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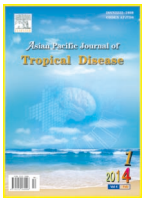

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Incidence of *Listeria* species in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay

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PEER REVIEW

Peer reviewer

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Comments

An extensive survey was carried out to detect the presence of *Listeria* spp.-both live bacteria and DNA-in different types of raw milk in Iran. Results are interesting in terms of the types of milk considered and the variation in levels of the pathogen detected by PCR in milk from different animal species.
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ABSTRACT

Objective: To determine the prevalence rate of *Listeria* species in bovine, ovine, caprine, camel and water buffalo milk in Iran.

Methods: From September 2010 to December 2011 a total of 260 bulk milk samples including 85 bovine, 37 camel, 34 water buffalo, 56 ovine and 48 caprine bulk milk samples were collected from commercial dairy herds, in Fars and Khuzestan provinces, Iran and were evaluated for the presence of *Listeria* species using cultural method and the PCR assay.

Results: Using cultural method, 19 samples (7.3%) were positive for *Listeria* spp. The highest prevalence of *Listeria* was found in raw water buffalo milk (11.8%), followed by raw bovine milk (10.6%), raw ovine milk (7.1%), and raw caprine milk (4.2%) samples. All 37 camel milk samples from 20 camel breeding farms were negative for *Listeria* spp. The overall prevalence of *Listeria* was 7.3%, in which *Listeria innocua* was the most recovered species (4.2%); the remaining isolates were *Listeria monocytogenes* (1.9%), *Listeria ivanovii* (0.08%) and *Listeria seeligari* (0.04%). The PCR assay could identify 8 *Listeria*-contaminated milk samples that were negative using the cultural method.

Conclusions: The results presented in this study indicate the potential risk of infection with *Listeria* in people consuming raw and unpasteurized milk.

KEYWORDS

Listeria spp., Milk, Ruminant, Foodborne pathogens

1. Introduction

The genus *Listeria* comprises six species: *Listeria monocytogenes* (*L. monocytogenes*), *Listeria innocua* (*L. innocua*), *Listeria ivanovii*, *Listeria welshimeri*, *Listeria seeligeri* (*L. seeligeri*) and *Listeria grayi*. Among the genus of *Listeria*, which cause the infection of listeriosis in both animals and man, *L. monocytogenes* is a major pathogenic microorganism, and bacterium *L. ivanovii* is rarely pathogenic for humans[1,2]. *L. monocytogenes* is an intracellular bacterium that has the capability to infect a range of cell types, including professional phagocytes and non-phagocytes (*e.g.* epithelial cells, endothelial cells, hepatic cells and fibroblasts), and to cross the intestinal, blood-brain and placental barriers. Due to its widespread nature and its ability to tolerate wide pH, temperature and salt ranges, *L. monocytogenes* readily enters food processing facilities and survives and grows in a variety of food stuffs

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such as milk, seafood, vegetables and meat products[3,4].

Human listeriosis is a sporadic disease, which is associated with consumption of contaminated milk, soft cheese, under-cooked meat, and unwashed raw vegetables and cabbage[5-7]. In human, the illness may range from mild flu-like sickness to severe manifestations (encephalitis, meningitis, septicemia, abortion, premature birth, stillbirth, and abscesses). Groups at highest risk are pregnant women, neonates, adults with underlying disease (cancer, AIDS, diabetes, chronic hepatic disorder, transplant recipients), the elderly and other immunocompromised individuals[1].

The importance of raw milk and dairy products as a vehicle for the transmission of various diseases has been documented; especially in countries where hygienic standards are not strictly enforced[8]. Milk and dairy products are two specific food categories with respect to the risk assessment for listeriosis. Currently there is limited information regarding the prevalence of *Listeria* spp. in foods in Iran. Therefore, the present study was undertaken to determine the prevalence rate of *Listeria* strains in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay in Fars and Khuzestan provinces, Iran.

2. Materials and methods

2.1. Collection of samples

Bovine, ovine, caprine, camel and water buffalo herds were randomly selected in Fars and Khuzestan provinces, Iran. These provinces are located in the southern part of Iran. From September 2010 to December 2011 a total of 85 bovine (Holstein cows), 37 camel and 34 water buffalo bulk milk samples were collected from 41, 20 and 16 commercial dairy herds, respectively. From March to April 2011 a total of 56 ovine bulk milk samples were collected from 25 sheep breeding farms and from September to October 2010 a total of 48 caprine bulk milk samples were collected from 20 goat breeding farms. The samples were immediately transported to the laboratory in a cooler with ice packs and were processed within an hour of collection. The samples were analyzed on the day they were collected.

