



## Prevalence and Characterization of *Listeria* Species from Raw Milk and Dairy Products from Çanakkale Province

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### ABSTRACT

The objective of this study was to determine the prevalence of *Listeria* species, specifically *Listeria monocytogenes*, in raw milk, pasteurized milk, white cheese, and homemade cheese. A total of 200 food samples were collected and analyzed to examine the presence of *Listeria* spp. The EN ISO 11290-1 method was used for isolation of *Listeria*. API *Listeria* test kit was used for biochemically characterization. *Listeria* spp. were isolated in 25 of the 200 samples (12.5%). The largest number of *Listeria* spp. was detected in homemade cheese (24%), followed by raw milk (18%), and white cheese (8%). *Listeria* spp. were not isolated from the pasteurized milk. The most common species isolated were *Listeria innocua* (5.5%); the remaining *Listeria* isolates were *Listeria ivanovi* (3.5%), *Listeria welshimeri* (3%), and *Listeria monocytogenes* (0.5%). *Listeria monocytogenes* was detected in only raw milk.

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### Introduction

*Listeria* spp. are Gram positive, and facultative anaerobic organisms. They are also non-spore forming, and rod-shaped bacteria (Momtaz and Yadollahi, 2013; Odetokun and Adetunji, 2017). The genus *Listeria* has been divided so far into 17 species and 4 subtypes on the basis of 16S rRNA sequences (Anon., 2017). *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. innocua*, *L. grayi* can be isolated from foods. These strains referred to as "classic" *Listeria* spp. Recently, 11 new species of the genus *Listeria* were identified. These are: *L. marthii*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia* and *L. booriae*. These newly identified species were isolated from foods and other environmental niches around the world (Barre et al., 2016). *L. monocytogenes* and *L. ivanovii* are the two known pathogenic species within this genus. Although *L. monocytogenes* may lead to illness and death in humans and other mammals, *L. ivanovii* is primarily associated with ruminant animals (Hellberg et al., 2013). *L. monocytogenes* is the principle reason of

listeriosis. It is transmitted to infect the susceptible individuals via consumption of the contaminated foods. The major risk population groups at risk for invasive listeriosis are the immunocompromised hosts such as pregnant women, unborn or newly delivered infants, organ transplant recipients, cancer and AIDS patients, and the elderly, with fatality rates of 20-30% (Jamali et al., 2013; Yehia et al., 2016; Phraephaisarn et al., 2017). *Listeria* species are widely distributed in many different environments. These environments are soil, surface water, sewage, animal feed, farm environments, food processing equipments and environments, urban and suburban settlements (Korsak and Szuplewska, 2016). In addition, this species can be found in a wide variety of raw and processed foods. These foods are milk and dairy products, various meats and meat products such as beef, fermented sausages, fish products, ready-to-eat foods, and vegetables. These contaminated foods have been implicated in several outbreaks of human listeriosis (Saludes et al., 2015).

*Listeria* spp. are the most frequently prevalent in the milk processing environment. Although pasteurization process destroys *L. monocytogenes* in raw milk, this process does not eliminate later risk of contamination of dairy products. Also, dairy products may become contaminated with *L. monocytogenes* during subsequent stages of production (Seyoum et al., 2015). The aim of the present work was to provide information about *Listeria* spp. strains isolated from raw milk and dairy products produced in Çanakkale (Turkey), focusing on their prevalence, phenotypic and biochemical characteristics.

## Materials and Methods

### Sampling

Between October 2016 and February 2017, a total of 200 food samples including raw milk, pasteurized milk, white cheese and homemade cheese were randomly purchased from various local bazaars and supermarkets in Çanakkale, Turkey. The samples were transported to the laboratory under cold conditions on the sampling day and processed immediately.

### Bacterial Strains and Culturing

Listerial strains isolated in this study and the references strains were propagated on Brain Heart Infusion (BHI) Broth (Merck, Germany) and Tryptone Soy Broth supplemented with 0.6% of yeast extract (TSB-YE) (Sigma, Germany). They were grown at 35°C for 24 h. The initial isolates of strains were stored at -20°C with 30% (v/v) glycerol (Merck, Germany).

The standard strain of *L. monocytogenes* ATCC 7644 was obtained from the culture collection of Prokaryote Genetics Laboratory, Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey.

