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ORIGINAL ARTICLE

# *Salmonella* prevalence and serovar distribution in healthy slaughter sheep and cattle determined by ISO 6579 and VIDAS UP *Salmonella* methods

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**Abstract** Prevalence of *Salmonella* in slaughter sheep and cattle was determined by International Organization for Standardization Method 6579 (ISO) and Vitek Immunodiagnostic Assay System UP *Salmonella* Phage Technology (VIDAS UP *Salmonella* SPT—VIDAS UP). A total of 400 healthy slaughter sheep (n = 200) and cattle (n = 200) carcass (C), fecal content (FC), mesenteric lymph node (MLN), liver (L), kidney (K), spleen (S) and gall bladder (GB) were randomly sampled and analysed. ISO and VIDAS UP results indicated 13 (3.25%) and 17 (4.25%) of 400 animals carried *Salmonella*, respectively, regardless of sample type. There was no isolation from L, S, GB, while 2 C (0.5%), 6 FC (1.5%), 7 MLN (1.75%), 3 K (0.75%) were contaminated with *Salmonella*. *S.* Typhimurium (27.8%), *S.* Enteritidis (22.2%), *S.* Newport (22.2%) were the three dominant serovars, followed by *S.* Kentucky (11.1%), *S.* Umbilo (5.6%), *S.* Corvallis (5.6%), and *S.* Albany (5.6%). Overall prevalence in 2800 samples was 0.46% by ISO and 0.61% by VIDAS UP. High relative trueness (RT: 99.79%) of VIDAS UP with a substantial agreement to ISO (κ value: 0.80) indicated its efficiency to accompany ISO to monitor *Salmonella* in slaughter animals. As the first report to evaluate ISO and VIDAS UP in detecting *Salmonella* from slaughter sheep and cattle, this current prevalence signifies a risk for public health in red-meat and related products in Turkey.

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**Keywords** *Salmonella* · Sheep · Cattle · ISO · VIDAS UP

## Introduction

Sheep and cattle meat are the two main red meat sources consumed in Turkey, with high demand for their products and edible offal, as well (FAO 2018a, b). Therefore, welfare of these animals and their hygienic slaughter has substantial role in preventing both transmissions of foodborne pathogens to meat and related food, and economic consequences for meat processors.

*Salmonella* is the second most commonly reported bacterial pathogen in gastrointestinal infections and foodborne outbreaks in the European Union (EU) (EFSA and ECDC 2016). Slaughter sheep and cattle can carry this pathogen with no particular sign of illness, therefore carcasses, internal organs and lymph nodes of such animals entering the food chain as contaminated meat, meat products and edible offal can cause significant threat to public health. Within farm animals, *Salmonella* prevalence is mostly studied in poultry as it is indicated as the primary source of infection in foodborne salmonellosis in humans. Although there are a number of studies performed in different countries reporting various prevalence rates of *Salmonella* in other possible carriers as sheep and cattle: on carcasses (Kuma et al. 2017; Loiko et al. 2016), in fecal samples (Hanlon et al. 2018; Bonardi et al. 2017), in edible organs as liver and spleen (Kuma et al. 2017; Moawad et al. 2017), in gallbladder (Zubair and Ibrahim 2012) and in mesenteric lymph nodes (Kuma et al. 2017; Hanlon et al. 2018), less information is available in the current literature investigating this pathogen in these important farm animals in Turkey.

As an important food safety indicator, Turkish Food Codex (TFC) Regulation on Microbiological Criteria (2011) indicates zero tolerance for *Salmonella* on carcasses of sheep and cattle tested with the reference culture method International Organization for Standardization Method 6579:2002 (ISO) (2002). In general, although referred as 'gold standard' in food diagnostics, standardized culture methods are known to be laborious and time-consuming (Chajecka-Wierzchowska et al. 2012; Lee et al. 2015). Besides, food matrices and their storage conditions could be quite stressful for the pathogen to survive. Also, environmental stress could reduce the metabolic rate, and therefore effect isolation and identification of *Salmonella* by culture, resulting in lower sensitivity and specificity of the test. Thus, rapid and reliable alternative methods are required to identify this bacterium, since prolonged test time in standard culture methods is an important barrier in food market, particularly for perishable products with short shelf-life, such as red meat and edible offal. One such method, Vitek Immunodiagnostic Assay System UP *Salmonella* Phage Technology (VIDAS UP Salmonella SPT—VIDAS UP) (Biomeriéux), which uses a recombinant phage protein-based technology enabling specific and rapid detection of *Salmonella* by ELFA (Enzyme Linked Fluorescent Assay Immunoassay), is a potential candidate to accelerate and fortify results to complement the culture-based methods. Since 2011, this technology has been applied and performed equivalent or superior to culture method in detecting *Salmonella* from various food matrices such as beef, pork and poultry meat (Zadernowska et al. 2014); sausage (Benetti et al. 2013; Jeuge et al. 2016); poultry meat (Chajecka-Wierzchowska et al. 2012); animal products (Gava et al. 2015); environmental samples (Bradbery et al. 2015). Up to our knowledge, there is no previous study in literature investigating the presence of *Salmonella* in the sample types that we used in this study using this novel technology. Therefore, our study aimed to determine current *Salmonella* prevalence in slaughter sheep and cattle in Turkey by ISO 6579 and VIDAS UP, and evaluate its effectivity to complement ISO in detecting *Salmonella* from these animals.

