Bangl. J. Vet. Med. (2008). 6 (1): 75–78

ISOLATION AND CHARACTERIZATION OF SHEEP SALMONELLAE IN AND AROUND BANGLADESH AGRICULTURAL UNIVERSITY CAMPUS

M. R. Karim[1], M. S. R. Khan[1], M. E. H. Kayesh[1], M. R. Islam[1] and M. O. Gani[2]

[1]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, [2]Goat and Sheep Production Research Division, BLRI, Savar, Dhaka, Bangladesh

ABSTRACT

The study was carried out for isolation of *Salmonella* spp. and their characterization from sheep during the period from November 2006 to May 2007. For this study a total number of 63 rectal swab samples of sheep were collected. Out of 63 samples, 9 samples were identified as positive for *Salmonella* and each positive sample was treated as one isolate. All the *Salmonella* isolates revealed the same morphological, cultural and biochemical characteristics. In the stab culture on TSI agar slant, all of the isolated *Salmonella* isolates produced acid (yellow) and gas in the butt, the production of hydrogen sulfide gas in both butt and slant, and the alkaline (red) reaction in the slant. For the slide agglutination test, *Salmonella* agglutinating antiserum (poly 'O' and poly 'H') was used which agglutinated all the isolates and thereby identified the organism as *Salmonella*. The pathogenicity of the isolates was studied in mice and it revealed that the isolates were pathogenic. After isolation of *Salmonella*, eight commonly used antibiotics were used for the antibiogram study of isolated *Salmonella*. Among the isolates, 77.78% were highly sensitive and 22.22% were moderately sensitive to ciprofloxacin and spiramycin, 66.67% were highly sensitive and 33.33% were moderately sensitive to gentamicin. 55.56% were moderately sensitive and 44.44% were less sensitive to oxytetracycline and streptomycin, 33.33% were moderately sensitive and 66.67% were less sensitive to amoxycillin, 11.11% were moderately sensitive, 33.33% were less sensitive and 66.67% were resistant to sulphamethoxazole, 11.11% were less sensitive and 88.89% were resistant to penicillin-G. From the results it may be concluded that the prevalence of *Salmonella* organisms in sheep of this country is remarkable and isolated *Salmonella* spp. are pathogenic for laboratory animals and highly sensitive to ciprofloxacin, spiramycin and gentamicin. From the results it also may be concluded that slide agglutination test can be used for the rapid detection of *Salmonella* organism.

Key words: isolation, characterization, pathogenicity, antibiogram study

INTRODUCTION

Salmonellosis is a disease caused by a wide variety of *Salmonella* serovars in various hosts including human being. This disease is also considered as an important bacterial disease in sheep (OIE Manual, 2006) which remain as a serious problem with public health significance throughout the world (Uzzau *et al*., 2000). Among domestic animals, sheep play a significant role in the subsistence economy of Bangladesh (FAO, 1991). *Salmonella* causes infection in all ages of sheep and is responsible for a considerable loss in lambs, even may cause abortion ranging from 22% to 38% during the last-third of gestation in adults (Habrun *et al*., 2006). Indiscriminate use of antimicrobial drugs to control economically important disease, Salmonellosis in sheep without prior testing of etiologic agent might have resulted emergence of multi-drug resistant *Salmonella* strains (Mirza *et al*., 1996) and thus it will limit the therapeutic success in the treatment of this disease. To select the suitable antibacterial agents for effective therapeutic use against Salmonellosis in sheep, the antibiogram study of isolated *Salmonella* should be performed. In order to prevention and control of any microbial disease prior isolation and characterization of that particular etiological agent is a pre-requisite. Therefore, the present research work was undertaken to isolate and identify *Salmonella* spp. from apparently healthy and diseased sheep through cultural, morphological and biochemical characterization and to study the pathogenicity and antibiogram of the isolated *Salmonella* spp.

