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SHORT COMMUNICATION

Isolation of *Listeria monocytogenes* from milks used for Iranian traditional cheese in Lighvan cheese factories

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Abstract

Traditional Lighvan cheese is a semi-hard cheese which has a popular market in Iran and neighboring countries. The aim of this study was evaluating the contamination of milks used for Lighvan cheese making with *Listeria monocytogenes*. Raw milk samples were randomly collected from different cheese producing factories (sampling carried out from large milk tanks used cheese making in factories). Isolation of *L. monocytogenes* was performed according to ISO 11290 and biochemical tests were done to identify and confirm *L. monocytogenes*. 9 samples (50%) of the 18 collected samples from milk tanks in Lighvan cheese producing factories were contaminated with *L. monocytogenes*. The concentration of *L. monocytogenes* in all 9 positive samples was 40 CFU/ml. This study is the first report of *L. monocytogenes* contamination in raw milks used for Lighvan cheese production in Iran. Regarding the fact that these cheeses are produced from raw milk and no heating process is performed on them its milk contamination can be a potential risk for consumers.

Key words

Listeria monocytogenes, raw milk, Lighvan cheese, Iran

INTRODUCTION

Listeria monocytogenes is an important bacterium for human and animals that causes public health problems. Listeriosis is an infection that can occur when a person eats foods (such as milk and dairy products, meat, egg, fruit and vegetable) that has been contaminated with *L. monocytogenes* [1]. The case fatality rate of Listeriosis in humans is between 20 to 30 percent that this rate reaches to 75% in high risk groups such as pregnant women, neonates and immune-compromised individuals [2]. Listeriosis outbreaks following the consumption of contaminated foods is usually associated with high case fatality rate and increase the concern about monitoring of this pathogen in foods [3]. It is shown that most human listeriosis outbreaks are linked to consumption of milks and dairy products [4]. With regard to the point that *L. monocytogenes* is able to survive and grow in different environments (different temperatures, salty, active water and osmotic conditions) [5], more attention should be paid to this pathogen in foods especially raw foods and productions that are made up of raw foods [4].

Traditional Lighvan cheese is a semi-hard cheese which has a popular market in Iran and neighboring countries [6]. This cheese is produced from raw sheep's milk or a mixture of raw sheep and goat milks without added starter in Lighvan region in East Azarbaijan province (Northwest of Iran). Annually, at this region, nearly 3150 tons of Lighvan cheese is produced [6]. The Optimum flavor of Lighvan cheese is attributed to its natural flora [7]. Since the Lighvan cheese is produced from unpasteurized raw milk and during its production there is no heating process [6], pathogenic bacteria may also grow and survive with this decline in these cheeses. Although there is a lot of evidence about the contamination of milks and dairy products by *L. monocytogenes* in the worldwide, there is no data about the contamination of Lighvan cheese and milks that are used for its production. So the aim of this study was evaluating the *L. monocytogenes* contamination of milks used for cheese making in Lighvan cheese factories.

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MATERIALS AND METHODS

Samples Collection. In Lighvan region near to Tabriz city (center of East Azarbaijan province, Northwest of Iran) there are about 50 factories producing Lighvan traditional cheese. Milk samples were randomly collected from different factories (sampling carried out from large milk tanks used cheese making in factories) in the zone of Lighvan, and were quickly transferred to the laboratory of food microbiology in faculty of veterinary medicine, Tabriz University.

Isolation and identification. Isolation of *L. monocytogenes* was performed according to ISO 11290. From each sample, two separate cultures were taken. For identification and confirmation of L. monocytogenes were used Gram staining and biochemical tests such as Catalase, Oxidase, Nitrate reduction, Methyl Red/Voges-Proskauer (MR/VP), hydrolysis of Esculin, Citrate test, fermentation of glucose, mannitol, α-methyl-D-mamoside and rhamnose, motility at 25 °C and 37 °C, gas production, β- hemolytic activity and CAMP tests [8].

Bacterial enumeration. Simultaneously with isolation of bacteria, enumeration of the *L. monocytogenes* in milk samples was performed using serial dilution and culturing

in PALCAM Listeria selective agar containing supplement (Merck – Germany). *L. monocytogenes* colonies were counted after incubation at 30 °C for 48h.

RESULTS

This study showed that nine samples (50%) of the 18 collected milks samples were contaminated with *L. monocytogenes*. The concentration of *L. monocytogenes* in all 9 positive samples was 40 CFUL/ml (Tab. 1).

Table 1. Concentrations (CFU/ml) of *L. monocytogenes* in all 9 positive milk samples

No of sample	CFU/ml
4	70
5	30
6	80
8	10
10	20
13	20
14	60
17	40
18	30
Mean	40

DISCUSSION

This study is the first report of *L. monocytogenes* contamination in raw milk used for Lighvan cheese production in Iran. The rate of contamination in this study (50%) was higher than of raw milk contamination levels in other studies in Iran, as in these studies the rate of contamination was 4% in northeast of Iran (Mashhad) [9], 2% in South of Iran (Noor Abad city) [10] and 1.6% in West of Iran (Shahr-e-Kord city) [11]. Also, in a study of the Isfahan city (central of Iran) in 2010, the rate of *L. monocytogenes* contamination in raw milk of sheep and traditional cheeses was 6 and 15% respectively [5]. The rate of *L. monocytogenes* contamination in raw milk also has been less than 5% in different studies in other countries [12, 13].

Regarding the fact that this type of cheese have produced from raw milk Without any heating process on them, so this is considered as a potential risk for consumer health [14]. If the raw milks used to produce unpasteurized cheeses like Lighvan cheese are contaminated with *L. monocytogenes* and other pathogen agents as in our study, this contamination may lead to an outbreak of listeriosis in humans through the consumption of these cheeses [4]. Although it is possible that storage of Lighvan cheese in high salt water (12%) and long-term maintenance (3–4 months) of this cheese before delivering to the consumers may partly cover this defect [6, 15] and affect the survival of pathogens, no studies has been conducted about this context yet.

One limitation of this study was the low number of samples, and more samples is required for this region to have a clear view about the current state of the contamination of milks used for Lighvan cheese production. This study shows that manufacturers of cheese processing should be continuously monitored and sanitary policies applied. It is recommended that complementary studies be conducted to investigate the contamination status of milks and Lighvan cheeses with *L. monocytogenes* and other food-borne pathogenic bacteria and based on the results suitable strategies should be taken in this case.

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