

# *Listeria monocytogenes*

## Class Notes PHR 150

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### Introduction

*L. monocytogenes* was first described in 1923. Prior to 1982 *L. monocytogenes* was recognized as a cause of abortions and encephalitis in many animals (particularly cattle and sheep) and was thought to be associated with contaminated animal feed or silage. While it was recognized as a cause of human illness in 1929 it was not until 1981 that a foodborne association was widely accepted.

The organism, its epidemiology, mechanisms of virulence, occurrence, and methods of detection in foods have been extensively reviewed (Bell and Kyriakides, 1998; Johnson et al., 1990; Farber and Peterkin, 1991; Farber, 1993; Pearson and Marth, 1990; Ryser and Marth, 1999).

### General characteristics

*L. monocytogenes* is a gram-positive ovoid to rod-shaped bacteria that is ubiquitous in the environment. It is a facultative anaerobe and acid but not gas is produced from glucose. The organism is capable of multiplying at temperatures between approximately 0 and 45°C (it is a psychrotroph), it is relatively resistant to NaCl (growth at 10%; survival at 20 to 30%) and low pH, is not inhibited by carbon dioxide, and can survive many processing techniques such as freezing and drying (Farber and Peterkin, 1991; Lammerding and Doyle, 1990). Table 1 indicates limits for growth of *L. monocytogenes* under ideal laboratory conditions. Limits for growth may be more restrictive in the conditions found in food systems.

**Table 1. Growth characteristics of *L. monocytogenes* under otherwise optimum conditions. (Adapted from ICMSEF, 1996.)**

	Minimum	Optimum	Maximum
Temperature (°C)	-0.4	37	45
pH	4.39	7.0	9.4
NaCl			growth at 10%
Water activity	0.92		

The Canadian regulatory policy on *L. monocytogenes* includes the following growth parameters for ready-to-eat products:

**RTE foods not supporting the growth of *L. monocytogenes* include the following (Farber and Harwig, 1996):**

- 1) pH 5.0 - 5.5 and water activity <0.95
- 2) pH < 5.0 regardless of water activity
- 3) water activity of less than or equal to 0.92 regardless of pH
- 4) frozen foods

**Table. 2.** Approximate lag and generation times for *L. monocytogenes* generated using the USDA Pathogen Modeling Program Version 6.0 for a pH of 6.0 and water activity of 0.98.

Temperature (°C)	Lag time (days)	Generation time (hours)
4	124.9	19.6
6	89.1	13.2
8	64.6	9.1
10	47.6	6.4
12	35.6	4.6
14	27.1	3.4
16	20.9	2.5

### **Taxonomy**

The genus *Listeria* is comprised of six species: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, and *L. ivanovii*. On rare occasions, *L. seeligeri* and *L. ivanovii* have been implicated in human infections. However, *L. monocytogenes* is the only species considered to be of public health significance.

### **Serology**

All 13 serotypes of *L. monocytogenes* may cause human listeriosis, however, 95% of human isolates are 1/2a, 1/2b or 4b. Serotype 4b strains are responsible for 33 to 50% of human listeriosis worldwide. This serotype has also been responsible for most recorded foodborne outbreaks.

### **Reservoirs**

*L. monocytogenes* is widely distributed in the environment. It can be found in decaying vegetation, in soils, animal and human feces, sewage, silage, and water.

*L. monocytogenes* has been isolated from a wide range of retail foods (Farber et al., 1989; Farber and Peterkin, 1991). Although numbers are often very low in these products, multiplication of *L. monocytogenes* can potentially occur during refrigerated retail and home storage. The psychrotrophic nature of *L. monocytogenes* makes it of particular concern in refrigerated foods with extended shelf life. Outbreaks of listeriosis have been associated with vegetable, dairy, and meat products.

It is estimated that 80 to 90% of listeriosis cases are linked to ingestion of contaminated food, however, demonstration of foodborne listeriosis is relatively rare and most cases are sporadic.

