

A Survey on the Presence of *Listeria monocytogenes* in Various Semi-Hard Cheeses from Different Regions of Turkey

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Abstract: Presence of *Listeria monocytogenes* in various semi-hard traditional cheeses including Van otlu (herby), Carra, Konya kuflu (mouldy) and Urfa tulum produced at different regions of Turkey was investigated. A total of 120 samples from these four cheese types were surveyed. *Listeria* sp. were detected in 50 of the 120 samples. Ratios of contaminated samples with *Listeria* sp. in four cheese types were Van otlu, 36.6% (11/30), Carra, 56.6% (17/30), Konya kuflu 43.3% (13/30), Urfa tulum, 30% (9/30). *L. monocytogenes* was determined in 34 of the 120 samples. Numbers of contaminated samples with *L. monocytogenes* in four cheese types were Van otlu, 9 (30%), Carra, 14 (46.6%), Konya kuflu, 7 (23.3%), Urfa tulum, 4 (13.3%). *Listeria innocua*, *Listeria welshimeri* and *Listeria grayi* were isolated from 2, 12 and 2 of the all samples, respectively. *Listeria ivanovii* and *Listeria seeligeri* were not detected in any of the samples. Any correlation was not observed between the presence of *Listeria* sp. and chemical composition of the cheeses.

Key words: *Listeria monocytogenes*, semi-hard cheeses, Van otlu, Carra, Konya kuflu, Urfa tulum, Turkey

INTRODUCTION

Cheese is an important component of Turkish diet (Yilmaz *et al.*, 2005). There are >100 varieties of cheese in Turkey (Hayaloglu *et al.*, 2002; Yilmaz *et al.*, 2005; Kamber and Terzi, 2008). In addition to three major cheese types, there are many traditional cheeses in Turkey (Yilmaz *et al.*, 2005). Van otlu (herby), Carra, Konya kuflu (mouldy) and Urfa tulum are common types of the traditional cheeses (Aygun *et al.*, 2005; Erkan *et al.*, 2007; Hayaloglu and Kirbag, 2007). Van otlu cheese is a semi-hard type of salty cheeses which is commonly consumed in the East and Southeast regions of Turkey. Approximately, twenty-five types of herb mixture could be added into curd at the range of 0.1-1.5% during production processes (Erkan *et al.*, 2007).

Carra cheese containing mostly black cummin (*Nigella sativa*) and/or garden thyme (*Thymus vulgaris*) is one of the most commonly consumed cheese in the Antakya region in Turkey. Its texture is semi-hard or hard (Aygun *et al.*, 2005). Konya kuflu cheese having semi-hard texture is a mould-ripened variety produced in around of the Konya in the central Anatolia region of Turkey.

Cheese blocks are ripened in the rooms until blue-green moulds spontaneously grown on the surface of the cheese (Hayaloglu and Kirbag, 2007). Urfa tulum cheese

produced in the Southeast regions of Turkey has semi-hard texture and is ripened in cotton bag. These traditional cheeses mentioned above are produced from raw milk in small dairy plants and home under unmechanised and/or nonhygienic conditions. Different process applications are applied for each and they have been consumed around the mentioned area until two decades. In spite of these known facts, they have also being produced from pasteurized milk in modern dairy plants and consumers' demands for these cheeses have been increasing recently in big cities such as Ankara, Istanbul and Izmir (Kivanc, 1989; Erkan *et al.*, 2007; Hayaloglu and Kirbag, 2007; Hayaloglu *et al.*, 2008).

In recent years, dairy products linked to both invasive and noninvasive listeriosis outbreaks have been associated with approximately half of the reported listeriosis outbreaks in Europe (Lunden *et al.*, 2004). An overview of food-borne diseases annual reports from seven countries, since 1980 indicated that sixty outbreaks and four single cases were described in the literature. *Listeria monocytogenes* were responsible for ten outbreaks and four well-documented single cases (De Buyser *et al.*, 2001).

