



**CONTAMINATION WITH *Listeria monocytogenes* (Murray, 1926) OF LIVE *Mytilus galloprovincialis* Lamarck, 1819 COLLECTED FROM BUTRINTI LAGOON (SOUTHERN ALBANIA)**

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**Synopsis**

**Key words:**

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Live bivalve mollusk (*Mytilus galloprovincialis*, Lamarck 1819) samples, collected from three stations in the lagoon of Butrinti, were analyzed for the presence of *Listeria monocytogenes* (Murray, 1926) during 2008 and 2009. From analytical control it was concluded that during 2008, 4 (5.1%) out of 78 samples (*M. galloprovincialis*) were positive for presence of *L. monocytogenes* and in 2009, 8 (7,2%) out of 98 samples were positive for this pathogen. The identified strains of *Listeria monocytogenes* (12) represent 6,1% of total number of samples analyzed in two years. 7 of them were isolated from the station in the northern part of the lagoon, confirming that this part is more polluted by microbiological contamination regarding to pathogen *L. monocytogenes*.

**INTRODUCTION**

*Listeria spp.* are ubiquitous organisms in water environment. *Listeria monocytogenes* is known as food-borne pathogen causing listeriosis in human, even thought by consumption of contaminated live bivalve mollusks (BAKER et al., 1993). This microorganism can be found in most of mammalian species, both domestic and wildlife, as well as in species of birds and possibly some species of fish and shellfish. It can be isolated from soil, silage, fresh and marine water, sediments and plants (BOERLIN et al., 1997; ERICSSON et al., 1997). Different attempts have been made to remove bacteria from the surface of fish by washing in either clean water or water containing chlorine. However, recent research on the effectiveness of washing, with or without chlorine indicates that while it can potentially reduce

*Listeria* numbers, it cannot be relied upon to completely eliminate *L. monocytogenes*. Further, washing with chlorine has raised concerns about allowable chlorine levels, the impact of washing on the quality of the finished product, and the cost of water purchase and disposal (FERRER & DE SIMON, 1993). Depuration process of live bivalve mollusk reduces the level of *L. monocytogenes* but it does not eliminate it, guaranteeing safe product for consumers. It means that live bivalve mollusks are permanently indicated by water contamination with this pathogen.

Contamination with *Listeria monocytogenes* of terrestrial and water environment is a common problem indicating higher incidence of animal and human listeriosis (GIANFRANCESCHI et al., 2003). In New Zealand, the incidence of listeriosis is generally around 15 reported cases a year, resulting in two to four deaths. Seafood has been implicated in 2% of cases over the last 8 years (BAKER et al., 1993).

*Listeria monocytogenes* is primarily of concern in products that have a long refrigerated shelf life and are normally eaten with little or no reheating. Inadequately cooked or improperly handled shellfish and cold smoked fin fish are two common examples.

*Listeria monocytogenes* can survive for 10-30 days in tap water at 28-30°C and for 7-110 days at 5-10°C. In pond water, *L. monocytogenes* has been reported to survive for more than 8 weeks (air temperature – 26 to 9°C) (BOERLIN et al., 1997).

*Listeria monocytogenes* has been reported to survive for at least 3 weeks and probably significantly longer in seawater. Survival in seawater was dependent on seawater temperature and *L. monocytogenes* strain. In conditions simulating those present in a processing environment (humidity 75% and temp. 15°C), a mixed population of *L. monocytogenes* and *Flavobacterium* spp. survived for more than 75 days on a stainless steel surface. Being present in sea water *L. monocytogenes* can possibly contaminate live bivalve mollusk. A number of publications indicate that mussels can frequently be contaminated with *L. monocytogenes* (BREMER & OSBORNE, 1997). The incidence rates from 2-35% according to some study reports from different countries (HARTEMINK & GEORGSON, 1991; MCCARTHY, 1997). Seawater samples, fish, live bivalve mollusks, shrimps, crabs and other water species are implicated by contamination of this pathogen. Considering the contamination of mussels (*M. galloprovincialis*), a possible risk for human and environment pollution, we undertake this study to evaluate the incidence of *L. monocytogenes* in production zones of Butrinti lagoon.

## MATERIALS AND METHODS

189 samples of mussels were collected during a two-year period, in the Lagoon of Butrinti located in south part of Albania according to respective sampling

points with geographical coordinates; (N: Latitude 20°18', Longitude 39°47', Latitude 20°18', W: Longitude 39°45', Latitude 20°12', S:Longitude 39°45', Latitude 20°28'). The samples have been ground at the same day of sampling and kept at the refrigerator. They were packed in sterile plastic bags, placed on ice, and transported under cooling conditions (4-6°C) to the laboratory of Department of Food Control at the Institute of Veterinary Research, Tirana. The same day of their arrival at the laboratory, bacteriological analysis of the samples for determination the presence of *Listeria spp.* was carried out.

