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Korrespondenzadresse:
tolgakah@istanbul.edu.tr

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey

Prevalence of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in meat and meat products in Turkey

Prävalenz von Salmonella spp., Escherichia coli O157:H7 und Listeria monocytogenes in Fleisch und Fleischerzeugnissen in der Türkei

Tolga Kahraman, Ali Aydin

Summary

The aim of the present study was to investigate the prevalence of *Salmonella* spp., *Escherichia* (*E.*) *coli* O157:H7 and *Listeria* (*L.*) *monocytogenes* in 691 samples of raw meat (100 raw beef, 100 raw sheep, 175 raw poultry, 178 ground beef) and meat products (138 sausages). All foods were obtained from producers and retailers in six provinces (Bursa, Balikesir, Canakkale, Bilecik, Sakarya and Yalova) of the southern Marmara region of Turkey. *Salmonella* spp. and *L. monocytogenes* were detected in 4.6% and 5.5% of the samples, respectively. *E. coli* O157:H7 was not detected in any meat and meat product samples. The results indicate that meat and meat products present a potential hazard for public health and essential precautions should be taken to ensure improving the quality of production technology.

Keywords: raw meat, meat products, *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*

Zusammenfassung

Ziel der vorliegenden Studie war, die Prävalenz von *Salmonella* spp., *Escherichia* (*E.*) *coli* O157:H7 und *Listeria* (*L.*) *monocytogenes* in 691 Proben von rohem Fleisch (100 rohes Rindfleisch, 100 rohes Schaffleisch, 175 rohes Geflügelfleisch und 178 Rinderhackfleisch) und Fleischprodukten (138 Würste) zu untersuchen. Alle Proben wurden entweder in den Herstellungsbetrieben oder im Einzelhandel in sechs Provinzen (Bursa, Balikesir, Canakkale, Bilecik, Sakarya und Yalova) der südlichen Marmara-Region der Türkei genommen. *Salmonella* spp. und *L. monocytogenes* wurden in 4,6 % bzw. 5,5 % der Proben festgestellt. *E. coli* O157:H7 wurde in keiner der Proben nachgewiesen. Die Ergebnisse zeigen, dass Fleisch und Fleischprodukte eine mögliche Gefahr für das öffentliche Gesundheitswesen darstellen und wesentliche Vorsichtsmaßnahmen getroffen werden sollten, um eine Verbesserung der Qualität der Produktionstechnologie sicherzustellen.

Schlüsselwörter: Frischfleisch, Fleischerzeugnisse, *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*

Introduction

Foodborne diseases represent a major public health concern worldwide in terms of human illness and economic loss resulting from the consumption of contaminated food. Each year millions of cases occur and these infections can cause mild illness, severe infections or serious complications including death (Notermans et al., 1995). Various food can serve as source of foodborne illness; meat and meat products are important sources of human infections with *Salmonella* spp., *Escherichia* (*E.*) *coli* O157:H7 and *Listeria* (*L.*) *monocytogenes*. All these foodborne pathogens can be present in the gastrointestinal tract of food-producing animals. The most frequent chain of events leading to meat borne illness involves food animals as healthy carriers of the pathogens; these organisms are subsequently transferred to humans through production, handling and consumption of meat and meat products (Norrung and Buncic, 2008).

Salmonella is one of the most important pathogenic genera implicated in foodborne bacterial outbreaks that include nausea, vomiting, septicemia and diarrhea. Beef and chicken are the foremost foods causing salmonellosis (Qiongzhen et al., 2004). *E. coli* O157:H7 is a neurotoxin producing enterohemorrhagic *E. coli*. It rarely produces clinical disease in animals but it is associated with hemolytic uremic syndrome, thrombocytic thrombocytopenic purpura and hemorrhagic colitis in humans. Cattle are one reservoir of the pathogen and consumption of meat of bovine origin is considered to be the main cause

of several outbreaks (Murphy et al., 2004; Dontorou et al., 2003). *L. monocytogenes* is an ubiquitous gram positive, intracellular bacterium with a high prevalence that may cause abortion in pregnant women and meningitis in newborns. Listeriosis in immuno-compromised may lead to septicemia, meningitis and central nervous system infections. It is a psychrotrophic microorganism that is capable of growing at refrigeration temperatures and at relatively low pH values. These characteristics make *L. monocytogenes* particularly difficult to control in food, therefore contamination could lead to this microorganism representing a high risk factor (Vitas and Garcia-Jalon, 2004; Farber et al., 1989).

