

The Presence of *Listeria* Species in Corn Silage and Raw Milk Produced in Southeast Region of Turkey

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Abstract

The objective of this study was to investigate the presence of *Listeria* species in the corn silage and raw milk samples. A total of 140 raw milk samples obtained from cows, sheeps and goats fed with silage and 90 corn silage samples collected from 10 dairy farms in South-Eastern Region of Turkey were analyzed for *Listeria* spp. In the result, *L. monocytogenes* and *L. innocua* were isolated from 2 (2.2%) and 5 (5.5%) silage samples and from 3 (2.1%) and 5 (3.5%) raw milk samples, respectively. The results indicates that these are a potential risk for animals and public health. Prevention of growth of *L. monocytogenes* in silage will also contribute to reduction of *Listeria* spp. in milk.

Keywords: *Listeria* spp., *L. monocytogenes*, Raw milk, Silage

Türkiye'nin Güneydoğu Bölgesi'nde Üretilen Mısır Silajı ve Çiğ Sütlerde *Listeria* Türlerinin Varlığı

Özet

Bu çalışmanın amacı, mısır silajında ve çiğ süt örneklerinde bulunan *Listeria* türlerinin varlığının araştırılmasıdır. Türkiye'nin Güneydoğu Bölgesi'nde bulunan 10 süt çiftliğinde mısır silajıyla yemlenen inek, koyun ve keçilerden elde edilen toplam 140 çiğ süt ve 90 mısır silajı örnekleri *Listeria* türleri bakımından analiz edildi. Silaj örneklerinin ikisinde (%2.2) ve çiğ süt örneklerinin üçünde (%2.1) *L. monocytogenes*, silaj örneklerinin beşinde (%5.5) ve süt örneklerinin de beşinde (%3.5) ise *L. innocua* izole edildi. Elde edilen sonuçlar, mısır silajları ve çiğ süt örneklerinin hayvan ve insan sağlığı açısından potansiyel bir risk taşıdığını göstermektedir. Silajlarda *L. monocytogenes*'in çoğalmasının önlenmesi, aynı zamanda sütlerdeki *Listeria*'ların azalmasına da katkıda bulunacaktır.

Anahtar sözcükler: *Listeria* spp., *L. monocytogenes*, Çiğ süt, Silaj

INTRODUCTION

Listeria spp. are widely distributed in the farm and in the industrial and human food environment (soil, plants, silage, faecal material, sewage and water) and they frequently contaminate foods [1,2]. Listeriosis is caused by generally *Listeria monocytogenes* and rarely by *Listeria ivanovii* in humans. It is well known that human listeriosis is largely attributable to foodborne transmission of *L. monocytogenes* [1-4]. In general, mild symptoms including headache, fever, diarrhoea and myalgia are seen in the majority of cases [5]. However severe symptoms including septicaemia, meningoenphalitis, abortion and stillbirth are also seen in humans and animals, primarily in certain

risk groups, such as, pregnant, new-borns, and immunocompromised individuals in the cases of invasive listeriosis [2,3].

The quality of silage depends on the competition between different groups of microorganisms. Lactic acid bacteria, responsible for the silage fermentation process, usually dominate the silage microflora. However a number of undesirable microorganisms existing at low levels on fresh plant materials may also grow during the storage of silage and lead to anaerobic or aerobic spoilage. Yeasts are generally responsible for the initiation of aerobic spoilage. These microorganisms oxidize the preservative acids present in silage. Then the pH rises and other aerobic microorganisms start to proliferate. This secondary aerobic



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spoilage flora consists of *Listeria*, moulds, bacilli and *Enterobacteriaceae*. The aerobic flora not only decreases the nutritional value of the silage, but also presents a risk to animal health and the quality and safety of milk [6,7].

An association between listeriosis and the feeding of silage to dairy cattle has been well documented, with most cases resulting from the consumption of low quality and improperly fermented silage with a pH of 4.0. Reports of bovine listeriosis from silage feeding and of subsequent asymptomatic shedding of *L. monocytogenes* in milk are of obvious concern to the dairy industry [8]. It has been reported that listeriosis in cattle is mainly feed-borne and *Listeria* spp. have been detected in a ratio of between 1.2% and 60% from the silage samples [9-11]. Furthermore, Taşçı et al. [12] reported that 6.6% of silage samples and 1.17% of milk samples obtained from cows fed with silage were contaminated with *L. monocytogenes*. Fenlon [13] has stated that 29-31% of cattle started to shed *L. monocytogenes* after silage feeding.

Therefore it is important to have information on the presence of this pathogen in milk and silage which may be a source of contamination and infection for animals and humans with *L. monocytogenes*. However, there are few local studies on the presence of this pathogen in the raw milk obtained from animals fed with silage and especially in silage in Turkey [12,14]. The aim of this study was to investigate the presence of *Listeria* spp. in the raw milks obtained from cows, sheeps and goats fed with silage and in silages produced in Southeastern Region of Turkey.