Previous reports on the prevalence of food-borne pathogens in various cheeses produced in Turkey have shown that *Salmonella* sp., *Staphylococcus aureus*, *L. monocytogenes*, *Campylobacter* sp., *Escherichia coli*

O157:H7 have been accepted as major pathogens for the safety of cheeses (Kivanc, 1989; Turantas *et al.*, 1989; Hayaloglu *et al.*, 2002; Aygun *et al.*, 2005; Tekinsen and Ozdemir, 2006; Colak *et al.*, 2007; Erkan *et al.*, 2007; Hayaloglu and Kirbag, 2007). There are few information on the presence of *L. monocytogenes* in semi-hard or hard cheeses produced in Turkey (Colak *et al.*, 2007; Erkan *et al.*, 2007), eventhough prevalence of *L. monocytogenes* in dairy products especially raw milk and soft cheeses has been investigated abundantly. While two researches have been done on the presence of *L. monocytogenes* in Van otlu cheese (Erkan *et al.*, 2007), no knowledge has been found in Carra, Konya kuflu and Urfa tulum cheeses. In this research, Van otlu, Carra, Konya kuflu and Urfa tulum cheeses were surveyed to obtain some knowledge on the presence of *L. monocytogenes* in these traditional semi-hard cheeses.

MATERIALS AND METHODS

About 30 samples from each cheese varieties were obtained from retail markets in Van, Hatay and Konya in Turkey. Samples were transferred to the laboratory in cold chain and held at 4°C until examination.

Microbiological analyses: Samples were evaluated for the presence of *L. monocytogenes* according to the Bacteriological analytical manual (FDA, 2003). The 25 g sample was aseptically weighed and homogenised with 225 mL Listeria enrichment broth (Merck, 1.11951.0500, Darmstadt, Germany) in a Colworth Stomacher Lab-Blender 400 (Seward Medical, London, UK) for at least 2 min. After the incubation at 30°C for 4 h for pre-enrichment, Listeria selective supplement (Merck, 1.07006.0001, Darmstadt, Germany) was added into and incubation was maintained at 30°C for 44 h.

The enrichment broth was then streaked on to Palcam Agar (Merck, 1.11755.0500, Darmstadt, Germany) with Palcam selective supplement (Merck, 1.12122.0001, Darmstadt, Germany) and Oxford Agar (Merck, 1.07004.0500, Darmstadt, Germany) with Oxford selective supplement (Merck, 1.07006.0010, Darmstadt, Germany) for isolation and the plates were incubated xat 35°C 24-48 h. Five presumptive colonies with a typical morphology were streaked on to Tryptone Soy Agar (TSA) (Oxoid, CM 131, Hampshire, UK) with yeast extract (1.03753.0500) for purification and plates were incubated at 30°C for 24-48 h.

To identify Listeria species, gram stain, catalase reaction, oxidase and indole tests, utilization of urea, H₂S, motility in SIM medium, methyl red, Voges-Proskauer, reduction of nitrate, hemolysis in blood agar,

carbohydrate fermentation tests (dextrose, esculine, maltose, ramnose, xylose and mannitol) and CAMP test were used.

Chemical analyses: Total dry matter and salt contents of cheese samples were determined by AOAC (1990) procedures. pH values of the samples were measured by using a digital pH meter (Ino Lab pH 720 model, WTW, GmbH, Germany). Total acidity as lactic acid was determined by Titrimetric method (Bradley *et al.*, 1993).

Statistics: Data obtained in the chemical analyses were subjected to one way ANOVA. SPSS statistic package program (16.0; SPSS, Inc., Chicago, IL, USA) was used (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

A total of 120 cheese samples were analyzed for the presence of *L. monocytogenes*. *Listeria* sp. were detected in 50 of the 120 samples. Ratios of contaminated samples with *Listeria* sp. in four cheese types were as follows: Van otlu, 36.6% (11/30), Carra, 56.6% (17/30), Konya kuflu, 43.3% (13/30), Urfa tulum, 30% (9/30). *L. monocytogenes* was detected in 34 (28.3%) of the 120 samples.

