Manual of Training Programme on Seafood Quality Assurance, CIFT Cochin, 14-26 Sep.: 96 (1998)

LISTERIA MONOCYTOGENES IN MARINE PRODUCTS

P.R.G. Varma

Interest in *Listeria monocytogenes* has grown in the last few years following several outbreaks in North America and Europe. However, reports on organisms resembling *Listeria* date back to the last century. Some of the earlier references about the organism are given in Table 1.

Table 1 References about *Listeria monocytogenes*

Year	Source	Proposed name
1891	Human tissue	-
1893	-do-	-
1911	Rabbit	*Bacillus hepatitis*
1926	Rodents	*Bacterium monocytogenes*
1927	Gerbil liver	*Listeria hepatitis*
1929	Human tissue	*Listeria monocytogenes*

Recent food borne outbreaks

The following are some of the recent food-borne outbreaks due to *L. monocytogenes* (Table 2). General characteristics of the organism are given in Table 3.

Table 2 Food-borne outbreaks due to *L. monocytogenes*

Year and Country	Vehicle	Number of cases	Number of deaths	Serotypes
1979 Boston	Raw tomato			
	Pasteurized milk	—	—	4b
1980 Newzealand	Shellfish	22	—	—
1981 Canada	—	41	18(44%)	4b
1983 Massachusettes	Pasteurized milk	49	14(29%)	4b
1985 California	Mexican stylechuse	142	47(33%)	4b
1987 Pheladelphia	Salami	—	14	4b

Table 3 General characters of the *Listeria*

Characteristic	Reaction
Gram character	Gram positive
Morphology	Small rods
Oxygen requirement	micro-aerophilic
Motility	Motile (20-25°C)
Peritrichous flagella	
Spore-formation	No spores
Catalase reaction	Positive
Temperature for growth	1 to 42° C
pH range for growth	5.6 to 9.2
Optimum temperature for growth	30° C
Capsule formation	Some produce a mucopolysac charide capsule
Factors affecting growth	Glucose and blood increase growth
Pigmentation	Never pigmented

Current taxonomy

Currently seven species of *Listeria* have been recognized.As given in Table 4.

Table 4 Taxonomy of *Listeria*

L. monocytogenes	A human pathogen
L. ivanovii	Rare in humans. Basically a mouse pathogen
L. welshimeri	Non-pathogenic
L. seeligeri	Non-pathogenic
L. innocua	Non-pathogenic
L. murrayi	Non-pathogenic
L. grayi	Non-pathogenic

L. denitrificans, which was previously grouped in *Listeria* is now recognized as a separate genus, *Jonesia* which is known as *Jonesia denitrificans*.

Significance of *L monocytogenes* in food

It is a pathogen. It is a tough organism and survives freezing and thawing. It is relatively heat resistant and if present in numbers more than 5.0×10^4 organisms/ml, it would survive and would be present in milk after pasteurization. It grows in refrigerated temperatures even 1°C and is relatively resistant to common salt and nitrate. It survives for 8 weeks in 20% sodium chloride at 4°C. High mortality rate is seen in the cases of human listeriosis (about 30%). Animal and human carriers without any symptom can shed the organism for more than three years.

Habitat

L. monocytogenes is widely distributed in nature. This organism has been isolated from different habitats as follows:

Habitats of L. monocytogenes

Sewage	Leaves, grasses
Soil	Cabbage, Lettuce
Mud	Tomatoes, fruits
Faecal matter	Insects
Fertilizer	Organic lesions (warm blooded animals)
Decaying vegetation	Cerebrospinal fluid (cold blooded animals)

Silage harbours this organism sometimes in populations greater than 12,000 cells/g. This bacterium is also known to persist in silage for 10-12 years. The organism has been isolated from silage having alkaline as well as acidic pH.