## Listeriosis

### Infective dose

The infectious dose is unknown but thought to be highly strain and host dependent. It is likely that <1000 CFU is of no concern to healthy adults. However, it is assumed that this level will cause illness in susceptible persons.

### Incubation period

24 hours to 91 days.

### Symptoms

Symptoms range from flu-like to septicemia and meningitis (Table 3). Listeriosis refers to the more serious life-threatening illnesses while gastroenteritis is the relatively mild illness experienced by healthy adults. Pregnant women, newborns, elderly and immunocompromised individuals are most susceptible and experience a more severe illness. Case fatality rates for these groups range from 13 to 34% (Farber and Peterkin, 1991).

### Virulence Factors

*L. monocytogenes* is an intracellular parasite. Abnormalities in T-cell immunity increase the risk of listeriosis. The T-cell response in the first few days following infection is important to the subsequent outcome of the disease. Considerable progress has been made in the past decade in understanding the pathogenesis of *L. monocytogenes*. Eight genes clustered on the chromosome are associated with virulence. *L. monocytogenes* cells cross the intestinal barrier via intestinal epithelial cells or the M cells of Peyer's patches. The organism is internalized by phagosomes. Surface proteins internalin and p60 are thought to aid internalization of *L. monocytogenes*. Once internalized within a phagosome, the vacuole membrane is lysed and *L. monocytogenes* is released to the cytoplasm where it can multiply. Listeriolysin O and a phosphatidylinositol-phospholipase C are involved in the lytic process. An essential component of virulence is the ability of *L. monocytogenes* to spread directly from cell to cell. To do this the organism uses the host cell actin machinery continuously assembling an actin tail at a pole of the bacterial cell surface. This serves to propel the bacterium across the cytoplasm pushing the organism against the host cell membrane thus forming a protrusion which can be ingested by an adjacent cell. Three genes, *mpl*, *actA*, and *plcB* have been linked to this process. The resulting vacuole is lysed releasing *L. monocytogenes* into the cytoplasm of the newly infected cell.

The phagosomes are transported via the blood to the lymph nodes, liver and spleen. Further dissemination of the organism via the bloodstream to the brain, or placenta in the pregnant woman, occurs giving rise to the various forms of the illness.

**Table 3. Illness caused by *L. monocytogenes*. (Adapted from Bell and Kyriakides, 1998)**

Type of Listeriosis	Nature of Infection	Severity	Time to Onset
Zoonotic infection	Local infection of skin lesions	Mild and self-resolving	1-2 days
Infection during pregnancy (listeriosis)	Acquired following the consumption of contaminated food	Mild flu-like illness or asymptomatic in the mother but serious implications for unborn infant including spontaneous abortion, fetal death, stillbirth and meningitis. Infection more common in third trimester	Varies from 1 day to several months
Neonatal infection (listeriosis)	Infection of new-born babies from infected mother during birth or due to cross-infection from one neonate in the hospital to other babies	Can be extremely severe, resulting in meningitis and death	1-2 days usually from congenital infection prior to birth
Infection of non-pregnant adults and children >1 month (listeriosis)	Acquired following the consumption of contaminated food	Asymptomatic or mild illness, which may progress to central nervous system infections such as meningitis. Most common in immunocompromised or elderly	Illness may occur within 1 day or up to several months
Gastroenteritis	Consumption of food with exceptionally high levels of <i>L. monocytogenes</i> , >10 <sup>7</sup> per ml	Vomiting and diarrhea, sometimes progressing to bacteremia but usually self-resolving	<24 h after consumption

Most cases of listeriosis are sporadic and not outbreak associated. Recent Foodnet data indicate that 2004 diagnosed infections range from 0.27 cases per 1,000,000 population (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5414a2.htm>).

