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Investigation of the Presence of *Salmonella* spp. and *Listeria monocytogenes* in Bovine Origin Foods*

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Summary: This study was conducted to investigate the presence of *Listeria monocytogenes* and *Salmonella* spp. in 200 bovine origin meat, milk and their products (minced meat, meatball, Inegöl meatball, sausage, pasteurized milk, Tulum cheese, fresh soft cheese and cecil cheese). *Salmonella* were isolated from 66 (33%) of 200 samples. While 45 (22.5%) of which were obtained from meat origin samples (16 minced meat, 10 inegöl meatball, 16 meatball and 3 sausage) and 21 (10.5%) of which was detected in cheese samples (6 tulum and 15 fresh soft cheeses). *L. monocytogenes* was detected in a total 6 samples (3%); 2 (1%) of the meat (one ground beef and one meatball) and 4 (2%) of the cheese samples (one tulum and 3 fresh soft cheeses). In contrast, *Salmonella* spp. or *L. monocytogenes* was not detected in pasteurized milk and sausage samples. The high prevalence of *Salmonella* spp. and presence of *L. monocytogenes* in the samples could pose public health risks for consumers. To avoid *Salmonella* spp. and *L. monocytogenes* contamination, hygienic rules of slaughter and meat processing or pasteurizing milk must be rigorously observed.

Key words: Food, *Listeria monocytogenes*, meat, milk, *Salmonella* spp.

Sığır Orjinli Gıdalarda *Salmonella* spp. ve *Listeria monocytogenes* Varlığının Araştırılması

Özet: Bu çalışma süt, et ve ürünlerini içeren 200 adet sığır orjinli gıdada (kıyma, kasap kötfe, inegöl köfte, sucuk, pastörize süt, tulum peyniri, taze beyaz peynir ve çeçil peyniri) *Salmonella* spp. ve *Listeria monocytogenes (L. monocytogenes)*'in prevalansını araştırmak amacıyla yapıldı. Toplanan örneklerin 66'sından (%33) *Salmonella* spp., 6'sından (%3) *L. monocytogenes* izole edildi. Ürünlere göre değerlendirildiğinde; et orjinli örneklerin 45'inden (%22.5) (16 kıyma, 10 inegöl köfte, 16 kasap köfte, 3 sucuk) peynir örneklerinin ise 21'inden (%10.5) (6 tulum peyniri, 15 taze beyaz peynir) *Salmonella* spp. saptandı. *Listeria monocytogenes*'in ürünlere göre dağılımı ise; 2'si (%1) et (kıyma ve kasap köfte), 4'ü (%2) peynir (1 tulum peyniri, 3 taze beyaz peynir) olarak belirlendi. Pastörize süt ve sucuk örneklerinden *Salmonella* spp. ve *L. monocytogenes* saptanmadı. Et, süt ve ürünlerindeki yüksek düzeydeki *Salmonella* spp. ve *L. monocytogenes* varlığının, halk sağlığı riski oluşturabileceği düşünüldüğünden, söz konusu etkenlerden kaynaklı kontaminasyonu önleme çalışmalarında, mezbaha ve et işleme aşamalarında hijyen kuralları ile pastörizasyon uygulamalarının zorunluluğu üzerinde önemle durulmalıdır.

Anahtar kelimeler: Et, gıda, *Listeria monocytogenes, Salmonella* spp., süt

Introduction

Salmonella spp. and *L. monocytogenes* are serious safety concerns for the food industry and public health. These pathogens colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals those for human consumption (15). Studies have implicated contaminated foods of animal origin such as milk, beef and their products in the transmission of the bacteria to humans.

Salmonellosis is one of the most common foodborne diseases. It has been recognized as human and animal pathogens for over a century. According to data, *Salmonella spp.* are estimated to cause about 1.03 million non-typhoidal infections in humans per year in the U.S. has been attributed with approximately 378 deaths and over 19,000 people requiring hospitalization. About 96% of these cases are believed to be food borne. *L. monocytogenes* is also an important food borne pathogen not because it causes large numbers of symptomatic cases but because of its relatively high case–fatality

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rate. About 94% of listeriosis cases are hospitalized and about 16% die (20). Recent years, incidence rates of *L. monocytogenes* ranging from 0.3 to 1.3 per 100.000 capita have been reported in European countries, the US, Canada and Australia (56). According to listeriosis outbreaks, between 2010 and 2012, in the US has been been attributed to imported ricotta cheese in the US (13). Similarly, raw milk, ice cream and cheddar cheese have implicated in salmonellosis in humans. Contamination of milk with these pathogens therefore poses a great health risks to humans (35).

