

1-1-2004

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AKMAN, DENİZ; DURAN, NİZAMİ; and DIĞRAK, METİN (2004) "Prevalence of Listeria Species in Ice Creams Sold in The Cities of Kahramanmaraş and Adana," *Turkish Journal of Medical Sciences*: Vol. 34: No. 4, Article 7. Available at: <https://journals.tubitak.gov.tr/medical/vol34/iss4/7>

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Prevalence of *Listeria* Species in Ice Creams Sold in The Cities of Kahramanmaraş and Adana

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Received: July 14, 2003

Abstract: In this study, the prevalence of *Listeria* spp. was investigated in the ice cream samples collected from the downtown stores of Kahramanmaraş (28 samples) and Adana (30 samples). A total of 58 ice cream samples were analysed by the Food and Drug Administration (FDA) two-stage enrichment techniques. Growth of *Listeria* spp. was determined in 14 (24.1%) of the samples collected from Kahramanmaraş and 10 (17.2%) samples from Adana. The results of the biochemical tests revealed that the bacterial growth in the samples from Kahramanmaraş was *Listeria grayi* whereas 8 (13.8%) samples of Adana were *Listeria grayi*, the other two samples being *Listeria innocua* and *Listeria welshimeri*. In this study, *Listeira monocytogenes*, a pathogenic bacteria which exists in the ice cream products manufactured using sheep and goat milk, could not be isolated. The overall incidence of *Listeria* species in raw milk was 41.4%. *Listeria grayi* was the most frequently isolated bacteria and was found in 37.9% (22/58) of the ice cream samples. In contrast, *Listeria innocua* and *Listeria welshimeri* were each determined in 1.7% (1/58) of the samples. The presence of other species of *Listeria* was not found in the samples under investigation. The findings of this study suggest that *Listeria* infections are not a potential risk in Kahramanmaraş and Adana, but it is strongly recommended that these products are monitored carefully.

Key Words: *Listeria monocytogenes*, *Listeria* spp, ice cream, food microbiology.

Introduction

The bacteria *Listeria* spp. has become a significant subject in biomedical research due to its central position in medical microbiology and food microbiology. The genus *Listeria* comprises six species, i.e. *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*. In humans, *L. monocytogenes* is the major pathogen, although very rare cases of infection due to *L. ivanovii* and *L. seeligeri* have been described (1,2). The presence of any *Listeria* species in food may be an indicator of poor hygiene. However, since *L. monocytogenes* is the major human pathogen, there is widespread agreement that the goal should be to exclude this organism from the food chain wherever possible, and to maintain conditions which will inhibit its multiplication in foods in which this bacterium can grow (3,4,5,6).

Ice cream, a dairy product consumed with pleasure by the general populace, especially children due to its

contents of sweeteners, aromatic compounds, and various fruits, is known to be the richest in terms of content among the dairy products (7,8)

Milk and dairy products under low temperatures, especially ice cream, constitute a potential risk for listeriosis (9,10). In several studies on food, *Listeria monocytogenes* has been demonstrated to have a widespread distribution (6,7,9,11,12).

In our country, there are numerous enterprenuers who, in an attempt to benefit from meat and dairy products, raise sheep and goats especially in the rural areas. Unfortunately, in our country most of the consumed milk and dairy products are not prepared under hygienic conditions. Ignorant producers utilize milk that is most often not boiled enough in the production of their products. This too, obviously affects the health of the consumer (13,14).

There are very few studies on the presence of *Listeria* species in ice cream, the dairy products consumed by the majority of the population in our country.

This study was therefore aimed at determining the level of contamination by isolating and identifying the *Listeria* species in 58 ice cream samples from across our region.

Materials and Methods

Collection of Ice Cream Samples

The study was conducted on ice cream sold in open containers. The ice cream samples were obtained from different pastry shops in the Kahramanmaraş and Adana provincial centers. In addition, the origins of the milk samples used for producing the ice creams were different. Upon collection of the ice cream samples into sterile containers and transport to the laboratory the isolation studies were commenced on the same day. In the isolation and enrichment of the *Listeria* species the method proposed by the Food and Drug Administration (FDA) was used (3).

Reference Species

With the aim of eliminating any possible source of error in the assessment of the tests used on possible colonies identified in the isolation studies, the reference *Listeria* species were also tested in parallel. As the reference species, *Listeria monocytogenes* serovar scott A (F4642) obtained from the Department of Biology, Faculty of Science of the Ege University was used.

