

Occurrence and Antibiotic Susceptibility of *Salmonella* Serotypes in Apparently Healthy Slaughtered Sheep in Van, Turkey

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Abstract: This study was conducted to investigate the occurrence of *Salmonella* sp. and to determine their susceptibility to antibiotics in apparently healthy slaughtered sheep. In this research, a total of 600 (300 rectal and 300 vaginal) swabs taken from sheep were used as materials. A total of 9 (1.5%) *Salmonella* sp. were isolated from samples. Of the 9 *Salmonella* sp., 6 (2%) were isolated from rectal swabs and 3 (1%) *Salmonella* serotypes from vaginal swabs. *Salmonella* strains were serotyped as *Salmonella saintpaul*. All *Salmonella* strains were found to be susceptible to ampicillin, enrofloxacin, tetracycline, oxytetracycline, gentamicin, neomycin, chloramphenicol, spectinomycin, cephalothin, amoxycillin/clavulanic acid, trimethoprim+ sulfamethoxazole, nalidixic acid and penicillin G (except 2 isolates) whereas, they were resistant to erythromycin and novobiocin. This is the first time, *S. saintpaul* was isolated in rectal and vaginal swab samples of the sheep slaughtered in abattoirs in Turkey.

Key words: Sheep, serotyping, *S. saintpaul*, occurrence, antibiotic susceptibility, Turkey

INTRODUCTION

Salmonella strains belonged to the Enterobacteriaceae family are most commonly found in nature. These organisms are Gram-negative facultative anaerobic bacilli of the genus *Salmonella* and they are most heterogeneous group of Gram-negative bacteria. The genus *Salmonella* includes into 2 groups as *Salmonella enterica* and *Salmonella bongori*. *S. bongori* group includes less commonly observed serovars (<10), while *S. enterica* group covers >2500 remaining serovars. *Salmonella* are classified into serogroups according to their somatic antigens (O) and serovars according to their flagellar (H) antigens (Le Minor, 1984; Holt *et al.*, 1994; Brenner *et al.*, 2000).

Salmonella strains cause infections both in humans and animals. Among these *S. abortus ovis* results in abortions in sheep, whereas certain *Salmonella* strains (*S. typhimurium*, *S. enteritidis*, *S. newport*, *S. anatum*, *S. infantis* etc.) cause diarrheal diseases (Holt *et al.*, 1994; Hjartardottir *et al.*, 2002; Kudaka *et al.*, 2006). *S. abortus ovis* is mostly responsible for infections resulting in abortions in sheep while, *S. dublin*, *S. enteritidis* and *S. typhimurium* have also been isolated from certain animals experiencing abortions (Plagemann, 1989;

Hjartardottir *et al.*, 2002). Possible causes for the occurrence of infections resulting from *Salmonella* are inclusion of infected sheep to the flocks, use of contaminated feed and water, wild birds, other animals and human movements. Apparently healthy animals can act as reservoirs, the source of infection is generally adult sheep, spread is reported to occur with the oral ingestion of feed and water contaminated by material like feces, aborted fetus, fetal membranes and vaginal discharges (Sandberg *et al.*, 2002; Woldemariam *et al.*, 2005). The causative agent localizes to the uterus, intestines, urinary bladder and gall bladder and is excreted via feces and urine (Linklater, 1991). If sufficient hygienic measures are not taken during slaughtering of the animals in abattoirs especially in critical control points, there is high risk for carcasses and internal organs to be contaminated with *Salmonella* and other pathogenic agents (Zweifel *et al.*, 2004; Woldemariam *et al.*, 2005).

It was informed that salmonellosis can be controlled with antimicrobial therapy (Molla *et al.*, 2006). There are several number of studies related the detection of susceptibility of *Salmonella* strains isolated from different sources to different antimicrobial agents (Threlfall *et al.*, 2003; Davies *et al.*, 2004; Intorre *et al.*, 2005; Molla *et al.*, 2006).

In this study, we aimed at serotyping the isolated *Salmonella* strains from rectal and vaginal swab samples of sheep slaughtered in abattoirs in Van and to test the antimicrobial resistance patterns.

MATERIALS AND METHODS

Materials were collected from 22 different sheep flocks, which represented 7.800 sheep. Three hundred rectal and 300 vaginal swab samples from apparently healthy sheep slaughtered in Van from February 2006 to May 2006.

