

RESEARCH REGARDING THE PRESENCE OF LISTERIA SPECIES IN MILK FOOD PRODUCTS

COL FLORICA, COL M.

University of Craiova, Faculty of Agriculture

Key words: selective media; raw milk; curd.

ABSTRACT

Out of the total 232 analysed samples, 12 (5,17%) were Listeria spp positive., and out of these latter ones 7 (3,02 %) Listeria monocytogenes stems, 4 (1,72 %) Listeria innocua stems and one (0,43 %) Listeria welshimeri stem were isolated. The presence of Listeria spp was found in the analysed products in the following percentages: in pasteurised cows' milk 6,45 %, in cows' milk obtained from unpasteurised milk 4,25 %, in cream obtained from unpasteurised milk 6,66 %, and in fresh ewe's curd 6,25 %. Listeria monocytogenes species was prevalent in the positive samples with a percentage of 58,33 %, while the other species were considered as having no pathogenic significance. Listeria monocytogenes was not isolated in the samples coming from pasteurised cows' milk and this makes us think that this thermal proceeding is efficient for destroying the listerias.

INTRODUCTION

Many studies in the field spotlighted the fact that *Listeria monocytogenes* is found in small quantities in many food products, less than 1 ufc/gram (Lund A.M., Zottola E.A., 1991). This indicates the fact that many people are daily exposed to small quantities of *Listeria monocytogenes*. Any food product, except for the ones thermally processed in a proper way, may be sporadically contaminated with *Listeria monocytogenes* and this is why it is necessary to prevent the increase of the number of microorganisms to a certain level that may be dangerous for the human body. (Jacquet C.H. 1995) Since it is pathogen both for the animals and the humans, *Listeria monocytogenes* causes food-borne infections with a mortality rate of 30%. An essential factor for the multiplication is related to the conservation of the milk food products for long periods of time (more than 8 days), under an insufficient refrigerating system (Lanceveld L.P.M., Corput P. Van de, Faasen M.Y.M.G. 1993). Milk, especially the unpasteurised type, but also some milk products such as: soft paste cheese, represent one of the main type of animal food products causing food-borne infections produced by *Listeria monocytogenes*. Raw milk may be contaminated with *Listeria monocytogenes* in a percentage of 1-45 %, depending on the country. The contamination is made endogenously, by the appearance of marmites or by the existence of healthy bearers (Nicolas J.A. 1996).

MATERIAL AND METHOD

The studies were accomplished at Sanitary Veterinary Direction for Food Safety of Dolj, during 2012-2014. The isolation and the identification of the species *Listeria monocytogenes* in the food products were made according to the standard SRISO 11290-1/2000. 232 samples were collected, among which: 30 samples of cow's pasteurised milk, 62 samples of cows' unpasteurised milk, 47 samples of cows' cheese obtained from unpasteurised milk, 45 samples of cream obtained from unpasteurised milk and 48 samples of ewe's fresh curd. The pasteurised milk was collected from the trade market and the other samples were collected from different private societies around Dolj county.

The method to identify and isolate the bacteria of *Listeria monocytogenes* was the following: before the enrichment, 25 grams/ml of product were homogenized in 225 ml demi-Fraser bouillon. After a 24-hour incubation at 30°C, there was a selective secondary enrichment by making 0,1ml of the pre-enrichment culture pass through 10ml of Fraser bouillon. After an incubation period at 35-37° C, for 24 hours, there was a selective isolation which was made by knurling 0,1ml of culture on the selective media of Palcam and Oxford. The Petriast tiles thus inseeded incubated at 35°C, for 24-48 hours. The confirmation of *Listeria monocytogenes* species was made by means of API-*Listeria* tests which examined 10 biochemical features.

RESULTS AND DISCUSSIONS

Considering the analysis of the data obtained and presented in table 1, we find that the frequency of the *Listeria* species in the examined products was the following: in cows' unpasteurised milk 6,45 %, in cows' cheese obtained from unpasteurised milk 4,25 %, in cream 6,66 %, in ewe's fresh curd 6,25 %, and in cows' pasteurised milk it was not isolated.

