

## The incidence of listeria monocytogenes in milk

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### Abstract

Raw milk samples obtained from three private farms near Buzau, were tested for the presence of *Listeria* species. A total of 100 raw milk samples were analyzed according SR ISO 11 290-1/2000 and two-stage enrichment techniques. Two-stage enrichment procedure was found to be more effective than SR ISO 11 290-1/2000 in isolating *Listeria* species from raw milk. The overall incidence of *Listeria* species in raw milk was 4%. *Listeria monocytogenes* was found in 3% of the raw milk samples, while *L. innocua* was found in 1% of the samples. One sample was revealed to contain two serotypes of *L. monocytogenes*.

**Keywords:** *Listeria*, milk, pathogen, contamination, isolation

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### 1. Introduction

*Listeria* microorganisms are widely distributed in nature and can be isolated from soil, water or vegetable. Of all *Listeria* species only *Listeria monocytogenes* has been regularly implicated as being pathogenic to humans and animals. *L. monocytogenes* is a motile, G<sup>+</sup> coccobacillus, non-sporeforming, aerobic to facultative anaerobic. Both the natural reservation and mode of transmission to man have been difficult to determine. Consumption of foods containing *L. monocytogenes* can lead to listeriosis in susceptible humans, adults with a compromised immune system, pregnant women and infants. *L. monocytogenes* has been causative agent of major food-borne epidemics in which dairy products including cheese, raw and pasteurized milk have been incriminated as the contaminated foods. Some investigators suggest that an intracellular stage may confer heat resistance of the organism and allow some *Listeria* within leukocytes to survive pasteurization. The bacteria can stay in injured state resulting from various food-processing operations that produce physical and/or chemical stresses and can also remain virulent upon recovery.

Various conditions in foods, including refrigeration, extended shelf life and lack of oxygen, may permit the resuscitation and growth of injured pathogens. Food associated outbreaks of listeriosis involving many deaths and because of presence of *L. monocytogenes* have prompted increased concern for detecting the bacteria in milk and dairy products.

Isolation of *Listeria* species is not unduly difficult, except from heavily polluted material because of overgrowth by other microbial species. Past experiences in clinical laboratories indicate that attempt to isolate the organism from positive biologic specimens by directly plating suspect material onto conventional media occasionally fail, but isolation ratio is improved when specimens undergo a period of cold enrichment.

The bacteria, contrary to other food-borne pathogens, are able to multiply at refrigeration temperatures (4-6°C). Because food typically contains large numbers of background bacteria, cold enrichment procedure is more successful than direct plating for detecting the organism in foods.

For confirmation procedures was used the optical method. In this method, the organism is first cultured for 18-24 h on a transparent medium, and then scanned by stereomicroscopy using oblique transmitted light. *Listeria* colonies were distinguished by characteristic blue-green edges when observed with low power magnification using obliquely transmitted light. The objective of this study was to determine the prevalence of *L. monocytogenes* in raw milk samples and two-stage enrichment techniques.

## 2. Materials and methods

*Raw milk samples.* They were obtained from three private dairy farms. We collected raw milk samples into bottles (100ml) from containers. All samples were kept refrigerated (4°C) prior to testing. Analysis was always begun on the same day of sampling.

The organisms were isolated from a specimen of contaminated milk. Three test suspensions were prepared: one with tryptic soy broth (TSB), one with whole pooled human serum, and one with pasteurized milk (2% fat). Aerobic plate counts of the serum and milk revealed no initial microbial load in these samples. The organisms were inoculated onto tryptic soy agar and incubated at 37°C. The organisms were harvested after 24 h of growth, and the cells were suspended in TSB, milk, or serum to obtain 10<sup>5</sup> CFU/ml. These test suspensions were used as the initial inocula for all tests.

*Enrichment procedures.* Raw milk sample was shaken thoroughly and 25 ml of each sample was added to 225 ml of Enrichment Broth (LEB) and incubated at 30°C. At intervals of 2 and 7 days, 0.1 ml of each culture was directly plated onto Modified McBride Agar (MMA) containing 5% sheep blood. In addition, 1.0 ml of LEB culture was diluted with 9 ml of 0.5 % KOH, mixed briefly, and streaked onto MMA. Plates were incubated at 35°C for 48 h in 5-10% CO<sub>2</sub>.

