JOURNAL OF PREVENTIVE MEDICINE
2005; 13 (3 - 4): 43 - 51

THE MAIN SOURCES OF LISTERIA MONOCYTOGENES CONTAMINATION IN MILK PROCESSING PLANTS

Luminița Minea[1], Oana Drug[1], Cristina Cîmpeanu[1] Marieta Vasilov[1], Doina Bucşă[2], Daniela Mircea[3],Cecilia Gafencu[4,] Lămâița Puiu-Berizințu[5], Manuela Cibi[6 1]I.S.P. Iaşi, [2]D.S.P. Botoşani, [3]D.S.P. Galați, [4]D.S.P. Neamț, [5]D.S.P. Bacău, [6]D.S.P. Iaşi

Abstract: The zoonotic Listeria monocytogenes is mainly transmitted to human trough the food-borne route. This bacterium is often found in the environment of food processing plants. In the period 2002-2004 it was studied the occurrence of Listeria monocytogenes. in 9 dairy processing plants in 5 counties of Moldova territory. The aim of the study was to identify the major sources and route for the dairy products contamination. Among the 196 analysed samples of dairy products, 20,4% were identified as being contaminated with Listeria spp., from which 3.57% with Listeria monocytogenes. The highest frequency of Listeria monocytogenes contamination was registered in raw milk (10.53%) and brining maturated cheeses (9.67%). There were analyzed 254 surface swabs. In 29.92% of samples it was noticed the presence of Listeria spp, from which Listeria monocytogenes in 6.3%, especially in the raw milk reception area (8.7%) The examination of working persons revealed that Listeria monocytogenes was most frequently isolated from feces comparing with nasal secretions and hands. This study indicates that Listeria monocytogenes is commonly in the dairy industrial environment including food handlers. Correct disinfection and hygiene will prevent or at least diminish cross-contamination of the food-products.

Key words: dairy-products, sources, food processing, environment, Listeria monocytogenes.

Rezumat: Studiul nostru, desfăşurat în perioada 2002-2004, a urmărit evidențierea Listeriei monocytogenes cu identificarea principalelor surse de contaminare în arealul de producție din 9 fabrici de produse lactate situate în 5 județe ale Moldovei. Din cele 196 probe de produse lactate analizate, 20,4% au fost găsite contaminate cu Listeria spp., din care 3,57% cu Listeria monocytogenes. Cele mai mari frecvențe ale contaminării cu Listeria monocytogenes au fost găsite în laptele crud (10,53%) şi în brânzeturile maturate în saramură (9,67%). Au fost analizate 254 tampoane recoltate de pe diverse suprafețe ale arealului de producție din unitățile investigate. În 29,92% din probe s-au identificat specii de Listeria, din care 6,3% de Listeria monocytogenes, în special, în zona de recepție a materiei prime (8,7%). Examinarea personalului a relevat faptul că Listeria monocytogenes a fost izolată mai frecvent în materiile fecale, comparativ cu exudatele nazale şi tampoanele palmare. Concluzia acestui studiu este că Listeria monocytogenes este frecvent izolată în mediul fabricilor de prelucrare a laptelui. Prezența acestui agent

patogen este determinată, în egală măsură, de materiile prime şi echipamentele contaminate şi, chiar, de purtătorii asimptomatici intestinali umani. Aplicarea unor măsuri de igienizare şi dezinfecție corecte şi ritmice pot preveni sau limita contaminarea alimentelor.

Cuvinte cheie: produse lactate, sursă, areal de producție, *Listeria monocytogenes*

INTRODUCTION

Listeria monocytogenes is one of pathogen agents with important involvements in the food safety issues in the last decade. The consumption of contaminated food with Listeria monocytogenes determines severe diseases, especially for some risk population groups (pregnant women, new-borns, elderly people, persons with immunodeficiency)(1).

Though the prevalence is low (0,4-0,8/100 000 inhabitants), various outbreaks of listeriosis were pointed out in industrialized countries of Europe and USA, the most frequent being associated with dairy, vegetables and meat products consumption (2).