2.2. Isolation and identification of Listeria

Twenty-five grams of each sample was aseptically taken, blended for 2 min in 255 mL of *Listeria* enrichment broth (UVM I) (Merck, Germany) and incubated at 37 °C for 24 h. One millilitre of primary enrichments were transferred to 9 mL of Frazer broth (UVM II) (Merck, Germany) and incubated at 37 °C for 24 h. Secondly enrichments were streaked onto Oxford agar (Merck, Germany) and Palcam agar (Merck, Germany) and incubated at 35 °C for 48 h. The plates were examined for *Listeria* colonies (black colonies with black sunken) and at least 3 suspected colonies were subcultured on Trypton Soy agar supplemented with 0.6% of yeast extract (Merck, Germany) and incubated at 37 °C for 24 h. All the isolates were subjected to standard biochemical tests including Gram staining, catalase test, motility test at 25 °C and 37 °C, acid production from glucose, manitol, rhamnose, zylose, α-methyl-D-mamoside, and nitrate reduction, hydrolysis of esculin, MR/VP test, β-hemolytic activity, and CAMP test[7].

2.3. DNA extraction and PCR condition for detection of Listeria spp.

DNA from a total of 260 milk samples was extracted from *Listeria* broth after the enrichment step using a genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's protocol. All oligonucleotide primers were obtained from a commercial source (CinnaGen, Iran). DNA amplification was performed in a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany). The amplification conditions, reagents and primers for identification of *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *Listeria welshimeri*, and *Listeria grayi* for the PCR assays were those described by Rahimi *et al*[9]. PCR products were analyzed by agarose gel electrophoresis and the specific DNA bands were visualized using ethidium bromide staining under UV illumination.

2.4. Statistical analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a *Chi*-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

3. Results

In the present study, a total of 260 bulk milk samples from 122 dairy bovine, ovine, caprine, camel and water buffalo herds in Fars and Khuzestan provinces of Iran were tested for *Listeria* spp. using cultural method and the PCR assay. Using cultural techniques, in total, 9 of 85 (10.6%) bovine milk samples and 4 of 34 (11.8%) water buffalo milk samples, 4 of 56 (7.1%) ovine bulk milk samples and only 2 of 48 (4.2%) caprine bulk milk samples were positive. All 37 camel milk samples from 20 camel breeding farmes were negative for *Listeria* spp. The most *Listeria* species isolated was *L. innocua* (4.2%). The remaining isolates were *L. monocytogenes* (1.9%), *L. ivanovii* (0.4%) and *L. seeligeri* (0.8%). Other species of *Listeria* were not isolated in this study (Table 1).

Overall, 27 milk samples were positive for *Listeria* spp. using the PCR assay (Table 2). The PCR assay could identify

Table 1
Prevalence of *Listeria* spp. in bovine, ovine, caprine, camel and water buffalo milk in Iran using cultural method.

Type of milk	No. of samples	No. (%) of *Listeria* spp.	No. (%) of *L. monocytogenes*	No. (%) of *L. innocua*	No. (%) of *L. ivanovii*	No. (%) of *L. seeligeri*
Bovine	85	9 (10.6)	3 (3.5)	5 (5.9)	1 (1.2)	–
Ovine	56	4 (7.1)	1 (1.8)	3 (5.4)	–	–
Caprine	48	2 (4.2)	1 (2.1)	1 (2.1)	–	–
Camel	37	0 (0.0)	–	–	–	–
Water buffalo	34	4 (11.8)	–	2 (5.9)	–	2 (5.9)
Total	260	19 (7.3)	5 (1.9)	11 (4.2)	1 (0.4)	2 (0.8)

Table 2
Prevalence of *Listeria* spp. in bovine, ovine, caprine, camel and water buffalo milk in Iran using PCR assay.

Type of milk	No. of samples	No. (%) of *Listeria* spp.	No. (%) of *L. monocytogenes*	No. (%) of *L. innocua*	No. (%) of *L. ivanovii*	No. (%) of *L. seeligeri*
Bovine	85	12 (14.1)	5 (5.9)	6 (7.1)	1 (1.2)	–
Ovine	56	7 (12.5)	2 (3.6)	4 (7.1)	1 (1.8)	–
Caprine	48	3 (6.3)	1 (2.1)	1 (4.2)	–	–
Camel	37	0 (0.0)	–	–	–	–
Water buffalo	34	5 (14.7)	1 (2.9)	2 (5.9)	–	2 (5.9)
Total	260	27 (10.4)	7 (2.7)	13 (5.0)	2 (0.8)	2 (0.8)

8 *Listeria*-contaminated milk samples that were negative using the cultural method.