Table 1

Frequency of listeriosis in the examined products

Name of the product	No. Of examined products	Positive samples		Negative samples	
		Number	%	Number	%
Cows' unpasteurised milk	62	4	6,45	58	93,55
Cows' pasteurised milk	30	-	-	30	100
Cows' cheese obtained from unpasteurised milk	47	2	4,25	45	95,75
Cream obtained from unpasteurised milk	45	3	6,66	42	93,34
Ewe's fresh curd	48	3	6,25	45	93,75
Total samples	232	12	5,17	220	94,83

Within *Listeria*, the species distribution depending on the product was the following (table 2) :

- out of 62 samples of cows' unpasteurised milk, 3 *Listeria monocytogenes* stems representing 4,83 %, and one *Listeria innocua* stem representing 1,62 % were auto-isolated;

- out of 47 samples of cows' cheese obtained from unpasteurised milk, one *Listeria monocytogenes* stem and one *Listeria welshimeri* stem (2,125%) were auto-isolated, representing 2,125% of the total analysed samples for each species;

- out of 45 samples of cream obtained from unpasteurised milk, 2 *Listeria monocytogenes* stem (4,44%) and one *Listeria innocua* stem (2,22%) were auto-isolated;

- out of 48 samples of ewe's fresh curd, two *Listeria innocua* stems representing 4,16% of the total analysed samples and one *Listeria monocytogenes* stem representing 2,08% of the total analysed samples were auto-isolated.

Table 2.

Distributing different *Listeria* species in positive samples

Name of the product	Number of analysed samples	Number of positive samples	Distribution of <i>Listeria</i> species in positive samples			Number of negative samples
			<i>Listeria monocytogenes</i>	<i>Listeria innocua</i>	<i>Listeria welshimeri</i>	
Cows' unpasteurised milk	62	4	3	1	0	58
Cows' pasteurised milk	30	-	0	0	0	30
Cows' cheese obtained from unpasteurised milk	47	2	1	0	1	45
Cream obtained from unpasteurised milk	45	3	2	1	0	42
Ewe's fresh curd	48	3	1	2	0	45

Therefore, the media of Palcam and Oxford proved to be intensely selective. On these media, after 24 hours of incubation, *Listeria monocytogenes* formed dark coloured colonies surrounded by a black halo due to the fact it produces esculin hydrolysis. For confirming and identifying the *Listeria monocytogenes* species, the stems that reacted typically to the tests above (Gram coloration, catalase and immobility at 25⁰C) were subjected to the haemolyse and CAMP tests and the final confirmation was made by means of API-*Listeria* biochemical tests.

CONCLUSIONS

Listeria monocytogenes was not isolated in the samples coming from cows' pasteurised milk and this makes us think that this thermal proceeding is efficient for destroying the listerias.

The final differentiation of the species of *Listeria monocytogenes* from other species within the genus was made by interpreting the tests of Api-*Listeria*, CAMP and producing dehaemolyse; the other tests used for identification allowed only the classification of the bacteria as part of the *Listeria* genus.

The frequency of the bacteria in the unpasteurised milk was close to the one of the examined milk products which shows a correlation between the unpasteurised milk and certain milk products obtained from whole milk.

The *Listeria monocytogenes* species was prevalent in the positive samples with a percentage of 58,33 %, while the other isolated species were considered as having no pathogenic significance.

BIBLIOGRAPHY

1. **Allerberger, F., Wagner, M.**, 2010 - *Listeriosis: a resurgent food-borne infection*. Clinical Microbiology and Infection 16:16-23. Food Standards.
2. **Ben Embarek, PK.**, 1994 – *Presence, detection and Growth of Listeria monocytogenes in sea foods: a review*. Int J. Food Microbiol 23:17-34.
3. **Catteau, M.**, 1991 - *Detection de Listeria monocytogenes dans les aliments*. ASEPT.
4. **Col , M, Col , F**, 2010 - *Researches regarding the alteration of milk fat acids secretions due to nutrition*. Analele Universitatii din Craiova, Seria Agricultură , Montanologie, Cadastru, 52-56.
5. **Col , M, Col , F**, 2010- *Monitoring milk quality and mammary gland health in the milk cows farm within Simnic research development station*. Analele Universitatii Din Craiova, Seria Agricultura, Montanologie, Cadastru. 49-52.
6. **Cox, L.J., Siebenga, A., Pedrazzani, C.**, 1991 - *Performance of enhanced haemolyse in agar compared to Oxford medium for the isolation Listeria monocytogenes from food enrichment broths*. 2009, Food Microbiol., 8.
7. **Jacquet, C.H.**, 1995 – *Listeria et listériose humaine*, In: Sécurité alimentaire du consommateur, Moll M et Moll N, éd., Tec et Doc Lavoisier Paris, p. 21-53