## Materials and methods

### Sample collection and preparation

In this study, 2800 samples of 7 types comprised of carcass (C), fecal content (FC), mesenteric lymph node (MLN), liver (L), kidney (K), spleen (S), gallbladder (GB) were collected randomly from 200 sheep and 200 cattle of various herds slaughtered in 4 slaughterhouses between 2013 and 2015 following the related requirements of TFC (2011). All samples were transferred to the laboratory in a + 4 °C cooler and prepared for analysis within maximum an hour.

For carcass sampling, a non-destructive sponge sampling method was applied to carcasses following the instructions indicated in ISO 17604:2003 (2003). For this, the sites of sheep and cattle carcasses with the highest prevalence of contamination/most consistently contaminated by high numbers of microorganisms as indicated in Annex A of the same document as abdomen (flank), lateral thorax, crutch, and lateral breast from sheep (4 sites); and as brisket, fore rib, flank, lateral of round and flank groin from cattle (5 sites) were selected. For each site, a separate sterile square template with a hollow internal area of 100 $cm^2$ (10 cm × 10 cm) was used to enclose the specific location. Then, the whole inner area was wiped for a total of ten times in vertical and ten times in horizontal direction with a sufficiently wetted sponge (Whirl Pak, B01351WA) with maximum recovery diluent (MRD, Oxoid, CM0733) using sterile sampling techniques. After swabbing, all sponges (total 4 sponge/bag for sheep, 5 sponge/bag for cattle) were placed back, and more diluent was added to the sample bag to keep all sponges wet. After transfer to the laboratory, sponge samples were prepared for analysis by ISO and VIDAS UP as follows: In order to obtain even distribution of samples for each analysis, sponges were vertically cut into two, and each half was placed into separate sterile stomacher bags following aseptic techniques. Thus, for each carcass, there were two sponge bags with 4 half sponges/bag for sheep, 5 half sponges/bag for cattle, each of which were then used for analysis by ISO and VIDAS UP in parallel.

In sampling of fecal content, whole colonic and rectal content was stripped from the anus and approximately 100 g thoroughly mixed content was placed into a sterile sampling bag as indicated by Ransom et al. (2002) and Milnes et al. (2008). After transfer to the laboratory, 25 g from each FC sample was divided into sterile stomacher bags, each of which were used for analysis by ISO and VIDAS UP in parallel.

Sampling of mesenteric lymph nodes, liver, kidney, spleen and gallbladder was performed by following sterile sampling procedures, and a minimum of 5 MLNs were excised and diced into smaller pieces. Swab samples were taken for VIDAS UP, and 25 g sample was placed into a sterile sampling bag (Alemu and Zewde 2012) for ISO analysis. Two separate swabs were taken from organs, each of which to be used in ISO and VIDAS UP analyses. L and K samples were taken from the entry site of vena porta, and from the orifice and surrounding of ureter, respectively, by a pre-wetted sterile swab (LP Italiana, L111598) with buffered peptone water ISO (BPW, Oxoid, CM1049) and placed into tubes containing 10 ml sterile BPW (Little

et al. 2008). S samples were taken by swabbing an approximately 5 $cm^2$ area of the arterial and venous orifice in the interior part of S, and the pre-wetted swab was then placed into tubes containing 10 ml of sterile BPW. For sampling from the GB, a pre-wetted sterile swab with BPW was inserted from the neck of the pre-emptied GB and rubbed against its walls, and then placed into a tube containing 10 ml sterile BPW (Akoachere et al. 2009).

