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RESEARCH ARTICLE

Seasonal Prevalence of *Escherichia coli* O157:H7, *Listeria* spp. and *Salmonella* spp. in Fermented Sausages, Sosis and Salami in Eastern Turkey

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ABSTRACT

The goal of our study was to characterize the proportions and seasonal prevalence of *Escherichia coli (E. coli)* O157:H7, *Listeria* spp. and *Salmonella* spp. in fermented sausages, sosis and salami. A total of 192 samples consisting of sausage (n=96), sosis (n=48) and salami (n=48) purchased from different butcher shops and markets in the Elazig Province of eastern Turkey for one year duration were processed and tested by culture and polymerase chain reaction (PCR) for the presence of *E. coli* O157:H7, *Listeria* spp. and *Salmonella* spp. *L. innocua* was found to be the most predominant *Listeria* spp. in fermented sausages and sosis whereas, *L. monocytogenes* (1.04%) and *Salmonella* spp. (1.04%) were detected at much lower prevalence in fermented sausage. No *L. monocytogenes* and *Salmonella* spp. were determined in salami samples. In addition, no *Salmonella* spp. was detected in sosis samples. *L. welshimeri* (12.5%) was observed most frequently on salami samples. No *E. coli* O157:H7 was isolated in any samples of fermented sausage, sosis and salami. Seasonal prevalence of *Listeria* spp. in fermented sausages, sosis and salami was highest in July, with no *L. monocytogenes* and *Salmonella* spp. being isolated during autumn, winter and spring months of the year. Although the proportion of *L. monocytogenes* in fermented sausages and sosis is much lower in the current study, the presence of *L. monocytogenes* in these samples in the Elazig Province of eastern Turkey could still present a major public health threat.

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INTRODUCTION

The most important foodborne pathogens such as *Salmonella, Listeria monocytogenes (L. monocytogenes)* and *Escherichia coli (E. coli)* O157:H7 have been observed in fermented sausages, sosis and salami. Although a coordinated effort by meat industry has had an appreciable reduction in the colonization of *L. monocytogenes* and *Salmonella* spp. in fermented sausage found in federally inspected plants, the United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) demonstrated that the prevalence of both bacteria in fermented meats was found approximately 1.4% and 3.3%, respectively (Porto-Fett *et al.,* 2010). Three thousand four hundred forty-five samples of dry and semi-dry sausages were indicated to be negative for *E. coli* O157:H7 from 1995 to 1999 (Porto-Fett *et al.,* 2010). In many countries, fermented sausages have been consumed in moderate rates, while the number of annual global infections per 100.000 people have calculated to be only 0.0000055 (Meloni, 2015; Huang *et al*., 2016).

Although the presence of *E. coli* O157:H7, *Listeria* spp. and *Salmonella* spp. in fermented sausages, sosis and salami is known in Turkey, there is little data regarding the presence of *E. coli* O157:H7, *Listeria* spp. and *Salmonella* spp. in sausages and no data on sosis and salami in Elazig. The goal of the current study was to analyze the presence and seasonal prevalence of *E. coli* O157:H7, *Listeria* spp. and *Salmonella* spp. in fermented sausages, sosis and salami purchased in Elazig Province in eastern Turkey.

MATERIALS AND METHODS

Samples: One hundred ninety-two samples comprised 96 fermented sausages, 48 sosis and 48 salami were purchased at different butcher shops and markets in Elazig in Turkey for one year duration. Samples were aseptically collected and instantly transported to the laboratory in a cooler that has been filled with ice.

Detection of *Listeria* species: *Listeria* isolation protocol was carried out according to USDA/FSIS (Anon, 2005). Presumptive colonies were plated onto blood agar with 5% horse blood (Oxoid, UK) and incubated for 24 h at 35°C, after which were screened for beta-hemolysis. Characteristic colonies were then streaked onto Tryptic Soy Agar-Yeast Extract (Difco) and confirmed biochemically through Gram staining, catalase activity, motility at 25°C for the typical umbrella shape, carbohydrate fermentation (mannitol, xylose, dextrose and rhamnose), nitrate reduction and beta-hemolytic activity (Yucel *et al.*, 2005).

