



ARTICLE

Nontyphoid *Salmonella* Prevalence, Serovar Distribution and Antimicrobial Resistance in Slaughter Sheep

Ece Cetin¹, Seran Temelli², and Aysegul Eyigor^{2,*}

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Namik Kemal University, 59030, Tekirdag, Turkey

²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059, Bursa, Turkey

OPEN ACCESS

Received August 7, 2019

Revised October 8, 2019

Accepted October 9, 2019

*Corresponding author : Aysegul Eyigor
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059, Bursa, Turkey
Tel: +90-224-294-1334
Fax: +90-224-294-1202
E-mail: aeeyigor@gmail.com

*ORCID
Ece Cetin
<https://orcid.org/0000-0002-8783-5507>
Seran Temelli
<https://orcid.org/0000-0002-8869-4929>
Aysegul Eyigor
<https://orcid.org/0000-0002-2707-3117>

Abstract This study aimed to determine the current prevalence, serovar distribution and antimicrobial resistance rate and patterns of nontyphoid *Salmonella* (NTS) in slaughter sheep and their edible offal. While filling the gap of up to date related information in Turkey, data presented is also of significance since contamination of ovine meat, its products and offal with this pathogen is threat to public health due to their considerably high consumption rates in our country. Current NTS carriage in 200 apparently healthy slaughter sheep by ISO 6579:2002, 6579:2002/A1:2007 standard bacteriology (ISO) was 5% (10/200) (4 fecal content - 2%, 3 mesenterial lymph node - 1.5%, 3 kidney - 1.5%) out of 1,400 samples (0.7%), with no isolation from carcass, liver, gallbladder, spleen. Real-time PCR was in substantial agreement to ISO in confirming *Salmonella*-suspect isolates (Relative Trueness: 93.6%). *S. Newport* (40%) was the predominant serovar, followed by the second prevalent serovars as *S. Typhimurium* and *S. Kentucky* (20%), and by *S. Umbilo* and *S. Corvallis* (10%). Four and 6 out of 10 NTS isolates were susceptible (40%) and resistant (60%) to 18 antimicrobials, respectively. *S. Typhimurium* isolates were multidrug resistant (MDR) to tigecycline and sulphamethoxazole/trimethoprim, with one also resistant to cefepime. *S. Corvallis* was MDR to ampicillin, ciprofloxacin, norfloxacin and pefloxacin. The predominance of *S. Newport* and first isolation of *S. Corvallis* in sheep in the world; first time isolations of Newport, Kentucky, Corvallis, Umbilo serovars from sheep in Turkey; and high antimicrobial resistance rates obtained in majority of the isolates highlights study findings.

Keywords nontyphoid *Salmonella*, sheep, ISO, serovar, antimicrobial resistance

Introduction

Turkey is the fifth largest producer (270.840 tons, 8%) and a significant consumer (6%) of sheep meat in the world (Colby, 2015; FAO, 2018a). According to 2017 Turkish Statistical Institute (TSI) data, the sheep meat production rate is 9.16% (99,874 tons) of the total 1,089,747 tons of red meat produced in our country (TSI, 2018).

Additionally, ovine meat (4.68 kg) accounts for almost one-third (28.66%) and 11.17% of annual 16.33 kg red meat and 41.89 kg overall per capita meat consumption, respectively (FAO, 2018b). Apart from being one of the primary red meat sources, there is also high demand and use of sheep meat products and edible offal (88.320 tons/year, particularly liver, kidney, spleen, brain, intestines - as grilled sheep intestines - kokorec), with 1.18 kg of annual consumption per capita (FAO, 2018a; FAO, 2018b). Therefore, in our country, the welfare and hygienic slaughter of sheep play a substantial role in preventing transmissions of foodborne pathogens to meat and related food destined for human consumption, which has economic consequences for meat processors.

In European Union (EU), *Salmonella* is a frequently reported pathogen from bacterial gastroenteritis and outbreak cases in humans due to contaminated meat, meat products and edible offal. In particular, nontyphoid *Salmonella* (NTS), which has the most significant disease burden and foodborne deaths worldwide, in carcasses, internal organs and lymph nodes of slaughter sheep entering the food chain could be a substantial threat to public health (EFSA and ECDC, 2018). Common practices, such as transport, consignment in multiple groups before sale, and feed and water deprivation, could cause stress and increased shedding of the pathogen prior to slaughter (Al-Habsi et al., 2018; Yang et al., 2017). Thus, assessment of the NTS serovars and their antimicrobial resistances isolated from ovine carcasses is of critical value for farm-level biosafety measures and epidemiology. Additionally, the Turkish Food Codex (TFC) Regulation on Microbiological Criteria (TFC, 2011) has zero tolerance for the detection of *Salmonella* from ovine carcasses assessed with EN/ISO 6579 (ISO, 2002) as the reference method. Accordingly, several studies have reported *Salmonella* prevalence in sheep carcasses (0%–26.7%) (Kuma et al., 2017; Ranucci et al., 2014; Stipetic et al., 2016), in fecal samples (0%–26.90%) (Dargatz et al., 2015; Hanlon et al., 2018; McAuley et al., 2014), in edible organs such as liver (0.9%–4.3%) and spleen (0%–2.27%) (Kuma et al., 2017; Molla et al., 2006; Woldemariam et al., 2005), in gallbladder (3%) (Zubair and Ibrahim, 2012) and in mesenteric lymph nodes (3.85%–13.6%) (Hanlon et al., 2018; Kuma et al., 2017; Tadesse and Tessema, 2014), where *S. Typhimurium* was reported as the predominant serovar regardless of the sample type.