### Standard *Salmonella* strains

*Salmonella enterica* subspecies *enterica* serovar Enteritidis 64 K (M.Y. Popoff, Institut Pasteur, Paris Cedex 15, France) and *S. enterica* subspecies *enterica* serovar Typhimurium NCTC 12416 (Refik Saydam National Public Health Agency, Ankara, Turkey) were used as positive controls in *Salmonella* isolation, identification and serotyping.

### Analysis of samples by ISO

As indicated in ISO 6579:2002 (2002), for pre-enrichment (PE) of C samples 225 ml BPW was added onto sponges in 25 ml MRD. Also, L, K, S and GB swabs in 10 ml BPW were placed into 90 ml BPW in 100 ml stomacher bags. All samples were then homogenized for 2 min at 230 rpm in Stomacher (Seward, 400 C), and incubated at 37 °C for 18 h. For selective enrichment (SE); (a) 1 ml from PE culture was transferred into 10 ml Mueller Kauffmann tetrathionate novobiocin (MKTTn Oxoid, CM1048) broth with novobiocin supplement (Oxoid, SR0181), and incubated at 37 °C for 24 h. (b) 0.1 ml from PE culture was transferred into 10 ml broth Rappaport–Vassiliadis soya peptone (RVS Oxoid, CM0866) broth, and incubated at 41.5 °C for 24 h. Selective plating was performed from each of the SE broths on xylose lysine deoxycholate (XLD, Oxoid, CM0469) agar and brilliance *Salmonella* (BS, Oxoid, CM1092) agar with *Salmonella* selective supplement (Oxoid, SR0194), and incubated at 35 °C for 24 h. FC samples: As indicated in ISO 6579/A1: 2007 (2007), for PE of the sample, 25 g FC was homogenized in 225 ml of BPW for 2 min at 230 rpm, and incubated at 37 °C for 18 h. After PE, 0.1 ml was transferred onto modified semisolid Rappaport–Vassiliadis (MsRV, Oxoid, CM1112) agar and incubated at 41.5 °C between 18 and 42 h until a defined zone was observed. Selective plating was performed from MsRV agar onto XLD agar, xylose lysine tergitol-4 (XLT4, Oxoid, CM1061) agar, and BS agar, and incubated at 37 °C for 24 h. After incubation, 1–5 *Salmonella* suspect colonies were selected and streaked onto MacConkey (MC, Oxoid, CM0115) agar for obtaining pure culture to be utilized in biochemical identification. Before biochemical identification, pure MC agar culture was transferred into brain heart infusion (BHI, Oxoid, CM1135) broth and incubated at 37 °C for 18 h. Then, this broth culture was grown on nutrient agar (NA, Oxoid, CM003) at 37 °C for 24 h, and tested for oxidase activity. The cultures verified as oxidase negative were further subjected to initial biochemical identification, and their urease activity (Urea Agar Base, Oxoid, CM0053), triple sugar utilization and $H_2S$ formation (Triple Sugar Iron Agar, Oxoid, CM0277), and lysine decarboxylase activity (Lysine Iron Agar, Oxoid, CM0381) were determined. Final identification was performed using API 20E (Biomerieux, 20100), and profile results were evaluated accordingly. *Salmonella* spp. positive cultures preserved in sterile 50% glycerol were stored at − 20 °C for serotyping.

### Analysis of samples by VIDAS UP

For carcass samples, as indicated in VIDAS UP product protocol, 30 ml BPW at 41.5 °C and 250 µl *Salmonella* supplement (Biomeriéux, 42650) was added onto carcass sponge samples in stomacher bag already in 30 ml of MRD, hand massaged from outside of the bag for 2 min, and left for incubation at 41.5 °C for 24 h. After incubation, 1.5 ml of this broth was transferred to a microfuge tube, heat treated for 15 min at 100 °C on block heater (Techne FBD02DD), while rest of the broth was stored at + 4 °C until confirmation. After cooling to room temperature, 500 µL from the heat-treated broth was placed into a VIDAS UP *Salmonella* (SPT) Strip (Biomeriéux, 30707) and then into the miniVIDAS (Biomeriéux) instrument for 48 min, and analyzed according to the manufacturer's instructions. A relative fluorescence value of $\geq 0.25$ for a sample was considered a presumptive positive result. The results were expressed as presence or absence of *Salmonella* in the broth. For the presumptive positive samples, confirmation was performed by streaking 10 µl of the broth culture onto the selective agar plate Chrom ID *Salmonella* (ChID, Biomerieux, 43621) as indicated in the protocol, and onto alternative XLD and BS plates that we selected. After incubation at 37 °C for 24 h, 5 typical colonies were picked for further biochemical identification by API 20E (Biomeriéux, 20100).