Numbers of contaminated samples with *L. monocytogenes* were Van otlu, 9 (30%), Carra, 14 (46.6%), Konya kuflu, 7 (23.3%), Urfa tulum, 4 (13.3%). Ratio of contaminated samples with *L. monocytogenes* was 68%. *Listeria innocua*, *Listeria welshimeri* and *Listeria grayi* were isolated from 2, 12 and 2 of the all samples, respectively. *Listeria ivanovii* and *Listeria seeligeri* were not detected in any of the samples (Table 1).

Total dry matter, salt content, pH and acidity values of the samples which are accepted as important factors on microbial behavior were shown in Table 2. It was shown from the Table 2 that wide ranges in the total dry matter, salt, pH and acidity values were observed and that Carra cheese samples had the highest total dry matter and salt contents and the lowest acidity value.

But when the chemical characteristics of the contaminated samples were compared with non-contaminated samples, no correlation was observed between the presence of *Listeria* sp. and chemical composition of the cheeses (Table 3).

Table 1: Number of contaminated samples with *Listeria* sp.

<i>Listeria</i> sp.	Van otlu	Carra	Konya kuflu	Urfa tulum	Total
Sample number	30	30	30	30	120
<i>Listeria monocytogenes</i>	9	14	7	4	34
<i>Listeria innocua</i>	-	-	-	2	2
<i>Listeria welshimeri</i>	1	3	5	3	12
<i>Listeria grayi</i>	1	-	1	-	2
Total	11	17	13	9	50

Table 2: Some chemical characteristics of the cheese types

Characteristics	Statistical analysis	Van otlu	Carra	Konya kufllu	Urfa tulum	F
Total dry matter (%)	Mean±SE	53.07±0.76 ^b	57.97±1.13 ^a	51.41±0.67 ^b	52.31±1.29 ^b	8.43
	Min.	43.77	46.87	44.84	45.15	-
	Max.	60.68	68.06	58.28	71.29	-
Salt (%)	Mean±SE	7.36±0.34 ^a	7.24±0.45 ^a	5.58±0.35 ^b	4.72±0.21 ^b	13.80
	Min.	4.33	2.76	1.90	1.90	-
	Max.	13.06	11.93	13.52	7.96	-
pH	Mean±SE	5.28±0.13 ^c	5.77±0.06 ^b	6.46±0.10 ^a	5.11±0.07 ^c	35.99
	Min.	4.29	5.25	5.59	4.10	-
	Max.	6.87	6.83	7.45	6.14	-
Acidity (% l.a.)	Mean±SE	0.69±0.06 ^b	0.45±0.04 ^c	0.61±0.05 ^{ab}	0.95±0.10 ^a	8.13
	Min.	0.21	0.07	0.27	0.24	-
	Max.	1.57	0.89	1.48	2.59	-

Values after±represent standard error; a-c: Different uppercase letters in a row show significant differences among the cheese types