Isolation of the *Listeria* species

Selective Enrichment

After the ice cream samples were thawed to below room temperature 25 ml each of a 225 ml *Listeria* Selective Enrichment Broth (Oxoid) was inoculated. For a homogenous distribution of the sample in the medium it was shaken for 2-3 minutes. Media so inoculated were then incubated at 30 °C for 24-48 hours.

The Isolation and Identification Stage (Selective Isolation)

After 24 and 48-hour pre-enrichment processing, the culture was made on the selective enrichment media

Listeria Selective Agar. The planted plates were incubated for 48 hours at 37 °C under aerobic conditions. At the end of the incubation, they were evaluated as typical colonies with black haloes of 1-3 mm in diameter. The typical *Listeria* colonies observed on the culture medium were selected and cultured separately in Tryptone Soya agar (Oxoid) medium containing TSYE agar (0.6% Yeast Extract (Oxoid). After the plates had been incubated for 24 hours at 37 °C, the colonies were checked morphologically and their purity controlled by gram staining.

The isolated serotypes from the TSYE agar were examined by the Henry technique by holding the petri dish at 45° to a mirror which reflects a strong light. The *Listeria* were identified by their transparent colonies with typical blue-green reflection.

Later, the colonies so separated were then subjected to gram staining, carbohydrate fermentation tests (maltose, glucose, mannitol, D-xylose, melibiose, sorbitol, salisin, cellulose, rhamnase, dextrose), catalase activity, oxidase activity, SIM motility, β-hemolysis, CAMP (*S. aureus*) test, CAMP (*R. equi*) test, methyl red, voges proskauer, indole production, H₂S production, nitrate reduction, citrate utilization, esculin and urea hydrolysis.

The colonies isolated as *Listeria* were then subjected to β-hemolysis and CAMP test in a 7% sheep blood agar for species identification.

Findings

This study was conducted on 58 ice cream samples with the aim of determining the level of contamination in ice cream by isolating and identifying the *Listeria* species.

The results of the biochemical and carbohydrate fermentation tests conducted on *Listeria monocytogenes* serovar scott A (F4642) used as the reference *Listeria* species and those of the isolated species are shown in Tables 1 and 2.

Analysis of the findings revealed that of the 58 ice cream samples, 22 (41.4%) were contaminated with 3 different *Listeria* species. In the study, whilst *Listeria grayi* with 37.9% was found to be the most widespread species among the identified *Listeria*, *Listeria innocua* was isolated in 1 (1.7%) and *Listeria welshimeri* in 1 (1.7%) of the samples. *Listeria monocytogenes* was not isolated in any of the samples (Figure 1).

Of the 28 ice cream samples collected from the Kahramanmaraş provincial center, *Listeria grayi* was isolated in 14 (50%) samples. Of the 30 samples collected from the Adana provincial center 10 (33.3%) were found to contain the various species of *Listeria*. Of the isolated 10 samples, 8 (26.6%) *Listeria grayi*, 1 (3.3%) *Listeria innocua* and 1 (3.3%) *Listeria welshimeri* were determined (Figure 2).

Discussion

Listeria species are found in almost every medium in the environment, soil, excrement, canalization waters, plants, animals, animal feeds and foods. They contaminate milk by various means from these sources (1,3,6,7). These microorganisms, because of their ability to grow at +4 °C makes contamination of ice cream, the basic structure of which is milk, by *Listeria* a frequent finding (15,16,17).

Table 1. The results of carbohydrate fermentation tests.

Carbohydrates	<i>Listeria monocytogenes</i> Scott A	<i>Listeria grayi</i>	<i>Listeria innocua</i>	<i>Listeria welshimeri</i>
Maltose	+	+	+	+
Glucose	+	+	+	+
Mannitol	-	+	-	-
D-Xylose	-	-	-	+
Melibiose	-	-	-	-
Sorbitol	+	-	-	-
Salisin	+	+	+	+
Cellulose	-	-	-	-
Rhamnose	+	-	V	V
Dextrose	+	+	+	+

V: Variable

Table 2. The results of biochemical tests.

