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Isolation and Characterization of *Salmonella* Serovars from Buffaloes in Mymensingh, Bangladesh

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A B S T R A C T

An investigation was carried out focusing the isolation and characterization of *Salmonella* serovars from buffaloes of some selected areas of Mymensingh district of Bangladesh. The objectives was to isolate and identify *Salmonella* serovars from diarrheic and apparently healthy buffaloes and to characterize the isolates by cultural and biochemical characteristics, serological tests and antibiotic sensitivity analysis. A total of 38 samples comprising rectal swabs and faeces were collected from 38 buffaloes originating from 3 selected areas of Mymensingh. Out of these 38 samples, 8 (20.63%) were found to be positive for *Salmonella* spp. All isolates fermented dextrose, maltose and mannitol with production of acid and gas but did not ferment sucrose and lactose. On the other hand, these isolates showed Indole and Voges-Proskaure test negative, Methyl-Red test positive. All these isolates subjected to rapid plate agglutination test with polyvalent "O" (poly 'O') and polyvalent "H" (poly 'H') antisera where positive agglutination were observed. All isolates were highly sensitive to ciprofloxacin, moderately sensitive to co-trimoxazole, gentamycin, tetracycline and less sensitive to erythromycin and resistant to furazolidone.

Keywords: Isolation, Characterization, Salmonella, Serovars, Buffalo, Mymensingh

Introduction

Salmonellosis is a disease condition caused by a wide variety of *Salmonella* spp. in various hosts including humans, cattle, sheep, goats, pigs, chickens, ducks and buffaloes (OIE, 2006) which remains as a serious public health problem throughout the world. Salmonellosis is manifested clinically in all hosts by one of three major syndromes: per-acute systemic infection, acute enteritis or chronic enteritis (Merchant and Packer, 1967). Among domesticated animals, buffaloes constitute one of the important reservoirs of Salmonella and are susceptible to disease caused by a wide variety of serotypes. Salmonella infection in buffalo occurs in all ages and is responsible for a considerable loss of buffalo calves, (Arruda et al., 2004). This infection may be a major problem in meat producing and buffalo rearing industries and areas where this will surely impair the fattiness and sound health of buffalo and give the poor quality and quantity of meat which leads to poor market value (Arruda et al., 2004). On the other hand, Salmonella organisms were isolated from chickens (Begum, 1992), goats (Rahman, 2006), Cattle (Islam 2007), Sheep (Karim, 2007), ruminants (Rahman, 2007) in the Department of Microbiology and Hygiene, Faculty of veterinary Science (FVS), Bangladesh Agricultural University (BAU), Mymensingh. Further details on Salmonella serovars infecting Buffaloes in Bangladesh including the antibacterial sensitivity pattern deemed important to design proper control measures. Present study was designed to isolate and characterize Salmonella serovars using cultural, morphological, biochemical and serological examination to know the prevalence of Salmonellosis in buffaloes of Bangladesh as well as to reveal the antibiotic sensitivity and resistance pattern of the isolates.

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Materials and Methods

Collection of samples

A total of 38 field samples comprising rectal swab from apparently healthy and diarrheic buffaloes, reared at BAU dairy farm, Trishal and Boyra area, Mymensingh, were collected under aseptic condition and carried out to the laboratory using Nutrient broth (NB).

Isolation and characterization

The collected samples from apparently healthy and diarrheic buffaloes were inoculated to Nutrient agar (NA), Salmonella-Shigella agar (SS), Brilliant green agar (BG), MacConkey agar (MC) and Eosin methylene blue agar (EMB). Petridishes were incubated at 37ºC for 24-48 hours and growing colonies were examined with Gram's staining method (Merchant and Packer, 1967) and motility test with hanging drop slide (Cowan, 1974).Biochemical characterization of the isolates were performed with Sugar fermentation test, Methyl Red test(MR) and Voges-Proskaucer test (V-P) (Cheesbrough, 1985).

Serological test

Salmonella agglutinating antiserum poly "O" and poly "H" (S & E reagents Lab, Bangkok, Thailand) was used to perform the serotyping of the isolated *Salmonella* spp. The macroscopic slide agglutination tests were performed. A single isolated colony from 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 one to two minutes were selected as positive for Salmonella and subjected to agglutination test with

Salmonella agglutinating antiserum (poly "H"). According to manufacturer's direction, it was noted that poly "O" antiserum gives positive agglutination reaction with any serovars for preliminary screening of Salmonellae and poly "H" antiserum gives specific agglutination reaction for motile *Salmonella* spp. (Buxton and Fraser, 1977).

Antibiotic sensitivity test

Susceptibility of the isolated Salmonellae to different antibacterial agents was performed following the disc diffusion method (Bauer et al, 1966) to determine the drug sensitivity pattern. Sensitivity to antibiotic was mostly determined on Nutrient agar with tetracycline, gentamycin, co-trimoxazole, furozolidone, erythromycin, ciprofloxacin. The inhibitory effect of the antibacterials to the growth of the culture was recorded (Bauer et al., 1966)

Result

Cultural and biochemical characterization of Salmonellae

All the isolates Salmonellae from three different areas showed characteristics colony morphology in NB agar, SS agar, MC agar, TSI agar, BG agar media. The isolates were Gram negative, small rod shaped, single or paired in arrangement and motile. In sugar fermentation test, all isolated Salmonellae fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose. In addition, all the isolates were found positive for MR test and negative to Indole test and V-P test (Cheesbrough, 1985).

