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Serotypes of *Salmonella* isolated from feces of cattle, buffalo, and camel and sensitivities to antibiotics in Turkey

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Abstract: In this study, *Salmonella* strains from dairy cows, calves with diarrhea, and buffalo and camel feces were isolated and serotyped. There were 869 feces samples (437 calves, 287 dairy cows, 100 buffalo, and 45 camels) collected from 13 provinces and 21 farms. Preenrichment feces samples were added to Rappaport-Vassiliadis medium. Samples were then subcultured on xylose lysine tergitol-4 agar and plates. Suspected colonies of *Salmonella* were confirmed by latex agglutination test. The isolates of *Salmonella* spp. were serotyped at the Etlik Central Veterinary Control Institute. In total, 40 *Salmonella* spp. were isolated, including 33 from calves, 5 from dairy cows, 1 from a buffalo, and 1 from a camel. *Salmonella* strains were serotyped as *S. Kentucky* (n = 23), *S. Muenchen* (n = 5), *S. Anatum* (n = 4), *S. Gaminare* (n = 4), *S. Typhimurium* (n = 1), *S. Muenster* (n = 1), *S. Enteritidis* (n = 1), and *S. Abony* (n = 1). The Kirby-Bauer disk diffusion method was used for the determination of antibiotic susceptibilities of isolates against 15 antibiotics. *Salmonella* isolates were determined as resistant to multiple antibiotics. In conclusion, 8 different *Salmonella* serotypes were found in dairy cows, calves, buffalo, and camels. Because of the occurrence of multiresistant isolates, biosafety measures and pathogen control processes are advised for *Salmonella*-associated risks to public health.

Key words: *Salmonella*, serotyping, dairy cow, calf, and buffalo

1. Introduction

Salmonellosis is a common bacterial enteric infection with significant economic losses for the intensive production of cattle, sheep, and poultry (1,2). *Salmonella* species are zoonotic and are transmitted to humans via ingestion of contaminated milk, eggs, and meat (3,4). Although *Salmonella* infections may occur at any age in cattle, the associated clinical symptoms are more severe in calves from the first 2 weeks to 3 months of their life (5,6).

Salmonella enterica subsp. *enterica* may cause infections associated with several clinical symptoms or systemic infections characterized by diarrhea and septicemia and may even lead to death in severe cases. It can be harbored by asymptomatic carriers (2,5). In the enteric form of salmonellosis, the stool is sticky and watery and has a putrid odor. It may contain flecks of mucus, shreds of the mucous membrane, and in some cases blood. Young calves and lambs frequently develop septicemia. Furthermore, marked depression, fever, symptoms of the central nervous system, pneumonia, and death within 2–3 days can also occur (7–9).

Clinical symptoms and necropsy findings alone are not sufficient for a definitive diagnosis of *Salmonella* infection. It is imperative to isolate and identify the causative *Salmonella* species (10–12). While the isolation of *Salmonella* is relatively easy via bacterial culture from samples taken from animals with septicemia, carcasses, and the organs of aborted fetuses, enrichment is needed to increase the chance of isolating *Salmonella* from feed samples or from fecal cultures used to detect carriers (7,13,14).

This study aimed to isolate and serotype *Salmonella* species from fecal samples of dairy cattle, calves with diarrhea, camels, and water buffaloes and to determine sensitivities of the isolates to antibiotics.

2. Materials and methods

2.1. Sampling

A total of 869 fecal samples were collected from 21 herds in 13 provinces (Konya, Kayseri, Düzce, Kahramanmaraş, Şanlıurfa, Amasya, Aksaray, Adıyaman, Afyonkarahisar, Aydın, Kırşehir, Niğde, and İzmir) in Turkey. Of these, 437

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were taken from calves, 287 from dairy cattle, 100 from water buffaloes, and 45 from camels. These samples were transferred under cold-chain conditions and within the shortest time possible to the laboratory of the Microbiology Department of Selçuk University, Faculty of Veterinary Medicine, in Turkey.

2.2. Microbiological examination

Detection of the presence of *Salmonella* spp. was carried out according to the ISO 6579 standard. For preenrichment, the fecal samples were cultured in 1:10 buffered peptone and water (2.5 g of feces and 22.5 mL of medium) and incubated at 37 °C for 24 h. Subsequently, selective enrichment was performed by culturing 1 mL of sample into Rappaport-Vassiliadis medium. The culture was incubated at 42 °C for 24 h. Finally, for selective culturing, the samples were passaged onto xylose lysine deoxycholate (XLD) and/or xylose lysine tergitol-4 (XLT-4) agar and incubated at 37 °C for 24–48 h. Colorless or light pink colonies with darker centers on XLD agar or black colonies on XLT-4 agar were suspected of being *Salmonella* (15,16).

