium for the isolation of *Salmonella* spp. MacConkey (MC) agar medium was used for culturing the organisms under the family

Enterobacteriaceae. Eosin methylene blue agar medium was used for the purpose of observing differential growth of *Salmonella* spp. and other enterobacters especially *Escherichia coli*. Triple sugar iron agar slant was used for the purpose of observing the colony characteristics of the isolated *Salmonella* spp. and for the preservation of bacteria. Blood agar medium was used to perform the antibiotic sensitivity study.

Morphological Characterization by Gram's Method

The representative *Salmonella* colonies were characterized morphologically using Gram's staining technique according to the method described by Merchant and Packer (1967).

Identification of Suspected *Salmonella* Isolates Using Biochemical Test

Sugar Fermentation Test

The carbohydrate fermentation test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into tubes containing the different sugar media (5 basic sugars such as dextrose, maltose, lactose, sucrose, and mannitol) and incubated for 24 h at 37°C. Acid production was indicated by the color change from pink to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 1985).

Methyl Red tTest

The test was conducted by inoculating a single colony from the pure culture of the test organisms in 5 ml sterile methyl red (MR) broth. After 5 days incubation at 37°C, 5 drops of methyl red solution were added and observed for color formation. Development of the red color was positive and indicated an acid pH of 4.5-6 resulting from the fermentation of glucose. Development of the yellow color indicated negative results (Cheesbrough, 1985).

Voges-Proskauer Test

Two ml of sterile glucose phosphate peptone water were inoculated with 5 ml of

the test organisms. It was incubated at 37°C aerobically for 48 h. A very small amount (a knifepoint) of creatine was added and mixed. Three ml of sodium hydroxide were added and shaken well. The bottle cap was removed and the bottle left for an hour at room temperature. It was observed closely for the slow development of the pink color in positive cases. In negative cases, there was no development of the pink color (Cheesbrough, 1985).

Indole Test

The test organisms were cultured in test tubes having 5 ml of peptone water containing tryptophan at 37°C for 48 h. Then, 1 ml of diethyl ether was added, shaken well and allowed to stand until the ether rose to the top. Then, 0.5 ml of Kovac's reagent was gently run down the side of the test tube so that it formed a ring in between the medium and the ether layer and observed for the development of the color of the ring. Development of a brilliant red colored ring indicated indole production. In a negative case there is no development of the red color (Cheesbrough, 1985).

Serotyping Through Slide Agglutination Test

Salmonella agglutinating antisera poly "O" and poly "H" (S & E Reagents Lab, Bangkok, Thailand) were used to perform the serotyping of the isolated *Salmonella* spp. The macroscopic slide agglutination tests were performed. The cultures to be tested were first checked with *Salmonella* poly "O" polyvalent antiserum. A single isolated colony from the SS agar was emulsified with physiological saline solution. A single drop of thick bacterial suspension was placed on a glass slide and a drop of polyvalent antiserum was added. The slide was gently rotated to mix the fluid thoroughly. These cultures, which agglutinated within 1 to 2 min, were selected as positive for *Salmonella* and subjected to an agglutination test with *Salmonella* agglutinating antiserum (poly "H"). According to the manufacturer's direction, it was noted that poly "O" antiserum gives a

positive agglutination reaction with any serovars for preliminary screening of *Salmonella* and poly "H" antiserum gives a specific agglutination reaction for motile *Salmonella* spp. (Buxton and Fraser, 1977).

Antibiogram Study of the Isolated *Salmonella* spp.

Susceptibility of the isolated *Salmonella* to different antibacterial agents was performed through the disc diffusion method to determine the drug sensitivity pattern according to the method described by Bauser *et al.* (1966).