There have been some reports on the prevalence of *Salmonella* spp. in Spain (Capita et al., 2003), in Northern Ireland (Madden et al., 2001), in England (Jorgensen et al., 2002) and in the USA (Cason et al., 1997); the prevalence of *E. coli* O157:H7 has for example been studied in Greece (Dontorou et al., 2003), in Italy (Conedera et al., 2004), in Scotland (Coia et al., 2001) and in Argentina (Oteiza et al., 2006) and that of *L. monocytogenes* in Italy (Cesare et al., 2007), in Portugal (Mena et al., 2004) and in Greece (Samelis and Metaxopoulos, 1999). In Turkey, the prevalence of these pathogens in meat and meat products has been determined by Aydin et al. (2006), Siriken et al. (2006), Cetinkaya et al. (2008) and Goncagul et al. (2005). Yet, the prevalence of these microorganisms in the southern Marmara region of Turkey has not been extensively investigated.

The present study was therefore undertaken to determine the presence of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* in meat and meat products obtained from retail markets and producers in the southern Marmara region (Bursa, Balikesir, Canakkale, Bilecik, Sakarya and Yalova), which has approximately an area of 45 200 km² (5.76% of the Turkish national territory) and a population of 4.7 million people. Furthermore, the Marmara region has a geographical importance due to its location representing a transit corridor between Europe and Asia.

Material and Methods

Sample Collection

Meat and meat products were collected at intervals between March 2007 and February 2008. A total of 691 meat (100 raw beef, 100 raw sheep, 175 raw poultry and 178 ground beef) and meat product (138 sausages) samples were investigated for the presence of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes*. The samples were obtained from producers and retailers in the southern Marmara region of Turkey (Fig. 1). A brief description of all the food types is given in Table 1. All samples were collected in sterile jars and immediately transferred to the laboratory in cold boxes at +4°C.

TABLE 1: *Prevalence of L. monocytogenes and Salmonella spp. contamination in various meat samples*

Provinces	Type of meat	Number of samples	*Salmonella* spp.	*L. monocytogenes*
Yalova	Ground beef	30	1 (3.3%)	3 (10%)
	Raw sheep meat	15	0	0
	Raw beef meat	15	0	0
	Raw poultry meat	20	0	0
	Sausage	30	3 (10%)	3 (10%)
	Total	110	4 (3.6%)	6 (5.5%)
Canakkale	Ground beef	30	2 (6.6%)	4 (13.3%)
	Raw sheep meat	20	1 (5.0%)	0
	Raw beef meat	20	1 (5.0%)	1 (5.0%)
	Raw poultry meat	20	2 (10%)	1 (5.0%)
	Sausage	30	6 (20%)	3 (10%)
	Total	120	12 (10%)	9 (7.5%)
Bursa	Ground beef	30	1 (3.3%)	3 (10%)
	Raw sheep meat	20	0	0
	Raw beef meat	20	0	0
	Raw poultry meat	38	1 (2.6%)	1 (2.6%)
	Sausage	30	2 (6.6%)	2 (6.6%)
	Total	138	4 (2.8%)	6 (4.3%)
Bilecik	Ground beef	30	0	2 (6.6%)
	Raw sheep meat	15	0	0
	Raw beef meat	15	0	0
	Raw poultry meat	20	0	1 (5.0%)
	Sausage	30	2 (6.6%)	1 (3.3%)
	Total	110	2 (1.8%)	4 (3.6%)
Sakarya	Ground beef	30	1 (3.3%)	4 (13.3%)
	Raw sheep meat	15	0	1 (6.6%)
	Raw beef meat	15	0	0
	Raw poultry meat	20	1 (5.0%)	0
	Sausage	30	3 (10%)	3 (10%)
	Total	110	5 (4.5%)	8 (7.2%)
Balikesir	Ground beef	28	1 (3.6%)	3 (10.7%)
	Raw sheep meat	15	1 (6.6%)	0
	Raw beef meat	15	0	0
	Raw poultry meat	20	0	0
	Sausage	25	3 (12%)	2 (8.0%)
	Total	103	5 (4.8%)	5 (4.8%)
General region	Ground beef	178	6 (3.3%)	19 (10.7%)
	Raw sheep meat	100	2 (2.0%)	1 (1.0%)
	Raw beef meat	100	1 (1.0%)	1 (1.0%)
	Raw poultry meat	138	4 (2.9%)	3 (2.1%)
	Sausage	175	19 (10.9%)	14 (8.0%)
	Total	691	32 (4.6%)	38 (5.5%)