4. Discussion

In this study, a low prevalence of *Listeria* spp. (7.3%) and *L. monocytogenes* (1.9%) was found in raw milk samples. This result is in agreement with the results reported by some authors. Moshtaghi and Mohamadpour examined 500 samples of raw cow milk obtained from the Milk Industry Foundation, five private dairy companies, and individual dairy farms in Shahrekord, Iran[10]. Of the analyzed samples, 8 (1.6%) were contaminated with *L. monocytogenes*, and 3 (0.6%) with *L. innocua*. Similarly, in a study in Isfahan, Iran, Rahimi *et al.* reported that 10 of 90 bovine (11.1%), 14 of 62 sheep (22.6%), 4 of 60 goat (6.7%) and 1 of 48 camel (2.1%) raw milk samples were positive for *Listeria* spp., in which 6 samples (20.7%) were positive for *L. monocytogenes*, 21 (72.4%) samples were positive for *L. innocua* and 2 samples (6.9%) were positive for *L. innocua*[9]. In contrast to our finding, results of another study in Iran indicate that all of raw cow milk samples were free from *Listeria* spp[11]. Vilar *et al.* found 3 (6.1%) positive samples from 98 bulk tank milk samples for *L. monocytogenes*[12]. In another study in Turkey, the incidence of *Listeria* spp., *L. ivanovii* and *Listeria grayi* was found to be 2.1% in 80 raw milk samples from Ankara[7]. In that study, *L. monocytogenes* was isolated in 1% and 5% of the raw milk samples and pasteurized milk samples, respectively. Of 1 300, raw milk samples from bulk tanks at dairy farms in Mexico City 299 (23%) were found to be positive for *Listeria* spp., 13% were positive for *L. monocytogenes*, 6% for *L. ivanovii*, 4% for *L. seeligeri* and 1% for *L. innocua*[13]. Yakuba *et al.* detected *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *Listeria welshimeri* and *L. seeligeri* in 8.9%, 20.3%, 7.3%, 2.1% and 1% of raw milk samples produced in Spain[14]. In the USA, 35 (7.8%) of 450 raw goat milk samples analyzed were positive for *Listeria* spp. in which *L. monocytogenes* was detected in 17 (3.8%) and *L. innocua* was detected in 26 (5.8%) samples[15].

In the present study, no *Listeria* isolate was detected in raw camel milk samples. The results of this study and previous studies in Iran show that raw camel milk is not an important source for *Listeria* infection[9,11]. It has been shown that camel's milk has a bacteriostatic effect against *L. monocytogenes* at 4 °C and 20 °C[16].

The sources of *Listeria* spp. in raw milk have been reported to be fecal and environmental contamination during milking, storage and transport, infected animals in dairy farms and poor silage quality[17]. Silage is not widely used as animal feed in the areas that the present study was conducted. Therefore we believe that the contamination source of *Listeria* spp. in raw milk is likely insufficient hygiene during milking, storage and/or transportation.

The PCR assay could identify 8 *Listeria*-contaminated milk samples that were negative using the cultural method. This could be due to the higher analytical and diagnostic sensitivities of the PCR assays. However, care must be taken to avoid false positive results arising from DNA contamination, as well as false negative results caused by inhibitory substances in foods or enrichment broths.

In conclusion, the presence of *Listeria* spp. has been shown in variety of raw milk samples in Iran. The incidence of *Listeria* spp. was 7.3% in raw milk samples in the present study. *L. monocytogenes* was found in 1.9% of raw milk samples. The results of this study indicate the potential risk of infection with *Listeria* in people consuming raw milk or unpasteurized milk and traditional dairy products in Iran. The information obtained from present study may be useful for the food producers at the dairy factory, in animal feeding, in the interest of public safety and for considerations for public health purposes, and for epidemiological and public health studies of *L. monocytogenes* and other *Listeria* spp.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Listeriosis caused by *Listeria* species, is one of the most important food-borne diseases in people. Milk and dairy products are two specific food categories with respect to the risk assessment for listeriosis.

Research frontiers

The present study was undertaken to determine the prevalence rate of *Listeria* strains in different types of raw milk (bovine, ovine, caprine, camel and water buffalo milk) using cultural method and the PCR assay in Iran.

Related reports

The results of the present study is in agreement with the results reported by some of authors in other countries[8-13]. In contrast, results of Rahimi *et al*., 2010 in Iran indicated that all of raw cow milk samples were free of *Listeria* spp.

Innovations and breakthroughs

There is limited information regarding the prevalence of *Listeria* spp. in foods in Iran. Therefore, the present study was undertaken to determine the prevalence rate of *Listeria* strains in different types of raw milk in Iran.

Applications

The presence of *Listeria* spp. in a variety of raw milk indicate the potential risk of infection with *Listeria* in people consuming raw milk, unpasteurized milk, or traditional dairy products in Iran. Therefore, high-risk groups should avoid previously prepared unpasteurized dairy products.

Peer review

An extensive survey was carried out to detect the presence of *Listeria* spp. both live bacteria and DNA- in different types of raw milk in Iran. Results are interesting in terms of the types of milk considered and the variation in levels of the pathogen detected by PCR in milk from different animal species.

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