Currently, the TFC Regulation on Pharmacologically Active Substances and Their Classification Regarding Maximum Residue Limits in Foodstuffs of Animal Origin (TFC, 2017) requirements have become mandatory for food producers. One driving force for this requirement was the high resistance rates (in descending order) to tetracycline, ampicillin, chloramphenicol, amoxicillin/clavulanic acid, sulfamethoxazole/trimethoprim, cefoxitin, sulfisoxazole, and streptomycin antimicrobials in NTS isolates of human/animal origin and multidrug resistance (MDR) to at least two or more antimicrobials with rates ranging between 7.5%–82.35% observed in these resistant serovars. Additionally, almost one third of the isolates from humans were indicated to carry resistance to sulfonamides, ampicillin and tetracyclines, and showed 26.5% MDR to these 3 antimicrobials (EFSA and ECDC, 2018). Recent antimicrobial resistance studies related to the *Salmonella* isolates from slaughter sheep indicate high ampicillin resistance, subsequently resistance to sulfamethoxazole-trimethoprim, chloramphenicol, gentamicin, amoxicillin-clavulanic acid, ciprofloxacin and sulfonamide, in decreasing order (Dargatz et al., 2015; Edrington et al., 2009; Igbinosa, 2015; Molla et al., 2006; Small et al., 2006). Therefore, infections with such resistant salmonellae isolated from foods of animal-origin could result in failures in management of public health risks.

In literature, there seems to be a scarcity of information on the current occurrence, serovar distribution and antimicrobial resistance of NTS in slaughter sheep and their offal in Turkey. Since ovine meat consumption has a considerably high share within red meat in Turkey, presence of NTS on carcasses and/or edible offal, or contamination of related products with this pathogen could be a significant threat to public health. Therefore, this study primarily focused on finding the NTS carrier rate of slaughter sheep, by using standard and internationally accepted methods, where real-time PCR aided in rapid identification

and confirmation of the *Salmonella* suspect isolates. Further, the study aimed to identify the serovars circulating among these animals and to determine their antimicrobial resistance rates and patterns for epidemiological and preventive purposes.

Materials and Methods

Sample collection and preparation

A total of 1,400 random samples comprised of 7 sample types (200 samples from each sample type) from carcass, fecal content, mesenteric lymph node, liver, kidney, spleen, and gallbladder were obtained from 200 sheep, which belonged to different herds, and slaughtered in 4 different slaughterhouses between 2013 and 2015. Sample collection strategy from above indicated sample types was applied in accordance with the requirements of the TFC (TFC, 2011) as follows: (1) Individual sterile sponges (Whirl Pak, B01351WA) wetted with buffered peptone water (BPW-ISO, Oxoid, CM1049) were used to wipe approximately a 100 cm² area from both sides of the abdomen, lateral thorax, crutch, and lateral breast of each carcass using a 10 cm×10 cm sterile square template, as instructed in the nondestructive sponge sampling method part of ISO 17604:2003 (ISO, 2003). (2) For fecal content collection, the methods indicated by Ransom et al. (2002) and Milnes et al. (2008) were applied, where the entire colonic and rectal content was stripped from the anus, carefully mixed, and about 25–50 g was placed into a sampling bag. (3) Mesenteric lymph nodes (3–5) were removed aseptically and collected into sampling bags as previously noted by Alemu and Zewde (2012). (4) Areas around the portal vein and the orifice of ureter were rubbed by sterile swabs (LP Italiana, L111598) prewetted with BPW (ISO, Oxoid, CM1049) for liver and kidney samples, which were inserted into tubes containing 10 mL of sterile BPW (Little et al., 2008), respectively. (5) The artery and vein openings on the renal surface of each spleen was swabbed with a prewetted swab to cover a 5 cm² area, which was then placed into 10 mL of sterile BPW. (6) Gallbladders were sampled following the procedure described by Akoachere et al. (2009), where the interior walls of the organ were rubbed with a BPW prewetted sterile swab, and placed into a tube containing 10 mL sterile BPW. All samples were transferred to the laboratory in icebox, and used in analyses within an hour.

Standard and quality control strains

As positive controls, we used *S. Typhimurium* NCTC 12416 (Refik Saydam National Public Health Agency, Ankara, Turkey) and *S. Enteritidis* 64K (M.Y. Popoff, Institut Pasteur, Paris, France) in all tests. *Escherichia coli* ATCC 25922 strain was utilized as the strain for quality control in antimicrobial resistance tests (EUCAST, 2015a).

Isolation and identification

For pre-enrichment of all sample types except fecal content was performed as follows: Carcass sponges and diced mesenteric lymph node samples close to 25 g, which were already in 25 mL BPW, were transferred into 225 mL BPW in individual 500 mL stomacher bags (LP Italiana, L177538). Liver, kidney, spleen and gallbladder swabs in 10 mL of BPW were allocated into stomacher bags containing 90 mL BPW. After a 2 min homogenization in a Stomacher Circulator (400C, Seward, West Sussex, UK) at 230 rpm, all samples were left for an 18 h incubation at 37°C. For selective enrichments, 1 and 0.1 mL of each pre-enrichment broth culture were inoculated into 10 mL of Mueller Kauffmann tetrathionate novobiocin (MKTTn, CM1048, Oxoid, Hampshire, UK) broth supplemented with novobiocin (SR0181, Oxoid) and into Rappaport-Vassiliadis soya peptone (RVS, CM0866, Oxoid) broth, and left for a 24 h incubation at 37°C and 41.5°C, respectively. Xylose lysine deoxycholate (XLD, CM0469, Oxoid) agar and brilliance salmonella (BS, CM1092, Oxoid) agar including

Salmonella selective supplement (SR0194, Oxoid) were used in selective plating. After inoculation from both selective enrichment broths, all plates were incubated for 24 h at 35°C (ISO, 2002).