Salmonella isolation: For the determination of *Salmonella* spp. from fermented sausages, sosis and salami, the method described by ISO 6579 was used, recognizing a few minor modifications (ISO 6579, 2002). Twenty-five g of samples were inoculated to 225 mL buffered peptone water (BPW, Oxoid, Basingstoke, UK), homogenized for 2 min, and then, incubated for 24 h at 37ºC. After incubation, 10 ml Rappaport Vassiliadis (RV, Oxoid, USA) was inoculated with 100 µl of BPW enrichment and incubated for 48 h at 42°C. RV enrichments were plated onto Xylose Lysine Deoxycholate (XLD) agar and incubated for 18 to 24 h at 37°C. Presumptive colonies were identified by the following biochemical tests: L-lysine decarboxylase, Urea agar (Christensen), β-galactosidase (ONPG), TSI agar, Voges Proskauer and Indole tests vs. (ISO 6579, 2002).

***E. coli* O157:H7 isolation:** For isolation of *E. coli* O157, 25 g of samples were supplemented to 225 mL of modified Tryptone Soya Broth (mTSB) (Oxoid, Basingstoke, UK), homogenized for 2 min and incubated at 37ºC for 24 h (Dontorou *et al.*, 2003). Modified Tryptone Soya Broth enrichments were plated on Sorbitol MacConkey Agar (Oxoid, Basingstoke, UK) added with Cefixime Tellurite Selective Supplement (Oxoid, Basingstoke, UK) and incubated at 37ºC for 18-24 h (Buyukunal *et al.*, 2016). Then, the colourless colonies were investigated by *E. coli* O157 latex kit (Oxoid, Basingstoke, UK) (Hitchins *et al.*, 2000). The purified colonies were morphologically identified by Gram stain and biochemical tests. Confirmed isolates were grown in 1 ml of the Brain Heart Infusion (BHI) overnight at 37ºC. Buffered glycerol (300 µl) was added to overnight BHI enrichments then stored at -80ºC.

DNA isolation and PCR: The isolation of genomic DNA from *L. monocytogenes* and *Salmonella* spp. isolates was conducted on using the QIAamp DNA mini kit (Qiagen, Hidelberg, Germany) as instructed by the manufacturer. Pathogen confirmation was based on the DNA sequence of the listeriolysin O and 16S rRNA genes, which are specific for *L. monocytogenes* and *Salmonella* spp., respectively (Border *et al.*, 1990; Lin and Tsen, 1996). PCR products of 701 bp and 572 bp were apparent with all *L. monocytogenes* isolates and *Salmonella* spp. isolates tested, respectively. The DNAs of *L. monocytogenes* and *Salmonella* spp. previously recovered from our studies and distilled water were utilized as positive and negative controls, respectively (Ozbey *et al.*, 2007; Ozbey *et al.*, 2013).

RESULTS

L. innocua was determined to be the most predominant *Listeria* spp. in fermented sausages and sosis while *L. monocytogenes* (1.04%) and *Salmonella* spp. (1.04%) were observed but at a much lower prevalence in fermented sausage. No *L. monocytogenes* and *Salmonella* spp. were determined from salami. *L. welshimeri* (12.5%) was found most frequently on salami samples (Table 1). We found that no *E. coli* O157:H7 was isolated in any samples of fermented sausage, sosis and salami. Positive *Salmonella* spp. and *L. monocytogenes* isolates as biochemically also tested positive by PCR.

The tables 2, 3 and 4 show monthly changes in the percentage of *Listeria* isolated from fermented sausage, salami and sosis samples. The most frequent isolation of *Listeria* spp. in fermented sausages was represented in July-September (25%), followed by April-June (12.5%) and October-December (4.17%). No *Listeria* spp. was detected in January-March. The isolation of *Salmonella* spp. in fermented sausages was represented in only July-September (4.17%), while no *Salmonella* spp. being isolated in other months (Table 2). The highest isolation rates of *Listeria* spp. in salami and sosis samples were also observed in July-September (25%).

DISCUSSION

Salmonella spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* are foodborne pathogens of global significance. Although the presence of *Salmonella* and *Listeria* spp. in fermented sausage, sosis and salami is known, knowledge regarding this subject in eastern Turkey is limited. Studies performed in the different countries of the world have indicated various prevalence of *L. monocytogenes* in the fermented sausage samples including 20.0% in Canada (Farber *et al.*, 1989), 15.0% in Switzerland (Jemmi *et al.*, 2002) and 11.6% in Turkey (Colak *et al.*, 2007). The prevalence (38.9%) of *L. monocytogenes* positive fresh sausages was detected at a higher percentage than that of fermented sausages (15.2%) (de Cesara *et al.*, 2007). A lower prevalence of *L. monocytogenes* (3.7% in 27 Spanish fermented sausages) was reported by Mena *et al.* (2004). Our study indicated similarity with the above mentioned result.