Test	<i>Listeria monocytogenes</i> Scott A	<i>Listeria grayi</i>	<i>Listeria innocua</i>	<i>Listeria welshimeri</i>
Gram-positive rod	+	+	+	+
Typical colony on LSA	+	+	+	+
Catalase activity	+	+	+	+
Oxidase activity	-	-	-	-
SIM motility	+	+	+	+
β-Hemolysis	+	-	-	-
CAMP (S.aureus)	+	-	-	-
CAMP (R.equi)	-	-	-	-
Methyl red	+	+	+	+
Voges Proskauer	+	+	+	+
Indole production	-	-	-	-
H ₂ S production	-	-	-	-
Nitrate reduction	-	-	-	-
Citrate utilization	-	-	-	-
Esculin	+	+	+	+
Urea hydrolysis	-	-	-	-

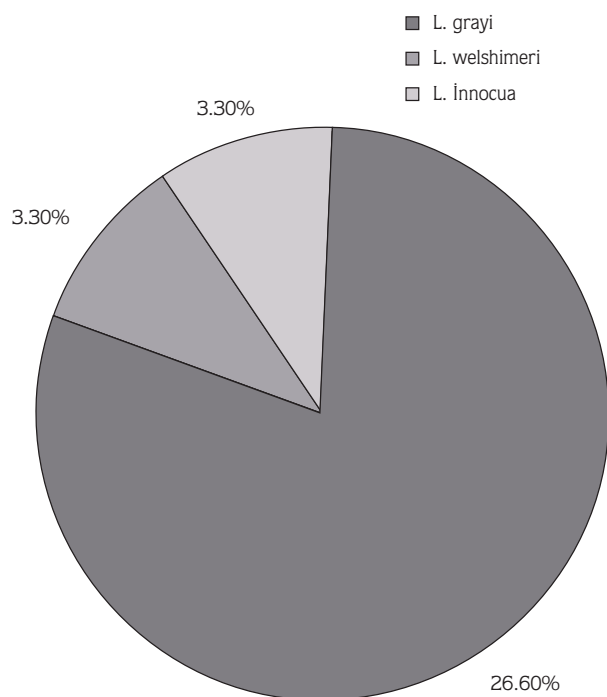


Figure 1. The distribution of isolated *Listeria* spp. from Adana.

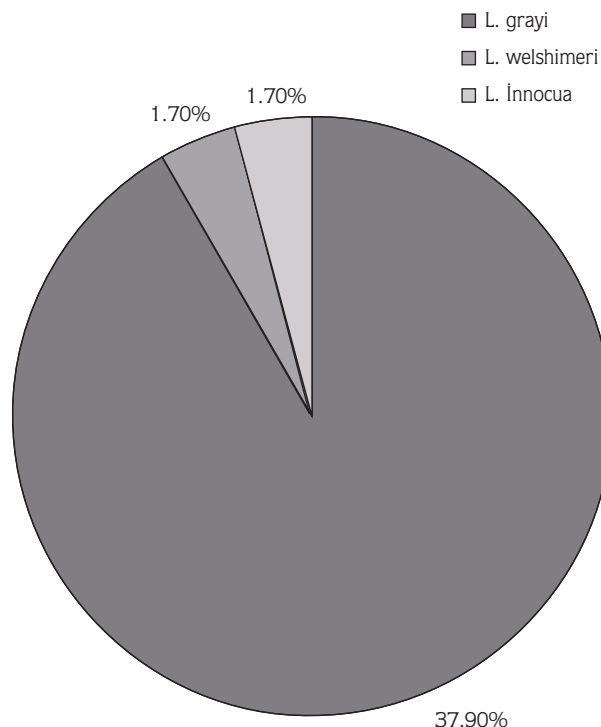


Figure 2. The distribution of isolated *Listeria* spp. from Kahramanmaraş and Adana.

In our country where studies on the prevalence of *Listeria* in ice cream are limited in number, a study conducted in Elazığ, in 1995 on the presence and distribution of *Listeria* in ice cream of the 73 samples studied 2 (%2.7) *Listeria monocytogenes*, 1 (1.4%) *Listeria innocua* and 1 (1.4%) *Listeria grayi* was found (13).

Ahrabi et al. (16), in a study conducted in 1998 investigated the presence of *Listeria* in 100 raw and 20 pasteurised milk samples from different regions of Anatolia. Whilst the presence of *Listeria monocytogenes* was found in 1% of the raw milk samples, it was not isolated from the pasteurised milk samples. In the same study, *Listeria innocua* was found in 5% and *Listeria seeligeri* in 8% of the raw milk samples. The total incidence of *Listeria* among the raw milk samples was 10% whilst that in the pasteurised milk samples it was found to be 5% (18).

In a similar study by Sharif et al. (19), where the incidence of *Listeria* in 77 raw and 22 pasteurised milk samples was investigated, the incidence of *Listeria monocytogenes* was 18.2% in the raw milk samples and negative for the pasteurised milk samples.