ISO 6579/A1: 2007 (ISO, 2007) requirements were followed in the processing of fecal content samples. Briefly, from the previously collected 50 g fecal content, a 25 g sample was weighed into a stomacher bag filled with 225 mL of BPW. This pre-enrichment mixture was then homogenized for 2 min at 230 rpm, and was incubated for 24 h at 37°C. Then, 0.1 mL aliquot from this pre-enrichment culture was dropped onto modified semisolid Rappaport-Vassiliadis (MSRV, CM1112, Oxoid) agar and incubated for 18–42 h at 41.5°C until a defined zone could be detected. A loopful of the growth zone was streaked onto xylose lysine tergitol-4 (XLT-4, CM1061, Oxoid), XLD, and BS agar plates, and left for 24 h incubation at 37°C. Then, 1–5 suspected colonies of *Salmonella* from these plates were inoculated onto MacConkey (MC, CM0115, Oxoid) agar for culture purification. This pure culture was used in *Salmonella* spp.-specific real-time PCR (RT-PCR) and in biotyping of the isolates. For RT-PCR template, we suspended pure culture from MC agar in sterile PCR-grade water (500 µL) which was then stored at –20°C. Urease activity test (Urea Agar Base, CM0053, Oxoid), triple sugar utilization and H₂S formation test (Triple Sugar Iron Agar, CM0277, Oxoid), and lysine decarboxylase activity (Lysine Iron Agar, CM0381, Oxoid) test were used for initial biotyping. For these tests, the subcultured brain heart infusion broth (BHI, CM1135, Oxoid) (37°C, 18–20 h) of the pure culture grown on MC agar was used. The same culture was also used in further identification tests using API20E (20100, Biomerieux, Marcy l’Etoile, France). After profile result evaluation, cultures identified as *Salmonella* spp. were stored at –20°C for further serotyping tests.

***Salmonella* spp.-specific real-time PCR**

In order to prepare the template to be used in RT-PCR, we extracted crude DNA from control strains, and *Salmonella* isolates as indicated by Carli et al. (2001). Shortly, 1 mL frozen culture was thawed and following the centrifugation (4 min at 4,600×g) (MicroCL 17, Thermo Scientific, Waltham, MA, USA) the pellet was washed in 0.85% saline and suspended in 20 µL of deionized water, which was then boiled at 95°C on a block heater (DB-2D-FDB02DD, Techne, Staffordshire, UK) for 10 min and centrifuged for 3 min at 18,000×g. In order to confirm suspected *Salmonella* colonies, two microliters of the supernatant were used as a template in RT-PCR.

We used the *Salmonella* *trrRBCA* locus-based primer pair and the target probe with previously determined specificity and sensitivity by Malorny et al. (2004). Additionally, we designed the *Escherichia coli* Lambda phage-specific Internal Amplification Control (IAC) with its specific primers and probe in this study (Way2Gene, BN 15-0001-01, Genmar, Turkey) (Table 1). The reaction volume and cycling parameters of the modified RT-PCR was based on a protocol indicated by Malorny et al. (2007) using Light Cycler 2.0 (03531414201, Roche Diagnostics, Basel, Switzerland) are as follows: The 10 µL reaction mix (Way2Gene, BN 15-0001-01, Genmar, Istanbul, Turkey) comprised of 2.5 µL *Salmonella* detection mix (0.5 µM of each primer and 0.25 µM each probe), 2 µL enzyme mix (0.5 U enzyme; dNTP mix, reaction buffer solution), 1 µL (approximately 100 copies) IAC template DNA, 2.5 µL template DNA (DNA for positive control and samples, PCR-grade water for negative control and 2 µL PCR-grade water. The cycling parameters started with an initial denaturation at 95°C for 11 min, which was followed by 40 cycles of 10 s denaturation at 95°C, 30 s of annealing at 58°C and 5 s of elongation at 72°C for. For the detection of fluorescence signals, 530 nm channel was used for *Salmonella* target signal and 560 nm channel was used for IAC.

Serotyping

Serological identification was applied to the isolates, which were confirmed as *Salmonella* by biotyping and RT-PCR.

Table 1. Primers, probes, and internal amplification control sequence used for *Salmonella* spp.-specific real-time PCR

Designation	Sequence	Positions ¹⁾
ttr-6 (forward)	CTCACCAGGAGATTACAACATGG	4287–4309
ttr-4 (reverse)	AGCTCAGACCAAAAGTGACCATC	4359–4381
ttr-5 (target probe)	FAM-CACCGACGGCGAGACCGACTTT-TAMRA	
IAC sequence ²⁾	CGTCAGTGTGAAGCGGTTATAAATC <u>TGCTCTTTCGCGGTATCCGTACCGAT</u> TTCGGTAAGGTAACCCCGTTTT TGTTTCGCTTACGTGGCAT	
IAC (forward)	CGTCAGTGTGAAGCGGTTATAA	
IAC (reverse)	ATGCCACGTAAGCGAAACA	
IAC probe	HEX-TGCTCTTTCGCGGTATCCGTACCGAT-TAMRA	

¹⁾ Positions correspond to Genbank accession no. AF282268.

²⁾ The sequences marked in boldface type are the primer binding sites for IAC, and the underlined sequence is the reverse complement IAC probe binding site.

IAC, internal amplification control.

Serotyping was performed by using O- and H- group specific antigens, according to the White-Kauffmann-Le Minor Scheme (Grimont and Weill, 2007), Guibourdenche et al. (2010) and Issenhuth-Jeanjean et al. (2014) using commercial antisera (Becton Dickinson, Franklin Lakes, NJ, USA). Slide agglutination test was utilized for the analysis of somatic antigens, while the flagellar phase antigens were assessed by using the tube agglutination test.