The different conditions in which this pathogen agent survive in environment, the remarkable resistance in the processing area, the multiplication capacity at refrigeration temperature, the long persistence in food even in hostile conditions, make from Listeria monocytogenes an important threat for the population health status.(1).

AIM

Taking into account that dairy products and their processing environments present a high potential of exposure to Listeria monocytogenes, we proposed to develop a study to know the relationship food-processing areas-workers in the milk processing units from Moldavian counties. We refer to the following aspects: the assessment of contamination frequency with Listeria monocytogenes in milk and dairy; the evaluation of Listeria monocytogenes spread in processing areas from the investigating units; the identification of main sources of contamination during the processing, and the identification of the possible deficiencies of hygienic and sanitary conditions.

MATERIAL AND METHOD

The presence of Listeria monocytogenes was investigated in 636 samples, during the last 2 years, from 9 plants with similar profile, having between 5 000 – 20 000 l/day processing capacity, with location in different territories of Moldavia (Botosani, Galati, Neamt, Bacau, Iasi). The analyzed samples included: food (raw milk, dairy products sampled from different stages of technological flow, and finite products), sanitation tests and human samples (fecals, nasal swabs, hand swabs). This 196 food samples

from different lots, in minimal weight of 100 g, were transported to laboratory in frigorific boxes (+ 4^0C) and analyzed in the next 24 hours.

The processing area from the studied plants was divided in 3 distinct zones: raw milk reception area; dairy processing area and maturating area. Each area includes both the surfaces in direct contact with food (work tables, tanks, vats, pools, centrifuges, strainers, sieves, recipients, knifes etc.), and surfaces which don't have any direct contact with food (floors, walls, sewer drains). The 254 swab samples were taken from different surfaces during the work time. The sample area was wiped with sterile swabs which, were afterwards introduced in tubes with 10 ml peptone water 0,1%. For the determination of Listeria monocytogenes presence, the workers from the respective units were investigated and there were taken 61 coprocultures, 62 nasal swabs and 63 hand swabs.

The detecting of Listeria monocytogenes in the food samples was done according to ISO 11290-1-1996 method. Each 25 g of food was aseptic introduced and homogenized with 225 ml enrichment broth demi-Fraser (CM 895, supplement SR 166, Oxoid).

After 24 hour incubation at 30^0C, 0,1 ml culture was transferred in 10 ml secondary enrichment Fraser Broth (CM 895, Oxoid) in which it was added the selective supplement SR 156 E. The respective cultures were incubated for 24 hours at 30^0C, with subsequent replication on Oxford selective medium (CM 856, SR 140, Oxoid). (3)

The samples from the different processing area surfaces were considered to be strong contaminated and were analyzed using a technique with two enrichment stages. Initially, the 10 ml peptone water 0,1% in which each swab was suspended, were introduced in 90 ml Tryptone Soy Broth with yeast extract, which was incubated for 24 hours at 30^0C. 1 ml was afterwards transferred in 10 ml Fraser Broth, as previously described. (2)

The swabs from human sources were immediately introduced in tubes with 10 ml Fraser Broth, incubated for 48 hours at 30^0C, and then, replicated on Oxford medium incubate for 24-48 hours at 35^0C.

To isolate Listeria monocytogenes in feces we used a culture technique based on enrichment (+4^0C), for 4-6 weeks in 10 ml Tryptone Soy Broth with yeast extract, followed by a selective enrichment in Fraser Base Broth, incubated for 48 hours at 30^0C, with a subsequent replication on Oxford agar (24-48 hours at 35^0C). (4)

The colonies with typical morphology (black hallo determinated by aesculine hydrolyses) were replicated on Tryptone Soy Agar. The identification by strains and species based on the next tests: Gram

coloration, mobility at 25^0C, cathalasis, oxidasis, β-hemolysis, Camp reaction with strains of Staphylococcus aureus and Rhodococcus equi, carbohydrates fermentation (xylosis, ramnosis, glucose, manythol), nitrates reduction and Voges-Proskauer test (3).

