

## INCIDENCE AND VIABILITY OF *L. MONOCYTOGENES* IN SEAFOOD HANDLING AND PROCESSING

By

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### ABSTRACT

*Listeria monocytogenes* was absent in all the 361 samples of frozen shrimps taken from the export trade. The organism survives freezing and further storage at  $-18^{\circ}\text{C}$  for over two years. In water *L. monocytogenes* survives 10-30 days at room temperature ( $28-30^{\circ}\text{C}$ ) and 70-110 days at refrigerated temperature ( $5-10^{\circ}\text{C}$ ) depending upon the initial load in the water sample. The process water in seafood processing factories should be chlorinated to a residual level of 10 ppm. to get full protection against *L. monocytogenes*.

### INTRODUCTION

During recent years, *Listeria monocytogenes* has been generally accepted as a food borne pathogen as it has been well established as a causative organism in several outbreaks of listeriosis in North America and Europe. Although the disease is relatively rare it is considered to be serious because of the high mortality rate, 30%, (Fuchs and Reilly, 1992). A number of outbreaks of listeriosis have been documented in which dairy and vegetable products were implicated as the source of infection (Fleming *et al.*, 1985; Lovett *et al.*, 1987). Seafoods are also involved in listeriosis. One outbreak of listeriosis has been linked to the consumption of shell fish and raw fish (Lennon *et al.*, 1984). Listeriosis from consumption of steamed fish containing *L. monocytogenes* has been documented (Facinelli *et al.*, 1989). Due to the involvement of the organism in food borne listeriosis several administrative and advisory bodies have started insisting that cooked and ready-to-eat food products have to be free from *L. monocytogenes*. The United States Food and Drug Administration (FDA) stipulates that *L. monocytogenes* is to be absent in 25 g samples of cooked and smoked seafoods. The U.S. National Advisory Committee on Microbiological Criteria for Foods has recommended that *L. monocytogenes* shall be absent in 25g samples of ready-to-eat shrimps and crab meat collected from a lot as a composite of five units of 50g (Buchanan, 1991). Monitoring of fish and fishery products for the presence of *L. monocytogenes* has been going on in many laboratories for the last 5-6 years. The presence of *L. monocytogenes* in frozen seafoods imported to the U.S. has been recorded by Weagant *et al.*, 1988, to the extent of 28% of the samples examined by them. On the other hand Fuchs and Surendran (1989) noted absence of *L. monocytogenes* in fresh and dried fish samples collected from Cochin, India. Hartemink and Georgsson (1991) isolated *L. monocytogenes* from 24% of raw fish samples examined by them. Buchanan *et al.*, 1989 isolated the pathogen from uncooked seafood samples. The organism has also been isolated from minced fish (Rorvik and Yndestad, 1991). Manoj and his colleagues (1991) on the other hand could not isolate *L. monocytogenes* from fish and fish handling areas at Mangalore, India. Review of literature indicates that only very limited work has been done

on the incidence of *L. monocytogenes* in seafoods of the export trade. India exports considerable quantities of frozen shrimps to the U.S.A. However, there is no recorded information on the incidence of *L. monocytogenes* in the seafoods processed and exported by India.

The characteristics of *L. monocytogenes* are different from those of other pathogens. It can grow at refrigerated temperatures and is known to survive in frozen products for a considerable length of time. However, the length of survival in frozen seafoods is not exactly known. Information on the level of chlorination to be adopted in seafood processing factories for disinfection of process water is also scanty. The Central Institute of Fisheries Technology has carried out studies on the incidence of *L. monocytogenes* in frozen shrimps from the export trade and also on the viability of the organism during freezing and frozen storage. Detailed studies have also been undertaken on survival in process water and viability during chlorination. The result of these investigations are presented in this paper.

## MATERIALS AND METHODS

361 samples of frozen shrimps collected from the processing factories at Cochin were used for monitoring the incidence of *L. monocytogenes*. The samples (25g. each) were brought to the laboratory in ice boxes under aseptic conditions. In all cases the analysis was started within 30 minutes of the sample collection.

To study the viability of *L. monocytogenes* during freezing and frozen storage, fresh peeled and deveined shrimps were inoculated with pure strains of *L. monocytogenes* ( $1.0 \times 10^4$  to  $1.0 \times 10^7$  cells/g) and were frozen and stored at  $-18^\circ\text{C}$ . The samples were withdrawn periodically and tested for the organism.

For the study on the viability of the organism in water, samples of water taken in glass containers of 5L capacity were inoculated with pure strains of *L. monocytogenes* and were stored at ambient temperatures (28 to  $30^\circ\text{C}$ ) and at refrigerated temperatures (5 to  $10^\circ\text{C}$ ). The samples were tested periodically for the organism.