Antimicrobial susceptibility test

In selection of the antimicrobials to be tested on the isolates, EUCAST (2015a) and TFC Regulation (TFC, 2017) recommendations were taken into consideration. Thus, 18 frequently used antimicrobials in veterinary and/or human medicine were used for testing the antimicrobial susceptibility of the isolates (Table 2). As the preparation step for the antimicrobial susceptibility test, a loopful of the *Salmonella* frozen stock culture was streaked onto nutrient (N, CM0003, Oxoid) agar (NA) and incubated at 37°C for 24 h. A 1–1.5 colony grown on NA was suspended in 5 mL 0.85% NaCl and streaked onto Mueller-Hinton (MH, CM0337, Oxoid) agar by 3-way streaking after adjusting the turbidity to 0.5 McFarland

Table 2. Antimicrobials used in this study for testing susceptibility of the isolates

No	Name (Abbreviation, quantity on disk, catalog no) ¹⁾	No	Name (Abbreviation, quantity on disk, catalog no) ¹⁾
1	Amikacin (AK, 30 µg, CT0107B)	10	Chloramphenicol (C, 30 µg, CT0013B)
2	Amoxicillin/clavulanic acid (AMC, 30 µg, CT0223B)	11	Ertapenem (ETP, 10 µg, CT1761B)
3	Ampicillin (AMP, 10 µg, CT0003B)	12	Gentamicin (CN, 120 µg, CT0794B)
4	Ampicillin/sulbactam 1:1 (SAM, 20 µg, CT0520B)	13	Norfloxacin (NOR, 10 µg, CT0434B)
5	Azithromycin (AZM, 15 µg, CT0906B)	14	Pefloxacin (PEF, 5 µg, CT0661B)
6	Ciprofloxacin (CIP, 1 µg, CT0623B)	15	Piperacillin/tazobactam (TZP, 110 µg, CT0725B)
7	Cefepime (FEP, 30 µg, CT0771B)	16	Sulphamethoxazole/trimethoprim 19:1 (SXT, 25 µg, CT0052B)
8	Cefotaxime (CTX, 30 µg, CT0166B)	17	Tigecycline (TGC, 15 µg, CT1841B)
9	Cefoxitin (FOX, 30 µg, CT0119B)	18	Tobramycin (TOB, 10 µg, CT0056B)

¹⁾ All disks had the same brand as Oxoid.

(Densimat, 21250, Biomerieux) and incubated for 16–20 h at 35°C (EUCAST, 2015b). Plates were examined in order to categorize the isolates as susceptible (S) and resistant (R) based on the complete inhibition zone diameters and the results were interpreted by comparing the zone diameter limit values given in EUCAST (EUCAST, 2015a).

Statistical analysis

Using the protocol by ISO 16140 (ISO, 2016), sensitivity for ISO and RT-PCR, relative trueness (RT) and the false positive ratio (FPR) of RT-PCR based on sample type were determined. Cohen's kappa test (Landis and Koch, 1977) was used in order to assess the accuracy of the agreement between ISO and RT-PCR results.

Results

Overall *Salmonella* prevalence was 5% (10/200) in all the sheep examined regardless of the sample type. On the basis of sample type, 2% (4/200) of the fecal contents, and 1.5% (3/200), of both mesenteric lymph node and kidney samples were found to harbor *Salmonella*. There was no *Salmonella* isolation from the carcass, liver, spleen, or gallbladder samples. The contamination rate in all 1,400 samples was 0.7% (10/1,400) (Table 3).

When the *Salmonella* confirmation results using RT-PCR and the ISO reference method from suspected isolates of various sample types were compared, only 2 and 5 false positives in the carcass and fecal content samples, respectively, yielded high confirmation rates in the RT for RT-PCR of 92% and 80.8%, respectively, which led to substantial agreement between RT-PCR and the ISO methods. Also, there was no false positivity in RT-PCR of suspected *Salmonella* isolates obtained from mesenteric lymph nodes and kidneys. This result suggested that RT-PCR was in perfect agreement with ISO (RT: 96%) to detect *Salmonella* from MLN samples. In addition, the test was in substantial agreement with ISO (93.8%) to identify

Table 3. Prevalence, serovar distribution and antimicrobial resistance profile of *Salmonella* in slaughter sheep

Sample type (n)	Number of positive samples (%)	Sample ID	Serovar	Resistance profile
Carcass (200)	0 (0.0)			
Fecal content (200)	4 (2.0)	F111	Newport	TGC
		F112	Newport	-
		F214	Newport	PEF
		F247	Typhimurium	TGC, SXT
Mesenteric lymph node (200)	3 (1.5)	M116	Newport	TOB
		M247	Umbilo	-
		M248	Typhimurium	FEP, TGC, SXT
Liver (200)	0 (0.0)			
Kidney (200)	3 (1.5)	K42	Corvallis	AMP, CIP, NOR, PEF
		K192	Kentucky	-
		K267	Kentucky	-
Spleen (200)	0 (0.0)			
Gallbladder (200)	0 (0.0)			
Total (1,400)	10 (0.7)			

Salmonella from kidney samples. Overall, when compared to ISO, the RT-PCR had 93.6% RT to detect suspected *Salmonella* isolates. This result points out that, overall, there was a substantial agreement between methods (Data not shown).

Study findings revealed the predominant serovar as *S. Newport*, since in total 40% (4/10 isolates; all 3 fecal content, and 1 out of 3 mesenteric lymph node) of the isolates were identified under this serotype. One fecal content isolate (25%) and one mesenteric lymph node (33.3%) isolate was found to be *S. Typhimurium*, while there were two *S. Kentucky* (1/3, 33.3%) isolates, both of which were isolated from kidneys. Overall, *S. Typhimurium* and *S. Kentucky* were the second-most dominant serovars (20%) within all isolates in the study. There was one *S. Umbilo* isolation from a mesenteric lymph node, and one *S. Corvallis* isolation from a kidney sample, bringing the overall isolation rates for these serovars to 10% (Table 3).