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Microbiological Analysis

The isolation of *Salmonella* spp. started with pre-enrichment by suspending 25 g of sample in 225 ml buffered peptone water (BPW; Oxoid, Basingstoke, UK) followed by incubation at 37°C for 16–20 h. Next, 0.1 ml of the over-night enrichment was transferred to Rappaport-Vassiliadis (RV; Oxoid) and Muller Kaufmann Tetrathionate Broth (MKTTn; Oxoid). MKTTn and RV broths were incubated for 24 h at 42°C. After incubation, samples were streaked on Hectoen Enteric Agar (HE; Oxoid) and XLD Agar (Oxoid), incubated for 24 h at 35°C. The typical colonies were identified by Triple Sugar Iron Agar (TSI; Oxoid), Lysin Iron Agar (LIA; Oxoid), urease test (Oxoid) and confirmed with *Salmonella* antiserum (O and H-Vi polyvalant antiserum) according to Harrigan (1998).

For detection of *E. coli* O157:H7, each sample was examined by combining 25 g of sample material with 225 ml of modified Tryptone Soy Broth with novobiocin (m-TSB; Oxoid) in a stomacher bag, homogenizing for at least 2 min in a stomacher and incubating at 37°C for 24 h. The enriched culture was streaked onto Sorbitol MacConkey Agar (Oxoid) supplemented with Cefixime Tellurite Selective Supplement (CT; Oxoid) and the plates were incubated at 37°C for 18–24 h. Following the incubation period, the colorless colonies were tested using the *E. coli* O157 latex kit (Oxoid) as described by Hitchins et al. (2000).

For detection of *L. monocytogenes*, 25 g of the food samples were combined with 225 ml of *Listeria* Enrichment Broth (LEB; Oxoid) containing *Listeria* Selective Supplement (Oxoid). Samples were homogenised in a stomacher bag for 60 s at normal speed and were incubated at 32°C for 24 h. A 0.1 ml portion of the enrichment broth was streaked onto Chromogenic *Listeria* Agar (OCLA; Oxoid) supplemented with *Listeria* Selective Supplement (Oxoid) and *Listeria* Differential Supplement (Oxoid). After incubation, typical colonies were transferred to Tryptic Soy Agar with Yeast Extract (TSYE; Oxoid) and incubated for 24–48 h at 30°C. The typical colonies were verified by Gram's staining, catalase reaction, tumbling motility at 20–25°C, Methyl Red-Voges Proskauer (MR-VP) reactions, CAMP test, nitrate reduction and fermantation of sugars (Hitchins, 1995).

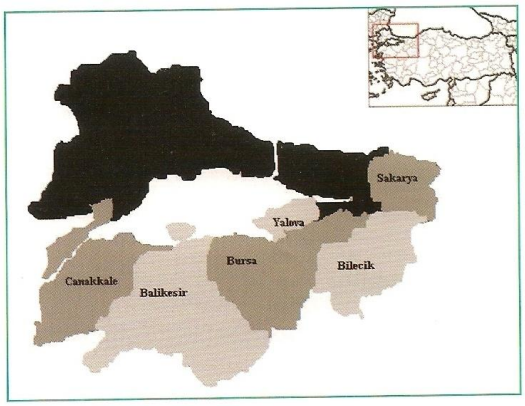


FIGURE 1: *Southern Marmara region of Turkey.*

Results and Discussion

A total of 691 raw meat and meat product samples were examined. *Salmonella* spp. and *L. monocytogenes* were detected in 4.6% and 5.5% of the samples, respectively (Tab. 1). All samples were negative for *E. coli* O157:H7. According to the Turkish Food Codex (TFC, 2006), the presence of *Salmonella* spp. in 25 g of raw meat and meat products, *E. coli* O157:H7 in 25 g of raw red meat and ground beef, *L. monocytogenes* in 25 g of meat products is not acceptable.