Each analytical sample, consisted of 25g ground meat taken from representative samples was homogenized with 225ml of primary enrichment broth medium (half Fraser Oxoid CM) in a horizontal mixer type "Stomacher 400" for 2 minutes, and was incubated for 24h at 30°C. After incubation, 0.1ml of pre-enriched cultures were transferred into tubes containing 10ml of secondary (full Fraser enrichment broth, Oxoid CM) and incubated for 24h – 48h at 37°C. Following incubations, a loopfull from each broth (primary and secondary enrichment culture) was streaked simultaneously on two selective solid plates, Oxford agar and PALCAM agar (Oxoid CM35). Incubation of inoculated Oxford agar plates was done at 30°C, inoculated PALCAM agar plates at 37°C. Examination of the plates for the presence of colonies presumed to be *Listeria* was carried out after overnight incubation or for an additional 18h to 24h. From each plate of each selective medium at least five colonies were selected for confirmation and transferred onto the surface of a pre-dried plates of TSYEA. Typical colonies of pure culture in TSYEA were tested further by catalase reaction, Gram staining, motility test, haemolysis test, and identified by biochemical tests using the API - *Listeria* system (Bio Merieux, Paris, France), as well as by CAMP test.

## RESULTS AND DISCUSSION

The lagoons are always indicated by effect of contamination of microbial agents targeting also and species of different aquatic animals. From pathogens as contaminants, *L. monocytogenes* is of high importance for the public health and protection of environment. Live bivalve mollusks during their biological activities are filtrating sea water and at the same time different cells of microorganisms including and *L. monocytogenes* (MOTES, 1991). In this context, considering live bivalve mollusk as an aquatic animal and food at the same time, the presence of *Listeria monocytogenes* compromises the quality and safety of this product when it is used as food.

As biggest reservoir for production of mussels (*Mytilus galloprovincialis*) lagoon of Butrinti is under the control of veterinary inspection. Control of pollution sources remains a permanent objective of veterinary and fishery inspectorates.

Analytical control of live bivalve mollusk for presence of *Listeria monocytogenes* is not yet required by the EU legislation for production zones of live bivalve mollusks. This study was carried out to evaluate the incidence of this pathogen in three sampling points. According to results of Table 1 and Table 2, *Listeria monocytogenes* was present during both years of this study. From the analytical control of 189 samples *Mytilus galloprovincialis* carried out from 2008 to 2009, 12 (6,1%) of them were identified positive for presence of *L. monocytogenes*. Incidence of *L. monocytogenes* was 5,1 % in 2008 and it grew higher in 2009 with 7,2% of samples collected in this time. The confirmed value of incidence of *L. monocytogenes* for both years comparing with analogue studies represents the same problem present in other countries (FARBER, 1991). According to the study reports, the incidence of this pathogen in sea water products ranges from 2 to 12% in the most of cases (BEN EMBARECK, 1994). From the study carried out by FERRER & DE SIMON (1993) it was concluded that *L. monocytogenes* was present in the 8,6% of the mussels. Other study confirmed that the incidence of *L. monocytogenes* in oysters was 2,5% (WEAGENT et al., 1988). Results found by this study showed the contamination of mussels with this pathogen in production zone of Butrinti as possible way of human infection.

**Table 1: Samples of live *Mytilus galloprovincialis* collected from Butrini lagoon and strains of *L. monocytogenes* isolated from their control from 2008 to 2009.**

On 2008			On 2009		
sampling points	No. samples	Presence of <i>L. monocytogenes</i>	sampling points	No. samples	Presence of <i>L. monocytogenes</i>
North	27	2	North	37	5
South	27	1	South	34	2
West	24	1	West	40	1
78 samples		4 strains of <i>L. monocytogenes</i>	111 samples		8 strains of <i>L. monocytogenes</i>

**Table 2. Incidence of *L. monocytogenes* isolated from samples of live *Mytilus galloprovincialis* on 2008 and on 2009.**

Incidence of <i>L. monocytogenes</i> on 2008			Incidence of <i>L. monocytogenes</i> on 2009		
North	27	2	North	37	5
South	27	1	South	34	2
West	24	1	West	40	1
78 samples		4 (5, 1%)	111 samples		8 (7, 2%)
189 samples /12 strains of <i>L. monocytogenes</i> (6, 1%)					

In the table 3 are presented the isolated strains of *L. monocytogenes* in *M. galloprovincialis* samples in different sessions of the years 2008 and 2009. It is clear that more strains are isolated in 2009 and during the winter period. This is explained by heavier rainfalls and discharge of waste waters from rural areas into the lagoon. This fact is mentioned by other authors in analogue studies (VARMA & LYER, 1993).

**Table 3: Strains of *L. monocytogenes* isolated in 2008 and in 2009.**

Sampling points	<i>L. monocytogenes</i> strains isolated on 2008				<i>L. monocytogenes</i> strains isolated on 2009			
	spring	summer	autumn	winter	spring	summer	autumn	winter
North	-	-	1	1	1	1		3
South	1	-	-	-			1	1
West	-	1	-	-			1	

## CONCLUSIONS

- Mussels produced in Butrinti lagoon are indicated for the contamination with *L. monocytogenes* in 6,1% of the cases. The high risk is coming from northern part of the lagoon where a canal of river Bistrica inflows bringing the fresh water and, in cases of rainfall, more contaminated water flow from rural areas.

- *Listeria monocytogenes* contaminates *Mytilus galloprovincialis* with higher incidence in 2009 (7,2%) confirming that risk remains permanent with the tendency to increase.

- Control enforcement of pollution sources of production zone of Butrinti lagoon and analytical control for *L. monocytogenes* guarantees the protection of public health and water environment.

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