Isolation and identification of *Salmonella*: Rectal and vaginal swabs obtained from the sheep were first placed into previously prepared and numbered tubes containing 10 mL of Buffered Peptone Water (BPW, Oxoid) and were immediately transferred to the laboratory under cold chain. For preliminary enrichment, rectal and vaginal swabs were incubated in BPW at 37°C for 18-24 h. At the end of this period, the culture media were well mixed with vortex, 0.1 mL of broth was inoculated into Rappaport Vassiliadis Enrichment Broth (Oxoid), this was incubated at 42°C for 24-48 h. Following the incubation, the media were well agitated with vortex, a loop full of culture was obtained to be inoculated onto *Salmonella* Shigella Agar (Oxoid) with streak method and was incubated at 37°C for 18-24 h. Suspect *Salmonella* colonies were confirmed biochemically and serologically, after which they were serotyped (Holt *et al.*, 1994; Carter *et al.*, 1995; Aksakal, 2003; Sandberg *et al.*, 2003).

Serotyping: The strains that were considered to be *Salmonella* depending on their biochemical characteristics were further tested with *Salmonella* polyvalent anti O serum (*Salmonella* O Antiserum Poly A-I and Vi, Becton, Dickens and Co. Sparks, MD, USA) according to test instructions. Positively reacting strains were identified as *Salmonella*, further serogrouping and serotyping was carried out by using polyvalent and monovalent O and H antisera (Refik Saydam National Hygiene Center, Ankara, Turkey and Statens Serum Institut, Hillerød, Denmark) according to Kauffmann-White scheme in National Reference Laboratory for Enteric Pathogens, Communicable Diseases Research Department in Refik Saydam National Hygiene Center (Popoff, 2001).

Antibiotic susceptibility testing: Antibiotic susceptibility testing was performed using a disk diffusion method on Mueller Hinton Agar (Merck) as previously described

according to the standards outlined by the National Committee for Clinical Laboratory Standards (NCCLS). The antibiotic discs contained the following antimicrobial agents: gentamicin (Bioanalyse, 10 µg), neomycin (Bioanalyse, 30 µg), tetracycline (Bioanalyse, 30 µg), oxytetracycline (Oxoid, 30 µg), enrofloxacin (Bioanalyse, 5 µg), nalidixic acid (Oxoid, 30 µg), ampicillin (Bioanalyse, 10 µg), amoxycillin/clavulanic acid (Oxoid, 30 µg), penicillin G (Oxoid, 10 IU), erythromycin (Bioanalyse, 15 µg), trimethoprim+sulfamethoxazole (Bioanalyse, 25 µg), spectinomycin (Bioanalyse, 100 µg), cephalothin (Oxoid, 30 µg), novobiocin (Oxoid, 30 µg) and chloramphenicol (Bioanalyse, 30 µg). Resistance was determined according to reference zone diameter interpretive standards (NCCLS, 2000).

Statistical analyses: The statistically significant differences among the rates of *Salmonella* isolation between rectal and vaginal swabs was calculated with chi-square (χ^2) test (Sumbuloglu and Sumbuloglu, 1997).

RESULTS

Isolation and identification: From a total of 600 swabs (300 rectal, 300 vaginal) 9 (1.5%) *Salmonella* strains were isolated and identified (Table 1).

Six *Salmonella* strains were isolated and identified from rectal swabs (6/300) and 3 from vaginal swabs (3/300) (Table 1). All the isolates were obtained from rectal and vaginal swabs pertaining to different animals.

The rate of *Salmonella* isolation was identified as 2% for rectal swabs (6/300) and 1% for vaginal swabs (3/300). The difference between the isolation rates was not statistically significant ($p > 0.05$; χ^2).

Serotyping: All of the strains isolated from rectal and vaginal swabs were identified to be B serogroup *Salmonella saintpaul* (Table 1).