## Outbreaks of Listeriosis

**Table 4. Examples of foodborne outbreaks of listeriosis**

Year	Country	Cases (deaths)	Food	Outbreak serotype	Food Isolate?
1980-81	Canada	41 (18)	Coleslaw	4b	Yes
1983	USA	49 (14)	Pasteurized milk	4b	
1983-87	Switzerland	122 (34)	Vacherin cheese	4b	Yes
1985	USA	142 (48)	Mexican-style soft cheese	4b	Yes
1987-89	UK	>350 (>90)	Belgian pate	4b	Yes
1992	New Zealand	4 (2)	Smoked mussels	1/2a	Yes
1992	France	279 (63)	Pork tongue in aspic	4b	Yes
1994	USA	45 (0)	Chocolate milk	1/2b	Yes
1995	France	20 (4)	Raw-milk soft cheese	4b	
1998/99	22 states (US)	>100 (20)	Hotdogs/deli meats	4b	Yes
1999	NY, CN, MD	11 (3)	Pate implicated		No
2000	10 states (US)	29 (7)	Deli turkey meat	4b	Yes

### Representative outbreaks

#### **Coleslaw – 1981, Maritime Provinces of Canada**

Product type: Chopped cabbage and carrot sold premixed at retail

Levels: not known

Serotype: 4b

Extent: 41 cases, 18 deaths, 2 adults and 16 fetal or newborn

Comments: Cabbage was suspected to be contaminated with *L. monocytogenes* from uncomposted sheep manure. Sheep were suspected to have had *Listeria meningitis*. Cabbage was stored for extended periods of time prior to shredding. No antilisterial processes were applied.

Control Options: Apply good agricultural practices. Control use of manure. Storage at temperatures to prevent the growth of *L. monocytogenes* (<1°C) or storage for short period of time (<10 days).

#### **Mexican-style white cheese – 1985, Los Angeles County, CA (Linnan et al., 1988)**

Product type: Soft cheese pH 6.6

Levels: not known

Serotype: 4b

Extent: 145 cases, 64 deaths

Comments: Environment and equipment were grossly contaminated with *L. monocytogenes* even after clean up. Raw milk deliveries allegedly exceeded pasteurization capacity of plant.

Control Options: Adequate pasteurization. Proper sanitation.

#### **Chocolate milk – 1995, Illinois**

Product type: Pasteurized chocolate milk

Levels in Implicated Food: 10<sup>7</sup> - 10<sup>9</sup> CFU/ml

Extent: 45 persons - gastroenteritis

Comments: Outbreak strain was isolated from stool samples of infected individuals, from a tank drain at the manufacturing plant and from unopened packs of the implicated milk. Milk was pasteurized and passed to a holding tank prior to filling. The jacket of holding tank was in poor state of repair and refrigerant could not be used. Lining of the holding tank jacket was not intact. Milk leaked into the jacket where it stayed until the tank emptied. Product could re-enter the vessel and contaminate the remaining product being filled. Sanitizer “spray-balls” were blocked and it was likely that insufficient cleaning took place. Cartons were taken to the picnic - 2 hours unrefrigerated, then refrigerated, then unrefrigerated for several hours.

Control Options: Properly functioning equipment. Good sanitation program. Temperature control.

### **Hot Dogs and Deli Meats August 1998 – January 99, Multi-state (CDC, 1998, 1999).**

Product type: Hot dogs and deli meats

Levels: unknown

Extent: Over 100 people, 15 deaths, 6 miscarriages

Comments: Construction dust at the plant is believed to have contaminated the product in the packaging room.

Control Options: Proper sanitation during packaging of product. Post-packaging pasteurization.

### **Sporadic Cases**

1986/87 CDC Case Control Study. Hotdogs not reheated, undercooked chicken

1988 – 1990 CDC Case Control Study. Soft cheese, food from delicatessin counters, undercooked poultry

### **Risk Assessment (FDA/USDA, 2003)**

Ranks 1 to 6 High Risk (per serving): Deli meats, frankfurters (not reheated), pate/meat spreads, unpasteurized fluid milk, smoked seafood, cooked ready to eat crustaceans.

Ranks 1 Very High Risk (per annum): Deli meats

Ranks 1 to 3 High Risk (per annum): Pasteurized fluid milk, high fat and other dairy products, frankfurters not reheated.