Antimicrobial resistance data of this study showed that 6 out of 10 (60%) salmonellae [3 of 4 *S. Newport* (F111, F214, M116), both *S. Typhimurium* (F247, M248), and one *S. Corvallis* (K42)] were resistant to one or more of the 18 antimicrobials. In contrast, *S. both Kentucky* isolates from kidney samples (K192, K267), one *S. Umbilo* isolate from mesenteric lymph node (M247) and the remaining *S. Newport* fecal content isolate (F112) were found as pansusceptible (40%) to all antimicrobials tested. Overall, 3 out of 6 resistant serovar isolates were MDR (50%), where *S. Typhimurium* and *S. Corvallis* (30%) showed multiresistance as follows: both *S. Typhimurium* were MDR to TGC/SXT, with one isolate's additional resistance to FEP, while MDR of *S. Corvallis* was AMP/CIP/NOR/PEF (Table 3).

Discussion

Within farm animals, *Salmonella* prevalence is mostly studied in poultry, as it is indicated to be the primary source of infection in foodborne salmonellosis in humans. In comparison, less information is available in the current literature on this pathogen in other possible carriers, such as sheep, particularly in Turkey. Therefore, in this study we mainly aimed to determine the *Salmonella* carrier status of slaughter sheep in our country by internationally accepted standard methods with a further focus on the determination of the serogroup/serotype and/or antimicrobial resistance of the isolates.

In this study, there was no *Salmonella* isolation from carcasses (Table 3). This lack of finding may be related to the slaughter being applied in monorail conveyors, the inverted de-pelting procedure involving injection of compressed air under the skin, and transfer of the carcass as a whole to cold storage, all of which contribute to less handling and lower microbial contamination. Additionally, there was less fecal contamination risk during evisceration due to the natural pellet form of sheep feces, which does not stick and smear to the carcass. Previous studies have indicated the absence (Kuma et al., 2017; Ranucci et al., 2014) or presence (Duffy et al., 2010; Stipetic et al., 2016; Tekelu and Negussie, 2011) of *Salmonella* in sheep carcasses. Differences observed in prevalence rates in these studies were reported to arise from the general hygiene status of slaughterhouses; whether slaughtering is applied in monorail conveyors or on the floor; sheep's initial pathogen carriage rate, increasing risk of shedding during transport stress leading to higher fecal contamination of carcass, and differences in sensitivity of *Salmonella* detection procedures. Our study results indicated that the fecal contents of apparently healthy slaughter sheep carried *Salmonella* (Table 3). Similar *Salmonella* contamination rates were reported in fecal contents as 3% by Chatzopoulos et al. (2016) in Greece, 3.6% by Yang et al. (2017) in Australia, and 6.08% by Hurtado et al. (2017) and Hanlon et al. (2018) in Spain. In Qatar, Stipetic et al. (2016) found a contamination rate of 22.5% in fecal content samples by PCR and attributed this finding to high cross-contamination between carcasses, poor sanitary handling by workers and equipment in the slaughterhouse. Likewise, Kuma et al. (2017) from Ethiopia linked their high *Salmonella* contamination rate of 11.36% in the fecal contents of sheep to their close contact during transport and prolonged holding time before slaughter.

Our *Salmonella* prevalence from mesenteric lymph nodes, which correlates with the isolation rate from fecal content, is in concordance with the previous 3.85% rate of Hanlon et al. (2018) from the USA. Additionally, there are studies from Ethiopia indicating considerably higher rates of 6.19% (Tadesse and Tessema, 2014) and 13.6% (Kuma et al., 2017). Examination of the edible offal (liver, kidney, and spleen), and gallbladder indicated that only kidney samples (1.5%) were contaminated with *Salmonella*. Within studies on *Salmonella* prevalence in internal organs of sheep, we came across only one study, by Little et al. (2008), that sampled from kidneys, reporting an overall prevalence of 3.1% (from liver, spleen and kidney samples). The detection of *Salmonella* from this sample type in our study indicates the requirement to examine kidneys as reservoirs for this pathogen and generates data for further research. Mainly, its common consumption as ‘rare grilled’ in Turkey poses a risk for salmonellosis. Additionally, contrary to our findings, there are previous studies indicating the presence of *Salmonella* at 2.0%–4.3% in liver, 3.0% in gallbladder, and 0%–1.0% in spleen (Kuma et al., 2017; Molla et al., 2006; Woldemariam et al., 2005; Zubair and Ibrahim, 2012).

There was only one common isolation from the fecal content (F247) and mesenteric lymph node (M247) of the same sheep, which indicated that this type of common isolation is not rare and can be caught only under exceptional conditions. Reasons for this rare common isolation may be mainly related to (1) the sheep’s carrier state at the time of sampling; (2) sample type (e.g., carcass may not be contaminated with feces carrying the bacterium; therefore, only feces and/or the lymph node can be positive); (3) sampling and/or isolation method (although standard and proper sampling and isolation was applied, it may not always be the optimum condition for the pathogen to survive); and (4) host/strain related factors affecting isolate/serovar’s survival (e.g., viable but not culturable state, occurrence of more than one isolate/serovar, and competition between strains/serovars).