Antibiotic susceptibility testing: All of the isolated strains were susceptible to ampicillin, enrofloxacin, tetracycline, oxytetracycline, gentamicin, neomycin, chloramphenicol, spectinomycin, cephalotin, amoxicillin/clavulanic acid, trimethoprim+sulfamethoxazole and nalidixic acid and they were resistant to erythromycin and

Table 1: *Salmonella* serotypes of isolated from rectal and vaginal swabs by sample type

Sample type	No.		
	isolation (%)	Serotype	Serogroup
Rectal swab (n = 300)	6 (2)	<i>Salmonella saintpaul</i>	B
Vaginal swab (n = 300)	3 (1)	<i>Salmonella saintpaul</i>	B

Table 2: Antimicrobial susceptibility among 9 *S. saintpaul* isolated from sheep

Antibiotic	Rectal isolates (n = 6)						Vaginal isolates (n = 3)		
	1	2	3	4	5	6	7	8	9
Ampicillin	S*	S	S	S	S	S	S	S	S
Enrofloxacin	S	S	S	S	S	S	S	S	S
Tetracycline	S	S	S	S	S	S	S	S	S
Oxytetracycline	S	S	S	S	S	S	S	S	S
Gentamicin	S	S	S	S	S	S	S	S	S
Chloramphenicol	S	S	S	S	S	S	S	S	S
Trimethoprim+sulfamethoxazole	S	S	S	S	S	S	S	S	S
Spectinomycin	S	S	S	S	S	S	S	S	S
Amoxycillin/clavulanic acid	S	S	S	S	S	S	S	S	S
Cephalothin	S	S	S	S	S	S	S	S	S
Nalidixic acid	S	S	S	S	S	S	S	S	S
Neomycin	S	S	S	S	S	S	S	S	S
Penicillin G	S	S	S	R**	R	S	S	S	S
Novobiocin	R	R	R	R	R	R	R	R	R
Eritromycin	R	R	R	R	R	R	R	R	R

*Susceptible, **Resistant

novobiocin. Two of the strains (rectal isolates No. 4 and 5) were resistant to penicillin G and others were susceptible (Table 2).

DISCUSSION

Salmonella infections in sheep influence animal health and animal breeding negatively as they result in significant economical losses. Moreover, certain *Salmonella* strains create an important public health problem by causing zoonoses. In the presence of factors like stress apparently healthy infected animals spread *Salmonella* to the environment with their feces. Thus, they act as reservoirs by spreading the causative agent to other animals and to the environment (Sandberg *et al.*, 2003; Woldemariam *et al.*, 2005; Molla *et al.*, 2006). Presence of *Salmonella* strains in the feces of animals slaughtered in abattoirs creates an important source of contamination for carcasses and edible internal organs; unless sufficient hygienic measures are taken during slaughtering, there is high risk of contamination reported for carcasses and internal organs (Zweifel *et al.*, 2004; Woldemariam *et al.*, 2005). In recent years, the importance of zoonoses in diseases of food origin has increased due to the fact that apparently healthy animals act as reservoirs for causative pathogens and that there is high risk of contamination to carcasses and internal organs at the time of slaughtering at critical control points where hygienic measures are weak (Zweifel *et al.*, 2004).

Gokcen and Erganis (1996), reported having isolated *Salmonella* at a rate of 1% from the intestinal contents of sheep slaughtered in abattoirs in Izmir, Turkey. Genc (2000), reported having isolated *Salmonella* at a rate of 2% from the intestinal contents of sheep slaughtered in abattoirs in Kars, Turkey. Jethon (1990), obtained fecal samples from animals slaughtered in abattoirs and reported isolating *Salmonella* at a rate of 5.3%.

Sandberg *et al.* (2002), reported having isolated *Salmonella* at a rate of 0.8% in rectal swabs obtained from sheep in Norway. Zweifel *et al.* (2004), reported having isolated *Salmonella* in 11% of the fecal samples of the sheep slaughtered in abattoirs in Switzerland. Davies *et al.* (2004), reported having isolated *Salmonella* in 0.1% of the fecal samples of the sheep slaughtered in the UK. Woldemariam *et al.* (2005), reported isolation of *Salmonella* in 2.1% of the fecal samples of the sheep slaughtered in abattoirs in Ethiopia, in another study carried out in Ethiopia Molla *et al.* (2006), reported an isolation rate of 4.8% from feces of sheep.

In this study, *Salmonella* was isolated in 2% of the rectal swab samples. This rate is close to those reported by Gokcen and Erganis (1996), Genc (2000), Sandberg *et al.* (2003) and Woldemariam *et al.* (2005) while, being lower than those of Jethon (1990) and Molla *et al.* (2006) and higher than that reported by Davies *et al.* (2004). Having isolation rates that are both similar as well as high and low might be related to the differences in the regions the samples were obtained from and also to the differences in the isolation techniques. In this study, *Salmonella* was isolated in 1% of the vaginal swab samples. In the study, search we have carried out there was no mentioning of *Salmonella* isolation from vaginal swabs. When *Salmonella* isolation rates from rectal and vaginal swabs were compared, the difference between the two was not found to be statistically significant.