### Isolation and Identification of *Listeria*

*Listeria* spp. were isolated from raw milk and dairy products according to the International Organization for Standardization (ISO 11290-1) procedure as described by Garedeew et al., (2015). Two-step method for enrichment of *Listeria* spp. was performed in accordance with the standard. Twenty five grams of cheeses were added to 225 ml of ½ Fraser broth (Merck, Germany) as the first selective enrichment medium. It was homogenized in a stomacher-400 (London, UK) at high speed for two minutes and incubated for 24±2 h at 30 ±1°C. Similarly, 25 ml milk was sampled and pH adjusted to neutral and thoroughly mixed with 1:10 ratio to ½ Fraser broth and incubated at 30±1°C for 24 h. After first enrichment step, 0.1 ml of ½ Fraser broth culture was transferred to 10 ml of Fraser broth as a secondary enrichment medium and incubated at 37°C for 48±2 h. At the same time, after primary enrichment incubation, a loop full of culture was streaked onto ALOA (Agar *Listeria* Ottaiani Agosti) agar (Merck, Germany) and PALCAM (Polmixin Acriflavin Lithium Chloride Cefazidime Aesculin Mannitol) agar (Merck, Germany) and incubated for 24-48 h at 37°C. After incubation, a loopful of secondary enrichment culture was streaked onto ALOA and PALCAM agar plates and incubated, at 37°C for 24-48 h. It was observed that grey-green colonies with black background on PALCAM agar plates, which is typical for *Listeria* spp.

Typical green-blue colored colonies with/without distinctive opaque colonies were determined on ALOA agar. Three to five presumptive colonies from ALOA and PALCAM agar were re-streaked on Tryptone Soy Agar supplemented with 0.6% of yeast extract (TSA-YE) (Sigma, Germany) at 37°C for 24-48 h. Typical colonies from TSA-YE (1 mm to 2 mm in diameter, convex, colourless and opaque) were subjected to standard biochemical tests including Gram staining, catalase activity, oxidase activity, and stabbed into a motility medium at 25°C and 35°C for observing the characteristics umbrella motility. The isolated and characterized strains were identified using API *Listeria* test system according to the manufacturer recommendations (BioMerieux, France). The reference strain *L. monocytogenes* ATCC 7644 was used in all biochemical tests.

## Results

A total of 200 food samples were analyzed for possible contamination with *Listeria* spp. according to ISO 11290-1 method, which is based on biochemical identification of suspected colonies on ALOA and PALCAM agar plates. Twenty five samples (12.5%) were found to be positive for *Listeria* spp. A total of 25 isolates were found to be Gram positive, catalase positive, oxidase negative, and the characteristics umbrella motility into a motility medium. All of the 25 isolates were also biochemically determined at species level using the API *Listeria* (data not shown). The incidence of *L. monocytogenes* and other *Listeria* spp. from raw milk and dairy products was given in Table 1. The counts of *Listeria* spp. was distributed as follows: 0.5% (1 of 200) to *L. monocytogenes*, 5.5% (11 of 200) to *L. innocua*, 3.5% (7 of 200) to *L. ivanovii*, and 3% (6 of 200) to *L. welshimeri*. None of the other *Listeria* species were determined. The highest prevalence of *Listeria* spp. was detected in homemade cheese (24%, 12 of 50), followed by raw milk (18%, 9 of 50), and white cheese (8%, 4 of 50).

The isolates *L. innocua* (8%, 4 of 50), *L. ivanovi* (6%, 3 of 50), *L. monocytogenes* (2%, 1 of 50) and *L. welshimeri* (2%, 1 of 50) were observed in raw milk. *L. monocytogenes* was detected in only raw milk (0.5%, 1 of 200). The most common species isolated in homemade cheese was *L. innocua* (10%, 5 of 50); the remaining *Listeria* isolates were *L. welshimeri* (8%, 4 of 50), and *L. ivanovi* (6%, 3 of 50). In white cheese, *L. innocua* (4%, 2 of 50), *L. welshimeri* (2%, 1 of 50), and *L. ivanovi* (2%, 1 of 50) were also detected. *Listeria* spp. was not isolated from the pasteurized milk.

## Discussion

In this study, *Listeria* spp. were isolated from 12.5% (25 of 200) raw milk and dairy products. *L. innocua* was known to be the highest prevalent *Listeria* spp. (Gebretsadik et al., 2011). In the present study, the dominant *Listeria* spp. isolated was *L. innocua* (5.5%). This finding was in agreement with earlier report (Abraham et al., 2008; Gebretsadik et al., 2011; Rahimi et al., 2012; Jamali et al., 2013).

Table 1 Incidence of *L. monocytogenes* and other *Listeria* spp. in raw milk and dairy products

Nature of Samples	Number of Samples	Number (%) of Positive Samples				Total (%)
		<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. welshimeri</i>	
Raw Milk	50	1 (2%)	4 (8%)	3 (6%)	1 (2%)	9 (18%)
Pasteurized Milk	50	-	-	-	-	-
White Cheese	50	-	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Homemade Cheese	50	-	5 (10%)	3 (6%)	4 (8%)	12 (24%)
Total	200	1 (0.5%)	11 (5.5%)	7 (3.5%)	6 (3%)	25 (12.5%) <sup>a</sup>