RESULTS AND DISCUSSIONS

From those 196 milk and dairy products analyzed samples in this study, 24.4% were contaminated with Listeria spp., with the next decreased hierarchy: 9.18% Listeria innocua, 3.57% Listeria monocytogenes, 3.57% Listeria ivanovii, 3.06% Listeria seeligeri şi 1.02% Listeria welshimeri (Fig.1).

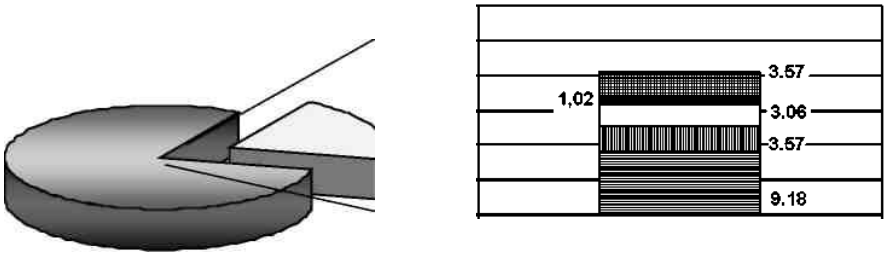


Fig. 1 The percentage repartition of Listeria spp. in the analyzed diary products

Taking into account the ubiquitary distribution, the more frequent presence of Listeria monocytogenes in raw milk (10.53%) in comparison to finite products (3.7%), is not surprising. There were not detected Listeria monocytogenes strains in dairy products sampled in different stages of the technological flow. This aspect depends on various thermic treatments (mainly pasteurization) applied to obtain dairy products, which efficiently destroyed the Listeria monocytogenes strains.(Fig. 2)(5)

Fig.2 The percentage repartition of Listeria monocytogenes in dairy products sampled in different stages of technological flow

Altough the dairy products present a high potential exposure to Listeria, during the different processing stages, some factors which prevent the survival and multiplication of this pathogen agent are developed. Many of these factors appear during the processing of acid dairy products, but also in certain stages of the un-fermented dairy products preparing cycle, being represented by: salt and fat content, humidity gradient and p_H level.

Other factors which we have to take into account, due to the inhibitor effect to Listeria viability, are the acids and bactericide substances released during starter cultures multiplication.(6) A conclusive example is the study developed by Silva (1998), who analyzed different types of cheese from Brasil and found that Minas Frescal range had the highest frequency of Listeria monocytogenes contamination (41.57%), because this range has a low pH (4.9-5.3) and a high humidity level (55-58%). (7)

In our study, the Listeria monocytogenes presence was identified only in salt maturated cheeses in percentage of 9.67%, depending on post-pasteurization cross – contamination from environmental sources and raw milk (10.53%) (Tab. 1).

Table 1 The frequency of Listeria monocytogenes contamination in the analyzed diary products

Foods	No. of samples	No. of strains of Listeria spp. (%)	No. of strains of L. monocytogenes (%)
Raw milk	38	15 (3.47)	4 (10.53)
Diary products sampled in different stages of technological flow	77	12 (15.58)	0
Salt maturated cheeses	31	7 (22.58)	3 (9.67)
Cream cheeses	28	5 (17.85)	0
Acid diary products	12	0	0
Melted cheese	10	1 (10.0)	0
Total	196	40 (20.4)	7 (3.57)

These results are similar with those of French specialists who reported that 10% (18 of 174) of analyzed light cheeses during 1995-1997 were contaminated by Listeria monocytogenes. Beckers (1987) and Gilbert (1988) found positive for Listeria monocytogenes 15% (10 of

69) and 14% (12 of 85) respectively of analyzed French light cheeses. Whereas, Farber & Co. (1987) reported that they isolated Listeria monocytogenes only in 1.9% of investigated cheeses.(8)

Studies developed in different periods by numerous researchers pointed out that Listeria monocytogenes is a frequent contaminant of the dairy processing area in such units.