*Two-stage enrichment procedure.* Ten ml of each raw milk sample were inoculated into 90 ml of Tryptose Broth (TSB) which was kept at 4°C. Cold enrichment broths were subcultured at the end of 7, 14st days as previously described. Briefly, 1 ml of TSB culture were inoculated into 9 ml of LEB. After incubation of the subculture at 35°C for 24 h in 5-10% CO<sub>2</sub>, 1 ml of the subculture was diluted to 9 ml with Sterile Ringer solution containing Lactate and 0.1 ml of this culture was plated onto MMA. The inoculated plates were incubated at 37°C for 24 h in 5-10% CO<sub>2</sub>.

MMA plates were examined for suspect colonies typical of those formed by *Listeria* species (translucent, slightly raised, bluish gray, 0.5-1.5 mm in diameter and/or weakly β-hemolysis). These colonies were examined by gram-stain reaction and in morphology (gram-positive, coccoid to diphtheroid-like rods). These colonies were streaked onto translucent tryptose-nalidixic agar and incubated for 48 h at 35°C. Plates were examined by the technique of Henry using obliquely transmitted light. Colonies of *Listeria* spp. were recognized by characteristic blue-green coloration. The suspect colonies were plated onto MMA plates and incubated at 35°C for 48 h in 5-10% CO<sub>2</sub>. The colonies were checked for motility, catalase production, oxidase production, metil red, Voges-Proskauer reaction, indole production, esculin hydrolysis, urease production, nitrate reduction, H<sub>2</sub>S production (TSI), citrate utilization, hemolytic activity, CAMP test, and ability to ferment glucose, rhamnose and mannitol, xylose with acid production.

## 3. Results and discussions

Isolation of *Listeria* spp. from raw milk were performed using the SR ISO 11 290-1/2000 and two-stage enrichment techniques. The results are shown in Table 1. Of the 100 raw milk samples, 3 (3%) were positive for *L. monocytogenes* and 1 (1%) for *L. innocua*. The use of two-stage enrichment procedure gave more isolates than SR ISO 11 290-1/2000.

One of the samples yielded two serotypes of *L. monocytogenes* (1/2b and 4b). Serotypes and phage types of isolated *Listeria* species are summarized in Table 2.

A lot of case reports suggested that foodborne transmission of listeriosis to human beings can occur by raw and pasteurized milk.

Table 1. Isolation of *Listeria* spp and two-stage enrichment procedures

<i>Listeria</i> spp	SR ISO 11 290-1/2000		Two-stage enrichment	
	1. day	7. day	7. day	14. day
<i>L. monocytogenes</i> (4b)	-	1	1	2
<i>L. monocytogenes</i> (1/2b)	1	1	1	1
<i>L. innocua</i> (6a)	1	1	1	1
Total	2	3	3	4

Table 2. Serotypes and phagetypes of isolated *Listeria* spp

<i>Listeria</i> spp	Serotype	Phagetype
<i>L. monocytogenes</i>	4b	nontyped
<i>L. monocytogenes</i>	4b	nontyped
<i>L. monocytogenes</i>	1/2b	475
<i>L. monocytogenes</i>	1/2b	nontyped
<i>L. monocytogenes</i>	1/2b	nontyped
<i>L. innocua</i>	6a	4286:3528
<i>L. innocua</i>	6a	4286:3528

#### 4. Conclusions

There was considerable variability in the efficacy of two enrichment procedures for isolating *L. monocytogenes* and other species. In this study, two-stage enrichment procedure was much more productive than SR ISO 11 290-1/2000 enrichment. Dilutions of the samples improve isolation of *L. monocytogenes* effecting of further reducing the concentrations of antagonistic microflora during course of cold enrichment. The number of isolates of *L. monocytogenes* and *L. innocua* increased from the samples incubated for 14 days, indicating that the organism may not survive well once, it has been resuscitated and/or grows among competitive microflora at 4°C. Using SR ISO 11 290-1/2000 enrichment procedure, prolonged incubation of samples for 7 days did not consistently improve recoveries over the initial 2 days incubation time of medium. *Listeria monocytogenes* was found in 3% of the raw milk.

In this study, all of tested pasteurized milk samples were found to be negative for *L. monocytogenes*. Other studies reported that 1.1 % *L. monocytogenes*, 8.2 % *L. innocua*, 1.3 % *L. welshimeri* and 0.4% *L. ivanovii*. were found in raw milk samples. Results of this investigation have revealed that raw milk which is used for making cheese could serve as vector for the transmission of human listeriosis. The specific role of milk could be defined by isolating the bacteria after pasteurization or production of cheese from raw milk. Conditions of prolonged cold storage give advantage to proliferation of *L. monocytogenes*. Consequently, all raw milk should be assumed to be contaminated with the organism, therefore, adequate measures to ensure proper pasteurization and to prevent post pasteurization contamination of processed dairy products must be adhered to by dairy manufacturers to ensure a safe product.

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