This study also indicated that *L. innocua* is the predominant species in fermented sausages in Elazig. This finding agrees with some studies performed in Turkey and some countries (Paziak-Domanska *et al.*, 1999; Sancak *et al.*, 2007). In contrast, another author have showed that *L. monocytogenes* in sausages was detected at a higher percentage (Berktas *et al.*, 2006).

Table 1: Isolation and identification of *Listeria* spp. by sample type

Type of sample	Number of samples	*Listeria* spp. n (%)	*L. monocytogenes* n (%)	*L. seeligeri* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)
Fermented Sausage	96	10 (10.4)	1 (1.04)	-	8 (8.3)	-	1 (1.04)
Salami	48	6 (12.5)	-	-	-	-	6 (12.5)
Sosis	48	6 (12.5)	1 (2.08)	-	3 (6.25)	1 (2.08)	1 (2.08)
Total	196	22 (11.2)	2 (1.02)	-	11 (5.6)	1 (0.5)	8 (4.08)

Table 2: Seasonal variations in the prevalence of *Listeria* spp. and *Salmonella* spp. isolates in fermented sausage samples

Months	Number of samples	*Listeria* spp.		*Salmonella* spp.	
		n	%	n	%
July-September	24	6	25	1	4.17
October-December	24	1	4.17	-	-
January-March	24	-	-	-	-
April-June	24	3	12.5	-	-
Total	96	10	10.4	1	1.04

Table 3: Seasonal variations in the prevalence of *Listeria* spp. isolates in salami samples

Months	Number of samples	*Listeria* spp.	
		n	%
July-September	12	3	25
October-December	12	1	8.3
January-March	12	-	-
April-June	12	2	16.7
Total	48	6	12.5

Table 4: Seasonal variations in the prevalence of *Listeria* spp. isolates in sosis samples

Months	Number of samples	*Listeria* spp.	
		n	%
July-September	12	3	25
October-December	12	1	8.3
January-March	12	1	8.3
April-June	12	1	8.3
Total	48	6	12.5

Studies related to the presence of *Salmonella* spp. in sausage have been reported in several research trials in Turkey. The prevalence of *Salmonella* spp. in the fermented sausage include 7% in Aydin (Ozbey *et al.*, 2007) and 1.52% in Istanbul, Adapazari, Afyon and Kayseri (Buyukunal *et al.*, 2016). In other studies, no *Salmonella* spp. was detected (Sancak *et al.*, 2007; Yoruk, 2012). Our findings (1.04%) were consistent with Buyukunal`s results (1.52%). The reasons of different detection rates may be due to varying isolation methods, sampling procedures and varying hygiene applications (Buyukunal *et al.*, 2016).

This study also agrees with other studies performed in Portugal and Turkey which indicated that *E. coli* O157:H7 in sausage was not isolated by Ferreira *et al.* (2007), Yoruk (2012) and Buyukunal *et al.* (2016). Oteiza *et al.* (2006) emphasized that *E. coli* O157:H7 was determined from 2% of morcillas, a typical Argentine sausage.

In studies involving sosis samples, *L. monocytogenes* prevalence has been determined to be 27.3% by Berktas *et al.* (2006) and 4.16% by Yoruk (2012). We found that the isolation rate (2.08%) of *L. monocytogenes* was lower than those to above mentioned studies but higher than 1.3% by Dontorou *et al.* (2003). Our result is in agreement with a previous data (Yoruk, 2012) that found no *Salmonella* spp. in sosis. *E. coli* O157:H7 prevalence in Turkey was determined to be 4.16% in sosis samples (Yoruk, 2012). Consistent with a previous study by Balpetek and Gurbuz (2010), *E. coli* O157:H7 was not observed in the current study.