Food producing animals may carry *Listeria*, and be a source of contamination for milk and meat. Biofilms containing *L. monocytogenes* in food production and processing facilities may constitute a persistent, ongoing, sometimes sporadic source of bacteria (52). Thermal processing of milk and meat will destroy *L. monocytogenes* but post-processing contamination does occur. Because this pathogen grows during refrigeration, simply keeping foods cold does not ensure their safety (20). *Salmonella* naturally live in the intestines of humans and other animals and therefore fecal material is usually the ultimate source of these bacteria. *Salmonella* are also present in the lymph nodes of some healthy cattle and other animals and this may be a source of *Salmonella* contamination of ground meat (29). It is reported that meat accounted for 29% of all outbreaks and 33% of outbreaks in the US and Canada with a known vehicle (20). Several studies have indicated that different prevalence rates of *Salmonella* spp. and *L. monocytogenes* are present in beef origin meats, milk and their products samples from Turkey and around of the world (18,21,31,34,41,46,51,54,55).

In the present study also was carried out to detect the prevalence of *Listeria monocytogenes* and *Salmonella* spp. in some bovine origin meat and milk and their products samples. For this purpose, a total of 200 bovine origin meat and milk and their products samples were analyzed using standard conventional culture methods.

Material and Methods

Sample collection

A total of 200 samples including 25 samples of minced meat, inegöl meatball, sausage, pasteurized milk, tulum cheese, fresh soft cheese and ceçil cheese marketed in the Middle- Aegean in Turkey were aseptically collected from the retail markets, restaurants and bazaar, between November 2011-February 2012, and examined the presence of *Salmonella* spp. and *L. monocytogenes*. Samples were transported to the laboratory under cold chain and analyzed within 2 h.

Isolation of Salmonella spp. and L. monocytogenes

Standard cultivation method was carried out for *Salmonella* spp. isolation as recommended by FDA (23). Twenty five g/mL of each food samples was transferred to plastic bags and homogenized with 225 mL of 1% (w/v) buffered peptone water (BPW) (Merck, Germany) and incubated at 37°C for 24h. After the overnight incubation, 0.1 mL aliquots were inoculated into tubes containing 10 mL Rappaport Vassiliadis (RV) broth and incubated for 24 h at 42°C. Xylose Lysine Deoxycholate (XLD) agar plates were inoculated from each of the RV- broths and incubated for 18-24 h at 37°C. Up to five suspect colonies with typical *Salmonella* morphology were confirmed biochemically by inoculating into lysine ıron agar (LIA), urea broth, tryptone broth, decorboxylase broth, MR-VP Medium, ONPG disk and triple sugar ıron agar (TSIA) slopes with confirmation carried out using specific *Salmonella* O and H agglutinating antisera (Difco 2537-47).

L. monocytogenes was isolated using FDA (24) procedure. In this study, twenty five g/mL representative portion from each sample was introduced aseptically into a sterile stomacher bag containing 225 ml of *Listeria* selective enrichment broth (Oxoid CM 862, SR 0141, UK) and incubated for 24 h at 30°C. After that a loopful of the enrichment culture was streaked on the surface of on *Listeria* selective (Oxford) agar (Oxoid, CM0856, suppl. SR0140, UK). These selective agars were then incubated for up to 48 h at 35°C. Suspected colonies were those that appeared grayish colonies surrounded by black halos with possible greenish sheen onto the plates. Up to 5 suspected colonies were streaked onto tryptone soya agar (Oxoid, CM0131, UK) supplemented by 0.6% of yeast extract powder (TSA-YE) (Oxoid, LP0021, UK) and incubated at 35°C for 24 h. All of the isolates were subjected to Gram staining, motility test, catalase test, oxidase test, hemolysis test, CAMP test, carbohydrate utilization, and biochemical identification by Microbact™ *Listeria* 12L Kit System (Oxoid, MB1128A, UK).

Results

According to analyzed results, *Salmonella* spp. were isolated from 33% (n=66) of these 200 samples. The rates of samples in which *Salmonella* were detected in 45% (45/100) of which isolated from meat samples (16 of minced meat, 10 of İnegöl meatball, 16 of meatball and 3 of sausage), and 21% (21/100) of which obtained from milk products (6 of tulum cheese and 15 of fresh soft cheese). In contrast, it was not isolated from cecil cheese and pasteurized milk samples.

L. monocytogenes was also isolated from total 6 (3%) samples; 2 (2%) of which from meat origin and 4 (4%) of which milk origin samples. According to samples distribution; it was isolated from one minced meat and one meatball samples, one of tulum cheese and three of fresh soft cheese samples. However, the bacterium was not isolated from cecil cheese and pasteurized milk samples. The results of this study are shown in Table 1.