<sup>a</sup>Calculated for total of 200 samples

*L. monocytogenes* was isolated from only raw milk (0.5%) in this study. Our findings were noticed that the frequency of isolation of *L. monocytogenes* was much lower. This observation also agrees with that of Vardar-Ünlü et al., (1998), Sağun et al., (2001), Aygun and Pehlivanlar, (2006), Taşçı et al., (2010), Abay et al., (2012), and Durmaz et al., (2015), who found 4% in Sivas, 1.2% in Van, 0% in Antakya, 2.4% in Burdur, 0% in Kayseri, and 2.2% in Şanlıurfa and Adıyaman, respectively. In other countries, as opposed to our study, the reported isolation rates of *L. monocytogenes* from raw milk samples were 16.7% in Brazil (Silva et al., 2003), 6.5% in United States (van Kessel et al., 2004), 4% in Iran (Jami et al., 2010), 22% in Ethiopia (Gebretsadik et al., 2011), 6.3% in Ireland (Fox et al., 2011), 41.6% in Syria (Al-Mariri et al., 2013), 21.7% in Malaysia (Jamali et al., 2013), and 16.6% in India (Nayak et al., 2015). Contamination rates of *L. monocytogenes* are affected seasonal variations (Taşçı et al., 2010). Samplings in our study were collected between October 2016 and February 2017. The low prevalence of *L. monocytogenes* in raw milk may be the result of seasonal factor. In this study, *L. innocua* was the main *Listeria* spp. isolated from raw milk which is agreement with earlier findings reported by Silva et al., (2003); Rahimi et al., (2012); Jamali et al., (2013), and Seyoum et al., (2015). Infected animals, poor silage quality, insufficient hygiene, and environmental condition which could occur during milking and storage are likely the most common causes of *L. monocytogenes* in raw milk. *Listeria* spp. were not detected from pasteurized milk. Similar results were reported by Vardar-Ünlü et al., (1998) and Sarker and Ahmed (2015). In contrast to our findings, Silva et al., (2003) detected *Listeria* spp. in pasteurized milk (16.7%). Thermal process like pasteurization does not in any way guarantee the absolute safety of milk and dairy products.

Among the samples tested in this study, homemade cheese had the highest percentage rate of *Listeria* spp. (24%), particularly *L. innocua* (10%), *L. welshimeri* (8%), and *L. ivanovii* (6%). Overall, 4 listerial strains were isolated from White cheese analyzed in this study, of which 2 (4%) were *L. innocua*, and 1 (2%) was *L. welshimeri* and the remaining 1 (2%) was *L. ivanovii*. *L. monocytogenes* was not detected from these sources in agreement with Demir and Öksüztepe, (2016). In contrast, contrary results were noted by some researchers. Arslan and Özdemir, (2008) detected *L. monocytogenes* in 9.2% cheese samples. Furthermore, Kaptan (2016) was determined *L. monocytogenes* in 15.77% of cheese samples. In other countries, as similar to our study, the reported isolation rates of *L. monocytogenes* from cheese

samples were 0% in Algeria (Bouayad et al., 2012), and Iran (Shamloo et al., 2015). In contrast to the present study, a high percentage of *L. monocytogenes* contamination to cheese samples was obtained: 6.7% in Brazil (Abraham et al., 2008) and 9.8% in Spain (Arrese and Arroyo-Izaga, 2012). The contamination reasons of *L. monocytogenes* in fermented milk products were: i) Insufficient heat treatment of milk to kill the organisms, ii) the resistance of *Listeria* to the decreased pH during cheese production, and iii) cross contamination through air, soil, equipment and processing units staff (Bouayad et al., 2012).

A total of 100 cheese samples (homemade and white cheese), 16% (16 of 100) were positive for *Listeria* spp. in this study. In other reports conducted with cheese in Turkey, isolation rates of *Listeria* spp. was 33.1% (Arslan and Özdemir, 2008) and 100% (Kaptan, 2016). These results mentioned above were higher than our findings. In contrast, a lower incidence of *Listeria* spp. was found by Aygun and Pehlivanlar, (2006). In countries other than Turkey, as similar to our study, the prevalence of *Listeria* spp. in cheese samples was reported as 16.7% in Brazil (Silva et al., 2003), 12.2% in Brazil (Abraham et al., 2008), 15% in Iran (Rahimi et al., 2012), and 10% in Egypt (Elshinaway et al., 2017). *L. ivanovii* is connected with listeriosis. Because of this, the presence of *L. ivanovii* in cheese samples is concerned. In this study, a higher frequency of *L. ivanovii* in homemade cheese than white cheese was observed.

## Conclusions

Outbreaks of listeriosis, caused by consumption of milk and dairy products indicate the danger to consumer health. We isolated the *Listeria* species from raw milk and dairy products and identified by API *Listeria* STREP. The results of this study provide information about the contamination status of raw milk and dairy products in Çanakkale with *Listeria* spp. The highest prevalence of *Listeria* spp. was detected in homemade cheese followed by raw milk, and White cheese. The most common species was *L. innocua*, the remaining *Listeria* isolates were *L. ivanovi*, *L. welshimeri* and *L. monocytogenes*. Considering of the low frequency of *L. monocytogenes* in this study, raw milk cannot be omitted as a potential source of food contamination for the population.

## Conflict of Interest

No conflict of interest associated with this work.

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