It needs to be set dot after a study conducted in Brazil also showed that the prevalence of *Listeria* spp. and *L. monocytogenes* in salami was 24.6% (32/130) and 6.2% (8/130), respectively (Martins and Germano, 2011). Studies related to *L. monocytogenes* and *Salmonella* prevalence in salami in Turkey reported various results. *L. monocytogenes* prevalence has been represented to be 10% in non-packaged salami by Sancak *et al.* (2007) and 8.33% by Yoruk (2012). Correlate with a previous study by Yoruk (2012), *E. coli* O157:H7 and *Salmonella* spp. was not observed in salami in the present study. Also, our results match previous finding by Berktas *et al.* (2006) who detected no *L. monocytogenes* in salami. This may be due to a longer cooking process required for salami (Sancak *et al.*, 2007). Because salami is thicker, they are cooked for 1-3 h in boilers containing 80°C hot water (Sancak *et al.*, 2007).

The present study found that *L. welshimeri* is the predominant species in salami samples. Pellicer *et al.* (2002) identified 1 strain (2%) as *L. monocytogenes* type 1, 7 strains (14%) as *L. innocua* and 2 strains (4%) as *L. welshimeri*. This study agrees with a study that reported *L. monocytogenes* and *L. innocua* were not isolated in salami while *L. welshimeri* (8%) was detected from salami samples (Bozkurt, 2003). Another study showed that *L. innocua* (10%) and *L. monocytogenes* (10%) were isolated from vacumme packaged salami and non-packaged salami, respectively (Sancak *et al.*, 2007). This study also agrees with a Turkish study which demonstrated that *E. coli* O157:H7 in salami was not isolated by Alisarli and Akman (2004).

In the current study, the highest isolation rates of *Salmonella* spp. (1.04%) and *L. monocytogenes* (1.04%) from fermented sausages were observed in July, with no *L. monocytogenes* and *Salmonella* spp. being isolated during autumn, winter and spring months of the year. Temperature increase is the most critical factor for microbial growth (Buyukunal *et al.*, 2016), thus explaining a study that reports an increase in the prevalence of *Salmonella* spp. (80%) and *L. monocytogenes* (75%) from fermented sausages in June, July and August (Buyukunal *et al.*, 2016). However, Colak *et al.* (2007) reported a decrease in the isolation of *L. monocytogenes* in June-July months. This may be due to hygienic conditions in applications such as slicing, packaging after temperature-time arrangements and heat treatment applied in the production of other foods with salami and sosis (Yoruk, 2012).

Conclusions: This study suggest there is a significant health threat from consumption of raw or under-cooked sausage and sosis. Although the prevalence of *L. monocytogenes* in fermented sausages and sosis is much lower in the current study, the presence of *L. monocytogenes* in these samples in Elazig Province in

eastern Turkey is not acceptable according to Turkish Food Codex (TFC, 2006). Further studies are required to clarify the relationship between the seasonal prevalence and infection in different regions in order to address the epidemiological importance of *L. monocytogenes* in rural populations. This may allow consumers to be aware of these gaps in knowledge and may open new avenues for prevention strategies.

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Authors contribution: OG conceived and designed the study, collected samples, analyzed the data and wrote the manuscript; OG and KF executed the experiment and analyzed the samples, OU performed DNA extraction and PCR and edited the manuscript; All authors interpreted the data, critically revised the manuscript and approved the final version.

REFERENCES

Alisarli M and Akman HN, 2004. Investigation of *Escherichia coli* O157 in retail minced meat. YYÜ Vet Fak Derg 15:65-9.

Anon, 2005. Listeriosis. Center for Food Security and Public Health. College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011. Available at: http://www.cfsph.iastate.edu/Factsheets/pdfs/listeriosis.pdf. (Assessed on 20 November, 2006).

Balpetek D and Gurbuz U, 2010. Investigations on the presence of *E. coli* O157:H7 in some meat products. Eurasian J Vet Sci 26:25-31.

Berktas M, Bozkurt EN, Bozkurt H, *et al.,* 2006. Isolation of *Listeria* species in meat and meat products. Van Tıp Derg 13:36-41.

Border PM, Howard JJ, Plastow GS, *et al*., 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett Appl Microbiol 11:158-62.

Bozkurt ENN, 2003. Et ve et ürünlerinden *Listeria monocytogenes*' in izolasyonu. Y.Y.Ü. Sağlık Bil Enst, PhD thesis, pp: 59.

Buyukunal SK, Sakar FS, Turhan I, *et al.,* 2016. Presence of *Salmonella* spp., *Listeria monocytogenes, Escherichia coli* O157 and nitrate-nitrite residue levels in Turkish traditional fermented meat products (sucuk and pastırma). Kafkas Univ Vet Fak Derg 22:233-6.