To study the effect of chlorination on the viability of *L. monocytogenes* in water, the water samples, inoculated with pure known cultures of *L. monocytogenes*, were chlorinated to 5 and 10 ppm. with a contact time of 3, 5 and 10 minutes and samples were subsequently tested for the organism. Oxoid Listeria selective enrichment broth (CM 863) with supplement SR 142 was used for enrichment of the samples. For presumptive identification Oxoid Listeria selective agar base CM 856 with supplement SR 140 was used. The identification of the suspected organism was done as per the method outlined by Lovett, 1988.

## RESULTS AND DISCUSSION

Details of samples collected and the incidence of *L. monocytogenes* are given in Table 1.

None of the 361 samples of frozen shrimp showed incidence of *L. monocytogenes*. However, a few samples showed presence of *L. murrayi* and *L. grayi*, species generally considered to be non-pathogenic. The results indicate that *L. monocytogenes* is not a serious

pathogen in the shrimps exported from India. There is no previous report of the isolation of *L. monocytogenes* in India either in fresh or frozen fish/shell fish.

Table 1

Incidence of *Listeria* in various samples

Samples	No. of samples	Number of samples positive for <i>L. monocytogenes</i>	Species of <i>Listeria</i> isolated
Frozen HL shrimps	35	NIL	murrayi-2; grayi-8
Frozen PD shrimps	127	NIL	murrayi-1; grayi-8
Frozen PUD shrimps	100	NIL	murrayi-1; grayi-8
Frozen CP shrimps	99	NIL	

Table 2 gives the viability of *L. monocytogenes* during freezing and frozen storage.

No. of months of frozen storage	Raw shrimps			Cooked shrimps		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
0	+	+	+	+	+	+
2	+	+	+	+	+	+
4	+	+	+	+	+	+
6	+	+	+	+	+	+
8	+	+	+	+	+	+
10	+	+	+	+	+	+
12	+	+	+	+	+	+
18	+	+	+	+	+	+
20	+	+	+	+	+	+
24	+	+	+	+	+	+

In the present study *L. monocytogenes* survived freezing at -40°C and remained viable for over two years in raw or cooked shrimps at -18°C. This indicates that *L. monocytogenes* is highly resistant to sub-zero temperatures. A detailed investigation on the behaviour of *L. monocytogenes* in shrimps stored at sub-zero temperatures has not been reported earlier. Hence the data obtained in the present study cannot be effectively compared with any earlier study. However, based upon the isolation of *L. monocytogenes* from several frozen seafoods (Weagant *et al.*, 1988; Fuchs and Surendran, 1989; Wong *et al.*, 1990; Motes, 1991), Fuchs and Reilly (1992) have concluded that the pathogen has an extended viability in seafoods kept in frozen storage.

The viability of *L. monocytogenes* in water is given in Table 3. It is evident from the Table that the pathogen is viable in water for 10 to 30 days at room temperature (28 to 30°C) and for 70 to 110 days at refrigerated temperatures (5 to 10°C) depending upon the initial load in the water sample. The results also indicate the comparatively extended viability at refrigerated temperatures, irrespective of the number of organisms initially present in the water.

Table 3

Viability of *L. monocytogenes* in water

Initial load of <i>L. monocytogenes</i>	Less than $2 \times 10^5$ /ml	$9.1 \times 10^5$ /ml
Viability at room temp. (28-30°C)	8 to 10 days	27 to 30 days
Viability at refrigerated temp. 5-10°C	60 to 70 days	105-110 days

The extended viability of the organism in water has great practical significance in the seafood processing industry. Any contamination of the product from water during processing may create public health problems. Further, it is also known that *L. monocytogenes* is fairly resistant to lower doses of chlorine in process water. The viability of *L. monocytogenes* in water during chlorination is shown in Table 4.

Table 4

Viability of *L. monocytogenes* in water during chlorination

Treatment time. min.	Cells/ml	Growth after exposure to different levels of chlorine		
		5 PPM	10 PPM	Control No. chlorine
3	32,000	-	-	+
5		-	-	+
10		-	-	+
3	65,000	+	-	+
5		+	-	+
10		-	-	+
3	234,000	+	+	+
5		+	-	+
10		+	-	+

+ = Growth      - = No growth

From the results it is clear that chlorination of process water to a residual level of 5 ppm chlorine is often insufficient to destroy viable cells of *L. monocytogenes*. Similarly it

is also evident that a contact time of three minutes is not sufficient to eliminate the pathogen. Therefore it is recommended that the process water is to be chlorinated to a residual level of 10 ppm giving a minimum contact time of five minutes to get full protection.

*L. monocytogenes* was not isolated from the samples of frozen shrimps intended for export. However, as the organism has extended viability during frozen storage it is recommended that the seafood industry should always be cautious about the organism and continue to monitor its presence throughout the processing line and in the finished product.

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