Table 1. Prevalence of *Salmonella* spp. and *Listeria monocytogenes* in food samples

Type of samples	*Salmonella* spp. (%)	*L. monocytogenes* (%)
Minced meat (n=25)	16 (64)	1 (4)
Meat ball (n=25)	16 (64)	1 (4)
Inegöl meatball (n=25)	10 (40)	0 (0)
Sausage (n=25)	3 (12)	0 (0)
Tulum cheese (n=25)	6 (24)	1 (4)
Fresh soft cheese (n=25)	15 (60)	3 (12)
Cecil cheese (n=25)	0 (0)	0 (0)
Pasteurized milk (n=25)	0 (0)	0 (0)
Total positive samples (n=200)	66 (33)	6 (3)

Discussion

In the present study, *Salmonella* spp. was isolated from a total 33% of the 200 samples. According to samples distribution; 45 (45%, n=100) *Salmonella* contaminations were detected in meat samples, whereas 21 (21%, n=100) contaminations were detected in cheese samples. There have been various *Salmonella* isolation ratio in beef and beef related products reported from different parts of the world and the results are ranging from 0.0% to 56.7% (1, 4, 10, 12, 17, 21, 33, 36, 38, 69). Two of the reports from Canada and China showed that the contamination ratio with *Salmonella* in beef samples were 0.0% and 17.0% (13/78), respectively (4, 59). In recent studies, *Salmonella* prevalence was reported as 3% (n=100) and 56.7% (n=238) in ground beef samples, respectively (13, 34). Also, *Salmonella* contamination ratio was reported between 0.0% and 20.0% in the meatball and ground beef samples in Turkey (2, 8, 18, 27, 50, 60, 61). The prevalence of *Salmonella* in ground beef samples were reported 10% in Afyon and Aydın (50), 11.1% in Istanbul (8), 8% in Amasya (61), and 16% in Samsun (2), respectively. In contrast these results, Cetinkaya et al. (14) and Direkel et al. (18) reported that *Salmonella* was not detected in any of the analyzed ground beef samples. Therefore, the results of present study show that *Salmonella* contaminations are higher than that of report relating *Salmonella* contaminations in different areas of Turkey. There have been limited data available about meatball in Turkey. It was detected in 5.4%, 4% and 24% ratio reported by Yıldız et al. (60), Yildirim et al. (61) and Al (2), respectively. The results of the present study indicated that *Salmonella* contaminations were the highest in Turkey in comparison to the other parts of the world's. However, the study of Cabrera-Diaz et al. (12) demonstrated a significant *Salmonella* contaminations in the world as well. Several earlier studies were conducted to determine the bacteria in the various types of sausages and the isolation *of Salmonella* spp. were ranged from 0.0 to 9.1% cases (21,37,39,40,51). Sırıken et al. (51) found that *Salmonella* spp. were detected about 7% in the 100 Turkish dry fermented sausage (sucuk) samples. There has been a wide variation in *Salmonella* spp. in beef meat and in beef meat related products as well as minced beef and meatball throughout the different areas in the world as reflected by the above-mentioned results. The differences could be due to the different geographical conditions, the number of ana-

lyzed samples, isolation methods, seasonal variations, and the cross contamination of meat from carcass to consumption steps as well as the number of salmonellosis case in cattle (may be via porter) etc. Cross contamination of *Salmonella* could occur during handling, processing, packing and distribution. Markets, butcher and other specialty food shops may offer a wide variety of specialist foods such as meats for sale directly to the consumer. We observed that beef meat and chicken meat samples present same table and contact each other particularly butcher shops. Therefore, the cross contamination of *Salmonella* spp. could occur during handling, processing, packing and distribution.

Salmonella spp. infections, besides poultry, have been also linked to outbreaks associated with the consumption of various types of cheese (36). There are wide variations among the contamination ratio of *Salmonella* spp. in among cheese samples according to cheese typing. In our country and other countries, various results were also showed the presence of *Salmonella* spp. in different types of cheeses. It was detected that 6 (2.4%) out of 250 cheese samples (16) and 3 (6%) of the 50 Van otlu (herby) cheese samples (54). Contrary to these findings, *Salmonella* spp. were not found in 80 white cheese and 40 cecil cheese samples by Gulmez and Guven (28) and 50 carra cheese samples by Aygun et al. (6). Twenty four tetilla cheese samples produced from raw cow's milk cheese, were not contaminated with *Salmonella* spp. (43). Similiarly, *Salmonella* has not been detected in any type of cheese samples (3). Also, *Salmonella* spp. has not been detected in any of the 4437 samples of fresh, ripened and semi-hard cheeses made from raw, thermized or pasteurized milk (39).