Reservoirs

A variety of animals can serve as hosts for *L. monocytogenes*. The bacterium is often associated with the intestinal tract of domestic animals, birds and humans. About 1% of

human population is known to carry *L. monocytogenes.* Animals from which *L. monocytogenes* has been isolated are as follows:

Cattle	Frog	Chicken
Sheep	Fish	Crane
Goat	Crustacean	Dove
Swine	Tick	Duck
Horse		Eagle
Cat		Hawk
Dog		Parrot
Deer		Pigeon
Vole		Seagull
Guinea pig		Sparrow
Lemming		Turkey
Chinchilla		

Incidence of *L. monocytogenes* in foods

Surveys have indicated the presence of *L. monocytogenes* in various kinds of foods, dairy products topping the list. Once present in foods the organism can successfully compete with other microflora, survive and grow. Both raw and pasteurized milk contains this organism. Cows shed this organism in milk at populations of about 10^3 cells/ml is found. The contaminated raw milk can induce bacterium into dairy products and foods made from raw milk.

Isolation of this pathogen from 57 % of fresh and frozen poultry meat has been reported. A survey in the United States indicated that about 70% of ground beef, 43% of pork and 48% of poultry were contaminated with *L. monocytogenes.* Fruits and vegetables are often mentioned as source of *L. monocytogenes* than other foods. Cabbage, fresh lettuce and tomatoes are implicated in human disease outbreaks.

Relatively very little is known about the incidence of *Listeria* species in fishery products. A case of listeriosis on consumption of shellfish and fresh Finfish has been documented in Newzealand in 1984. In a recent study (1988) the U.S. Food and Drug Administration has reported isolation of *Listeria monocytogenes* from 15 out of 57 seafood samples tested by them. Results of the study are given below:

1.	Products analysed	Raw shrimps, cooked and peeled shrimps, cooked crab meat, raw lob ster tails, squids, langostinose, oysters, scallops, finfish and surimi based seafoods.
2.	Country of origin	Canada *, Chille *, China, Equador, Japan *, Korea *, Mexico, Philippines *, Singapore, Taiwan *, Thailand, U.S.A.*
3.	Number of samples showing incidence of *L.monocytogenes*	15 out of 57 samples (26%) Raw shrimps - 2 (Finfish 1) cooked shrimps - 2 cooked crab meat - 7, surimi based seafoods- 2, lobster tail -1 * *L. monocytogenes* was isolated from the samples from these countries.

Listeriosis

Listeriosis occurs sporadically throughout the year. Sometimes the occurance increases during summer. *Mortality due to infection is as high as 30% but in untreated cases it may go* up to 70%. Everyone who gets infected with *Listeria monocytogenes* need not necessarily become sick. In some cases the infection produces only mild flu-like symptoms. But in some cases it can even be a threat to life particularly in pregnant women, newborns and adults with a compromised immune system.

Forms of listeriosis

1 *Listeriosis of women during pregnancy*. This results in abortion or still birth. Such women after recovery may carry the organism for some more time

2 *Listeriosis of newborn*: Symptoms in such cases include respiratory distress, vomiting, mucus stool and heart failure.

3 *Meningo encephalitic listeriosis:* Clinically it will be exactly such as meningitis. Fatality is as high as 70% in such cases.

4 *Cutaneous listeriosis*: These are primarily skin lesions usually common among farmers and veterinarians who deal with infected animals.

5 *Septicemic listeriosis*: Symptoms include severe fever and pharyngitis: Sometimes the disease turns into meningitis.

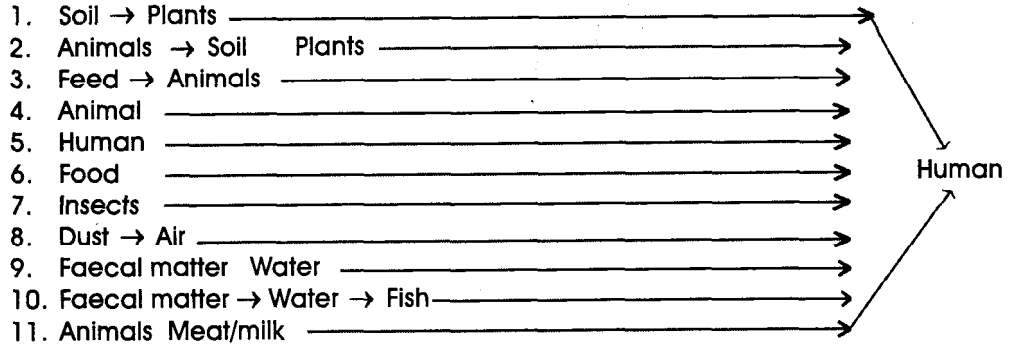
6 *Oculoglandular listeriosis* : Basically this is conjunctivitis. Complications similar to meningitis are possible

7 *Pneumoni listeriosis* : Symptoms are similar to those of pneumonia and typhoid.