For fecal content samples, 225 of BPW at 41.5 °C and 2 ml *Salmonella* supplement was added onto 25 g of FC in sterile stomacher bag, hand massaged from outside for 2 min, and incubated at 41.5 °C for 18 h. Then, 1 ml of this broth was transferred into *Salmonella* Xpress (SX2) (Biomeriéux, 42121) broth and incubated at 41.5 °C for 24 h. A 1.5 ml aliquot was transferred into a microfuge tube, and the same procedure was followed as indicated above in the analysis for carcass samples.

For mesenteric lymph node, liver, kidney, spleen and gallbladder samples, 40 µl of *Salmonella* supplement was

added into 10 ml BPW including swab samples, and incubated at 41.5 °C for 18 h. Then, 1 ml of this broth was transferred into SX2 broth, which was incubated at 41.5 °C for 24 h. Subsequently, same steps were followed as indicated above in the analysis for carcass samples to the 1.5 ml transferred aliquot.

### Serotyping

Serological identification of the isolates was performed that were confirmed as *Salmonella* after isolation and identification. Serotyping was applied by reaction with O- and H-group antigen, according to the White–Kauffmann–Le Minor Scheme (Grimont and Weill 2007), to Guibourdenche et al. (2010), and to Issenhuth-Jeanjean et al. (2014) by using commercial antisera (Becton–Dickinson). Slide agglutination and tube agglutination tests were applied for the analyses of somatic and flagellar phase antigens, respectively.

### Statistical analysis

Sensitivity for ISO and VIDAS UP, relative trueness (RT) and the false positive ratio (FPR) of VIDAS UP were calculated according to the protocol described in ISO 16140 (2016). Reliability of the agreement between method results was determined by Cohen's kappa test (Landis and Koch 1977).

## Results

In this study, 13 (3.25%) and 17 (4.25%) out of 400 animals were found positive for *Salmonella* by ISO and VIDAS UP, respectively. There was no *Salmonella* isolation from L, S, GB, while 2 C (0.5%) (C86, C94), 6 FC (1.5%) (F111, F112, F133, F158, F214, F247), 7 MLN (1.75%) (M53, M116, M132, M133, M136, M247, M248), and 3 K (0.75%) (K42, K192, K267) samples were contaminated with *Salmonella* (Table 1). Overall *Salmonella* prevalence in 2800 samples was calculated as 0.46% by ISO and 0.61% by VIDAS UP (data not indicated in tables).

ISO and/or VIDAS UP positive *Salmonella* isolates' serotyping results indicated that the predominant serovar was *S.* Typhimurium (27.8%). The second dominant serovars were *S.* Enteritidis and *S.* Newport (22.2%), which was followed by *S.* Kentucky (11.1%). Additionally, there were single isolations of *S.* Umbilo, *S.* Corvallis, and *S.* Albany (5.6%) serovars (Table 1).

From a total of 18 *Salmonella* positive samples, 12 were positive by both methods, whereas 6 samples were positive at least with one method (Table 1). Tables 2 and 3 present the processes followed during ISO and/or VIDAS UP in detail for the detection, isolation and identification of *Salmonella* from FC and other sample types, respectively.

Statistical analysis results revealed that sensitivities of ISO and VIDAS UP were 72.22% and 94.44%,