Table 3: Chemical characteristics of the contaminated samples

<i>Listeria</i> sp.	Cheese types	Sample no.	Total dry matter (%)	Salt (%)	pH	Acidity (% l.a.)	
<i>Listeria monocytogenes</i>	Van otlu	1	54.42	9.36	5.780	0.340	
		2	45.94	8.56	6.060	0.470	
		3	52.41	6.22	4.850	1.060	
		4	55.81	5.76	4.290	1.080	
		5	50.66	7.67	4.370	1.170	
		6	48.83	5.38	5.800	0.290	
		7	56.33	9.83	4.990	0.310	
		8	51.68	9.83	6.000	0.210	
		9	52.76	8.66	4.420	1.570	
		Carra	1	46.87	2.76	5.400	0.430
			2	59.92	4.26	5.700	0.250
			3	62.40	10.53	5.960	0.250
			4	61.52	5.24	5.940	0.220
			5	57.68	4.21	5.730	0.290
6	54.35		5.15	5.780	0.500		
7	58.65		5.62	5.850	0.370		
Konya kufllu	8	59.40	6.08	5.550	0.850		
	9	59.96	4.45	5.680	0.500		
	10	64.35	11.93	5.520	0.720		
	11	63.68	9.83	5.580	0.810		
	12	54.79	6.08	5.540	0.590		
	13	51.44	7.72	6.220	0.140		
	14	50.36	6.55	6.830	0.090		
	1	48.15	4.91	5.770	0.470		
	2	52.72	5.78	6.440	0.520		
	3	58.08	5.24	7.380	0.470		
	4	58.00	6.22	6.760	0.660		
	5	52.77	1.89	6.260	0.450		
	6	49.80	6.53	6.730	0.500		
	7	55.51	5.33	7.020	1.080		
Urfa tulum	1	54.20	4.91	5.280	0.310		
	2	50.20	6.81	4.930	0.880		
	3	53.34	4.91	5.200	2.050		
	4	48.49	4.45	4.850	1.040		
<i>Listeria innocua</i>	Urfa tulum	1	50.49	4.44	4.870	1.510	
		2	47.84	4.68	5.100	0.720	
<i>Listeria welshimeri</i>	Van otlu	1	55.33	6.32	5.580	0.360	
		Carra	1	64.90	8.84	5.390	0.610
			2	50.20	6.79	5.580	0.230
	3		54.82	5.38	6.620	0.070	
	Konya kufllu	1	53.30	5.62	7.290	1.480	
		2	49.20	5.15	7.050	0.520	
		3	49.80	4.09	5.900	0.684	
		4	50.19	4.09	6.050	0.650	
		5	44.84	4.16	5.590	0.580	
	Urfa tulum	1	52.28	4.56	5.740	0.500	
		2	48.34	4.14	4.930	1.010	
		3	52.56	4.68	5.300	0.630	
	<i>Listeria grayi</i>	Van otlu	1	55.66	5.15	4.720	0.900
		Konya kufllu	1	48.99	5.94	7.170	0.500

All cheeses can be classified as soft, semi-hard, hard or very hard (Farkye, 2004). Despite the fact that soft and

semi-soft cheeses which have higher water activity than hard cheeses, allow growth of *L. monocytogenes*, it can

survive in semi-hard cheeses with high salt concentration and low water activity (Lunden *et al.*, 2004). The present results confirmed these information stated by Lunden *et al.* (2004). Similarly, Durmaz and Sagun reported that *L. monocytogenes* inoculated to Van otlu cheese at 10^2 , 10^3 , 10^4 and 10^5 cfu mL⁻¹ survived for 60 days. Larson *et al.* (1999) stated that *L. monocytogenes* survived in cheese brines from >7 days to >259 days and survival did not correlate with pH and salt content. In addition, it is well known that in several countries, the use of raw milk for cheese making is still prevalent (Farkye, 2004) and also although, normal pasteurization of milk would prevent the contamination of cheeses with *Listeria* sp., recontamination during production could be occurred (Beckers *et al.*, 1987; Lunden *et al.*, 2004).

All of these mentioned results/factors are noteworthy for listeriosis outbreaks. Because of the fact that the main forces that drive cheese technology are economics, equipment/engineering, consumer demands and regulatory standards (Farkye, 2004) in Turkey, there has been growing interest in the consuming of the traditional semi-hard cheeses recently for their characteristics taste and flavour (Erkan *et al.*, 2007; Hayaloglu and Kirbag, 2007; Hayaloglu *et al.*, 2008). Although, these traditional cheeses are also being produced from pasteurized milk in modern dairy plants recently, important part of the production is realized in uncontrolled and nonhygienic small plants and home. According to the microbiological criteria of cheese in Turkish Food Codex, *L. monocytogenes* must not be found (zero tolerance) in cheese samples to be analyzed (Turkish Food Codex, 2009). Thus in the present study, the high numbers of contaminated samples with *L. monocytogenes* (34/120) and other *Listeria* sp. (16/120) could be assessed as noteworthy. The present results would be compared previously with the findings on the presence of *L. monocytogenes* in semi-hard cheeses because this investigation reports the results of a survey on the presence of *L. monocytogenes* in semi-hard cheeses.