An evaluation of 39 Californian diary plants, developed in 1991 by Walker & Co. showed that 12% of samples where positive for Listeria. (5). Klausner (1991) and Charlton (1990) reported a global incidence between 12.6-17.5% for Listeria in the processing area of some dairy plants. The highest percent of contaminated samples (35.5%) was obtained by Pritchart & Co., who analyzed 9 dairy plants in 1995 (6).

The previous data are similar with those from this study, though it is difficult to compare these data, because of some variations regarding the number and the types of investigated samples. The examination of 254 samples from different processing area zones revealed the Listeria species and Listeria monocytogenes presence in 29.92% and 6.3% samples respectively.(Fig. 3)

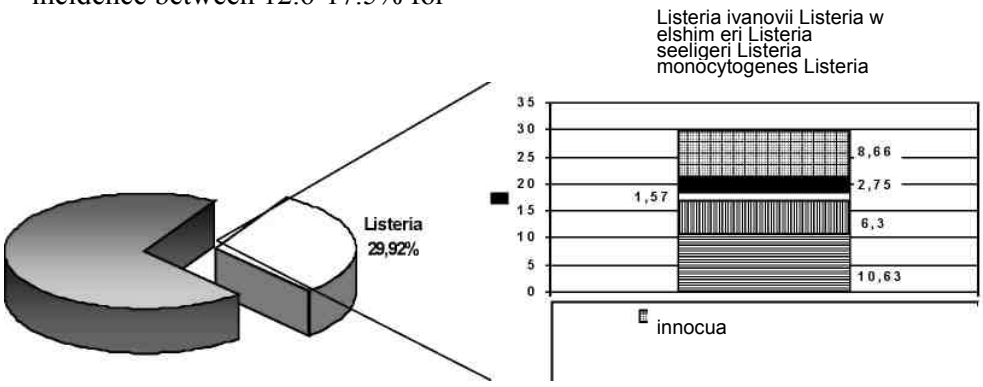
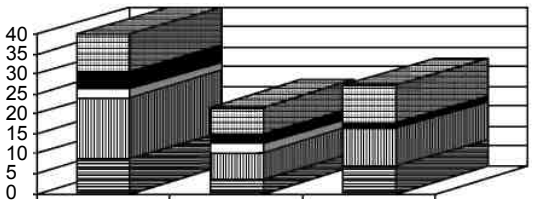


Fig. 3 The percentage repartition of Listeria species isolated from the investigated units

We can remark a higher percentage of Listeria monocytogenes isolated in swabs from the raw milk area reception (8.7%) in comparison with the samples from finite products processing area (3.41%), especially on the surfaces which don't have direct contact with food (14.41%).

The Listeria monocytogenes presence on the surfaces from all technological flow demonstrates that

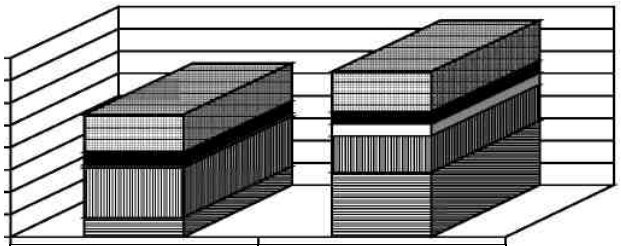
a food post-processing contamination is possible (Fig. 4 and 5).



