

SEARCH FOR *LISTERIA MONOCYTOGENES* ACCORDING TO STANDARDIZED METHOD ISO-11290-1 IN BELIEVED MILK OF COWS IN THE AREA IS ALGERIA (SETIF, BATNA & BISKRA)

Belabed¹⁾ Hana Nedjma, R.R. Triki-Yamani²⁾, M. Bachir Pacha³⁾

¹⁾University ISV-Blida-1

²⁾University lecturer in Parasitology parasitic E diseases ISV-Blida-1

³⁾Professor in Microbiology and pathologies ISV-Blida-1

Abstract. 252 taking away of believed milk of cows were collected starting from 9 sales outlets, distributed on three common to knowing, Bougaa (W.Sétif), Djerma (W.Batna) and Sidi Okba (W. Biskra). The results of the analysis by standardized method NF IN ISO 11290, confirmed the presence of *Listeria spp* (2.8%) with a prevalence of *L. innocua* (1.58%) and *L. monocytogenes* (1.19%) similar to those reported by the literature. In order to guarantee the safety of the consumer (risk of mortality = 20-30%) the search for *L. monocytogenes* in the dairy products is an obligation.

Keywords: Food listeriosis of origin, *L. monocytogenes*, believed, bovine milk

INTRODUCTION

Table 1

Results of the bacteriological analyzes for the search for *Listeria spp*.

Area	Month	Taking away (N)	Positive	Rate (%)
Bougaa	January	12	0	0.00
Djerma		12	2	0.79
Sidi okba		12	0	0.00
Bougaa	February	12	3	1.19
Djerma		12	0	0.00
Sidi okba		12	0	0.00
Bougaa	March	12	0	0.00
Djerma		12	0	0.00
Sidi okba		12	1	0.40
Bougaa	April	12	0	0.00
Djerma		12	0	0.00
Sidi okba		12	1	0.40
Bougaa	May	12	0	0.00
Djerma		12	0	0.00
Sidi okba		12	0	0.00
Bougaa	August	12	0	0.00
Djerma		12	0	0.00
Sidi okba		12	0	0.00
Bougaa	October	12	0	0.00
Djerma		12	0	0.00
Sidi okba		12	0	0.00
	Total	252	7	2.77

Milk is a complete and ideal medium for the microbial growth. When it is secreted by the cow, it is theoretically sterile. A first contamination occurs at the time of its passage in the channel of the trayon (which can be minimized by a good disinfection of this one before the draft). Throughout the line production, that it is with the farm or the factory, the contamination is omnipresent. Even with all the precautions “the cold storage combined with the machine milking, believed milk is found nevertheless contaminated during its production and of its handling in a more or less important way. [1] [2].

Table 2

Frequency of *Listeria spp* per season.

Season	Positive	Frequency (%)
Winter	5	1.98
Spring	2	0.79
Summer	0	0.00
Fall	0	0.00

This contamination can have consequences going of the simple deterioration of the product (loss of organoleptic qualities and the commercial value, with the appearance of serious toxoinfections. [3]

Table 3

Frequency of *L. monocytogenes* and *L. innocua*.

Frequency of <i>L. monocytogenes</i> and <i>L. innocua</i> . (n=252)Area	<i>L. monocytogenes</i>	<i>L. innocua</i>
Bougaa	2 (0.8%)	1 (0.4%)
Djerma	1 (0.4%)	1 (0.4%)
Sidi okba	0	2 (0.2%)
	3 (1.2%)	4 (1.6%)

Among the pathogenic bacteria found in milk, *Listeria monocytogenes*, is responsible for serious infections in the consumers. *It east* is the causal agent of the human listeriosis, a fatal infection of food origin. The clinical demonstrations vary feverish gastroenteritises with invasive severe forms including septicaemias, meningitides, rhombencéphalites, antenatal infections and abortions. Differently the other pathogenic ones of the food infections of origins, the listeriosis is associated with cases of mortality which can reach values of 20-30% [3].

In Algeria, these last years the breeding of the milch cows starts to extend, whereas few studies were led on *Listeria*.

This work precisely aims at highlighting the presence of *Listeria* in bovine milk believed in the sales outlets located in the communes of three willayas: Sétif (Bougaa), Batna (Djerma) and Biskra (Sidi Okba). The interest of the search for *L. monocytogenes* is confirmed by the importance that grants official journal (JORADP) to him N°3, The objective of this work is to seek *L. monocytogenes* in the believed milk of cow, in order to confirm its presence in the zone of study and to evaluate its importance.

