

## Prevalence of *Salmonella* in Edible Offal in Afyonkarahisar Province, Turkey

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### Summary

In this study, the prevalence of *Salmonella* sp. was examined using a total of 205 edible bovine offal samples collected from different abattoirs (n=105) and butcheries (n=100) by immunomagnetic separation (IMS) method. The isolation rate of *Salmonella* was found to be 8.57%, 5.71% and 5.71% for the liver, kidney and tripe samples collected from the abattoir, respectively. Of the offal samples obtained from the butcheries, the detection rate of *Salmonella* sp. was 16% in the liver and 4% in the kidney, tripe and brain samples. Overall, it was found that 8.29% of all the offal samples obtained from the abattoirs and butcheries were determined to be contaminated with *Salmonella* sp. The results of this study shows that edible offal are cross-contaminated by *Salmonella* sp. at the abattoirs and retail sale points until they reach to the consumer. It is recommended that adequate hygienic and sanitary measures be taken in these kind of places in order to protect public health.

**Keywords:** *Salmonella*, Edible offal, Abattoir, Butcher, IMS

## Afyonkarahisar İlindeki Yenebilir Sakatatlarda *Salmonella*'nın Prevalansı

### Özet

Bu çalışmada, farklı kesimhane (105 adet) ve kasaplardan (100 adet) temin edilen toplam 205 adet yenebilir sakatat örneği, *Salmonella* türlerinin prevalansını belirlemek amacıyla immunmanyetik ayırma tekniği (IMS) kullanılarak incelendi. *Salmonella* izolasyon oranı kesimhaneden alınan karaciğer, böbrek ve iškembe örnekleri için sırasıyla %8.57, %5.71 ve %5.71 olarak saptandı. Kasaplardan temin edilen sakatat örneklerinde ise *Salmonella* saptama oranı karaciğerde %16, böbrek, iškembe ve beyin örneklerinde ise sırasıyla %4 olarak belirlendi. Genel olarak, bu çalışma kapsamında kesimhane ve kasaplardan temin edilen tüm sakatat örneklerinin %8.29 oranında *Salmonella* ile kontamine olduğu belirlenmiştir. Bu çalışmanın bulguları, yenebilir sakatatların tüketiciye ulaşmaya kadar kesimhane ve perakende satış noktalarında *Salmonella* ile çarpaz kontaminasyona maruz kaldığını göstermektedir. Halk sağlığını korumak amacıyla bu gibi yerlerde yeterli hijyen ve sıhhi önlemlerinin alınması tavsiye olunur.

**Anahtar sözcükler:** *Salmonella*, Yenebilir sakatat, Kesimhane, Kasap, IMS

### INTRODUCTION

*Salmonella* cause a variety of zoonotic infections in humans and animals. *Salmonella* spp. are naturally present in the intestines of humans and animals and contracted

by humans through ingestion of contaminated foods and water <sup>1,2</sup>. Salmonellosis is manifested by enteric fever (typhoid), septicemia and gastroenteritis in humans <sup>3</sup>. The

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micro-organisms are disseminated by the carrier animals which do not manifest any clinical sign and therefore the infected animals can not readily be detected at the abattoirs<sup>4</sup>. Salmonellosis is reported as one of the leading causes of foodborne diseases in most countries such as Germany, France, Spain, Sweden, England, the US and Canada<sup>3,5-8</sup>, which also cause increasing big economic losses<sup>5</sup>.

Animal offal which are edible parts of internal organs such as liver, kidneys and spleen are widely consumed in Turkey by tradition<sup>9</sup>. Although the offal are rich at mineral and vitamin contents they have high loads of micorganisms<sup>10</sup>. They can be contaminated more frequently than animal carcasses by *Salmonella*. It has been reported that offal samples (36%) were more frequently contaminated with *Campylobacter* and *Salmonella* than muscle tissue (7%)<sup>11</sup>. Sinell<sup>12</sup>, found very high *Salmonella* contamination of raw offal meat sold in retail concluding that a health risk may arise due to consumption or direct contact or inadequately heat process. This was confirmed by Cornell and Neal<sup>13</sup>, who reported an food poisoning outbreak of *Salmonella typhimurium* related to tripe and chitterlings in UK.

This investigation was instigated by the scarcity of the published information at determining the prevalence of *Salmonella* sp. in edible bovine offal obtained from different abattoirs and butcheries in Afyonkarahisar province of Turkey.