MATERIALS AND METHODS

The research work was carried out in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh during the period from November 2006 to May 2007. A total number of 63 rectal swab samples were aseptically collected from the sheep of selected areas for the investigation of *Salmonellae*. Bacto selenite broth (SB), Nutrient broth (NB), MacConkey agar (Mc), Salmonella-Shigella (SS) agar, Brilliant green (BG) agar, Eosin methylene blue (EMB) agar, Nutrient agar (NA) and Blood agar, Sugar media (dextrose, maltose, lactose, mannitol and sucrose) and Triple sugar iron (TSI) agar slant were used for conducting the study. Gram's stain, Methyl Red-Voges Proskauer (MR-VP) solution, Kovac's reagent, and Phosphate buffered saline (PBS) solution were also used. *Salmonella* polyvalent antiserum (Poly "O" & Poly "H") was used for the serological identification of *Salmonella* spp.

Day old white mice were obtained from the Department of Microbiology and Hygiene, BAU, Mymensingh and were used for the pathogenicity study of the isolated organism. Eight different antibacterial discs were used for the antibiogram study of isolated *Salmonella*. After collection of samples, each of the rectal swabs was inoculated into each of the freshly prepared SB and incubated aerobically at 37^0C for 24 hours in bacteriological incubator. The incubated tubes were then examined for growth of bacteria and from all incubated tubes a loopful was streaked separately into the Mc agar plates and then incubated aerobically at 37^0C for 24 hours in bacteriological incubator for the growth of bacteria. Lactose non fermenter colorless colonies from Mc agar were sub-cultured on SS agar. Translucent round and colorless colonies on SS agar were suspected to be *Salmonella*. Then, a loopful of bacterial culture from SS agar were streaked separately into the EMB agar and BG agar plates and incubated aerobically at 37^0C for 24 hours in bacteriological incubator. Then the plates were examined and studied carefully for the presence of characteristic colonies of *Salmonellae*. The characteristic colonies produced by the isolated *Salmonellae* on SS agar and BG agar plates which were later confirmed by biochemical test and the motility test. The incubated tubes containing bacterial growth were preserved at refrigerator for further use. The smears were prepared from each of the agar plates. The smears were then fixed and stained with Gram's method of staining and examined under microscope at 100 magnifications for the presence of Gram negative rods. BG agar plates containing characteristic colonies of *Salmonellae* were selected for sub culturing in order to obtain a pure culture of the organisms. The organisms thus obtained as pure culture were selected for subsequent studies. The representative *Salmonella* colonies were characterized morphologically using Gram's stain according to the method described by Merchant and Packer (1967).

The motility test was performed to differentiate motile bacteria from non-motile one as per method described by Cheesbrough (1985). Methyl Red test, Voges-Proskauer test, Indole test and Sugar fermentation test were performed as per method described by Cheesbrough (1985). Pathogenicity test was performed as per method described by Merchant and Packer (1967). Susceptibility of the isolated *Salmonellae* to different antibacterial agents was performed through disc diffusion method to determine the drug sensitivity pattern. This method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that result from different rates of diffusion of the agent into the medium surrounding the disc.

RESULTS AND DISCUSSION

The results of isolated of *Salmonella* are shown in Table 1. *Salmonella* isolated from one animal was considered as one isolate. The organisms were isolated from 63 samples and identified as isolate number R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9. Among the positive samples, 3 (R_1, R_2 and R_3,) were from Sheep farm, BAU; 2 (R_4 and R_5) were from Animal Nutrition field lab, BAU and 4 (R_6, R_7, R_8 and R_9) were from Veterinary Clinic, BAU, Mymensingh. The percentages of prevalence of *Salmonella* from those areas were 14.29, 11.11 and 16.67%, respectively.

In Gram's staining, the morphology of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which is supported by Gene (2002) and Freeman (1985). In motility test, all the isolates showed swinging movements which differentiate the motile bacteria from non-motile bacteria and also from *E. coli* which had forward movement, that support the findings of Buxton and Fraser (1977) and Merchant and Packer (1967). In this study, colony characteristics of *Salmonella* on Mc agar, SS agar and BG agar were similar to the findings of Buxton and Fraser (1977) and Merchant and Packer (1967).