MATERIAL AND METHODS

252 taking away of believed milk of cow were carried out in 03 communes (Bougaa, Djerma, Sidi okba), during one 07 months period going from January in October 2014 and, according to the rules of hygiene in 09 sales outlets of a capacity of sale day

laborer which borders the 100 L each one, They are then conveyed in a refrigerator at the military laboratory of El-Harrach (Algiers). To be done, various culture media were used: half Fraser, Fraser, Palcam, of Oxford and TSYEA. The analysis of the taking away was made according to the Standard **ISO 11290-1** which consists in of the search for listeria leaving the foodstuffs.

The identification of the kind: Test of the catalase then Coloring of gram and observatio with to the immersion with the objective X 100: the listeria are small positive bacilli with Gram, in the shape of “V” or “L”. The identification of the species is carried out thanks to the gallery Biochimique API Listeria:API Listeria is a system of identification of using Listeria of the standardized and miniaturized tests, as well as a database specific. It allows the characterization of the Listeria kind by elimination of the species not belonging pasau Listeria kind. The API gallery Listeria comprises 10 micro tubes or cups containing of the substrates in dehydrated form, which allow the realization of the enzymatic tests.

An statistical analysis according to the test of independence of the Khi-square (X²) of Pearson (1857-1936) was used.

RESULTS

On 252 analyzed taking away, one found 7 positive cases (2.77%. The rate of *Listeria spp* is most important in no one and February (1.19%) during August, May, October (Table 1). The rate of *Listeria spp* is more important in winter (1.98%) that in summer and autumn (0%). Table 2). The statistical analysis does not show a significant difference of the effect of the season with a probability $P=0.070999$ ($P>0,05$). *L.innocua* is overall more frequent (1,6%) that *L.monocytogenes*.(1,2%).Table 3). Moreover, its frequency is different from an area to another. *L. monocytogenes* detected forever in the area of Sidi okba (W. Biskra), contrary to *L.innocua* which is omnipresent in the two other studied communes.(Figure 3)

DISCUSSION

This study undertaken with an aim of highlighting the presence of *Listeria monocytogenes* in bovine milk believed by the ISO method 11290-1. Has requires 2 successive stages of enrichment (primary and secondary), a stage of insulation and one of confirmation:

The primary education enrichment made on a bubble half-Fraser, made it possible to obtain after 24:00 of incubation a blackening of certain sachets following the hydrolysis of the esculine, which reacts with the ferric ions. Then one passes to secondary enrichment and insulation on gélose Palcam and Oxford. Then, one incubates with 37°C. The secondary enrichment made on selective medium of Fraser enabled us to obtain after 48H incubation the same results. Insulation is carried out on medium Palcam and Oxford, is incubated with 37°, after 24 with 48H.

Thus, on the 252 analyzed samples we suspecté 07échantillons being able to contain the Listéria kind. On medium Oxford, the colonies of these suspect samples, are present in the form of small colonies grisâtres surrounded by a halation noirâtre after 24:00 of incubation and, become darker with greenish reflections. They have a diameter of approximately 2mm surrounded by a halation noirâtre and present a central depression.

after 48 hours. Blackening is also allotted to the hydrolysis of the esculine, which reacts with the ferric ions.

On Palcam medium, the colonies are presented in the form of small colonies grisâtres or green olives with sometimes a center noirâtre always surrounded of a halation noirâtre after 24:00 of incubation. *Listeria* is presented in the form of the green colonies of 1,5mm to 2mm of diameter, with a central depression surrounded by a halation noirâtre, rough 48heures.

Before passing to the identification one carried out the second insulation on medium TSYEA.

On medium TSYEA, the typical colonies have a diameter of 1mm with 2mm, convex, colorless, translucent, on board regular after 24:00 of incubation to 37°C

The 07 suspect samples presented typical colonies of *Listeria* on the 3 preceding mediums which were the subject of a confirmation and a biochemical identification on gallery Api *Listeria*. For the Confirmation: *Listeria spp.* is catalase positive and positive Gram