## MATERIAL and METHODS

### Samples

A total of 205 edible bovine offal (liver, kidney, brain and tripe) samples were collected from 5 abattoirs (n=105) and from 5 different butcheries (n=100) present in Afyonkarahisar province of Turkey. The samples were examined by immunomagnetic separation method (IMS). The abattoir samples consisted of liver, kidney and tripe (35 of each) and the samples obtained from butcheries included liver, kidney, brain and tripe samples (25 of each). The samples were kept in cool environment (+4°C) and immediately transported to laboratory for analyses.

### Detection of *Salmonella* sp.

For the isolation of *Salmonella* sp., 25 g of each sample was homogenised in 225 ml of Buffered Peptone Water (BPW) (Oxoid, CM0509) and incubated at 37°C for 24 h. All the samples enriched in BPW were subjected to IMS technique in order to concentrate *Salmonella* sp. The IMS technique was performed according to manufacturer's instructions<sup>14</sup>. Briefly, 1 ml of the enriched culture and 20 µl of Dynabeads<sup>14</sup> were mixed in an eppendorf tube, and incubated with continuous mixing for approx. 10 min at room temperature and rotated about 3 min using a vortex<sup>14</sup>. A magnetic field was applied to the side of the tube for about 3 min, and the beads with any adherent of *Salmonella* sp. were drawn to the side of the tube. The

supernatant was carefully removed by aspiration and the beads were re-suspended in 1 ml phosphate-buffered-saline-Tween (PBS, Sigma) by mixing for a few min. This washing step was repeated twice. After the final washing, the beads were re-suspended in 100 µl of washing diluent and resuspended bead-bacteria complexes were transferred into Rapoport Vasilliadis Soya Peptone Broth (RVSB, Oxoid, CM866) and incubated at 42°C for 18-24 h. After post selective enrichment of the bead-bacteria complexes, 50 µl of the Dynabeads-*Salmonella* complexes were streaked onto Modified Brilliant Green Agar (MBGA, Oxoid, CM0329) plates and Xylose-Lysine-Desoxycholate (XLD, Oxoid, CM469) agar plates and incubated at 37°C for 18-24 h. Three suspected colonies per plate were picked up and re-streaked onto Tryptone Soya Agar (TSA, Oxoid, CM131) in order to purify the colonies. The isolated colonies were biochemically characterized by inoculating into Urea Broth (Oxoid, CM0071), Lysine Decarboxylase Broth (Oxoid, CM0308) and Triple Sugar Iron (TSI, Oxoid, CM0277) slants. Microbact™ TM 24E Gram-Negative Identification System (Oxoid, MB1074A) was employed for further characterization of the isolates according to manufacturers' instructions<sup>15</sup>.

## RESULTS

Out of 105 edible bovine offal samples examined, seven (6.66%) of them were found positive for *Salmonella* sp. The prevalence of *Salmonella* sp. was determined to be 8.75%, 5.71% and 5.71% in the liver, kidney and tripe samples, respectively. The recovery rate of *Salmonella* sp. was 10% (10 of the 100 examined) for the samples obtained from the butcheries. The contamination rates of *Salmonella* spp. were found to be 16% in the liver and 4% in each of the kidney, tripe and brain samples obtained from the local butcheries. In total, the overall prevalence of *Salmonella* sp. was 8.29% (17 positive out of the 205 examined) for all the samples examined in this study. The contamination level of the liver was determined slightly higher than that of the other samples (see Table 1 for details).

## DISCUSSION

It is reported that consumption of meat and meat products constitutes 54.7% of all food poisoning cases<sup>5</sup>. The existence of *Salmonella* in foods are not permitted and their presence in foods pose a significant risk for public health<sup>16,17</sup>. Animal carcasses and their offal are unavoidably contaminated during evisceration from the intestinal tracts of the slaughtered animals and the extent of microbial contamination of the meat and meat products also depend on a number of other factors such as the level of implementation of hygienic standards during slaughter, dressing, transport, storage and further processing, and other environmental<sup>18</sup>. The prevalence of *Salmonella* sp. in various types of meat and meat products was found to be very common in studies conducted in different countries<sup>4,11,18-20</sup>.