S. Newport was identified as the predominant serovar in our study regardless of the sample type, whereas *S. Typhimurium* and *S. Kentucky* were isolated at lower rates. This finding is in contrast to previous reports indicating *S. Typhimurium* to be either the predominant (Kidanemariam et al., 2010; Molla et al., 2006; Moussa et al., 2012; Stipetic et al., 2016; Tadesse, 2015; Wani et al., 2013; Yang et al., 2014) or the second-most dominant (Lewerin et al., 2011; Tadesse and Tessema, 2014) serovar isolated. There are only a few reports that mention *S. Newport* as the second-most dominant serovar and one of the lesser isolated serovars in sheep FC (Dargatz et al., 2015; Stipetic et al., 2016; USDA, 2013). Additionally, parallel to our findings, *S. Kentucky* was indicated as the second-most predominant serovar by Dargatz et al. (2015). Despite previous reports on the isolation of *S. Kentucky* in sheep-related samples from South Africa (Kidanemariam et al., 2010), Egypt (Moussa et al., 2012), and the USA (USDA, 2013), there is only one study on the isolation of this serovar from a human salmonellosis case in our country (Cilo Dalyan et al., 2015). Interestingly, one of the serovars in this study was *S. Umbilo* isolated from mesenteric lymph nodes, and the other was *S. Corvallis* isolated from a kidney. To the best of our knowledge of the previous isolation of either of these serovars from sheep, there is one study, by Hurtado et al. (2017), reporting *S. Umbilo* isolation from sheep feces, while there is no study indicating *S. Corvallis* detection from sheep. In our country, these two serovars were previously isolated only from human salmonellosis cases (Cilo Dalyan et al., 2015). The reasons behind our isolation of these ‘rare serovars’ from the above sample types may be mainly related to the scarcity of sheep *Salmonella* seroprevalence studies in our country. There may also be unreported isolations of these serovars, while differences in serotyping methods and technical applications, as well as changes in serovars due to geographical and physical conditions where sheep are raised would all account for these types of variations.

An additional benefit in our study is the use of both RT-PCR and ISO in the fast and accurate confirmation of suspected *Salmonella* isolated from sheep, and especially from mesenteric lymph nodes, as this was proven by an overall substantial

agreement between methods (Data not shown).

Based on antibiotyping findings, more than half of the salmonellae (60%) in this study were resistant to one or more antimicrobials tested, with a 40% MDR rate (Table 3). Similarly, Edrington et al. (2009) indicated 80% resistance in their *Salmonella* isolates with 20% MDR. Within *S. Newport* isolates, all but one (75%) was resistant to only one out of 18 antimicrobials tested. Within previous literature, we could not come across a study reporting antimicrobial susceptibility of a *S. Newport* isolated from sheep. Thus, we were unable to discuss the resistance profiles of these isolates to any earlier relevant data. Both of the *S. Typhimurium* isolates were MDR to TGC and SXT, with one additional resistance to FEP. Formerly, SXT resistance and susceptibility of this serovar were reported by Molla et al. (2006) and Scott et al. (2012), respectively. Additionally, the AMP, AMC, C, CIP, AK, FOX, and CN susceptibility of the *S. Typhimurium* serovar isolated from the fecal content was indicated by Molla et al. (2006) and Scott et al. (2012). Two *S. Kentucky* isolates from kidney samples were pansusceptible to all of the antimicrobials tested. In a study by Dargatz et al. (2015), where 8.8% of isolated salmonellae were found resistant, the *S. Kentucky* isolate was susceptible to AMC, AMP, AZM, FOX, C, CIP, CN, and SXT. Similar to our *S. Kentucky* isolates, the *S. Umbilo* serovar isolated from mesenteric lymph nodes was also susceptible to all antimicrobials tested, parallel to an earlier finding by Hurtado et al. (2017). Another serovar isolated in our study was *S. Corvallis*, with an AMP/CIP/NOR/PEF resistance profile, which indicated that this serovar showed resistance to all fluoroquinolones tested. We were not able to directly compare this unique result of *S. Corvallis* isolation from sheep and its high MDR, since we could not come across relevant data in the past studies. However, one possible source for this resistant serovar in sheep could be related to poultry (a source for transmission if sheep and poultry are bred in close contact), since poultry is an important reservoir for fluoroquinolone resistant *S. Corvallis* (Veldman et al., 2008).

Conclusion

Contaminated carcass, fecal content, mesenteric lymph nodes and edible offal of apparently healthy slaughter sheep carrying NTS is of significant health risk to public in Turkey, where ovine meat covers the second highest annual red meat consumption quota with high demand to edible offal. In this study, current predominance of *S. Newport*, particularly in fecal contents; the presence of *S. Typhimurium* and *S. Kentucky* as the second dominant serovars; detection of *S. Corvallis* for the first time in sheep in the world, first time isolations of *S. Newport*, *S. Kentucky*, *S. Corvallis*, *S. Umbilo* serovars from sheep in Turkey; and high antimicrobial resistance rates obtained in more than half of the isolates showing 20% MDR highlights the findings of this study. Isolation of such diverse and antimicrobial resistant serovars from healthy sheep indicates its non-negligible reservoir and shedding capacity of *Salmonella* to the environment. Hence, the inclusion of sheep in national-based *Salmonella* surveys for safer food production and processing lines is strongly recommended.

Conflict of Interest

The authors declare no potential conflict of interest.

Acknowledgements

Funding for this project was provided in part by the Uludag University Scientific Research Unit Grant, Project No: OUAP(V)-2013/29.

Author Contributions

Conceptualization: Temelli S, Eyigor A. Data curation: Cetin E. Formal analysis: Cetin E. Methodology: Cetin E, Temelli S, Eyigor A. Software: Eyigor A. Validation: Temelli S, Eyigor A. Investigation: Cetin E, Temelli S. Writing - original draft: Temelli S, Eyigor A. Writing - review & editing: Cetin E, Temelli S, Eyigor A.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