2.3. Serological confirmation and serotyping

Microorganisms suspected of being *Salmonella* strains were tested with a *Salmonella* latex agglutination kit (17,18). The *Salmonella* isolates that gave positive results by the latex agglutination test were serotyped at the Etlik Central Veterinary Control Institute.

2.4. Antibiotic sensitivity test

The antibiotic sensitivity tests of the *Salmonella* isolates were performed by the Kirby-Bauer disk diffusion method using oxytetracycline (30 µg), gentamycin (10 µg), erythromycin (15 µg), cefuroxime (30 µg), chloramphenicol (30 µg), danofloxacin (5 µg), ampicillin (25 µg), amoxicillin (25 µg), cephalexin (30 µg), kanamycin (75 µg), spectinomycin G (10 U), ceftiofur (30 µg), lincomycin+spectinomycin (10

µg), enrofloxacin (5 µg), and rifamycin (30 µg) disks and Mueller-Hinton agar (19). The results were evaluated after the media were incubated at 37 °C for 48 h.

3. Results

Salmonella strains were isolated from 8 herds (61.5%); 40 (4.60%) *Salmonella* isolates were recovered from 869 fecal samples (Table 1). *Salmonella* strains were isolated from calves (7.5%, 33/437), dairy cows (1.74%, 5/287), a buffalo (1%, 1/100), and a camel (2.22%, 1/45).

Salmonella isolates in calves were serotyped as *S. Kentucky* (8,20:i:z₆) (n = 19), *S. Muenchen* (6,8:d:1,2) (n = 5), *S. Gaminare* (16:d:1,7) (n = 4), *S. Anatum* (3,10,[15],[15,34]:e,h:1,6) (n = 3), *S. Enteritidis* (1, 9,12:g,m:-) (n = 1), and *S. Muenster* (3,10[15][15,34]:e,h:1,5) (n = 1). *Salmonella* isolates in dairy cows were serotyped as *S. Kentucky* (8,20:i:z₆) (n = 3), *S. Anatum* (3,10,[15],[15,34]:e,h:1,6) (n = 1), and *S. Typhimurium* (1,4,[5],12:i:1,2) (n = 1). In addition, 1 isolate in buffalo was *S. Kentucky* (8,20:i:z₆) and the 1 isolate in camel was *S. Abony* (1,4,[5],12,27:b:e,n,x) (Table 1).

All *Salmonella* isolates were resistant to one or more antibiotics. All *Salmonella* serotypes were resistant to erythromycin and rifamycin. In addition, all *Salmonella* serotypes (except *S. Abony*) were resistant to amoxicillin. *S. Enteritidis* was resistant to 10 antibiotics; *S. Gaminare* to 8; *S. Abony* to 6; *S. Kentucky*, *S. Anatum*, *S. Typhimurium*, and *S. Muenchen* to 4; and *S. Muenster* to 3. However, all *Salmonella* serotypes were susceptible to cefuroxime and ceftiofur (Table 2).

4. Discussion

Salmonella enterica subsp. *enterica* is a food pathogen that may cause disease in both people and animals (3,5,20). Some of the *Salmonella* species are host-specific. They

Table 1. *Salmonella* serotypes.

Serotype	Calf	Dairy Cow	Buffalo	Camel	Total
<i>S. Kentucky</i>	19	3	1	-	23
<i>S. Anatum</i>	3	1	-	-	4
<i>S. Muenchen</i>	5	-	-	-	5
<i>S. Typhimurium</i>	-	1	-	-	1
<i>S. Abony</i>	-	-	-	1	1
<i>S. Enteritidis</i>	1	-	-	-	1
<i>S. Gaminare</i>	4	-	-	-	4
<i>S. Muenster</i>	1	-	-	-	1
Total	33	5	1	1	40

Table 2. The sensitivity of *Salmonella* serotypes to antimicrobial agents.