Table 1. Isolation of *Salmonella* from rectal swab samples collected from selected areas

Source of samples	No. of sample collected	No. of samples positive for *Salmonella*	Percentage of S*almonella* prevalence
Sheep farm, BAU	21	3	14.29
Animal nutrition field lab, BAU	18	2	11.11
Veterinary Clinic, BAU	24	4	16.67
Total	63	9	14.29

In sugar fermentation test, all of the isolated *Salmonella* fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which satisfy the statement of Buxton and Fraser (1977). Again, all the isolates were positive to methyl red test and negative to indole test. TSI agar was used which was one of the best biochemical media for the identification and differentiation of *Salmonella* from the other allied organisms (Merchant and Packer, 1967). In this study, slide agglutination test was performed with commercially available agglutinating polyvalent antiserum which is very simple and sensitive (Avakian *et al*., 1988).

The pathogenicity test revealed that the broth culture of the isolates was pathogenic in mice by oral administration but more pathogenic when administered intra-peritonially. These were indicated by no lethal effects of the isolates in mice within 36 hours of oral administration and more than 23% death of mice within 48 hours of intra-peritonial injection of isolates. The results are similar to those of Jack (1968), Khan (1961) and Schnelder (1951) who demonstrated lethal and non-lethal effects in mice by parenteral and oral administration of the broth culture of *Salmonella*, respectively.

Among the isolates R_1, R_2 and R_3, 100% were highly sensitive to gentamicin, 66.66% were moderately sensitive to spiramycin, ciprofloxacin, streptomycin, oxytetracycline, 33.33% were moderately sensitive to spiramycin, ciprofloxacin and amoxycillin and 33.33% were less sensitive to streptomycin, oxytetracycline, 66.66% were less sensitive to amoxycillin and 100% were resistant to penicillin-G (Table 2). Between the isolates R_4 and R_5, 100% were highly sensitive to spiramycin, 50% were highly sensitive and 50% were moderately sensitive to ciprofloxacin and gentamicin, 50% were moderately sensitive and 50% were less sensitive to streptomycin, sulphamethoxazole, amoxycillin, 100% less sensitive to oxytetracycline and 100% were resistant to penicillin.

Table 2. Results of antibiogram study of isolated *Salmonellae*

Salmonella isolates	Highly sensitive (+++)	Moderately sensitive (++)	Less sensitive (+)	Resistant (-)
R_1	CIP, SP, GN	S, OT	AML	RL, P
R_2	SP, GN	CIP, OT,	AML, S	RL, P
R_3	CIP, GN,	SP, S, AML,	OT,	RL, P
R_4	CIP, SP	GN, S,	OT, AML, RL	P
R_5	SP, GN,	CIP, RL, AML	OT, S	P
R_6	CIP, SP, GN	OT, AML,	RL, S	P
R_7	CIP, SP,	GN, OT,	S, AML, P	RL,
R_8	CIP, GN,	SP, S, OT,	AML, RL	P
R_9	CIP, SP	GN,	S, OT, AML,	RL, P

R_1- R_3 = Rectal swab sample of Sheep farm, BAU; R_4-R_5= Rectal swab samples of Animal Nutrition field Lab, BAU; R_6-R_9 = Rectal swab samples of Veterinary Clinic, BAU; GN=Gentamicin, CIP= Ciprofloxacin, SP= Spiramycin, AML= Amoxycillin, S= Streptomycin, OT= Oxytetracyline, RL= Sulphamethoxazole, P= Penicillin-G.

Among the isolates R_6, R_7, R_8 and R_9, 75% were highly sensitive and 25% moderately sensitive to spiramycin, 100% were highly sensitive to ciprofloxacin, 50% were highly sensitive and 50% moderately sensitive to gentamicin, 25% were moderately sensitive and 75% were less sensitive streptomycin and amoxycillin, 75% were moderately sensitive and 25% were less sensitive to oxytetracycline, 25% were less sensitive and 75% were resistant to penicillin-G, 50% were less sensitive and 50% were resistant to sulphamethoxazole. Moreover, among all the isolates, 77.78% were highly sensitive and 22.22% were moderately sensitive to ciprofloxacin and spiramycin respectively, 66.67% were highly sensitive and 33.33% were moderately sensitive to gentamicin, 55.56% were moderately sensitive and 44.44% were less sensitive to oxytetracycline and streptomycin, 33.33% were moderately sensitive and 66.67% were less sensitive to amoxycillin, 11.11% were moderately sensitive, 33.33% were less sensitive and 66.67% were resistant to sulphamethoxazole, 11.11% were less sensitive and 88.89% were resistant to penicillin-G. Most of the isolated *Salmonellae* were highly sensitive to ciprofloxacin, spiramycin, and gentamicin, moderately sensitive to oxytetracyline, streptomycin, and amoxycillin, less sensitive to sulphamethoxazole and resistant to penicillin-G. These findings are in conformity with the result of Yadav *et al*. (2006) and Habrun *et al*. (2006). Isolate R_7 was less sensitive to penicillin-G which correlates with the findings of Roy *et al*. (2002). The authors found *S. berta* was moderately sensitive to penicillin. The antibacterial resistance observed here in the isolated *Salmonellae* might be due to routine indiscriminate use of those antibacterial agents in field condition in study areas and or rapid chromosomal mutation and presence of specific plasmid DNA.