**Table 1** *Salmonella* positive samples in slaughter sheep and cattle by ISO and/or VIDAS UP and their serovars

| Animal (n) | Sample type | Sample ID | N | Result (%) | | Serovar |
|---|---|---|---|---|---|---|
| | | | | ISO | VIDAS UP | |
| Sheep (200) | Fecal content | F111 | 4 | + | + | Newport |
| | | F112 | | + | + | Newport |
| | | F214 | | + | + | Newport |
| | | F247 | | + | + | Typhimurium |
| | Mesenteric lymph node | M116 | 3 | + | − | Newport |
| | | M247 | | + | + | Umbilo |
| | | M248 | | + | + | Typhimurium |
| | Kidney | K42 | 3 | + | + | Corvallis |
| | | K192 | | + | + | Kentucky |
| | | K267 | | + | + | Kentucky |
| Cattle (200) | Carcass | C86 | 2 | + | + | Typhimurium |
| | | C94 | | + | + | Typhimurium |
| | Fecal content | F133 | 2 | − | + | Enteritidis |
| | | F158 | | − | + | Albany |
| | Mesenteric lymph node | M53 | 4 | + | + | Typhimurium |
| | | M132 | | − | + | Enteritidis |
| | | M133 | | − | + | Enteritidis |
| | | M136 | | − | + | Enteritidis |
| Total (400) | | | 18 | 13 (3.25) | 17 (4.25) | |

**Table 2** Evaluation of *Salmonella* positive fecal content samples by ISO and/or VIDAS UP from sheep and cattle

| Animal | Sample ID | ISO | | | | | | VIDAS UP | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Salmonella*-suspect colony on selective plate | | | Differential plating to MC from | BCH ID | | ISO result | VIDAS SPT presumptive | VIDAS confirmation | | | | VIDAS result |
| | | | | | | Initial | Final | | | Confirmed colony on selective plate | Alternative plating on | BCH ID | | |
| | | MsX | MsT4 | MsB | MC | OX/TSI/LI/U | API | | | ChID | BS | Initial OX/TSI/LI/U | Final API | |
| Sheep | F111 | + | + | + | X, T4, B | +, +, + | +, +, +, + | + | + | + | + | + | + | + |
| | F112 | + | – | + | X, B | +, + | +, + | + | + | + | + | + | + | + |
| | F214 | + | – | – | X | + | + | + | + | + | + | + | + | + |
| | F247 | + | + | – | X, T4 | +, + | +, – | + | + | + | + | + | + | + |
| Cattle | F133 | – | – | – | ND | ND | ND | – | + | + (2nd sc) | + | + | + | + |
| | F158 | – | – | – | ND | ND | ND | – | + | + | + | + | + | + |

*MsX* modified semisolid Rappaport–Vassiliadis agar (Ms) and Xylose lysine deoxycholate agar (X), *MsT4* modified semisolid Rappaport–Vassiliadis agar (Ms) and Xylose lysine tergitol-4 agar (T4), *MsB* modified semisolid Rappaport–Vassiliadis agar (Ms) and brilliance *Salmonella* agar (B), *MC* MacConkey agar, *OX* oxidase activity, *TSI* triple sugar utilization and $H_2S$ formation, *LI* lysine decarboxylase activity, *U* urease activity, *ChID* Chrom ID *Salmonella* agar, *BS* brilliance *Salmonella* agar, *ND* not determined, *2nd sc* positive after second subculture

respectively. Relative trueness (RT: 99.79%) of the alternative method, VIDAS UP, resulted a substantial agreement to ISO (κ value: 0.80) (Table 4).

## Discussion

Overall *Salmonella* prevalence rate from slaughter animals determined by ISO standard culture method and VIDAS UP were 3.25% and 4.25%, respectively. FCs and MLNs of both sheep and cattle were found to carry *Salmonella,* while there was no isolation from any of the L, S, GB samples tested. Additionally, sheep K and cattle C were contaminated with the pathogen (Table 1). Up to our knowledge, there is no previous prevalence study by VIDAS UP on the detection of *Salmonella* in the sample types that we used in our study. Within previous studies, which used ISO as their method in the isolation of *Salmonella* from sheep and cattle samples, there are recent sheep reports with lower (0–2%) (Kuma et al. 2017; Zubair and Ibrahim 2012), similar (3%) (Chatzopoulos et al. 2016), or higher (5.6–13.6%) (Kuma et al. 2017; Hurtado et al. 2017), as well as cattle reports with rates ranging between 1.2 and 5.9%, mostly of which are carcass *Salmonella* isolation rates and similar to (Alemu and Zewde 2012; Pacheco da Silva et al. 2014), or lower than (Wieczorek and Osek 2013), and slightly higer than (Alemu and Zewde 2012; Hurtado et al. 2017) our findings. Variations in isolation rates in these ISO-based studies can be mainly related to environmental (country, region, season, feeding), animal (hygiene management and health status, age, gender, animal's carrier state at the time of sampling), sample (type, microbial load, isolate/serovar's survival/competition capability). Regardless, the most predominant serovar in this study was *S.* Typhimurium, followed by *S.* Enteritidis and *S.* Newport. The sources of our *S.* Typhimurium isolates are C and MLN of cattle, and sheep FC and MLN, indicating that both of these animals carry this pathogen, whereas *S.* Enteritidis was isolated only from cattle. *S.* Typhimurium and *S.* Enteritidis serovars are known as the two most commonly reported *Salmonella* serovars in EU/European Economic Area (EEA) in 2015 representing 45.7% and 15.8%, respectively, of all reported serovars in 69.663 confirmed human cases, and sources for these serovars are attributed mostly to cattle and sheep (EFSA and ECDC 2016).