Results and Discussion

Isolation of *Salmonella* spp. from Faecal Samples

The results of isolation of *Salmonella* from different faecal samples were shown in Table 1. Out of 47 samples examined 6 were found to be positive for *Salmonella*. Among the positive samples, 3 were from BAU Veterinary clinic, 2 were from the BAU goat farm, and one was from Savar goat farm. The prevalence rate of *Salmonella* in goats of these study areas were 25%, 15.38%, and 10%, respectively. In Babul goat farm, no sample was found to be positive for *Salmonella*.

Prevalence of *Salmonella* Infection

The epidemiological investigation revealed that the prevalence of *Salmonellosis*

was found to be higher in young (16.67%), sick (44.44%), and locally bred (16%) goats with equal susceptibility of both male (50%) and female (50%) goats, which was similar to the findings of Mudit *et al.* (2007). The prevalence of *Salmonellosis* was found to be higher in summer (19.04%), which also satisfied the findings of Mahendra *et al.* (2006). On the other hand, the prevalence of *Salmonellosis* is lower in adults and Black Bengal breed goats with good body conditions. *Salmonella* infection in goats occurs in all ages, all seasons both in males and females, is responsible for considerable loss in kids and even may cause abortion ranging from 22% to 38% during the last third of gestation in adults (Habrun *et al.*, 2006). This infection may be a problem in the goat rearing industry and areas where this will surely impair the fattiness and sound health of goats and give poor quality and quantity of goat meat which leads to poor market value (Arruda *et al.*, 2004). Infection by *Salmonella* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). The prevalence rate of the *Salmonella* organism in the goats of this country is not insignificant and should not be overlooked, because of the public health significance and the possibility of dissemination of diseases in man, animals, and birds.

Isolation and Identification of *Salmonella*

Specific enrichment media and biochemical tests were used for the isolation and identification of *Salmonella* which was previously suggested by a number of

Table 1. Results of isolation of *Salmonella* from faecal samples of apparently healthy and diarrheic goats

Serial No.	Source of animal faecal samples	No. of positive samples	No. of collected samples	Prevalence rate (%)	Overall prevalence rate (%)
1	BAU Veterinary clinic	12	3	25	12.76 (6/47)
2	BAU goat farm	13	2	15.38	
3	Babul goat farm	12	0	0.0	
4	Savar goat farm	10	1	10	

researchers (Ruiz *et al.*, 1992; Dhruba *et al.*, 1999; Habrun *et al.*, 2006). In this study, the colony characteristics of *Salmonella* from different species of animals and birds on MC agar, SS agar, and BG agar were similar to the findings of other authors (Shaffer *et al.*, 1964; Merchant and Packer, 1967).

In Gram staining, the morphological characteristics of the isolated *Salmonella* exhibited Gram negative, small, rod shaped,

single, or paired in arrangement under the microscope which was supported by other researchers (Burrows and Freeman, 1985; Jones *et al.*, 1997; Gene, 2002). In the motility test, all of the isolates of goat were found to be motile. This result correlated with the results of Merchant and Packer, (1967) and Buxton and Fraser, (1977) .

Differentiation of *Salmonella* into species level was difficult based on their sugar

Table 2. Results showing isolation of *Salmonella* and their prevalence rate in different epidemiological parameters

Epidemiological parameter	Level of pattern	No. of animals examined	No. of animals affected	Prevalence rate (%)
Age	1-12 months	12	2	16.67
	13-24 months	20	3	15
	25-36 months	15	1	6.67
Sex	Male	19	3	15.78
	Female	28	3	10.71
	Breed Local	25	4	16
	Black Bengal	22	2	9.09
Season	Rainy	14	1	7.14
	Winter	12	1	8.33
	Summer	21	4	19.04
Health status	Apparently healthy	38	2	5.26
	Sick	9	4	44.44