In the present study, *L. monocytogenes* was also isolated from total 6 (3%) of the samples that 4 (2%) of these samples were from meat origin. It has been reported that the contamination rate of *L. monocytogenes* in gound beef was 12.2% in Japan, 7.2% Turkey, 19% in Jordan and 37% in Argentina (5, 26, 30, 33). According to studies reported around the world, the prevalence of *L. monocytogenes* in different types of sausage was found to vary from 2.6% to 19% ratio. It was detected in 7% in Turkish sausage (51) and 11.6% in Turkish style fermented sausage (16), 19% in unpacked dry sausages (45), 8.5% in fresh meat (32), 14% in uncooked sausage, and 3.7% in cooked sausages; 15% in sausages; 3.7% in Spanish-style sausages; 2.6% (42, 25) in whole or sliced (loose sold) fermented meats on retail sale samples. Moisture levels, protein content and salt concentrations also affect growth of this pathogen (53). Likewise, Diez et al. (19) reported that thirty days of drying of a fermented sausage, chourico de vinho, and reduced water activity are sufficient to destroy all pathogens. Cured meats also contain several added ingredients that restrict microbial growth, including salt, lactate, and nitrate/nitrite (53). However, natural and organic foods do not contain the addition of nitrite and some other antimicrobials. Therefore, *L. monocytogenes* could grow better in these products. When compared the present study to many other studies, the different prevalence rates detected in these studies might be due to variations in livestock farm management, sampling and isolation methods, human activity, hygienic conditions in slaughterhouse as well as food-processing environments. The production process of cooked meats includes a heating step that is probably sufficient to eliminate any *L. monocytogenes*, therefore, the presence of *L. monocytogenes* is most likely due to post-process contamination. Unpasteurized milk and dairy products made from raw milk serve as vehicles for transmission of pathogenic bacteria from cattle to humans. *Salmonella* spp. and *L. monocytogenes* were detected in the tulum and fresh soft cheese in contrast the cecil cheese and pasteurized milk. The tulum cheese and fresh soft cheese are made from raw milk. During these types cheese making, heated procedure has not been applied. Whereas, cecil cheese is one of cooked-curd chesses. The same situation is valid for pasteurized milk. Poppe et al. (47)'s studies showed that 3-6% of raw milk samples and 19% of milk filters were positive for *Salmonella* spp. and soft cheeses made from unpasteurized or insufficiently pasteurized milk may also be contaminated with *Salmonella* spp. In this study, *Salmonella* spp. (21%) and *L. monocytogenes* (4%) were isolated from some milk products (tulum cheese and fresh soft cheese) in contrast to pasteurized milk. Our results are in agreements with Sagun et al. (49), Colak et al. (16) and Bouayad et al. (9). However, Rudolf and Scherer (48), (15.8%) and Torres-Vitela et al. (57) (15%) reported that

significantly higher contamination rate was noted for *L. monocytogenes* for cheese samples. In contrast these data, Lambertz et al. (37) found it in 0.4% ratio. The behaviour of *L. monocytogenes* in different kinds of cheese during ripening has been widely studied by some authors around the world. Although *L. monocytogenes* does not grow or survive in Mozzarella (11) or in pressed cooked cheeses (7), it grows in soft cheeses and washed smear cheeses (44). It can decrease but nevertheless survive more or less according to length of ripening in hard cheeses (58). Another studies, it is also reported that *L. monocytogenes* can be found more frequently in raw milk samples and soft cheeses. In soft and semi-soft cheeses, the water activity is higher than in hard cheeses, allowing growth of *L. monocytogenes* (22).

Conclusion

The results of this study demonstrated that the ground beef, meatball and cheese samples were contaminated with two major food-borne pathogens bacteria; *Salmonella* spp. and *L. monocytogenes.* Therefore, these kinds of samples may be a potential vehicle for the transmission of these two bacteria to humans. The presence of *L. monocytogenes* and *Salmonella* spp. was in analyzed some bovine origin foods seems to be related with the use of raw milk, and non-hygienic production processes and the hygienic rules of slaughter processing must be rigorously observed. Therefore, it is essential to ensure the high safety standards such as raw milk quality, the process of effective pasteurization, storage condition, proper cleaning and sanitation processes in milk and dairy production places. Pre-slaughter and processing interventions prevent pathogenic bacterial contamination that may improve the health of the cattle reduce the presence and/or concentrations of the bacteria in the feces and hides of the cattle and consequently reduce the prevalence of beef contamination.

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