According to the results of this study, the highest rates of *Salmonella* spp. (10%) and *L. monocytogenes* (7.5%) were detected in Canakkale, where the highest temperature values in all seasons among the provinces have been reported. The lowest rates of *Salmonella* spp. (1.8%) and *L. monocytogenes* (3.6%) were determined in Bilecik, where the lowest temperature values in all seasons have been observed (Turkish State Meteorological Service, 2008). There were no certain differences between the other provinces concerning *Salmonella* spp. and *L. monocytogenes* detection levels. Temperature is being considered the most critical factor for the microbial quality of meat at the stage of manufacture, distribution and consumption. Microbial growth is seen mainly parallel with temperature increase (Aydin et al., 2006).

TABLE 2: *Prevalence of Salmonella spp. in meat and meat products in Turkey and other countries*

Meat	Number of samples	Incidence	Country	References
Ground beef	120	4 (3.3%)	Turkey	Erol (1997)
Ground beef	48	1 (2%)	Turkey	Gokalp et al. (1982)
Ground beef	27	3 (11.1%)	Turkey	Baskaya et al. (2004)
Ground beef	100	3 (3%)	Turkey	Gokmen and Alisarli (2003)
Beef	200	3 (1.5%)	N. Ireland	Madden et al. (2001)
Beef	240	0	Turkey	Aydin et al. (2006)
Chicken	315	27 (8.57%)	Turkey	Goncagül et al. (2005)
Chicken	288	52 (18%)	Brazil	Cortez et al. (2006)
Chicken	40	22 (55%)	Spain	Capita et al. (2003)
Chicken	99	19 (19%)	South Africa	Nierop et al. (2005)
Chicken	241	60 (25%)	England	Jorgensen et al. (2002)
Chicken	210	42 (20%)	USA	Cason et al. (1997)
Chicken	168	1 (0.5%)	Turkey	Cetinkaya et al. (2008)
Sheep	1117	0	Australia	Phillips et al. (2006)
Sheep	100	4 (4%)	Turkey	Kahraman et al. (2005)
Sausages	140	86 (61.5%)	Mexico	Escartin et al. (1999)
Sausages	120	31 (26%)	Africa	Mrema et al. (2006)
Sausages	336	82 (24.4%)	Brazil	Murmann et al. (2008)
Sausages	100	7 (7%)	Turkey	Siriken et al. (2006)

Ground beef, raw lamb, raw beef, poultry meat and sausage samples have been examined in several countries for the presence of *Salmonella* spp. (Tab. 2). Gokalp et al. (1982) collected 48 ground beef samples from Erzurum, Turkey and 2% were found to be positive for *Salmonella* spp. Gokmen and Alişarlı (2003) reported 3% occurence of *Salmonella* spp. in ground beef purchased from the retail outlets in Van, Turkey; whereas Erol (1997) reported a prevalence rate of 3.3% in 120 ground beef samples in Ankara, Turkey. Our findings were about similar to these results (6/178). Contrary to this, studies showing higher

results (11.1% and 15%) than ours were carried out by Baskaya et al. (2004) and El-Leithy and Rashad (1989).

In the present study, of the analyzed 100 raw sheep meat and 100 raw beef meat samples analyzed, 2% and 1%, respectively, were positive for *Salmonella* spp. In Northern Ireland, 1.5% of 200 (Madden et al., 2001) and in Australia 0.22% of 1063 (Vanderlinde et al., 1998) beef samples were contaminated with *Salmonella* spp. In another study in Turkey, 4% of the 100 sheep samples were detected positive (Kahraman et al., 2005). Overall, a major survey for *Salmonella* spp. on 1117 sheep samples found none (Phillips et al., 2006).

In relation to sausages, a study performed in Brazil reported *Salmonella* spp. in 24.4% of the sausages examined (Murmann et al., 2008). In Africa, 26% of 120 sausages (Mrema et al., 2006) and in Mexico, 61.5% of 140 sausages (Escartin et al., 1999) were tested positive for *Salmonella* spp. In Turkey, Siriken et al. (2006) reported seven positive samples out of 100 sausages. These frequencies were higher than that described in our study (2.9%, 4/138). The authors mentioned above explained the high *Salmonella* spp. prevalence with different hygiene levels in slaughterhouses and meat markets.