In our study, 12 out of 18 samples were found be *Salmonella* positive by both ISO and VIDAS UP, in contrast to a positive detection observed in the remaining 6 samples by either method. Within these, there were 5 cattle FC (F133, F158), and MLN samples (M132, M133 and M136) from which *Salmonella* could not be detectected by ISO but by VIDAS UP (Table 1). From these, the two FC

**Table 3** Evaluation of *Salmonella* positive MLN and kidney samples of sheep, and carcass and mesenteric lymph node samples of cattle by ISO and/or VIDAS UP

| Animal | Sample type | Sample ID | ISO | | | | | | | | VIDAS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Salmonella*-suspect colony on selective plate | | | | Differential plating to MC from | BCH ID | | ISO result | VIDAS SPT presumptive | VIDAS confirmation | | | | VIDAS result |
| | | | | | | | | | | | | Confirmed colony on selective plate | Alternative plating on | BCH ID | | |
| | | | | | | | | Initial | Final | | | | | Initial | Final | |
| | | | RX | RB | MX | MB | MC | OX/TSI/LI/U | API | | | ChID | BS | OX/TSI/LI/U | API | |
| Sheep | MLN | M116 | + | + | − | − | RX, RB | +, + | +, + | + | − | ND | ND | ND | ND | − |
| | | M247 | + | − | + | − | RX, MX | +, + | +, + | + | + | + | + | + | + | + |
| | | M248 | + | − | − | − | RX | + | + | + | + | + | + | + | + | + |
| | Kidney | K42 | + | + | + | − | RX, RB, MX | +, +, + | +, +, + | + | + | + | + | + | + | + |
| | | K192 | + | + | + | + | RX, RB, MX, MB | +, +, +, + | +, +, +,+ | + | + | + | + | + | + | + |
| | | K267 | + | − | + | − | RX, MX | +, + | +, + | + | + | + | + | + | + | + |
| Cattle | Carcass | C86 | + | + | + | − | RX, RB, MX | +, +, + | +, +, + | + | + | + | + | + | + | + |
| | | C94 | + | + | − | − | RX, RB | +, + | +, + | + | + | + | + | + | + | + |
| | MLN | M53 | + | − | + | + | RX, MX (3rd sc), MB | +, +, + | +, +, + | + | + | + | + | + | + | + |
| | | M132 | − | + | + | + | RB, MX, MB | − | ND | − | + | + (2nd sc) | + | + | + | + |

*RX* Rappaport–Vassiliadis soya peptone broth (R) and Xylose lysine deoxycholate agar (X), *RB* Rappaport–Vassiliadis soya peptone broth (R) and Brilliance *Salmonella* agar (B), *MX* Mueller Kauffmann tetrathionate novobiocin broth and Xylose lysine deoxycholate agar (X), *MB* Mueller Kauffmann tetrathionate novobiocin broth and brilliance *Salmonella* agar (B), *MC*: MacConkey agar, *OX* oxidase activity, *TSI* triple sugar utilization and $H_2S$ formation, *LI* lysine decarboxylase activity, *U* urease activity, *ChID* Chrom ID *Salmonella* agar, *BS* brilliance *Salmonella* agar, *ND* not determined, *3rd sc* positive after third subculture, *2nd sc* positive after second subculture

**Table 4** Sensitivity, relative trueness and false positive ratio of VIDAS UP with respect to ISO in detecting *Salmonella* from sheep and cattle samples

| Reference method ISO | | | Alternative method VIDAS UP | | | | | Kappa index value |
|---|---|---|---|---|---|---|---|---|
| Positive (n) | Negative (n) | SE ISO (%) | $False_{neg}$ (n) | $False_{pos}$ (n) | SE VIDAS UP (%) | RT (%) | FPR (%) | |
| 13 | 5 | 72.22 | 1 | 5 | 94.44 | 99.79 | 0.18 | 0.80[a] |