Table 1. Cultural, morphological and biochemical characteristics of the isolated Salmonellae from buffaloes

No. of samples (n)	Cultural examination		Biochemical examination		Gram staining examination		Prevalence
	Positive for Salmonella	Negative for Salmonella	Positive for Salmonella	Negative for Salmonella	Single	Paired	
BAU dairy farm (12)	3	9	3	9	2	1	25%
Trishal(14)	4	10	4	10	2	2	28.57%
Boyra (12)	1	11	1	11	-	1	8.33%
Total (38)	8	30	8	30	4	4	21.05%

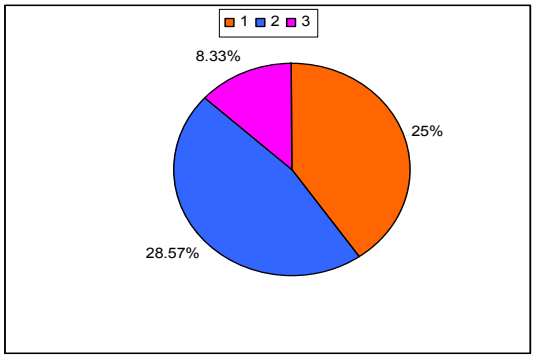

Fig. 1. Pie diagrammatic presentation of overall prevalence (as shown in Table 1) of buffalo origin Salmonella in three different regions. Legends: 1= BAU dairy farm, 2= Trishal, 3= Boyra, Mymensingh

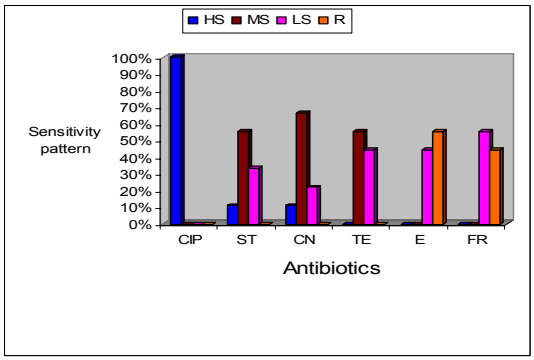

Fig. 2. Diagrammatic presentation of Antibiotic Sensitivity and Resistance pattern of buffalo Salmonellae isolates (CIP= Ciprofloxacin, ST= Co-trimoxazole, CN= Gentamycin, TE= Tetracyclin, E= Erythromycin, FR= Furozolidone, HS = highly sensitive, MS= Moderately sensitive, LS = Less sensitive, R = Resistant)

Serological characteristics of isolated Salmonellae

Out of 38 samples, 8 samples were isolated and all isolates were positive to agglutination tests confirming *Salmonella* spp.

Antibiotic sensitivity test of isolated Salmonellae

The isolated Salmonellae from all three areas were highly sensitive to Ciprofloxacin, gentamycin; moderately sensitive to Co-trimoxazole, tetracycline, gentamyin. They are less sensitive to erythromycin, tetracycline and nearly resistant to furozolidone.

Discussion

The isolated Salmonella serovars produced opaque, translucent, colorless and smooth, round colonies on SS agar; pale colorless smooth, transparent, raised colonies on MC agar, pale pink color colonies in BG agar (Buxton and Fraser, 1977; Merchant and Packer, 1967 & Shaffer et al., 1964). In Gram staining, the isolated Salmonellae showed Gram negative, small rod shaped, single or paired in arrangement which was supported by other researchers (Gene, 2002, Jones et al., 1997 and Freeman, 1985). In motility test, all isolates of buffalo have shown swinging movement with forward movement (Buxton and Fraser, 1977 & Merchant and Packer, 1967). All the isolated Salmonellae fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose (Buxton and Fraser, 1977). All the isolated were positives to MR test and negative to Indole test. Slide agglutination test was performed with commercially available agglutinating polyvalent antisera. The isolates gave positive result to agglutination test with both poly "O" and poly "H" antisera which indicated that the isolates were of *Salmonella spp*. ELISA can also be done but expensive and gives non-specific reaction and time consuming (Begum, 2005) whereas slide agglutination test is very simple, sensitive (Avakian et al., 1988).

The study also showed that the isolated Salmonellae were highly sensitive to Ciprofloxacin, gentamycin; moderately sensitive to Co-trimoxazole, tetracycline, gentamyin. They were less

sensitive to erythromycin, tetracycline and nearly resistant to furozolidone. This finding is similar to the result of Chugh and Suheir, 1983; Banani et al., 2003; Zhang et al., 2006 and Kobayashi et al., 2007.

Conclusion

The overall prevalence of *Salmonella* spp. was 21.05% from buffalo fecal samples as detected by cultural, morphological and biochemical examination and by slide agglutination test. This method may used for the rapid detection of Salmonella in field cases in buffaloes. Ciprofloxacin, co-trimoxazole, gentamycin appears to be the choice of drug from the list of antibacterial agents we tested. The antibacterial resistance observed here in the isolated Salmonellae might be due to indiscriminate use of these antibacterial agents in field condition in study areas and/or rapid chromosomal mutation and presence of specific plasmid DNA.

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