The results agree with Erkan *et al.* (2007) who reported that of the 50 samples of Van otlu cheese, 8 (16%) samples were found to be contaminated with *L. monocytogenes* and Colak *et al.* (2007) who stated that *L. monocytogenes* was detected in 12 of the 250 (4.8 %) of the tulum cheese samples and Sagun who reported that of the 254 Van otlu cheese samples examined, 10 proved to be positive for *L. monocytogenes*, 1 *L. ivanovii*, 1 *L. innocua* and 1 *L. welshimeri*. However, Cetinkaya stated that a total number of 52 tulum cheese samples, *L. monocytogenes* was not isolated and Sengun reported

that *L. monocytogenes* was isolated only one of the 15 mouldy earthenware cheese samples. The discrepancies observed in the mentioned researches may be attributed to a variety of factors such as quality of milk, application of heat treatment and different manufacturing protocols, hygienic condition in production plants, ripening condition and time and inadequacy in the quality control, etc. A lot of conclusions related to this explanation have been reported by Farkye (2004) who stated that quality of the starting material is major rule for good quality cheese and by Aygun *et al.* (2005) who recommended that for improving the microbiological quality of Carra cheese, pasteurized milk should be used and the processing, ripening and storage should be carried out under good hygienic conditions and by Kivanc (1989) who indicated the lack of quality control and satisfactory sanitary conditions during the manufacture and storage of cheeses and by Erkan *et al.* (2007) who reported that to prevent public health risks standardization and modernization of Van otlu cheese production could be a necessity.

It has been found few researches related to *L. monocytogenes* in cheese in Turkey. The presence of *L. monocytogenes* in soft cheese was investigated by Gonc and Kilic who reported that *L. monocytogenes* was isolated from 13.4% of the white pickled cheese samples and by Uysal and Ang who determined that of the 271 white pickled cheese samples, 11 were found to have been contaminated with *L. monocytogenes*.

The effect of ripening on the surviving of *L. monocytogenes* was investigated by Yildirim and Sarimehmetoglu who observed that complete inhibition of *L. monocytogenes* inoculated at 10^3 and 10^5 to in Turkish white cheese with starter and probiotic cultures, occurred on days 30th and 60th of ripening and by Durmaz and Sagun who concluded that *L. monocytogenes* inoculated to Van otlu cheese at 10^2 - 10^5 cfu mL⁻¹ survived for 60 days but it was not isolated after 90 days of ripening.

Similarly, Pinto *et al.* (2009) stated that the time and the dose of bacteria inoculated affected the survival of *L. innocua* in Minas traditional Serro cheese during ripening. Solano-Lopez and Hernandez-Sanchez (2000) concluded that *L. monocytogenes* was able to survive during the manufacture and ripening processes of Manchego and Chihuahua Mexican cheeses.

CONCLUSION

By this study, it was determined that important parts of these traditional semi-hard cheese samples analyzed had been found contaminated with *L. monocytogenes* and

Listeria sp. and did not meet the microbiological criteria in Turkish Food Codex. In order to prevent possible listeriosis outbreaks associated with consuming of these types of cheese, it is important to create standardization and modernization in all manufacturing stages and to take all preventive measures to avoid recontamination during production, storage and distribution of cheese. It is considered as vital that official control must be implemented by authorized members at regular intervals in production and marketing plants.

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