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RESEARCH ARTICLE

Investigations of *Listeria* Species in Milk and Silage Produced in Burdur Province [1]

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Summary

The aim of this study was to investigate the presence of *Listeria* species in the milk and silage samples obtained from fifteen different farms in Burdur. A total of 250 samples (silage and cow's milk obtained from animals fed and not fed with silage) were analyzed. *L. monocytogenes* was isolated in 6 (2.4%) out of the 250 samples. Five (6.66%) of the 75 silage samples and 1 (1.17%) of the 85 milk samples obtained from cows fed with silage were contaminated with *L. monocytogenes,* whereas no *Listeria* spp. were isolated from the 90 milk samples from cows not fed with silage. The isolation of *L. monocytogenes* from milk and silage samples in Burdur indicates that these products could create a serious risk to the public health.

Keywords: *Cow's milk, Silage, Listeria spp.*

Burdur Yöresinde Üretilen Süt ve Silajlarda *Listeria* Türlerinin Araştırılması

Özet

Bu çalışmada, Burdur yöresinde onbeş farklı çiftlikte üretilen süt ve silajlarda *Listeria* türlerinin varlığının araştırılması amaçlanmıştır. Toplam 250 örnek (silaj ile beslenen ve beslenmeyen inek sütleri ve silaj) analize alınmıştır. İki yüz elli örneğin 6'sında (%2.4) *L. monocytogenes* izole edilmiştir. Yetmiş beş silajın 5 (%6.66)'inde, 85 silaj verilen inek sütünün 1 (%1.17)'inde *L. monocytogenes* olduğu belirlenmiştir. Silaj ile beslenmeyen 90 inekten alınan sütlerde ise *Listeria* spp. izole edilememiştir. Sonuç olarak, Burdur'da üretilen süt ve silajlarda *L. monocytogenes*'in izole edilmesi bu ürünlerin halk sağlığı açısından bir risk oluşturabileceğini göstermektedir.

Anahtar sözcükler: *İnek sütü, Silaj, Listeria spp.*

INTRODUCTION

Listeria spp. are widely distributed in nature and found in soil, silage, decaying vegetation, animal feces, sewage water, and other environmental sources [1]. *Listeria monocytogenes* may contaminate milk because of mastitis, encephalitis, or abortion related to *Listeria* spp. in animals [1,2]. Listeriosis is a severe and often fatal illness with clinical manifestations such as sepsis or meningitis in immunocompromised patients or neonatal babies and flu-like illness or abortion during pregnancy in women. The major outbreaks of listeriosis have been associated with the consumption of foods of animal origin [3]. The genus *Listeria* contains 6 species: *L.*

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monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii, and *L. grayi* [4]. In addition to *L. monocytogenes, L. seeligeri* and *L. ivanovii* may be pathogenic in humans [1,5]. *L. monocytogenes* can frequently be isolated from unpasteurized milk and milk products [6-10]. Except in modern cheese production plants, raw milk is widely used in cheese production by small and medium domestic and commercial plants in Turkey. Also, isolation rate of *L. monocytogenes* in cheese samples in Turkey has been reported from 2% to 5% [6-8,11].

Silage is produced by harvesting a forage crop with a high moisture content (greater than 50%) and subsequently fermenting. In general, good silage remains stable, with no change in composition or heat, once air is eliminated and the silage has achieved a low pH [12]. *Listeria* spp. are most commonly recovered from improperly fermented silage [13,14]. It has been reported that listeriosis in cattle is mainly feed-borne [1] and *Listeria* spp. have been detected from 1.2% to 60% of the silage samples [15-17]. Also, *Listeria* spp. have been isolated from 2% to 6.1% milk samples from cows fed with silage [17]. In a study by Fenlon et al.[18] has been stated that 29-31% of cattle started to shed *L. monocytogenes* after silage feeding.

The aim of this study was to investigate the presence of *Listeria* species in the milk and silage samples obtained from fifteen different farms in Burdur. The milk obtained from cows fed and not fed with silage were compared in terms of *Listeria*, and its importance in contamination of silage was put forward.

MATERIAL and METHODS

Sampling

Five research centers in Burdur were determined for sampling. Three different farms in every research center were visited every month between December 2007 and May 2008. In fifteen farms, seventy five silage samples, 85 milk samples obtained from cows fed with silage and 90 milk samples obtained from cows not fed with silage were collected. The samples were collected in sterile plastic bags and transported to the laboratory in boxes containing ice.

Isolation and Identification of Listeria spp.