Antibiotics	S. Kentucky			S. Muenchen			S. Anatum			S. Typhimurium			S. Abony			S. Enteritidis		S. Gaminare			S. Muenster		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	R	S	I	R	S	I	R
SEF	23	-	-	5	-	-	4	-	-	1	-	-	-	1	-	1	-	4	-	-	1	-	-
ENR	-	1	22	5	-	-	4	-	-	1	-	-	1	-	-	1	-	4	-	-	1	-	-
AML	-	-	23	-	-	5	-	-	4	-	-	1	1	-	-	-	1	-	4	-	-	-	1
C	23	-	-	5	-	-	4	-	-	1	-	-	1	-	-	-	1	4	-	-	1	-	-
AMP	1	-	22	-	-	5	-	-	4	-	-	1	1	-	-	-	1	-	4	-	-	1	-
E	-	-	23	-	-	5	-	-	4	-	-	1	-	-	1	-	1	-	-	4	-	-	1
CL	6	11	6	5	-	-	1	3	-	-	1	-	-	1	-	-	1	-	-	4	1	-	-
LCS	-	-	23	5	-	-	-	4	-	1	-	-	-	-	1	-	1	-	-	4	1	-	-
OT	1	-	22	5	-	-	2	1	1	1	-	-	1	-	-	-	1	-	-	4	-	1	-
K	5	14	4	5	-	-	2	2	-	1	-	-	-	-	1	-	1	-	-	4	1	-	-
S	-	1	22	3	2	-	-	2	2	-	1	-	-	-	1	-	1	-	-	4	1	-	-
DFX	-	1	22	5	-	-	4	-	-	1	-	-	1	-	-	1	-	4	-	-	1	-	-
EFT	23	-	-	5	-	-	4	-	-	1	-	-	1	-	-	1	-	4	-	-	1	-	-
RA	-	-	23	-	-	5	-	-	4	-	-	1	-	-	1	-	1	-	-	4	1	-	-
CN	2	-	21	2	2	1	2	1	1	1	-	-	-	-	1	1	-	-	-	4	-	-	1
Total	23			5			4			1			1			1		4			1		

SEF: Cefuroxime, ENR: enrofloxacin, AML: amoxicillin, C: chloramphenicol, AMP: ampicillin, E: erythromycin, CL: cephalixin, LCS: lincomycin+spectinomycin, OT: oxytetracycline, K: kanamycin, S: spectinomycin, DFX: danofloxacin, EFT: ceftiofur, RA: rifamycin, CN: gentamycin.

can be asymptomatic or can cause death (9,21). There are over 2500 *Salmonella* serovars and, due to the diversity of host types, *Salmonella* control is compulsory in animal production (22). Infected animals may spread the agent through feces without showing any clinical symptoms (23).

For *Salmonella* isolation, three cultural procedure stages are generally used: preenrichment, selective enrichment, and selective culture (15,16). In the preenrichment stage, the most commonly used media are buffered peptone water (BPW) and lactose broth (15). In selective enrichment, media that support production of *Salmonella* and inhibit production of other bacteria are used. Media such as tetrathionate broth, selenite-cysteine broth, selenite broth, and Rappaport-Vassiliadis broth are widely used (15). In selective culture, MacConkey agar, *Salmonella-Shigella* agar, XLD agar, Rambach agar, and XLT-4 agar could be used (15,16). In this study, BPW

was used for preenrichment. For selective enrichment, Rappaport-Vassiliadis broth was used, and XLT-4 and XLD agar was used for the isolation of *Salmonella* species.

The source of *Salmonella*-based diseases in people is often subclinically infected local dairy cattle (22,24). Cattle are the most important *Salmonella* reservoir agents and consumption of infected cattle meat is related to salmonellosis epidemics (25). Various studies were carried out to isolate and serotype *Salmonella* from dairy cattle and calves (4,7,14,18,21,22,24). Several researchers (4,7,14,18,21,22,24) reported that *Salmonella* isolates were identified as *S. Typhimurium*, *S. Dublin*, *S. Agona*, *S. Orion*, *S. Aintpul*, *S. Braenderup*, *S. Muenchen*, *S. Croft*, *S. Kentucky*, *S. Telaviv*, *S. Montevideo*, *S. Kpeme*, *S. Infantis*, *S. Abadina*, *S. Cerro*, *S. Mismarhaenek*, *S. Enteritidis*, *S. Guildford*, *S. Anatum*, *S. Gozo*, *S. Mbandaka*, *S. Senftenberg*, *S. Newport*, *S. Give*, and *S. Muenster*.

Fecal *Salmonella* studies in Turkey are usually based on slaughterhouse samples or case-based notices. Gökçen et al. (26) isolated *S. Typhimurium* from the small bowel contents of only one cow out of 298 cattle. Akbarut (27) did not isolate *Salmonella* from fecal samples of 119 cattle. Genç (14) isolated *Salmonella* in 0.7% of 1000 feces samples obtained from cattle slaughtered in Kars. They were serotyped as *S. Enteritidis* (n = 5) and *S. Typhimurium* (n = 1). Erganiş et al. (10) isolated *S. Typhimurium* from the internal organs of 2 feedlot calves with septicemia. Canpolat and Akan (28) isolated only 1 (0.9%) *Salmonella* sp. from fecal samples of 106 cattle. Hadimli et al. (9) reported that 3 *S. Dublin* specimens were isolated from liver, spleen, and mesenteric lymph node samples of dead calves.