*SE* sensitivity, *RT* relative trueness, *FPR* false positive ratio

[a]Substantial agreement between ISO and VIDAS UP results

samples were negative even in the beginning of the isolation, failing to produce no suspect colony on any of the selective plates used in ISO (Table 2). Other three MLN samples, although had *Salmonella*-suspect colonies in their plates, were found as *Salmonella* negative in the initial biochemical identification (Table 3). There may be several reasons for the negativities observed in the ISO standard culture method. Firstly, the pre and/or primary enrichment steps in ISO was/were insufficient to resuscitate the possibly injured/stressed *Salmonella* cells present in the sample, therefore leading to absence of typical growth on selective plates. Secondly, colonies formed on selective plates were not distinctive enough due to overgrowth of competing microflora, yielding false negatives (Lee et al. 2015). This instance, although could be related to the low number of initial cells in the sample, may also be in part due to problems in the selectivity capacity of the method, particularly the culture media used in ISO, as we have observed dominancy of *Citrobacter* and/or *Pseudomonas* spp. on the entire plate making it impossible to distinguish any other suspect colonies. Also, possible coexistence of several serovars in the sample with different growth dynamics and competition for nutrients could cause problems both in the isolation and/or selection for pure culture and storage. Additionally, from the aspect of VIDAS UP result for these 5 ISO negative isolates could be explained by the successful capture of the exposed *Salmonella* antigens of even injured/stressed cells by SPT antibodies in the initial phase. Also, the selectivity of the media used in VIDAS UP confirmation worked better than the ones used in ISO, thus propagating the growth of *Salmonella* while supprressing contaminant flora. All these findings are in concordance with the previously reported observations of Junillon et al. (2014), Chajecka-Wierzchowska et al. (2012), and Zadernowska et al. (2014).

There was only one sheep MLN sample (M116), from which *Salmonella* could not be detected by VIDAS UP, but by ISO (Table 3). In fact, reasons for this false negativity by VIDAS UP requires further inquiry. However, in one study by Raman (2017) analysis of such VIDAS UP negative/standard culture positive strains showed that these strains' flagella genes (*fli*B and *fli*C-the genes of the antigen target used in the assay) significantly differed or was putatively reppressed from other *Salmonella* isolates that tested positive. Both of these observations were the likely reasons for the lack of complete flagella on the surface of the strains, which might be responsible for our false negative VIDAS UP result. Another reason for this negativity might be linked to a variation in the surface antigen of this *S. enterica* strain, which hindered binding of the antibodies to their selective target receptor (Raman 2017).

Overall, the high sensitivity and (94.44%) relative trueness (RT: 99.79%) rates obtained from VIDAS UP results suggest that this test has the potential to complement ISO in the true detection of *Salmonella* from these sample types. Thus, the substantial agreement between our methods (Table 4) is in accordance with the findings of Gava et al. (2015), who reported equivalent detection rate of *Salmonella* from animal products by VIDAS UP compared to ISO (SE: 90%); with Benetti et al. (2013) indicating VIDAS UP's 100% sensitivity of *Salmonella* detection from sausage samples in 92.9% agreement to ISO; and with Zadernowska et al. (2014), who found VIDAS UP's sensitivity as 100% in detecting the pathogen from meat samples, thereof a substantial agreement to ISO. Moreover, in studies using various isolation methods other than ISO, Bradbery et al. (2015) indicated that USDA FSIS and VIDAS UP were similar in their ability to detect the presence of *Salmonella* from environmental samples; and Junillon et al. (2014) reported 80% sensitivity and 88% overall agreement in the detection of *Salmonella* between their specific enrichment broth that they had developed and VIDAS UP.

In conclusion, considerably high prevalence of *Salmonella* in apparently healthy slaughter sheep and cattle is of significant concern to public health. Presence of this pathogen in carcasses, organs and lymph nodes denotes the importance of efficient veterinary meat inspection practices, and application of proper and hygienic carcass/organ handling procedures in slaughterhouses coupled by routine monitoring of samples from suspect/apparently healthy animals. Thus, introduction of *Salmonella* to red meat

industry as contaminated raw material would be prevented. This study reports the first-time evaluation of VIDAS UP to complement ISO in detecting *Salmonella* from slaughter sheep and cattle samples, and strongly suggests implementation of such rapid and reliable test practices for red-meat and meat products in our country.

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