Table 3. Antibiotic sensitivity pattern of *Salmonella* isolates from goats

Source of sample	Isolated <i>Salmonella</i>	Highly sensitive (+++)	Moderately sensitive(++)	Less sensitive (+)	Resistant (-)
G ₁	G _{1a}	CIP, SP, GN	S, OT	AML	RL, P
	G _{1b}	SP, GN	CIP, OT	AML, S	RL, P
	G _{1c}	CIP, GN	SP, S	OT, AML	RL, P
G ₂	G _{2a}	CIP, SP	GN, S, OT	AML, RL	P
	G _{2b}	CIP, SP, GN	S, RL, AML	OT	P
G ₃	G _{3a}	CIP, SP	OT, AML, GN	P, S	RL

G_{1a}, G_{1b}, G_{1c} = Isolated *Salmonella* from BAU Veterinary clinic, G_{2a}, G_{2b} = Isolated *Salmonella* from BAU goat farm, G_{3a} = Isolated *Salmonella* from Savar goat farm, CIP = Ciprofloxacin, SP = Spiramycin, GN = Gentamycin, S = Streptomycin, OT = Oxytetracycline, AML = Amoxicillin, RL = Sulphamethoxazole, P = Penicillin-G

fermentation pattern (Burrows and Freeman, 1985). In the sugar fermentation test, all of the isolated *Salmonella* fermented dextrose, maltose, and mannitol and produced acid and gas but did not ferment sucrose and l lactose which satisfied the statement of Buxton and Fraser (1977). Again all of the isolated *Salmonella* were positive to the MR test and negative to the indole test and Voges-Proskauer test.

In this study the slide agglutination test was performed with commercially available agglutinating polyvalent antisera which are very simple and sensitive (Avakian *et al.*, 1988), whereas the enzyme-linked immunosorbent assay test is very expensive and time consuming (Begum, 2005). All the isolates were agglutinated with both poly "O" and poly "H" antisera which indicated that the isolates were *Salmonella* spp.

Results of Antibigram of the Isolated *Salmonella*

Isolated *Salmonella* spp. showed various degrees of sensitivity to oxytetracycline, gentamycin, sulphamethoxazole, spiramycin, streptomycin, amoxicillin, penicillin-G, and ciprofloxacin. Out of 6 *Salmonella* isolates from goats, 83.33% were highly sensitive and 16.67% were moderately sensitive to ciprofloxacin and spiramycin, respectively; 66.67% were highly sensitive and 33.33% were moderately sensitive to gentamycin; 66.67% were moderately sensitive and 33.33% were less sensitive to oxytetracycline; 66.67% were moderately sensitive and 33.33% were less sensitive to streptomycin; 33.33% were moderately sensitive and 66.67% were less sensitive to amoxicillin; 16.67% were moderately sensitive, 16.67% were less sensitive, and 66.66% were resistant to sulphamethoxazole; and 16.67% were less sensitive and 83.33% were resistant to penicillin-G. (Table 3). The antibiotic sensitivity tests were performed by the disc diffusion method using 8 different antibiotic discs. Most of the *Salmonellae* isolates were highly sensitive to ciprofloxacin, spiramycin, and gentamicin; moderately sensitive to oxytetracycline, streptomycin, and amoxicillin;

less sensitive to sulphamethoxazole; and resistant to penicillin-G. These findings satisfy the results of Yadav *et al.* (2006) and Habrun *et al.* (2006). However, the authors found that *S. berta* was moderately sensitive to penicillin. The antibacterial sensitivity pattern of the *Salmonella* isolates recorded in this study might be due to indiscriminate use of those antibacterial agents in field conditions. This provides a guideline to veterinarians for selecting appropriate antibiotics. Data of the antibacterial sensitivity pattern indicated that antibiotic resistant *Salmonella* isolates were present in goats, which might affect public health.

Conclusions

Since only *Salmonella* commonly plays the major role for salmonellosis, the slide agglutination test can be used for the rapid detection of *Salmonella* in field cases. Salmonellosis occurred most frequently in young, sick, and local breeds of goat. The prevalence of Salmonellosis is higher in summer. Ciprofloxacin, spiramycin, and gentamicin were the best choices of drugs among the antibacterials available in the market. Variation of the antibacterial sensitivity or resistance patterns was observed in this study.

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