References

- Akoachere JF, Tanih NF, Ndip LM, Ndip RN. 2009. Phenotypic characterization of *Salmonella* Typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. *J Health Popul Nutr* 27:612-618.
- Al-Habsi K, Yang R, Abraham S, Ryan U, Miller D, Jacobson C. 2018. Molecular characterisation of *Salmonella enterica* serovar Typhimurium and *Campylobacter jejuni* faecal carriage by captured rangeland goats. *Small Rumin Res* 158:48-53.
- Alemu S, Zewde BM. 2012. Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. *Trop Anim Health Prod* 44:595-600.
- Carli KT, Unal CB, Caner V, Eyigor A. 2001. Detection of *Salmonella* in chicken feces by a combination of tetrathionate broth enrichment, capillary PCR, and capillary gel electrophoresis. *J Clin Microbiol* 39:1871-1876.
- Chatzopoulos DC, Sarrou S, Vasileiou NGC, Ioannidi KS, Peteinaki E, Valiakos G, Tsokana CN, Papadopoulou E, Spyrou V, Mavrogianni VS, Giannakopoulos A, Sbiraki A, Lacasta D, Bueso JP, Athanasiou LV, Billinis C, Fthenakis GC. 2016. Dissemination of intestinal pathogens between lambs and puppies in sheep farms. *Small Rumin Res* 141:5-10.
- Colby L. 2015. World sheep meat market to 2025. AHDB Beef & Lamb, Warwickshire, UK. Available from: <http://beefandlamb.ahdb.org.uk/wp-content/uploads/2016/01/World-sheep-meat-market-to-2025.pdf>. Accessed at Jan 16, 2019.
- Dalyan Cilo B, Ozmerdiven GE, Efe K, Gulesen R, Levent B, Agca H, Sınırtas M, Ozakin C. 2015. Serotype distribution and antimicrobial susceptibility profiles of *Salmonella* isolates from South Marmara Region, Turkey. *J Turk Soc Microbiol* 45:122-127.
- Dargatz DA, Marshall KL, Fedorka-Cray PJ, Erdman MM, Koprak CA. 2015. *Salmonella* prevalence and antimicrobial susceptibility from the National Animal Health Monitoring System sheep 2011 study. *Foodborne Pathog Dis* 12:953-957.
- Duffy LL, Small A, Fegan N. 2010. Concentration and prevalence of *Escherichia coli* O157 and *Salmonella* serotypes in sheep during slaughter at two Australian abattoirs. *Aust Vet J* 88:399-404.
- Edrington TS, Long M, Ross TT, Thomas JD, Callaway TR, Anderson RC, Craddock F, Salisbury MW, Nisbet DJ. 2009. Prevalence and antimicrobial resistance profiles of *Escherichia coli* O157:H7 and *Salmonella* isolated from feedlot lambs. *J Food Prot* 72:1713-1717.
- EFSA [European Food Safety Authority], ECDC [European Centre for Disease Prevention and Control]. 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J* 16:5500.
- EFSA, ECDC. 2018. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. *EFSA J* 16:5182.

- EUCAST [The European Committee on Antimicrobial Susceptibility Testing]. 2015a. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0. EUCAST, Basel, Switzerland.
- EUCAST. 2015b. Antimicrobial susceptibility testing EUCAST disk diffusion method. Version 5.0. EUCAST, Basel, Switzerland.
- FAO [Food and Agricultural Organisation]. 2018a. FAOSTAT: Livestock primary. Available from: <http://www.fao.org/faostat/en/#data/QL>. Accessed at Jan 16, 2019.
- FAO. 2018b. FAOSTAT: Food supply - livestock and fish primary equivalent. Available from: <http://www.fao.org/faostat/en/#data/CL>. Accessed at Jan 16, 2019.
- Grimont PAD, Weill FX. 2007. Antigenic formulae of the *Salmonella* Serovars. 9th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, France.
- Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemuhl J, Grimont PA, Weill FX. 2010. Supplement 2003–2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Res Microbiol 161:26-29.
- Hanlon KE, Miller MF, Guillen LM, Echeverry A, Dormedy E, Cemo B, Branham LA, Sanders S, Brashears MM. 2018. Presence of *Salmonella* and *Escherichia coli* O157 on the hide, and presence of *Salmonella*, *Escherichia coli* O157 and *Campylobacter* in feces from small-ruminant (goat and lamb) samples collected in the United States, Bahamas and Mexico. Meat Sci 135:1-5.
- Hurtado A, Ocejo M, Oporto B. 2017. *Salmonella* spp. and *Listeria monocytogenes* shedding in domestic ruminants and characterization of potentially pathogenic strains. Vet Microbiol 210:71-76.
- Igbinosa IH. 2015. Prevalence and detection of antibiotic-resistant determinant in *Salmonella* isolated from food-producing animals. Trop Anim Health Pro 47:37-43.
- ISO [International Organization for Standardization]. 2002. Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp. ISO 6579:2002. ISO, Geneva, Switzerland.
- ISO. 2003. Microbiology of food and animal feeding stuffs: Carcass sampling for microbiological analysis. ISO 17604:2003. ISO, Geneva, Switzerland.
- ISO. 2007. Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp. Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. ISO 6579:2002/A1:2007. ISO, Geneva, Switzerland.
- ISO. 2016. Microbiology of food and animal feedingstuffs protocol for the alternative validation methods. ISO 16140:2016. ISO, Geneva, Switzerland.
- Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, Guibourdenche M, de Pinna E, Nair S, Fields PI, Weill FX. 2014. Supplement 2008–2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Res Microbiol 165:526-530.
- Kidanemariam A, Engelbrecht M, Picard J. 2010. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. J S Afr Vet Assoc 81:37-44.
- Kuma F, Lakew M, Koran T, Olani A, Tamiru M, Yimesgen L, Zenebe T, Gerbi F. 2017. A cross sectional study on *Salmonella* in apparently healthy sheep and goats slaughtered at Elfora and Luna export abattoirs, Ethiopia. Afr J Microbiol Res 11:530-536.
- Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. Biometrics 33:159-174.
- Lewerin SS, Skog L, Frossling J, Wahistrom H. 2011. Geographical distribution of *Salmonella* infected pig, cattle and sheep herds in Sweden 1993–2010. Acta Vet Scand 53:51.