From the above findings it may be concluded that the prevalence of *Salmonella* organisms in sheep of this country is remarkable, and isolated *Salmonella* spp. are pathogenic for laboratory animals and that might be pathogenic for other animals also. And the isolated *Salmonella* spp. are highly sensitive to ciprofloxacin, spiramycin and gentamicin.

REFERENCES

1. Avakian AP, Kleven SH and Glisson JR (1988). Evaluation of the specificity and sensitivity of two commercial enzyme linked immunosorbent assay kits, the serum plate agglutination test and haemagglutination inhibition test for antibodies formed in response to *Salmonella* spp. *Avian Diseases* 32: 262-272.
2. Buxton A and Fraser G (1977). *Animal Microbiology*. Vol. 1. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne. pp. 85-86.
3. Cheesbrough M (1985). *Medical laboratory manual for tropical countries*. Vol. 2. Microbiology. pp. 400-480.
4. FAO (1991). Asian Livestock. *Monthly Technical Magazine of the FAO Animal Production and HealthCommission for Asia and the Pacific (APHCA)* 16 (8): 85-87.
5. Freeman BA (1985). *Burrows Textbook of Microbiology*. 22nd edn., WB Saunders Company, Philadelphia, USA. pp. 464-472.
6. Gene O (2002). The isolation nand identification and serotyping of *Salmonella* isolated from domestic poultry in Kars district. *Kafkas Univarsikesi Veteriner Fakultesi Dergisi* 8 (1): 23-30.
7. Habrun B, Listes E, Spicic S, Cvetnic Z, Lukacevic D, Jemersic L, Lojkic M and Kompes G (2006). An outbreak of *Salmonella abortusovis* abortions in sheep in South Croatia. *Journal of Veterinary Medicine Series* 53 (6): 286–290.
8. Jack EJ (1968). *Salmonella abortus ovis*, an atypical *Salmonella*. *Veterinary Record* 82: 558- 561.
9. Khan AQ (1961). Isolation of *Salmonella* from a sheep and a goat. *Research in Veterinary Science* 2: 130.
10. Merchant IA and Packer RA (1967). *Veterinary Bacteriology and Virology*. 7th edn., The Iowa University Press, Ames, Iowa, USA. pp. 286-306.
11. Mirza SH, Beeching NJ and Hart CA (1996). Multi-drug resistant typhoid: a global problem. *Journal of Medical Microbiology* 44: 317-319.
12. OIE (2006). Salmonellosis. http://www.oie.int/chapter X.4.
13. Roy P, Dhillon AS, Lauerman LH, Schaberg DM, Bandli D and Johnson S (2002). Results of *Salmonella* spp. isolation from poultry products, poultry, poultry environment and other characteristics. *Avian Diseases* 46 (1): 17-24.
14. Schnelder MMD (1951). Pathogenecity of *Salmonella typhimurium* administered subcutaneously to mice in mixture with each Polynucleotide complex from *Salmonella typhimurium*, *Salmonella typhosa*, *Salmonella neoport. American Journal of Veterinary Research* XII: 340-344.
15. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesús J, Platt DJ and Olsen JE (2000). Host adapted serotypes of *Salmonella enterica*. *Epidemiology and Infection* 125: 229-255.
16. Yadav MM, Sham Tale, Rakesh Sharda, Varsha Sharma, Sheela Tiwari, Umesh K and Garg (2006). Bacteriological quality of sheep meat in Mhow town of India. *International Journal of Food Science & Technology* 41 (10): 1234–1238.