8 *Other forms of listeriosis*: Infection can also result in endocarditis, arthritis, osteomyelitis, spinal or brain abscess, peritonitis and the such as.

Transmission of *L. monocytogenes* to humans

As *L. monocytogenes* are widely distributed in nature, human beings can be exposed to this bacterium in various ways. The possible routes of infection are given below:



Drugs of choice

1. Penicillin
2. Ampicillin

Effect of environmental and processing conditions on *L. monocytogenes*

Temperature

- Grows at refrigerated temperature
- Temperature range for growth lies between 3°C and 45 °C

pH

- Can grow in acidic pH up to 5.0
- Can grow in alkaline pH up to 9.6

Carbon dioxide

- Growth is enhanced under decreased oxygen concentration.

Sodium chloride

- Quite tolerent to sodium chloride
- Can survive 10% sodium chloride for 15 days at 37°C
- Can survive 20-30% sodium chloride for 5 days
- Can survive 10% sodium chloride for 30 days at 22°C
- Can survive 10-30% sodium chloride for 100 days at 4°C
- Can survive and grow in 10% sodium chloride for one year at pH 6.0

Nitrite

- Not inhibited by the usual concentrations permitted in foods.

Effect of pasteurization

- Several reports allude to the possibility that *L. monocytogenes* can survive the milk pasteurization temperature.
- In Massachusetts an outbreak of listeriosis on consumption of pasteurized milk has been reported. Survey carried out in the concerned factory has not shown any evidence of improper pasteurization.
- In Spain, 21% of the pasteurized milk are reported to contain *L. monocytogenes*.
- *L. monocytogenes* are known to survive pasteurization particularly when the thermal inactivation studies were carried out by the open-tube method.

  It is, therefore, recommended that the pasteurization may be done 72.2°C for 16.4 seconds instead of 71.7°C for 15 seconds.

High temperature processing

The organism is heat resistant.

- In eggs - survived frying
- In cheese - survived 75-76°C
- In milk - survived 57.2° C for 30 minutes
- In meat balls survived grilling for 15 minutes.
- In spray dried milk - survived.

Low temperature processing

L. monocytogenes has the ability to multiply in temperatures up to 1°C.

- In milk the organism survived up to 20 days at -10°C and -20°C.
- The organism has been isolated from several imported seafood samples frozen and stored at sub-zero temperatures.

L. monocytogenes in many respects is unique. The organism is capable of growing in refrigerated temperatures. It is also resistant to pasteurization temperatures. The organism is not inactivated in cheese within the ripening period of 60 days whereas many other pathogens do. In aged cheese it survives for more than 400 days.

Methods for identification of *Listeria monocytogenes* in seafoods

Procedure

Stage 1 Enrichment

Inoculate 25 g sample in 225 ml Oxoid *Listeria* Enrichment Broth base (UVM formulation) (CM 863) containing Oxoid *Listeria* Primary Selective Enrichment Supplement (UVMI) (SR 142E). Incubate for 24 hours at room temperature.

Stage 2 Selective media

Streak 1 loopful from *Stage 1* to plates of Oxoid *Listeria* Selective Agar Base (CM 856) containing Oxoid *Listeria* Selective Supplement (Oxford formulation) (SR 140E) and incubate at room temperature for 24-48 hours.

Listeria monocytogenes colonies will be brown and transparent and the surrounding media brown.

Stage 3

Transfer (stab) typical colonies to SIM media and incubate at Room temperature for 48 hours. *L. monocytogenes* will show umbrella motility.