**Table 1.** Prevalence of *Salmonella* sp. in edible bovine offal**Tablo 1.** Yenebilir sığır sakatatlarında *Salmonella* sp. varlığı

Abattoirs	Liver	Kidney	Rumen	Brain	Total
A1	1	-	-	-	
A2	1	1	-	-	
A3	-	1	1	-	
A4	-	-	1	-	
A5	1	-	-	-	
<b>Total A</b>	<b>%8.57 (35/3)</b>	<b>%5.71 (35/2)</b>	<b>%5.71 (35/ 2)</b>	-	<b>%6,66 (105/ 7)</b>
<b>Butcheries</b>					
B1	-	-	-	-	
B2	1	-	-	-	
B3	1	-	-	2	
B4	1	1	-	-	
B5	1	1	2	-	
<b>Total B</b>	<b>%16 (25/4)</b>	<b>%4 (25/2)</b>	<b>%4 (25/2)</b>	<b>%4 (25/2)</b>	<b>%10 (100/10)</b>
<b>Total (A+B)</b>	<b>%11.66 (60/7)</b>	<b>% 6.66 (60/4)</b>	<b>%6.66 (60/4)</b>	<b>%4 (25/2)</b>	<b>% 8.29 (205/17)</b>

A: Abattoir Sample; B: Retail Butcheries Sample

as well as in Turkey<sup>21,22</sup>. The incidence of *Salmonella* sp. in edible offal was also investigated by many researchers. Samuel et al.<sup>23</sup> detected 32% of the liver samples collected from the abattoirs to be positive for this organism. The level of *Salmonella* contamination was reported to be 68.9% and 28.9% in bovine lung and tripe samples, respectively, in Germany<sup>24</sup>. Fifty-three percent of the calf liver were found positive for *Salmonella* by Van Klink et al.<sup>25</sup>. The level of contamination was 6.1, 3.1 and 23.6% in bovine, sheep and pig offal samples, respectively, in a study conducted by Little et al.<sup>11</sup>. In a study performed by Ulutürk<sup>26</sup>, in Turkey, none of the liver and tripe samples collected from abattoirs were determined to be positive, however, the contamination level of *Salmonella* was 33.3% in the liver and tripe samples obtained from the local markets. Keven and Ay<sup>27</sup>, also reported the same findings from Turkey that none of the liver, tripe and brain samples from abattoirs were found positive, however, 2.2%, 2.1% and 3.3% of the liver, tripe and brain samples, respectively, obtained from markets were positive and 3.3%, 2.2% and 7.7% of the liver, tripe and brain samples obtained from local butcherries were positive for *Salmonella* sp. The recovery rates of *Salmonella* sp. were 10% for the liver and tripe samples and 20% for the brain samples obtained from local butcherries in another study performed in Turkey<sup>28</sup>.

The highest level of contamination of *Salmonella* sp. was reported from the brain samples in some studies conducted by Ulutürk<sup>26</sup>, Keven ve Ay<sup>27</sup> and Oflaz<sup>28</sup> and from the lung samples by Sinell et al.<sup>24</sup> and from the liver samples by Samuel et al.<sup>23</sup> and Van Klink et al.<sup>25</sup>. The highest recovery rate of *Salmonella* sp. was from the liver samples obtained both from the abattoirs (8.57%) and butcherries (16%) in the current study.

In the present study, the prevalence of *Salmonella* sp. was determined to be slightly higher in the samples collected from the local butcherries (10% versus 6.66%) than that of the samples from abattoirs. The presence of *Salmonella* sp. in the samples from two places indicates that there are multiple sources of contamination that cross-contaminate the offal during handling and processing post-slaughter and also reflects the intestinal bacterial load of slaughtered animals and hygienic standards of the premises. The level of microbial contamination at abattoirs varies depending on the application of quality control systems such as HACCP (Hazard Analysis and Critical Control Point) and GMP (Good Manufacturing Practice). In a study conducted at five different abattoirs in the same area, the prevalence of *Salmonella* was 11.4, 16.0 and 3.4 % in samples taken from abattoir workers, equipments and abattoir environment, respectively<sup>27</sup>. A relatively lower level of contamination encountered in the current investigation implies better application of hygienic procedures from slaughter to retail trade until it reaches to consumer in this area. In another study undertaken by Akkaya et al.<sup>29,30</sup> in Afyonkarahisar province, processes such as slaughter, cooling and transport play important roles on the cross contamination of cattle carcasses. Studies performed on butcher personnel and their equipments revealed that hygiene principles are not followed properly by the personnel and the equipments used by those people have important effect on cross contamination of meat, which pose a significant risk for human health<sup>31,32</sup>.

In conclusion, it is essential that HACCP systems and Good Manufacturing Practice (GMP) involving the whole processes from farm to table should be introduced and properly implemented in order to control *Salmonella*

infections of humans that result from edible offal. In this regard, control strategies such as prevention of contamination of animal feed and abattoir environment by *Salmonella*, strict implementation of antemortem and postmortem analyses in abattoirs and application of proper hygienic conditions by personnel working in meat industry and cleaning/disinfection of the equipments and machines used for processing are important to minimize and/or prevent infections caused by these pathogenic microorganisms.

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