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Prevalence of *Listeria monocytogenes* in traditional cheeses obtained from food sale centers of Tehran, Iran

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ABSTRACT

The bacteria *Listeria monocytogenes* can contaminate food and cause disease, not just in underdeveloped, but also in developed countries. Cheese is one of the dairy products that can cause *listeriosis* if it is consumed with contaminated milk. Sixty samples of traditional unpasteurized white cheese weighing approximately 100 g were obtained from sales centers in Tehran for three consecutive weeks. Then, 25 g of each weighed sample was added to 225 ml of initial enrichment medium and incubated at 30°C for 24 h. In the next step, they were cultured in Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol medium and incubated at 35°C for 24 h. The growth and non-growth of *Listeria monocytogenes* bacterial colonies were examined, and additional tests were performed. The results of this study showed that out of 60 traditional cheese samples that were examined, based on the results of culture and supplementary tests, one cheese sample (1.6%) was infected with *Listeria monocytogenes*. In general, our study showed a lower level of contamination by *Listeria monocytogenes* compared to other studies. Iranian cheeses can be found in many varieties and there are differences in processing traditional cheese in different regions. Thereby, continuous monitoring is required.

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1. Introduction

Human beings are trying to increase food production because the population is growing (1). Due to the growth of mass production in food and food hygiene, bacteriologists are now discovering previously unknown aspects of this science. Bacterial contaminants in food are one of the aspects of food hygiene control.

These contaminants can cause disease (2). Due to cultural poverty and low levels of health care, the number of foodborne illnesses in the Third World is higher than in developing countries (3). *Listeria monocytogenes* is one of the pathogens that causes food contamination and subsequent disease, both in developed and developing countries (4).

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Pasteurization cannot eliminate *L. monocytogenes*, because contamination can occur after processing (5). The Psychrotrophic property of this microorganism is another reason for the importance of food contamination to this bacterium because it survives in the refrigerator and grows and multiplies (9).

A bacterium cannot cause disease in a healthy individual and can only be harmful if certain conditions are met (5). Decreased levels of the immune system, which can occur during pregnancy, diseases of the immune system, and long-term use of certain drugs, can allow pathogens to thrive (6,7,8).

On the other hand, milk and dairy products have traditionally been used as sources of protein (10). Cheese is one of the milk products that, if not contaminated and heated insufficiently before production, can cause the spread of *Listeria* sporadic or epidemic (11,12). *Listeria* genus contains small, coccobacilli, and gram-positive bacteria which rotate at room temperature and do not produce endospores. Catalase-positive, oxidase-negative organisms with a fermented glucose metabolism without gas production and acid production (13).

Listeriosis causes diseases such as; Neonatal listeriosis, meningitis, meningoencephalitis, pneumonia and septicemia of the *Listeria* and cutaneous and ocular forms (13). This study aimed to investigate the prevalence of Listeria monocytogenes in traditional cheeses of Tehran, Iran.

2. Materials and methods

During this study, 60 samples of traditional unpasteurized white cheese were collected from dairy sales centers and supermarkets in Tehran for three consecutive weeks. The samples, which weighed approximately 100 g, were transferred to the food quality control laboratory of the Faculty of Veterinary Medicine, University of Tehran, along with ice. For culture and isolation of *Listeria monocytogenes* from the samples taken, in this study, a modified Canadian method provided by the FDA was used. In this way:

After transferring the samples to the laboratory, 25 g of each weighed sample was added to the initial enrichment medium next to the flame. In this method, Listeria enrichment broth medium (containing 37.5 g / l potassium thiocyanate and 50 µg / l nalidixic) was used as the primary enrichment medium. 225 ml of *Listeria* enrichment broth was poured into 500 ml bottles (these glass jars had already been sterilized in an autoclave) and 25 g of the previously weighed sample was added to the enrichment medium.

The medium was incubated for 30 h at 30°C to incubate at this temperature. After this step, one of the media that was incubated for 24 h on the selected listeria medium, which is modified in the Canadian method, this medium is PALCAM, LSA), was cultured linearly under the flame and under sterile conditions, and this medium was The incubator was transferred to 35°C for 24 h and then the growth and non-growth of Listeria monocytogenes colonies were examined on them and then the following additional tests were performed on all suspected black colonies.

2.1. Wet Mount Test

In this experiment, the rotational motion of the organism was observed with a phase microscope at 1000 times magnification.

2.2. Tumbling Motion test

The motion test was performed at 25 and 35°C, but the bacterium showed umbrella-like motion only at 25°C.

2.3. Fermented sugar test

This bacterium was only able to ferment rhamnose among the sugars of rhamnose, xylor and mannitol.

Then, the positive samples were serotyped in the microbiology department of the Faculty of Veterinary Medicine, University of Tehran, and using Difco antigen. To count *Listeria monocytogenes* in suspicious samples, 25 g of positive *Listeria monocytogenes* samples were prepared from 10-slice serials and then, using MPN method, five practical counting tubes were performed using the selected *Listeria* broth media, which was described before.

3. Results

Out of 60 samples studied, only one sample was positive, indicating a ratio of 1.6%. The results are available in Table 1.

4. Discussion

In 2020, in Belgium, out of 134 samples of traditional cheese, only 2 cases (1.49%) of cheese made from two factories with raw milk were reported to be infected with *Listeria monocytogenes* (14). In Qazvin province, from autumn 2017 to summer 2018, 128 samples of traditional cheeses were randomly purchased and 14 samples (10.9%) of traditional cheeses were positive for *Listeria monocytogenes*, of which white cheeses were the most contaminated (7). Also, the highest infection rate was in summer and winter (3.1%). It was concluded that the presence of *Listeria monocytogenes* in traditional cheeses in Iran has been proven, and food safety officials and officials in Iran should develop an effective method to test for the presence of *Listeria* in foods because people who eat traditional cheese are exposed to it and there is a risk of becoming ill (15).