MATERIAL and METHODS

Sampling Procedure

Total 140 raw milk samples obtained from cows (50), sheeps (75) and goats (15) fed with corn silage and 90 corn silage samples were collected from 10 farms in Sanliurfa (6) and Adiyaman (4) regions in Turkey. Milk samples were taken from the bulk storage tanks in the same farms. Silage samples were taken from the surface, interior of silos and in manger. All samples were kept at 4°C until examination.

Isolation and Identification

Listeria spp were isolated according to standard method

recommended by Food and Drug Administration [15]. Twenty five gram silage or 25 mL of milk sample was homogenized in a stomacher with 225 mL *Listeria* Enrichment Broth (Oxoid, CM0862) supplemented with *Listeria* Selective Enrichment Supplement (Oxoid, SR0141) and incubated at 30°C for 48 h. A loopful of the enriched culture was streaked onto Oxford Agar (Oxoid, CM0856) supplemented with *Listeria* Selective Supplement (Oxoid, SR0140) and incubated at 35°C for 48 h. Five selected colonies were confirmed by streaking cultures onto Tryptone Soya Agar (Oxoid, CM0131) and testing isolated colonies for catalase production and for the following characteristics: tumbling motility at 25°C, carbohydrate fermentation (maltose, dextrose, mannitol, xylose and rhamnose), nitrate reduction, Methyl Red-Voges Proskauer reactions, umbrella motility in SIM medium at 25°C, β -hemolysis and Gram staining.

Physicochemical Analysis

A total of 25 g of fresh corn silage was macerated with 100 mL distilled water with a high-speed blender. The macerated silage samples were filtered through two layers of cheesecloth and the pH values of the filtrates were measured with a laboratory pH meter (Model 890, Nel Instruments Inc., Ankara, Turkey).

Statistical Analysis

Data were statistically analyzed by a one-way analysis of variance, and the means were compared by the Duncan's multiple-range test by using the software package [16].

RESULTS

The results of the study are presented in *Table 1* and *Table 2*. The analysis showed that 5.7% of raw milk and 7.7% of corn silage samples were contaminated with *Listeria* spp. *L. monocytogenes* and *L. innocua* were isolated from 2 (2.2%) and 5 (5.5%) silage samples and from 3 (2.1%) and 5 (3.5%) raw milk samples, respectively (*Table 1* and 2).

DISCUSSION

The presence of *Listeria* spp. in silage samples was examined by several studies [10-12,14]. In a study, *Listeria*

Table 1. The presence of *Listeria* spp. isolated from silage samples

Tablo 1. Silaj örneklerinde bulunan *Listeria* türleri

Parameters	Silage Interior	Silage Surface	Silage in Manger	Total
Number of samples	30	30	30	90
pH	4.05±0.16*	5.77±0.12*	5.57±0.04*	-
<i>L. monocytogenes</i>	nd**	1 (3.3%)	1 (3.3%)	2 (2.2%)
<i>L. innocua</i>	nd**	3 (10.0%)	2 (6.7%)	5 (5.5%)
Total	nd**	4 (13.3%)	3 (10%)	7 (7.7%)

* Different letters show significant differences between the rows ($P < 0.001$), ** nd: not detected

Table 2. The presence of *Listeria* spp. isolated from raw milk samples**Tablo 2.** Çiğ süt örneklerinde bulunan *Listeria* türleri

Parameters	Cow's Milk	Sheep's Milk	Goat's Milk	Total
Number of samples	50	75	15	140
<i>L. monocytogenes</i>	1 (2.0%)	2 (2.7%)	nd*	3 (2.1%)
<i>L. innocua</i>	nd*	5 (6.7%)	nd*	5 (3.5%)
Total	1 (2.0%)	7 (9.3%)	nd*	8 (5.7%)