- Little CL, Richardson JF, Owen RJ, de Pinna E, Threlfall EJ. 2008. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003–2005. *Food Microbiol* 25:538-543.
- Malorny B, Made D, Teufel P, Berghof-Jager C, Huber I, Anderson A, Helmuth R. 2007. Multicenter validation study of two blockcycler- and one capillary-based real-time PCR methods for the detection of *Salmonella* in milk powder. *Int J Food Microbiol* 117:211-218.
- Malorny B, Paccassoni E, Fach P, Bunge C, Martin A, Helmuth R. 2004. Diagnostic real-time PCR for detection of *Salmonella* in food. *Appl Environ Microbiol* 70:7046-7052.
- McAuley CM, McMillan K, Moore SC, Fegan N, Fox EM. 2014. Prevalence and characterization of foodborne pathogens from Australian dairy farm environments. *J Dairy Sci* 97:7402-7412.
- Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJ, Evans SJ, Teale CJ, Smith RP, McNally A, Toszeghy M, Futter R, Kay A, Paiba GA. 2008. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiol Infect* 136:739-751.
- Molla W, Molla B, Alemayehu D, Muckle A, Cole L, Wilkie E. 2006. Occurrence and antimicrobial resistance of *Salmonella* serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. *Trop Anim Health Pro* 38:455-462.
- Moussa IM, Ashgan MH, Mahmoud MH, Al-Doss AA. 2012. Rapid detection and characterization of *Salmonella enterica* serovars by multiplex polymerase chain reaction assay. *Afr J Biotechnol* 11:3452-3458.
- Ransom JR, Belk KE, Bacon RT, Sofos JN, Scanga JA, Smith GC. 2002. Comparison of sampling methods for microbiological testing of beef animal rectal/colonial feces, hides, and carcasses. *J Food Prot* 65:621-626.
- Ranucci D, Branciarri R, Miraglia D, Stocchi R, Rea S, Loschi AR. 2014. Evaluation of carcass hygiene in sheep subjected to gas de-pelting with different skinning procedures. *Ital J Food Saf* 3:4143.
- Scott L, Menzies P, Reid-Smith RJ, Avery BP, McEwen SA, Moon CS, Berke O. 2012. Antimicrobial resistance in fecal generic *Escherichia coli* and *Salmonella* spp. obtained from Ontario sheep flocks and associations between antimicrobial use and resistance. *Can J Vet Res* 76:109-119.
- Small A, James C, James S, Davies R, Liebana E, Howell M, Hutchison, M, Buncic S. 2006. Presence of *Salmonella* in the red meat abattoir lairage after routine cleansing and disinfection and on carcasses. *J Food Prot* 69:2342-2351.
- Stipetic K, Chang YC, Peters K, Salem A, Doiphode SH, McDonough PL, Chang YF, Sultan A, Mohammed HO. 2016. The risk of carriage of *Salmonella* spp. and *Listeria monocytogenes* in food animals in dynamic populations. *J Vet Med Sci* 2:246-254.
- Tadesse G, Tessema TS. 2014. A meta-analysis of the prevalence of *Salmonella* in food animals in Ethiopia. *BMC Microbiol* 14:270.
- Tadesse G. 2015. A meta-analysis of the proportion of animal *Salmonella* isolates resistant to drugs used against human salmonellosis in Ethiopia. *BMC Infect Dis* 15:84.
- Teklu A, Negussie H. 2011. Assessment of risk factors and prevalence of *Salmonella* in slaughtered small ruminants and environment in an export abattoir, Modjo, Ethiopia. *Am Eurasian J Agric Environ Sci* 10:992-999.
- TFC. 2011. Turkish food codex regulation on microbiological criteria. *Turkish Food Codex, Official Gazette*: 28157, 29.12.2011.
- TFC. 2017. Turkish food codex regulation on pharmacologically active substances and their classification regarding

- maximum residue limits in foodstuffs of animal origin. Turkish Food Codex, Official Gazette: 30000, 07.03.2017.
- TSI [Turkish Statistical Institute]. 2018. Meat production. Retrieved from: <https://biruni.tuik.gov.tr/medas/?kn=79&locale=tr>. Accessed at Jan 16, 2019.
- USDA [United States Department of Agriculture]. 2013. *Salmonella* on U.S. Sheep Operations, 2011. USDA, Fort Collins, CO, USA.
- Veldman K, van Pelt W, Mevius D. 2008. First report of qnr genes in *Salmonella* in the Netherlands. J Antimicrob Chemother 61:452-453.
- Wani SA, Hussain I, Beg SA, Rather MA, Kabli ZA, Mir MA, Nishikawa Y. 2013. Diarrhoeagenic *Escherichia coli* and *Salmonellae* in calves and lambs in Kashmir absence, prevalence and antibiogram. Rev Sci Technol 32:833-840.
- Woldemariam E, Molla B, Alemayehu D, Muckle A. 2005. Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. Small Rumin Res 58:19-24.
- Yang R, Abraham S, Gardner GE, Ryan U, Jacobson C. 2017. Prevalence and pathogen load of *Campylobacter* spp., *Salmonella enterica* and *E. coli* O157/O145 serogroup in sheep faeces collected at sale yards and in abattoir effluent in Western Australia. Aust Vet J 95:143-148.
- Yang R, Jacobson C, Gardner G, Carmichael I, Campbell AJ, Ryan U. 2014. Longitudinal prevalence, faecal shedding and molecular characterisation of *Campylobacter* spp. and *Salmonella enterica* in sheep. Vet J 202:250-254.
- Zubair AI, Ibrahim KS. 2012. Isolation of *Salmonella* from slaughtered animals and sewage at Zakho abattoir, Kurdistan region, Iraq. Res Opin Anim Vet Sci 3:20-24.