In Tabriz province, in 1396 (SC), a study was conducted to investigate the extent of contamination of traditional cheeses with *Listeria* species. Out of 111 samples of traditional cheeses in Tabriz region, 12 samples were contaminated with *Listeria* species. Seven cases were related to *Listeria Ivanovi* and 5 cases were related to *Listeria monocytogenes*, however, all these results were isolated in the secondary enrichment stage, which indicates the low number of this bacterium in the traditional cheese samples of Tabriz (16).

During a study conducted in Iran in 1997-2008, 30 cheese factories were randomly selected and sampled three times at three months distance from each factory depending on the amount of production and sent to the Microbiology and Food Chemistry Laboratory. This study was transferred to the Institute of Nutrition and Food Industry Research. Chemical tests included pH, moisture content and salt content. In this sampling, 3 samples of white cheese, 3 samples of pizza cheese, 1 sample of gouda cheese, 1 sample of smoked cheese, 1

Table 1. 5 tubes each with 0.1, 0.01 and 0.001 g MPN and 95% confidence interval

Pos. Tubes			MPN/g	Conf. lim		Pos. Tubes			MPN/g	Conf. lim	
0.1	0.01	0.001		Low	High	0.1	0.01	0.001		Low	High
0	0	0	1.8 <	-	6.8	4	0	2	21	6.8	40
0	0	1	1.8	0.09	6.8	4	0	3	25	9.8	70
0	1	0	1.8	0.09	6.9	4	1	0	17	6	40
0	1	1	3.6	0.7	10	4	1	1	21	6.8	42
0	2	0	3.7	0.7	10	4	1	2	26	9.8	70
0	2	1	5.5	1.8	15	4	1	3	31	10	70
0	3	0	5.6	1.8	15	4	2	0	22	6.8	50
1	0	0	2	0.1	10	4	2	1	26	9.8	70
1	0	1	4	0.7	10	4	2	2	32	10	70
1	0	2	6	1.8	15	4	2	3	38	14	100
1	1	0	4	0.7	12	4	3	0	27	9.9	70
1	1	1	6.1	1.8	15	4	3	1	33	10	70
1	1	2	8.1	3.4	22	4	3	2	39	14	100
1	2	0	6.1	1.8	15	4	4	0	34	14	100
1	2	1	8.2	3.4	22	4	4	1	40	14	100
1	3	0	8.3	3.4	22	4	4	2	47	15	120
1	3	1	10	3.5	22	4	5	0	41	14	100
1	4	0	11	3.5	22	4	5	1	48	15	120
2	0	0	4.5	0.79	15	5	0	0	23	6.8	70
2	0	1	6.8	1.8	15	5	0	1	31	10	70
2	0	2	9.1	3.4	22	5	0	2	43	14	100
2	1	0	6.8	1.8	17	5	0	3	58	22	150
2	1	1	9.2	3.4	22	5	1	0	33	10	100
2	1	2	12	4.1	26	5	1	1	46	14	120
2	2	0	9.3	3.4	22	5	1	2	63	22	150
2	2	1	12	4.1	26	5	1	3	84	34	220
2	2	2	14	5.9	36	5	2	0	49	15	150
2	3	0	12	4.1	26	5	2	1	70	22	170
2	3	1	14	5.9	36	5	2	2	94	34	230
2	4	0	15	5.9	36	5	2	3	120	36	250
3	0	0	7.8	2.1	22	5	2	4	150	58	400
3	0	1	11	3.5	23	5	3	0	79	22	220
3	0	2	13	5.6	35	5	3	1	110	34	250
3	1	0	11	3.5	26	5	3	2	140	52	400
3	1	1	14	5.6	36	5	3	3	180	70	400
3	1	2	17	6	36	5	3	4	210	70	400
3	2	0	14	5.7	36	5	4	0	130	36	400
3	2	1	17	6.8	40	5	4	1	170	58	400
3	2	2	20	6.8	40	5	4	2	220	70	440
3	3	0	17	6.8	40	5	4	3	280	100	710
3	3	1	21	6.8	40	5	4	4	350	100	710
3	3	2	24	9.8	70	5	4	5	430	150	1100
3	4	0	21	6.8	40	5	5	0	240	70	710
3	4	1	24	9.8	70	5	5	1	350	100	1100
3	5	0	25	9.8	70	5	5	2	540	150	1700
4	0	0	13	4.1	35	5	5	3	920	220	2600
4	0	1	17	5.9	36	5	5	4	1600	400	4600
						5	5	5	>1600	700	-

Sample of blue cheese, and 1 sample of buttercup were tested, all of which were negative in terms of contamination with this bacterium (13).

5. Conclusion

There was a much lower level of contamination in this study than the level of contamination of traditional and unpasteurized cheeses elsewhere in the world, perhaps due to a lack of diversity in our country and this study in particular. There is a climate difference between Iran and the countries as well as the cheese consumed there. Furthermore, in comparison with developed countries, less research has been done on the contamination of other foods in our country, and because food contamination with *Listeria monocytogenes* and the occurrence of sporadic substances and even widespread cases of *Listeria monocytogenes* constitute a major health concern. More extensive and comprehensive research should be conducted on the contamination of other foods with this bacteria.

Conflict of interest

The authors declare that there is no Conflict of interest.

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