All procedures were applied according to the FDA-Bacteriological Analytical Manual [19]. All media used were obtained from Oxoid (Oxoid Ltd., Hampshire, UK). Each sample (25 g/ml) was taken and placed in a stomacher bag to which 225 ml of sterile *Listeria* Selective Enrichment Broth (Oxoid) was added and homogenized with a stomacher (Masticator, IUL Instruments-Spain) for 1-3 min and incubated at 30°C for 48 h. A loopful of homogenate was surface streaked in duplicate on Palcam agar (Oxoid) and Oxford agar (Oxoid). The Palcam plates were incubated at 37°C for 48 h under microaerophilic conditions and Oxford plates at 35°C for 48 h under aerobic conditions. All colonies surrounded by a brownish green and/or black halo were taken as possible *Listeria* spp. One suspected *Listeria* spp. colony from each plate was chosen and purified on tryptic soy agar (Oxoid CM 131) with 0.6% yeast extract (Oxoid L 21) and incubated at 30°C for 24-48 h for further biochemical characterization. Presumptive *Listeria* isolates were confirmed and identified at the species level based on Gram staining, typical umbrella motility in SIM medium (Oxoid CM 435), H_2S production, indole, urease, catalase, oxidase reaction, β-hemolysis, nitrate reduction, methyl-red/voges-proskauer (Oxoid CM 43), CAMP tests and fermentation of mannitol, L-rhamnose, D-ksilose, sorbitol, dextrose, maltose, esculin, dulcitol and salicin [4,20,21]. Serotyping of isolates was performed with Bacto-*Listeria*-O-antisera types 1 and 4 and poly (Difco Laboratories, Detroit, MI) by the slide agglutination test [4,21].

Measurement of pH Values of the Samples

After the samples were collected for microbiologic analysis, the pH values of the milk samples were measured with an electronic pH meter (Metrohm 704 pH Meter). A 25-g aliquot silage sample was blended with 100 ml of deionized water for 2 min and filtered through four layers of cheesecloth. Then the pH of the extract was measured [22].

Statistical Analysis: The results were analyzed using Minitab-15 with the chi-square analysis.

RESULTS

Overall, *L. monocytogenes* was found in 6 (2.4%) out of 250 samples. Five (6.66%) of the 75 silage samples and 1 (1.17%) of the 85 milk samples obtained from fed with silage were contaminated with *L. monocytogenes,* whereas no *Listeria* spp. were isolated from the 90 milk samples from cows not fed with silage. The differences between isolation rates of *L. monocytogenes* were statistically significant (χ^2=8.02; P=0.018; P<0.05) *(Table 1).* Two selective plating media Palcam and Oxford were compared for isolating *L. monocytogenes* from the samples, and the isolation rates from these media were found to be equal.

In the present study, the pH values of the milk samples varied between 6.6 and 7.1, and the pH values

Table 1. *The isolation rate of L. monocytogenes isolated from milk and silage* [a]

Tablo 1. *Süt ve silajlarda L. monocytogenes izolasyon oranı* [a]

Sample Type and Number (n)	*L. monocytogenes*	
	n	%
Silage (n: 75)	5	6.66
The milk of cows fed with silage (n: 85)	1	1.17
The milk of cows not fed with silage (n: 90)	-	-
Total (n: 250)	6	2.4

[a] *Chi-square statistic is significant, χ^2=8.02 ; P=0.018; P<0.05*

of the silage varied between 4.1 and 8.7. In the silage samples contaminated with *L. monocytogenes,* the pH values varied between 5.1 and 8.3, and the pH value of the milk sample contaminated with *L. monocytogenes* was 6.9.

As the collection period of the milk and silage samples was compared in isolation, the contamination of *L. monocytogenes* was found higher in March (3 silage samples) than in January (2 silage samples) and February (1 milk sample from the cows fed with silage).

In this study, for the serotype determination of 6 isolates defined as *L. monocytogenes* Difco Bacto O Antiserum type 1 and type 4, and type poly were used. The results were as follows: 5 isolates (1 milk and 4 silage samples) type poly and type 4, 1 isolate (1 milk sample) type poly.

DISCUSSION

The isolation rates of *Listeria* spp. in silage has been demonstrated in several studies carried out in Turkey and in other countries [15-17,23,24]. In this study, *L. monocytogenes* was detected in 6.66% of the 75 silage samples. This percentage is lower than the results reported by Oliveira et al.[25] and Grønstøl [26], but similar to the 6.1% obtained by Vilar et al.[17] In Turkey, Aslantaş and Yıldız [23] isolated *L. monocytogenes* from 1 of 11 silage samples. However, Şahin et al.[24] did not isolated *L. monocytogenes* from the silage, but isolated *L. welshimeri* and *L. grayi.* In this study, the low isolation rate of *L. monocytogenes* in silage may be accounted that high-quality silage is produced by mostly producers. However, in our study, silage samples contaminated with *L. monocytogenes* was obtained only from wet silage. In the illumination of this result, we cold say that and rainy weather conditions are the cause of this result.