For the biochemical identification: the API gallery *Listeria* comporte 10 microphone-tubes or cups containing of the substrates in dehydrated form, which allow the realization of the enzymatic tests. For test DIM, the identification is obtained by seeking the numerical profile in the list of the numerical profiles (cf Appendix II). For the 07 suspect samples, after use of a biochemical gallery especially designed for the species *Listeria* (API *Listeria*), we cancelled the presence of *Listeria spp.*, in a rate of 2.77%. Moreover, the biochemical identification showed the prevalence of *Listeria innocua* (1.59%) and, *L. monocytogenes* (1.19%). This prevalence is explained in a European study undertaken in 19 laboratories of 14 different countries, in order to measure the performances of the standardized method and in particular the precision. Each laboratory analyzed 3 types of artificially contaminated matrices on 3 levels [5 to 100 Units Forming Colonies/25g]. All in all, the results showed that the method had a sensitivity of 85,2% and one specificity of 97,4%. It was not shown to differences in the performances of the two selective mediums employees, Oxford and PALCAM. Moreover of the negative forgeries were obtained when the categories of analyzed food contained strong rates of *L. innocua*. Thus, it was shown that during the stages of enrichment, *L. innocua* became the dominant population and could mask the presence of *L. monocytogenes* after insulation on selective medium. In the majority of the cases, *L. monocytogenes* was not detected at the stage of the first enrichment but only after the one second enrichment. Moreover, even of small quantities of *L. innocua* can mask the presence of *L. monocytogenes* at the conclusion of the procedure of enrichment [11, 12,13] *L. innocua* would have a “selective advantage” on *L. monocytogenes* by a faster growth in selective bubbles (Fraser Type) what leads to reports/ratios of concentration *L. very weak monocytogenes/L. innocua*. According to Beumer [120], this difference would be due to the acriflavine, to which *L. monocytogenes* would be more sensitive than *L. innocua*. Other authors suggest that this advantage during the procedure of enrichment could be due to an inhibition of *L. monocytogenes* by *L. innocua* [14]. An inhibiting effect of *L. innocua* was well shown against *L. monocytogenes* [14,15]. The mechanism would be a production of bactériocine, or more probably of phagic particles. One can suppose that this action is expressed in a stronger way in a liquid medium of enrichment (free diffusion of the particles) that in situ in solid food.

The rate of 1,09%, *Listeria monocytogenes*, obtained at the time of this study is on the one hand, similar to those reported in France by Larpent J.P in 1995 (1,90%); in Spain by Soriano J.M in 2000 2,9 (0%); in Thèquie by Holko et al. in 2001 (2,56%) and, in Algeria by Lebres and Guetarni in 2000 (1,96%). On the other hand, our rate is lower than that

reported in Turkey by Vardar- Ünlü and Al in 2000 (4%) and, in the USA by Van Kessel J.S. in 2004 (6,5%).

At all events, such a frequency of contamination makes these foodstuffs of the products at the risk as regards listeriosis, of which microbiological stability depends primarily on their conditions of conservation. The percentage of listeria, obtained during the winter is of 7.5% superior to that obtained during spring (5%) and the summer (0%). Taking into account the geographical location, the incidence of *Listeria spp.* is weaker in Biskra (4,41%) than in Mila (8,33%). That could be associated with the climatic differences rather than with the factors related on the draft or the conditions in hygiene. The season effect was not quoted by the authors, contrary to the effect of the temperature of growth. The seasonal variations of the incidence of *Listeria spp.* in believed milk were reported by Rea and Al (1992), Sanaa and Al (1993) and Gaya and Al (1996). Thus, our study brought additional information as for the seasonal incidence of *L. monocytogenes*. Indeed, the incidence is more marked during the winter (16,12%), than in spring (3,33%). On the other hand, in autumn and summer, there is complete absence of *Listeria spp.* in the analyzed samples. Certain authors [125] reported that the contamination of the milk believed by *Listeria spp.* is usually more current in winter, probably because of the food by the ensilage, widespread during this season in the world. Kalac (2011) affirms that the ensilage is a rich person source of contamination by undesirable bacteria such as *L. monocytogenes*, *Bacillus cereus* and *Clostridium tyrobutyricum*. He adds that the occurrence of *L. monocytogenes* in the ensilage is associated with the anaerobic bacterial processes which damage hygienic and nutritional quality ensilages and which the growth of the bacterium becomes considerable when the oxygen tension is raised and when the values of the pH border 4,4. He adds to these factors, the bad quality of the ensilage due to a low acidity because of an inappropriate lactic fermentation, or an aerobic deterioration. According to the statistical analyzes of Vilar and Al (2007), the relation between the bad quality of ensilage (high pH) and the presence of *L. monocytogenes* in the ensilage is confirmed. They note rates of insulation of 29,5% compared with 6,2% for a pH above or in lower part of 4,5 respectively. These observations are also confirmed by many authors who showed an increase in the bearing of *Listeria spp.* in winter and in spring (from October at June, according to the countries), times when the animals, are nourished with ensilage generally: The bearing of *Listeria spp.* exist in the majority of the herds (cows), with a variable rate between 0,5 to 10% of the animals, according to the seasons [5].