Colak H, Hampikyan H, Ulusoy B, *et al*., 2007. Presence of *Listeria monocytogenes* in Turkish style fermented sausage (sucuk). Food Cont 18:30-2.

de Cesare A, Mioni R and Manfreda G, 2007. Prevalence of *Listeria monocytogenes* in fresh and fermented Italian sausages and ribotyping of contaminating strains. Int J Food Microbiol 120:124-30.

Dontorou C, Papadopoulou C, Filioussis G, *et al.,* 2003. Isolation of *Escherichia coli* O157:H7 from foods in Greece. Int J Food Microbiol 82:273-9.

Farber JM, Sanders GW and Johnston MA, 1989. A survey of various foods for the presence of *Listeria* species. J Food Prot 52:456-8.

Ferreira V, Barbosa J, Silva J, *et al.,* 2007. Characterisation of alheiras, traditional sausages produced in the North of Portugal, with respect to their microbiological safety. Food Cont 18:436-40.

Hitchins AD, Feng P, Watkins WD, *et al.,* 2000. *Escherichia coli* and the coliform bacteria. In, Food and Drug Administration. 8th ed., pp:401-429, AOAC International, Washington DC.

Huang YS, Wu YC, Hu CW, *et al*., 2016. Isolation and characterization of *Salmonella* spp. in sheltered wild birds in Taiwan. Pak Vet J 36:472-6.

International Organization for Standardization (ISO) 6579, 2002. Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella* spp.

Jemmi T, Pak S and Salman MD, 2002. Prevalence and risk factors for contamination with *L. monocytogenes* of imported and exported meat and fish products in Switzerland, 1992-2000. Prev Vet Med 54:25-36.

Lin CK and Tsen HY, 1996. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. J Appl Bacteriol 80:659-66.

Martins EA and Leal Germano PM, 2011. *Listeria monocytogenes* in ready-to-eat, sliced, cooked ham and salami products, marketed in the city of São Paulo, Brazil: Occurrence, quantification, and serotyping. Food Cont 22:297-302.

Meloni D, 2015. Presence of *Listeria monocytogenes* in Mediterranean-style dry fermented sausages. Foods 4:34-50.

Mena C, Almeida G, Carneiro L, *et al.,* 2004. Incidence of *Listeria monocytogenes* in different food products commercializedin Portugal. Food Microbiol 21:213-6.

Oteiza JM, Chinen I, Miliwebsky E, *et al*., 2006. Isolation and characterization of Shiga toxin-producing *Escherichia coli* from precooked sausages (morcillas). Food Microbiol 23:283-8.

Ozbey G, Kok F and Muz A, 2007. Isolation of *Salmonella* spp. in camel sausages from retail markets in Aydin, Turkey, and polymerase chain reaction (PCR) confirmation. Turk J Vet Anim Sci 31:67-71.

Ozbey G, Icyeroglu A and Muz A, 2013. Prevalence of *Listeria* species in raw hamburger meatballs and chicken burgers in eastern Turkey. Afr J Microbiol Res 7:4055-8.

Paziak-Domanska B, Boguslawska E, Wieckowska-Szakiel M, *et al.,* 1999. Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS Microbiol Lett 171: 209-14.

Pellicer K, Copes J, Malvestiti L, *et al.,* 2002. Isolation and identification of *Listeria monocytogenes* and *Listeria* spp. in dry sausages obtained in markets in the city of La Plata, Argentina. Rev Argent Microbiol 34:219-21.

Porto-Fett AC, Call JE, Shoyer BE, *et al.,* 2010. Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes, Escherichia coli* O157:H7*, Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. Int J Food Microbiol 140:61-75.

Sancak YC, Isleyici O and Sagun E, 2007. Presence of *Listeria monocytogenes* in some meat products consumed in Van. YYU Vet Fak Derg 18:93-9.

Turkish Food Codex (TFC), 2006. Mikrobiyolojik kriterler tebligi. Resmi Gazete, Teblig No 2006/23. Ankara, Basbakanlik Press.

Yoruk NG, 2012. Investigation of frankfurter sausage, mortadella type cooked sausage, fermented dry sausage and hamburger patty for foodborne pathogens produced by meat plants having ISO food safety regulation. PhD Thesis, Selcuk University, Turkey.

Yucel N, Cıtak S and Onder M, 2005. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. Food Microbiol 2:25-31.