The present study demonstrated that *Salmonella* spp. was isolated from 10.9% of the poultry samples. Studies concerning chicken, the detection rates of which were higher than the *Salmonella* spp. occurrence detected in this study, were reported from the USA, where 20% of 210 chicken samples (Cason et al., 1997), from England where 25% of 241 (Jorgensen et al., 2002), from South Africa where 19% of 99 (Nierop et al., 2005), from Spain where 55% of 40 (Capita et al., 2003), from Brazil where 11% of 288 samples (Cortez et al., 2006) were contaminated with *Salmonella* spp. In previous works done in Turkey, Cetinkaya et al. (2008) and Goncagül et al. (2005) reported occurrence rates for *Salmonella* of 0.5% and 8.57%, respectively. These were much lower than in this present study. Poultry products are frequently contaminated with *Salmonella* spp. and are consequently thought to be major sources of the pathogen in humans. Our findings in this study showed similarity with this idea as the frequency of isolation of *Salmonella* spp. from poultry samples was higher than that from the other raw meat and meat product samples.

In the present study, *E. coli* O157:H7 was not detected in any of the samples. Similar results in 100 lamb carcasses were stated by Kahraman et al. (2005) in Istanbul (northern area of the Marmara region). Also, *E. coli* O157:H7 was not detected in sausages (Ferreira et al., 2007), ground beef (Dontorou et al., 2003) and raw beef (Fantelli and Stephan, 2002). *E. coli* O157:H7 is present sporadically at very low levels together with very high levels of competitor organisms which is the reason why it is difficult to detect.

In contrary, Conedera et al. (2004) and Heuvelink et al. (1998) demonstrated that *E. coli* O157:H7 was isolated from 0.43% (4/931) and 1.1% (6/571) of ground beef samples, respectively. However, the isolation of *E. coli* O157:H7 was reported in 5.9% (26/431) (Chapman et al., 1996) and 0.21% (1/484) of lamb minced beef (Chapman et al., 2001). In the USA, 3.5% of ground beef samples, 1.5% of pork samples, 1.5% of poultry samples and 2.0% of lamb samples were contaminated with *E. coli* O157:H7 (Doyle and Schoeni, 1987). In Argentina, 4.8% of the 83 fresh sausages tested and 3.3% of the 30 dry sausages tested were positive for *E. coli* O157:H7 (Chinen et al., 2001).

The prevalence of *L. monocytogenes* in raw (red) meat and poultry tested in this study (1.0% and 8.0%) was lower in comparison to that detected by Vitas and Garcia-Jalon (2004) in Spain (34.9% and 36.1%) and Mena et al. (2004) in Portugal (17.7% and 60.0%). In Northern Ireland, 26% of 79 poultry samples were contaminated with *L. monocytogenes* (Lawrence and Gilmour, 1994). In a study that was conducted by Kalorey et al. (2005) in India, *L. monocytogenes* was detected in 8 out of 94 (8.5%) poultry meat samples. Our findings showed similarity with this result.

Regarding the contamination rates of ground beef and sausages, our results (10.7% and 2.1%) were in accordance with the study of Ciftcioglu (1992), who collected 100 ground beef and 100 sausage samples from Istanbul and found 11% and 2% of the samples, respectively, to be positive for *L. monocytogenes*. In Turkey, 22% (22/100) of beef minced meat and 11% (11/100) of lamb minced beef were contaminated with *L. monocytogenes* (Gokmen and Alisarh, 2003). In contrary, Farber et al. (1989) demonstrated that *L. monocytogenes* was isolated from 15 of 96 (15.63%) sausages. In another study, the prevalence of *L. monocytogenes* was found to be 73% and 76% in ground beef and sausages from the Van province of Turkey (Berktas et al., 2006). These differences may originate from different detection methods, sampling procedures, regions, geographical conditions, slaughtering techniques and sanitation applications.

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In conclusion, the results indicate that meat and meat products present a potential hazard for public health. Therefore, it is essential to ensure an improvement of the quality of production technologies for controlling food-borne pathogens and enhancing the safety of food.

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Address for correspondence:
Dr. Tolga Kahraman
Department of Food Hygiene and Technology
Faculty of Veterinary Medicine
Istanbul University
34320 Avcilar-Istanbul
Turkey
tolgakah@istanbul.edu.tr