Many researchers have investigated *L. monocytogenes* contamination of milk [6,9,11,23,27,28] and *Listeria* species have been detected from 0.40% to 10% of milk samples [6,9,11,23,29]. In Turkey, the isolation rates from raw milk samples have been reported 0.45% in İstanbul [7], 0.94% in Ankara [27], 1.20% in Van [6], 3% in West Anatolia [28] and 5% in Ankara [29]. In other countries, the reported isolation rates from bulk tank milk samples were 1.2% in Pennsylvania [30], 4.9% in Ireland [31] and 6.5% in the United States [10]. The sources of *Listeria* spp. in raw milk have been reported to be fecal [32] and environmental contamination during the milking, storage, and transport of infected cows on dairy farms, and poor silage quality [33].

In the present study, *Listeria* species were not found from cow's milk samples not fed silage. But, 1.17% of the milk samples obtained from cows fed with silage were contaminated with *L. monocytogenes.* However, Şahin et al.[24] have reported that *L. monocytogenes* was not isolated from the silage and milk samples of cows fed with silage, but *L. welshimeri* and *L. grayi* were isolated. Vilar et al.[17] detected *Listeria* spp. in 33.7% of silage samples and in 16.3% of milk samples. Donnelly [34] observed that 8 of 44 Holstein cows fed *Listeria*-contaminated silage shed the organism in their milk. Furthermore, milk from these animals was free of *L. monocytogenes* one month after feeding of contaminated silage ceased.

In our study, two selective plating media Palcam and Oxford were compared for isolating of *L. monocytogenes* from the samples, and the isolation rates from these media were found to be equal, which is consistent with the reports by Art and Andre [35], Capita et al.[36] and Uysal and Anğ [7].

L. monocytogenes has thirteen serotypes, but, only three serotypes-4b, 1/2a and 1/2b-are responsible for the majority of veterinary and human listeriosis cases [37]. In this study, for the serotype determination of 6 isolates defined as *L. monocytogenes,* O Antiserum type 1 and type 4, and type poly were used. The results were as follows: 5 isolates (1 milk and 4 silage samples) type poly and type 4, 1 isolate (1 milk sample) type poly. Van Kessel et al.[10] isolated *L. monocytogenes* from 56 (6.5%) of 861 bulk-tank milk samples, and serotyping of these isolates yielded 5 serotypes (1/2a, 1/2b, 3b, 4b, and 4c). Jayarao and Henning [38] reported isolating *L. monocytogenes* in 6 (4.6%) of 131 bulk-tank milk samples and all isolates of *L. monocytogenes* belonged to O antigen type 1.

Multiple studies have reported seasonal variations of *Listeria* spp. isolation, some report that contamination rates increase during the summer months [39], while others [40] reported increased rates during winter. Gaya et

al.[41] found that raw caprine milk contamination by *Listeria* spp. was seasonal; the incidence in the autumn (9.33%) and winter (5.14%) samples was higher than the incidence in the spring (0.85%) and summer (0.85%) samples. Uraz and Yücel [27] isolated 1 of the *L. monocytogenes* in winter whereas the other one was isolated in the spring season. Two factors may explain the increased isolation rate during March in our study: (1) March is usually very rainy in Burdur, and water is moisturised silage. Therefore, the quality silage is changed. (2) Seasonal differences in the incidence of *Listeria* spp. in raw milk may also be related to breeding practices. Dairy cattle typically bear their young in late winter or early spring. During winter gestation, dairy cattle develop a weakened immune system as a direct result of pregnancy, which, in turn, makes these animals more susceptible to listerial infections and abortions [42].

The pH values of the silage samples from which *Listeria* spp. were isolated ranged from 5.1 to 8.3. Different range from those observed in other studies were 3.8 to 5.2 in Rea et al.[31], 5.78 to 5.89 in Ryser et al.[16], and 4.47 to 6.97 in Vilar et al.[17]. A variety of studies have confirmed that *L. monocytogenes* contamination is most frequently associated with poor-quality silage [17]. Poorly fermented silage, which has a pH greater than 5.5, is ideal for *Listeria* growth [16,17]. However, Fensterbank et al.[43] identified *Listeria* spp, including *L. monocytogenes,* in 11 of 31 high-quality silage samples with pHs of 3.6 to 4.0. In our study, the pH value of the milk and silage samples contaminated with *L. monocytogenes* was greater than 6.6. We believe that the contamination sources of *Listeria* spp. are the consumption of bad-quality silage, subjected to inadequate fermentation, with pH values higher than 4.0, which allows the multiplication of *Listeria* spp.

As a conclusion, the isolation of *L. monocytogenes* from milk and silage samples in Burdur indicates that these products could create a serious risk to the public health and could have a potantial risk for animals. Correct practices with respect to silage production and milking are essential for preventing introduction of *Listeria* into the herd, its spread within the herd, and its entry into milk. The risk of contamination of milk by *Listeria* spp. increased when animals were fed low-quality silage, notably silage with pH ≥4.5. Although the contamination ratio is very low in this research, *Listeria* contamination must be obstructed or minimized to achieve standard conditions.

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