In studies carried out for the serovars isolated from sheep slaughtered in abattoirs different serovars were reported. Gokcen and Erganis (1996), reported having isolated *S. typhimurium* from the intestinal contents of the sheep slaughtered in Izmir, Turkey. Genc (2000), reported having isolated *S. enteritidis* from the intestinal contents of sheep slaughtered in abattoirs in Kars, Turkey. Jethon (1990), Sandberg *et al.* (2003) and Zweifel *et al.* (2004), reported having isolated *S. subspecies enterica* IIIb

61: k:1,5,(7). Woldemariam *et al.* (2005), reported having isolated *S. infantis* and *S. butantan* from the feces of the sheep slaughtered in abattoirs in Ethiopia. Molla *et al.* (2006), reported having isolated *S. enteritidis*, *S. typhimurium*, *S. reading* and *S. heidelberg* from the feces of sheep slaughtered in Ethiopia. In this study, *Salmonella* strains isolated from rectal and vaginal swab samples were serotyped as *S. saintpaul*. In the study, we have searched we have not come across any study regarding the isolation of *S. saintpaul* from rectal and vaginal swabs. This serovar has been isolated for the first time from rectal and vaginal swabs in Turkey.

Antibiotics have a widespread use for the treatment of bacterial infections in animals. However, irrational use of antibiotics results in bacteria developing resistance. Davies *et al.* (2004), reported that *Salmonella* strains they had identified from feces of animals slaughtered in abattoirs were sensitive to amoxycillin/clavulanic acid, ampicillin, chloramphenicol, gentamicin, neomycin, trimethoprim + sulfamethoxazole and nalidixic acid. Threlfall *et al.* (2003), reported that *Salmonella* strains isolated from fecal samples were sensitive to nalidixic acid. Molla *et al.* (2006), identified that *Salmonella* strains isolated from feces of sheep slaughtered in abattoirs in Ethiopia were susceptible to gentamicin, neomycin and nalidixic acid, whereas they were resistant to amoxycillin/clavulanic acid, ampicillin, cephalotin, chloramphenicol, trimethoprim + sulfamethoxazol, spectinomycin and tetracycline. Intorre *et al.* (2005), reported that *Salmonella* strains isolated from sheep were resistant to penicillin and erythromycin. Kudaka *et al.* (2006), stated that *S. saintpaul* strains identified from chicken were resistant to tetracycline. All of the *Salmonella* strains isolated in this study were resistant to eritromycin and novobiocin while, they were susceptible to ampicillin, enrofloxazine, tetracycline, oxytetracycline, gentamicin, neomycin, chloramphenicol, amoxicillin/clavulanic acid, cephalotin, trimethoprim + sulfamethoxazol, spectinomycin and nalidixic acid. Furthermore, 2 of the strains (isolates No. 4 and 5) were penicillin resistant whereas others were susceptible. Having different antimicrobial resistance patterns in the isolated *Salmonella* strains was related to differences in the serovars isolated and the differences in the regions they were isolated. This might be easily related to using the antibiotics without performing antimicrobial susceptibility test in those regions.

CONCLUSION

This study demonstrated the presence of *S. saintpaul* in rectal and vaginal swab samples obtained from sheep in Van and its environs. This is the first time, *S. saintpaul* was isolated in rectal and vaginal swab samples of the

sheep slaughtered in abattoirs in Turkey. The causative agent being isolated from rectal and vaginal swab samples is also important in demonstrating the fact that apparently healthy sheep might as well act as reservoirs.

When we take into account the fact that *Salmonella* serovars differ in countries and regions, we anticipate that the results obtained from this study will further contribute to the study to be performed at country wide for the identification of *Salmonella* profile. For the characterization of isolated *Salmonella* serovars detailed epidemiological and molecular studies are required. For preventing the development of further antimicrobial resistance, antibiotics should be administered at relevant doses for required period after taking into account the antimicrobial susceptibility test results. For minimizing the risk of *Salmonella* and other microorganism contamination to carcasses and internal organs in abattoirs attention should be paid to scrutinized use of hygienic measures.

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