In our study, fecal samples were collected from calves and dairy cattle with diarrhea from 13 provinces and 21 different enterprises. *Salmonella* spp. was isolated from 8 (38.09%) out of 21 enterprises and 40 (4.6%) out of 869 fecal samples. Thirty-three isolates were isolated from calves, 5 from dairy cows, 1 from buffalo, and 1 from camel. Nineteen of the calves' isolates were *S. Kentucky*, 5 were *S. Muenchen*, 4 were *S. Gaminare*, 3 were *S. Anatum*, 1 was *S. Enteritidis*, and 1 was *S. Muenster*. Three isolates from dairy cattle were *S. Kentucky*, 1 was *S. Anatum*, and 1 was *S. Typhimurium*. *S. Kentucky* was the most commonly isolated serotype.

Any clinical signs and death for salmonellosis were observed in sampling dairy cows and buffaloes. Sampling was done considering that these animals may have had salmonellosis as reservoirs. Three isolates and one isolate were isolated from dairy cows and buffalo, respectively. Three different *Salmonella* serotypes (*S. Kentucky*, *S. Anatum*, and *S. Typhimurium*) were determined. *S. Gaminare*, *S. Muenchen*, *S. Enteritidis*, and *S. Muenster* isolates were separately isolated each from a single enterprise. Any clinical signs and death for salmonellosis were observed in sampling calves. *S. Anatum* isolates were recovered from different enterprises, and 2 isolates were isolated from calves and one isolate from a dairy cow. *S. Kentucky* isolates in an enterprise including dairy cows, calves, and buffaloes were isolated from calves with widely observed cases of diarrhea and death. In this enterprise, 19, 3, and 1 *S. Kentucky* isolates were recovered from calves, dairy cows, and buffaloes, respectively. *S. Typhimurium* was also isolated from the same enterprise. Moreover, *S. Abony* was isolated from a camel.

Salmonella infections have a subclinical course. There are isolates with resistance against multiple antibiotics (5,29,30). Vella and Cushieri (21) stated that all of 131 *Salmonella* isolates were susceptible to all antibiotics; the most resistance was observed with trimethoprim. McEvoy et al. (20) stated that *S. Typhimurium* isolates were

resistant to ampicillin, chloramphenicol, streptomycin, sulfafurazole, and tetracycline and *S. Dublin* and *S. Agona* isolates were sensitive to antibiotics. Addis et al. (30) reported that *Salmonella* strains isolated from fecal and milk samples of dairy cattle were resistant to ampicillin (100%), streptomycin (66.7%), and nitrofurazone (58.3%) and sensitive to ciprofloxacin (91.7%), clotrimazole (87.5%), and cephexon (75%). Gorski et al. (25) stated that *Salmonella* isolates were sensitive to 12 different antibiotics. Wiczorek and Osek (24) stated that some of the *S. Dublin*, *S. Enteritidis*, and *S. London* strains were resistant to sulfamethizole. *S. Typhimurium* is resistant to multiple antibiotics (ampicillin, streptomycin, tetracycline, and sulfamethizole) (24). Rodriguez-Rivera et al. (3) reported that 90 *Salmonella* strains (23.6%) out of 381 from subclinically infected dairy cattle and environmental samples were resistant to antibiotics varying from 1 and 11. All of the isolated strains were sensitive to amikacin and ciprofloxacin, and the highest resistance was against ampicillin (72%), tetracycline (63%), and amoxicillin+clavulanic acid (58%). Moreover, different serotypes or serovars may be included in strains that are resistant to antibiotics.

In the present study, we found that *Salmonella* strains were resistant to multiple antibiotics. All *S. Kentucky* isolates were resistant to amoxicillin, erythromycin, lincomycin+spectinomycin, and rifamycin; the *S. Muenchen* isolates were resistant to amoxicillin, ampicillin, erythromycin, and rifamycin. The *S. Typhimurium* isolate was resistant to amoxicillin, ampicillin, erythromycin, and rifamycin; the *S. Abony* isolate was resistant to lincomycin+spectinomycin, kanamycin, spectinomycin, erythromycin, rifamycin, and gentamycin; *S. Enteritidis* was resistant to amoxicillin, chloramphenicol, ampicillin, erythromycin, cephalixin, lincomycin+spectinomycin, oxytetracycline, kanamycin, spectinomycin, erythromycin, and rifamycin; all the *S. Gaminare* isolates were resistant to erythromycin, cephalixin, lincomycin+spectinomycin, oxytetracycline, kanamycin, spectinomycin, rifamycin, and gentamycin; and *S. Muenster* was resistant to amoxicillin, erythromycin, and gentamycin.

In conclusion, *Salmonella* is present in cows, calves, buffaloes, and camels in Turkey. For public health, biosafety measures and pathogen control processes are needed.

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