Stage 4 Biochemical tests

1	Gram Reaction	Positive rods
2	Motility	Tumbling motility
3	TSI	Acid butt and slant, no gas, no H_2S
4	Urea hydrolysis	Negative
5	MR	Positive
6	VP	Positive
7	Dextrose Fermentation	Positive
8	Maltose Fermentation	Positive
9	Aesculin Fermentation	Positive
10	Mannitol Fermentation	Negative
11	Rhamnose Fermentation	Positive
12	Xylose Fermentation	Negative
13	Camp test	S. *aureus* positive

Composition of media

Listeria Enrichment Broth Base (UVM formulation)
Oxoid No.CM 863

Proteose Peptone	5.0g
Tryptone	5.0g
Lab. Lemco Powder	5.0g
Yeast extract	5.0g
Sodium chloride	20.0g
Di. Sod. hydrogen Phosphate	12.0g
Potassium dihydrogen phosphate	1.35g
Aesculin	1.0g
pH :	7.4 ± 0.2

Autoclaving 121°C for 15 minutes.

To 500 ml of the above suppliment add due vial of *Listeria* primary selective enrichment Supplement (UVMI) S.R. 142 E.

Composition of SR 142 E.

One vial contains -

Nalidixic Acid	-	10 mg
Acriflavine hydrocholoride	-	6.0 mg

Dissolve one vial in 2ml sterile distilled water

Listeria selective agar base

Oxoid formulation Oxoid code	CM 856
Blood agar base	39.0 g/l
Aesculine	1.0 g
Ferric amonium citrate	0.5 g
Lithium chloride	15.0 g
Final pH -	7.0 ± 0.2

Autoclaving 121°C (15 lbs) for 15 minutes.

Blood agar base

Peptone	23.00g
Starch	1.00g
Sodium chloride	5.00g
Agar	10.00g

To 500 ml of the *Listeria* selective agar base add the content of one vial of *Listeria* selective supplement (Oxoid formulation Oxoid code) SR 140 E

One vial of SR 140 E contains

Cycloheximide	200 mg
Colistine sulphate	10 g
Acriflavine	2.5 mg
Cefotetan	1.0 mg
Fosfomycin	5.0 mg

Dissolve the above contents in 5 ml of 1:1 mixture of Ethonol and sterile distilled water.

SIM (Sulphide - Indole - Motility) medium

Tryptone	20.0g
Peptone	6.1g
Ferrous amonium sulphate	0.2g
Sodium thiosulphate	0.2g
Agar	3.5g
pH	7.3

Autoclaving 121°C (15 lbs) for 15 minutes.

Triple Sugar Iron Agar (TSI agar)

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	15.0 g
Proteose peptone	5.0 g
Glucose	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
$FeSO_4$	0.2 g

Sodium chloride	5.0 g
$Na_2S_2O_4$	0.3 g
Phenol red	0.024 g
Agar	12.0 g
Distilled water	1 litre

Urea Agar

Basal ingredients

Peptone	1.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Monobasic potassium phosphate	2.0 g
Phenol red (6 ml of a 1:500 solution)	0.012 g
Agar	15.0 g
Distilled water	900 ml
Urea concentrate	
Urea	20.0 g
Distilled water	100.0 ml

Adjust to pH 6.8-6.9. Filter-sterilize.

Dissolve 15 g agar in 900 ml distilled water, add other ingredients and sterilize 15 min at 121°C. Cool to 50-55°C; then add 100 ml urea concentrate. Mix and distribute in sterile tubes. The medium is slanted with a deep butt.

Glucose Broth (MR-VP Broth) - Buffered

Proteose peptone	7.0 g
Glucose	5.0 g
Dipotassium phosphate (K_2HPO_4)	5.0 g
Distilled water	1 litre
Voges-Proskauer (VP) Test Reagents	
Solution 1	
a - Naphthol-	5.0 g
Alcohol (absolute) -	100 ml
Solution 2	
Potassium hydroxide -	40.0 g
Creatin -	0.3 g
Distilled water -	100 ml

Perform the Voges-Proskauer (VP) test at room temperature by transferring 1 ml of 48-hour culture to a test tube and adding 0.6 ml of a-Naphthol (solution 1) and 0.2 ml of 40% KOH (solution 2); shake after the addition of each solution. Read the results 4 hour fafter adding the reagents. A positive VP test is the development of an eosin pink color.