%

	Milk raw reception area	Finite product processing area	Maturated cheeses area
L.ivanovii	9,78	6,82	9,46
L.welshimeri	4,35	2,27	1,35
L.seeligeri	2,17	2,27	0
L.innocua	15,21	6,82	9,46
L.monocytogenes	8,7	3,41	6,76

Fig% The occurrence of Listeria spp.in different spaces of production area



	Surfaces with direct contact	Surfaces whithout direct contact
L.ivanovii	8,4	9
L.welshimeri	2,8	2,7
L.seeligeri	0,7	2,7
L.innocua	11,2	8,1
L.monocytogenes	4,2	14,41

Fig.5 The Listeria species distribution depending of different surfaces of processing area

In this study, Listeria monocytogenes was detected in the most investigated units, especially on the floors and drain sewers. This is not surprisingly, because it is well known that Listeria monocytogenes is an ubiquitary bacteria, presenting a remarkable resistance in relative discordant conditions. The high contamination level of drain sewers represents an alarm signal, because the spread pathogen agent can determine the subsequent contamination of the surfaces which is in direct contact with food.(9).

The workers can be asymptomatic carriers (intestinal, pharynx, nasal), being important sources of the food contamination during the handling and processing work. This study results pointed out a higher frequency of isolated Listeria monocytogenes strains in the workers coprocultures, in comparison with nasal secretions and the swabs from the hands, but which are situated in the intestinal asymptomatic carriers limits of the general population (5-10%) (Tab.2) (9, 10).

Table 2 The contamination frequency of Listeria monocytogenes in the workers samples from the investigated units

Samples	No. 61	No. of Listeria monocytogenes strains(%)
Coprocultures	62	4 (6.56)
Nasal swabs	63	2 (3.2)
Hand swabs		1 (1.6)

CONCLUSIONS

Listeria monocytogenes was detected in most investigated diary processing units. The initial sources of contamination seems to be the raw milk from the ill or asymptomatic carrier animals. The equipments are involved in the finite products post-pasteurization contamination. The drain sewers can be considered high fidelity indicators of the Listeria monocytogenes presence in the processing zone from these units. We can't neglect the importance ot the asymptomatic carrier workers from the unit, who can contaminate the food during the un-hygienically handling.

The Listeria food contamination prevention can be achieved by: epizootological active surveillance with illness cases diagnosis and applying of differential control measures; the HACCP programme implementation to identify and estimate the associated risks for different stages of processing flow; an adequate design

of the processing equipment, which allows a correct and regular hygiene and disinfection associated with the cross-contamination avoidance in different critic points of the technological flow.

REFERENCES

1. J.Kozak, T.Balmer, R.Byrne – Prevalence of Listeria monocytogenes in foods: incidence in dairy products, Food Control, volume 7, 1996
2. Roberts D. - Listeria monocytogenes and foods: The UK approach, Dairy Fd. Env. Sanit., 1994, 14, 202-204
3. Allerberger F – Listeria: growth, phenotypic differentiation and molecular microbiology, FEMS Immunology and Medical Microbiology, volume 35, 2003, 183-189
4. Erdogan H.M., Cripps P.J. – Optimization of a culture technique for the isolation of Listeria monocytogenes from faecal samples, J.Vet.Infect, Dis., volume, 49, 2002, 502-506
5. Teufel P.- European perspectives on Listeria monocytogenes, Dairy Fd. Env. Sanit., 1994, 14, 212-214
6. J.Kozak, T.Balmer, R.Byrne – Prevalence of Listeria monocytogenes in foods: incidence in dairy products, Food Control, volume 7, 1996
7. Farber J.M, Sanders G.W., Johnston M.A. – A survey of various foods for the presence of Listeria species, J.Food Protect., 1996, 52, 456-458
8. Isabella M.M. Silva, R..C.Almeida - Occurence of Listeria spp. in critical control points and the environment of Minas Frescal cheese processing, International Joural of Food Microbiology, volume 81, 2003, 241-248
9. Loncarevic S., M.L. Danielsson-Tham - Occurence of Listeria monocytogenes in soft and semi-soft cheeses in rerail outlets in Sweden, International Joural of Food Microbiology, volume 26, 1995, 245-250
10. El-Shenawy M.- Sources of Listeria spp. in domestic food processing environment, International Journal of Environmental Health Research, 1998, 8, 241-251