* nd: not detected

spp. (*L. monocytogenes*, *L. innocua* and *L. welshimeri*) were isolated from 10% of corn silage samples, 28% of hay silage samples, and 60% of grass silage samples [10]. These values were higher than the results for *Listeria* spp. in silage samples obtained from our study (Table 1). In the same study [10], *L. monocytogenes* was isolated from 2.3% of corn samples and 2.6% of hay silage samples, which are similar with our results. Vilar et al. [11] detected *Listeria* spp. (*L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. grayi* and *L. seeligeri*) in 33.7% of total 83 grass and corn silage samples and *L. monocytogenes* in 6.0% of silage samples. Taşçı et al. [12] isolated *L. monocytogenes* in 6.66% of silage samples. These results on *L. monocytogenes* in silage samples are higher than the result obtained from our study. Şahin et al. [14] detected *Listeria* spp. (*L. welshimeri* and *L. grayi*) but not *L. monocytogenes* in silage samples. These authors have stated that the low prevalence of *L. monocytogenes* in silage samples may be attributed to the high-quality of the silage (as indicated by pH \leq 4.0). It has been reported that *L. monocytogenes* rapidly disappear under strictly anaerobic conditions and at a pH value lower than 4.4 [7]. Therefore the growth and survival of *Listeria* spp. in silage depends on the degree of anaerobiosis and on the pH value of the silage. The results obtained by our study on the *Listeria* spp. in interior, manger and surface silage samples (Table 1) support this suggestion. Şahin et al. [14] indicated an inhibitory effect of the cold seasons on the growth of *L. monocytogenes*.

In present study, *L. monocytogenes* was detected in one milk sample (2.0%) obtained from cows and two milk samples (2.7%) obtained from sheep, while in goat's milk samples *L. monocytogenes* was not detected (Table 2). Soyutemiz et al. [18] have found three positive samples (3%) from 100 raw milk samples for *L. monocytogenes* in West Anatolia. Sağun et al. [17] determined that 2.4% of raw milk samples obtained in Van province located in eastern Turkey, were positive for *Listeria* spp., where *L. monocytogenes* was found in 1.2%, *L. innocua* and *L. welshimeri* in 0.4% of those samples. Vilar et al. [11] detected *Listeria* spp. in 16.3% of bulk-tank milk samples, where *L. monocytogenes* was found in 6.1%, *L. innocua* in 7.1%, *L. welshimeri* in 1.0% and *L. grayi* in 2.0% of those samples. They emphasized a relationship between low silage quality by high pH and high prevalence (33.7%) of *Listeria* spp. in silage. In a study [12], *L. monocytogenes* was not found in milk samples obtained from cows not fed with silage,

however, *L. monocytogenes* was isolated from 1.17% of milk samples obtained from cows fed with silage. In another study [14], *L. welshimeri* and *L. grayi* were isolated from milk samples obtained from cows fed with silage, whereas *L. monocytogenes* was not isolated in the milk samples. The authors have reported that *Listeria* spp. began to be seen in the milk samples together with the occurrence of *Listeria* spp. in silage samples. On the other hand, Aygun and Pehlivanlar [21] found one (2.12%) positive sample for *L. ivanovii* and *L. grayi* among 47 raw milk samples. Faecal or environmental contamination during milking, storage and transport, infected cows in dairy farms and poor silage quality have been reported [7,14,17,19-21] as contamination sources of *Listeria* spp. to raw milk. It was reported that the poor quality silage is one of the primary sources of contamination of raw milk by *L. monocytogenes* which presents a serious risk to the quality and safety of milk and animal health [7]. In present study, the contamination of raw milk samples with *Listeria* spp. may be due to the reasons mentioned above, especially poor quality silages.

Infection of animals with *L. monocytogenes* has been associated most frequently with poor-quality silage [11,12]. Low-quality silage with a pH value higher than 5.5 supports the growth of *Listeria* spp. [10]. In our study, the pH values of the *Listeria* spp.-positive silage samples ranged from 4.05 to 5.77 and the pH value of the silage samples contaminated with *L. monocytogenes* was higher than 5.5. In other studies, pH values were reported between 5.1 to 8.3 by Taşçı et al. [12], 3.8 - 5.2 by Rea et al. [22], <4 - 5.89 by Ryser et al. [10] and 4.47 - 6.97 by Vilar et al. [11]. However, Ryser et al. [10] could not identified *L. monocytogenes* in 3 *Listeria* spp.-positive grass silage samples were all of poor quality, ranging in pH from 5.78 to 5.89. It could say that one of the contamination sources of *Listeria* spp. was the consumption of low-quality silage with pH values higher than 5.5 by milking animals in parallel with studies by Driehuis and Oude Elferink [7], Vilar et al. [11] and Taşçı et al. [12].

In conclusion, the isolation of *L. monocytogenes* from corn silage and raw milk examined by this study indicates that these are a potential risk for animals and public health. To prevent the growth of the bacteria in silage it is important the controll of the silage fermentation process with a low pH value (<5.5). Application of special cultures of lactic acid bacteria or chemical additives can aid silage fermentation and improve aerobic stability of silage.

Prevention of growth of *L. monocytogenes* in silage will contribute to reduction of *Listeria* spp. in milk. Infected animals in dairy farms should be insulated, the milking animals not fed with poor-quality silage and the hygienic conditions should be improved in order to minimize the contamination risk of *L. monocytogenes* in raw milk during the milking, storage and transport process in farm and dairy plant.

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