From the studies on the prevalence of *Listeria* species in milk and dairy products, it is seen that various *Listeria* species including *Listeria monocytogenes* have been isolated. Ünlü et al.(14), in their study on 100 raw milk samples of sheep, goats and cows collected from the Sivas area reported that in 6% of the milk *Listeria* were isolated and of these 4% was *Listeria monocytogenes* and 2% *Listeria innocua* (14).

However, in, 1994 100 raw milk samples collected from milk tanks in farms in and around İstanbul, the ratio of *Listeria monocytogenes* isolation was 4%, of *Listeria innocua* 9%, and of *Listeria welshimeri* 2% (20).

Yüce et al. (20), in another study carried out on raw milk samples collected from barns and farms in İzmir and surroundings however, found *Listeria monocytogenes* 2%, *Listeria murrayi* 1% and *Listeria dentrificans* 1% (21).

In Eastern Anatolia where livestock farming is widely practised, İnce et al. (22), with the aim of determining the presence of *Listeria monocytogenes* in raw milk and white cheese in Elazığ found 28% of the 72 raw milk samples obtained from the milk factory to be *Listeria* spp.

positive. Out of the examined 35 white cheese samples however, only 11% contained *Listeria monocytogenes* (22).

In another study in 1997 on raw milk and cheese from sheep and goats in Elazığ and its surroundings, *Listeria monocytogenes* could not be isolated. Of the isolated samples, 3% was *Listeria welshimeri*, and 1% *Listeria dentrificans* (23).

A look at studies on this topic in other countries, shows that in a factory dealing in frozen milk and dairy products in California, of the 922 samples taken from the different divisions of the factory, *Listeria* spp was isolated in 111 (12.04%) samples, and of the 39 examined factories *Listeria monocytogenes* was encountered, in only 5, whilst 13 of them contained *Listeria innocua*, in 9 of them both species were encountered, and in 12 factories no isolates were encountered (15).

Farber et al. (12), in a study in which 394 ice cream samples were examined, found the incidence of *Listeria monocytogenes* to be 0.25% (12).

Pak et al. (24), in a study on dairy products in 2002, found the incidence of *Listeria monocytogenes* to be 4.9%. In the same study, whilst the incidence of *Listeria monocytogenes* was 7.6%, Laciari et al. (25), in their study in Spain in 1999, investigating the presence of *Listeria* species in different food items, failed to isolate *Listeria* species in pasteurised milk, cheese and chocolate milk products but *Listeria welshimeri* was isolated from ice cream samples (25).

However Gaya et al. (26), in a study in Spain in 1996 investigated the presence of *Listeria* in the milk of sheep. In this study, whereas the incidence of *Listeria monocytogenes* was 2.56% and that of *Listeria innocua* as 1.73% the incidence of *Listeria seeligeri* and *Listeria ivanovii* was 0.07% and 0.21% respectively (26).

A study by Greenwood et al. (28), in England on milk and dairy products found the incidence of *Listeria monocytogenes* to be 8.2% (27).

In our study, the findings show the incidence of *Listeria* as 41.4% in our country, a rather high value

compared to values obtained from around the world. However, in our study *Listeria monocytogenes*, which is pathogenic to man, was not isolated. In studies conducted by Sharif et al. (19), and Ahrabi et al. (18), *Listeria monocytogenes* could not be isolated in samples of pasteurised milk in line with our findings. In a similar manner, Casedei et al. (28), in 1998 and Manifreni et al. (10), in 1993 in their studies on ice cream samples could not isolate the known pathogen *Listeria monocytogenes*. In 1999, Laciari et al. (25), in their study on ice cream samples reported in a similar manner that *Listeria monocytogenes* could not be isolated. These results are in conformity with our findings. However, Arslan et al. (13), in their study on ice cream found the incidence of *Listeria monocytogenes* to be 2.7%, whilst Faber et al. (12), reported an incidence of 0.25%, contrary to our results.

In conclusion, whereas *Listeria* species (*Listeria grayi*, *Listeria innocua* and *Listeria welshimeri*) were found to a large extent in the analysed ice cream samples, *Listeria monocytogenes* was not encountered. It should be mandatory to perform routine controls to screen ice cream samples for detection of *Listeria* spp.

It is pleasing that the known pathogen *Listeria monocytogenes* was not isolated in this study of ours. This condition demonstrates that consumption of ice cream made from the milk of sheep and goats and consumed by all and sundry does not pose a potential health hazard in our region.

The findings of this study suggest that *Listeria* infections are not a potential risk in Kahramanmaraş and Adana. It is strongly recommended however that these products are monitored carefully.

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