Preparation of sugar media for *Listeria monocytogenes*

Basal media

Peptone	10.0 g
Sodium chloride	5.0 g
Distilled water	1 litre
pH -	7.2

After adjusting the pH add 3.2 ml of 1% Bromocresol purpule per litre of the basal media. Prepare 0.5 % of the Sugars (Dextrose, Maltose, Aeculine, Mannitol, Rhamnose & Xylose) in the basal media, transfer into sugar tubes with Durham tubes inside and sterilise at 10 lbs for 20 minutes.

Camp test

The christie-Atkins-Munch-Peterson (CAMP) test is useful in confirming species, particularly when blood agar stab test results are equivocal. To perform the test, streak a beta-hemolytic *Staphylococcus aureus* and a *Rhodococcus equi* culture in parallel and diametrically opposite each other on a sheep blood agar plate. Streak several test cultures parallel to one another, but at right angles to and between the *S. aureus* and *R. Equi* streaks. After incubation at 35°C for 24-48 hours, examine the plates for hemolysis. Hemolysis is more easily read when the blood agar is thinner than usual. The *L. monocytogenes* reaction is often optimal at 24 hours rather than 48 hours. To obtain enough *R. Equi* and to give a good streak of growth, incubate the inoculum slant culture longer than 24 hours. Use of known control *Listeria* spp. on a separate sheep blood agar plate is recommended. Sheep blood agar plates should be as fresh as possible.

Streak weakly *beta*-hemolytic *S. aureus* strain *R. Equi* vertically on sheep blood agar. Separate vertical streaks so that test strains may be streaked horizontally between them without quite touching them. After 24 and 48 hours incubation at 35°C, examine plates for hemolysis in the zone of influence of the vertical streaks. CAMP test cultures are available from several national culture collections.

Hemolysis of *L. monocytogenes* and *L. seeligeri* is enhanced near *S.aureus* streak; *L. ivanovii* hemolysis is enhanced near *R. equi* streak. Other species are nonhemolytic and do not react in this test. The CAMP test differentiates *L. ivanovii* from *L. seeligeri* and can differentiate a weakly hemolytic *L. seeligeri* (that may have been read as nonhemolytic) from *L. welshimeri*. Isolates giving reactions typical of *L.monocytogenes* excepting for hemolysin production should be CAMP-tested before they are identified as nonhemolytic *L. innocua*. A factor easily prepared from *S. aureus* cultures may be used to enhance hemolysis by *L. monocytogenes* and *L.seeligeri* fin sheep blood agar plates.

CAMP test reactions of Listeria species

	Hemolytic interaction	*Rhodococcus*
Species	*Staphylococcus aureus* (S)	*equi* (R)
L. monocytogenes	+	-
L. ivanovii	-	+
L. innocua	-	-
L. welshimeri	-	-
L. seeligeri	+	-

Rare strains are S+ and R+, but the R+ reaction is much less pronounced than that of *L. ivanovii.* (Table 5).

Table 5 Identification of *Listeria*

Species	Gram reaction	Motility	SIM mortality medium	Catalase	Beta Haemolyels	NO_3 reduction	TSIA slant	Urea hydrolysis	CAMP test	MR	VP	Dextrose	Maltose	Esculin	Mannitol	Rhamnose	Xylose	
L. monocytogenes	+	+*	+[u]	+	+	–	AB AS	–	S+	+	+	+	+	+	–	+	–	
L.lvanovii	+	+	+	+	+	–	AB AS	–	R	+	–	–	+	+	–	–	–	
L. innocua	+	+	+	+	–	–	AB AS	–	+	+	–	–	+	+	–	V	–	
L.welshimeri	+	+	+	+	–	–	AB AS	–	–	+	–	–	+	+	–	V	+	
L.seeligeri	+	+	+	+	+	–	AB AS	–	–	+	+	–	+	+	–	–	–	
L.irayi	+	+	+	+	–	–	AB AS	–	–	+	+	–	+	+	–	–	–	
L.murrayi	+	+	+	+	–	+	AB AS	–	–	+	+	–	+	+	–	V	–	

*Turbidity
[u] Umbrella motility
AB, AS – Acid butt, acid slant, No H_2S
V Variable