CONCLUSION

Listeria is a bacterium ubiquitaire, largely widespread in the environment. It can develop with temperatures as low as +1°C and, can of this fact of contaminating strongly certain food, in particular believed milk. With a rate of 1,64%, certainly weak but similar to that brought back by the international literature, it comes out from our work the following essential points:

- Taking into account the ubiquity of *L.monocytogenes*, its absence in believed milk is illusory.
- Without having reliable data, the presence of the listeriosis in our country is certain,
- The presence of *Listeria spp.* in our food is a real risk.

It is thus convenient, to redouble vigilance while setting up, an adequate regulation as well as a good communication on the risks in order to as well as possible sensitize the population sensitive exposed to the risk of a possible listeriosis.

REFERENCES

1. Morse, TOKEN ENTRY, Jackson, H., McNaughton, C.H, Leggat, A.G., Landerkin, G.B. and Jons, C.K., 1968. Investigation off factors contributing to the bacterial count off bulk tank milk II. Bacteria in milk from individual cows. *J. Dairy Sci.* 51:1188 - 119 1.
2. Thomas, S.B. and Thomas, B.F., 1978. Content The bacteria off milking machines and pipeline milking seedlings. With review: Thermoduric organisms. *Dairy Ind. Int.* 43: 17-2 1,25
3. Allerberger F., Wagner Mr.: Listeriosis: resurgent foodborne infection has. Covering joint. *Microbiol. Repugnant.*, 2010,16,16-23.
4. Larpent J.P. (2004). *Listeria*. 3rd edition. Technique and documentation. London-Paris New York. Lavoisier. ISBN: 2-7430-065769. 227 p.
5. Augustin (jc). Resistance of *Listeria monocytogenes* to the heat treatments. *Pathology Biology. flight 44, n°9, 1996, PPP 790-807.*
6. Larpent J.P. (1995) *Technical Listeria and Documentation*, ED. Lavoisier, 102 pages
7. Larpent J.P. (2000) *Technical Listeria and Documentation*, Zd. Lavoisier, 106 pages
8. Sanaa Mr., Audurier A., Poutrel B., Menard J.L., Serieys F. (1996). Origin off bovine milk contamination by *Listeria monocytogenes*. *Int. Dairy EDF.* 25:163 - 179. *L.monocytogenes*. Thesis Univ Doctorate. Paris XI. 207 p.
9. Sanaa Mr. (1993). Epidemiology of the contamination of milk to the farm by *Listeria*
10. Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, TOKEN ENTRY & Tauxe, R.V. (1999). Food-related illness and death in the United States. *Emerg Infect Say.* 5,607-625.
11. Curiale, M.S. and Lewus, C. (1994). Detection off *Listeria monocytogenes* in samples containing *Listeria innocua*. *J. Food Protect.* 57:1048 - 1051.
12. McDonald, F. and Sutherland, A.D. (1994). Important differences between generation times off *Listeria monocytogenes* and *Listeria innocua* in two enrichment broths. *J. Res. Dairy* 61:433 - 436.
13. Beumer, R.R., Te Giffel, M.C. Spoorenberg, E. and Rombouts, F.M. (1996). *Listeria* species in domestic environments. *Epidemiol. Repugnant.* 117 (3): 437-442.
14. Kalmokoff, M.L., Daley, E., Austin, J.W and Farber, J.M. (1999). Bacteriocin-like inhibitory activities among various species off *Listeria*. *Int. J. Food Microb.* 50 (5): 191-201.
15. Rea M.C., Cogan T.M., Tobin S. (1992). Incidence off pathogenic bacteria in raw milk in Ireland. *J.Appl. Bacteriol* 73: 331-336.
16. Sanaa Mr. (1993). Epidemiology of the contamination of milk to the farm by *Listeria monocytogenes*. Thesis Univ Doctorate. Paris XI. 207 p.
17. Gaya P., Saralegui C., Medina Mr., Nunez Mr. (1996). Occurrence off *Listeria monocytogenes* and Other *Listeria* spp. in Raw Caprine Milk. *J. Dairy Sci.* 79:1936 - 1941.
18. Waak E., Tham W., Danielsson-Tham M.L. (2002). Prevalence and fingerprinting off *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy seedling receiving tanks. *Appl. Approximately. Microbiol.* 68: 3366-3370.
19. Vilar, Mr. J., Yus, E., Sanjuan, Mr. L., Dieguez, F.J., Rodriguez-Otero, J.L. (2007). Prevalence off and risk factors for *Listeria sp* one dairy farms. *J. Dairy Sci.*90: 5083-5088.