### **Impact of Foodborne Illness**

With improvements in medical technology, the proportion of the population susceptible to foodborne listeriosis continues to rise. The numbers and types of foods in which *L. monocytogenes* is able to survive and grow (ready-to-eat, modified atmosphere packaged and chilled foods) also continue to increase. The economic impact of *L. monocytogenes* is enormous in terms of human health (medical costs, loss of life) and loss of revenue to the food industry (recalls, zero-tolerance).

Estimated foodborne cases in the U.S. annually: 1,526 – 1,767. Estimated deaths: 378 - 485. Annual estimated medical costs and productivity losses are 0.2 to 0.3 billion dollars (Buzby et al., 1996).

### Zero tolerance

FSIS and FDA don't necessarily set "tolerances" for pathogens. Pathogens are considered an adulterant in RTE foods. Zero is defined by sample size and sensitivity of the diagnostic test.

In 1985, the FDA began monitoring dairy products for *L. monocytogenes* after a particularly large outbreak of listeriosis associated with cheese. In 1989, the CDC published a report of a case of listeriosis from the consumption of turkey franks. In response, the USDA initiated a microbiological surveillance program for *L. monocytogenes* in ready-to-eat meats and initiated a zero tolerance policy prohibiting the sale of ready-to-eat meat products contaminated with *L. monocytogenes*. Shortly thereafter this policy was expanded to include all ready-to-eat foods, in practice this is interpreted as "processed" ready-to-eat foods. RTE foods are those products that have been processed so that they may be safely consumed without further preparation by the consumer.

This policy designated *L. monocytogenes* an "adulterant". Any ready-to-eat food that contains this organism (in a 50 g sample) can be considered adulterated and subject to a Class I recall and/or seizure. Class I recalls are classified as a situation in which there is a reasonable probability that the use of, or exposure to, a product will cause serious adverse health consequences. In the US from October 1, 1991 to September 30, 1992, 16% of all recalled products and 57% of class I recalls could be attributed to *L. monocytogenes* (Venugopal et al., 1996). It has been suggested that these procedures as well as consumer educational efforts lead to a reduction in illness and death associated with this organism (Tappero et al., 1995). When 1989 and 1993 data were compared, a reduction in illness and death of 44% and 48%, respectively was observed. Similar trends were noted in the U.K. Other countries, including Canada, have adopted more moderate approaches based on risk (see Farber and Harwig, 1996).

**Table 5. Canadian Compliance Criteria for *Listeria monocytogenes* in ready-to-eat (RTE) foods**

Category	Action Level for LM	GMP Status	Immediate Action	Follow-up Action
1. RTE foods causally linked to listeriosis. This list presently includes: soft cheese, liver pate, coleslaw mix with shelf life >10 days, jellied pork tongue	>0 CFU/50 g	N/A	Class I recall to retail level.  Consideration of Public Alert.  Appropriate follow-up at plant level.	
2. All other RTE foods supporting growth of LM <u>with refrigerated shelf-life &gt;10 days</u>	>0 CFU/25 g	N/A	Class II recall to retail level.  Health alert consideration  Appropriate follow-up at plant level.	
3. RTE foods supporting growth of LM with refrigerated shelf-life ≤ 10 days and all RTE foods not supporting growth	≤100 CFU/g	Adequate GMP	Allow sale	Appropriate follow-up at plant level.
	≤100 CFU/g	Inadequate or no GMP	Consideration of Class II recall or stop sale.	Appropriate follow-up at plant level.
	>100 CFU/g	N/A	Class II recall or stop sale	Appropriate follow-up at plant level

**Control in the Food Processing Environment**

1. Separation of raw product and processing areas.
2. Processing controls monitored, records maintained.
3. Dry processing areas.
4. Cleaning and sanitizing. Human hygiene
5. Environmental and product sampling.



**Further Reading:**

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**Suggested Web Sites (always look for dates on materials):**

<http://www.fsis.usda.gov/OA/topics/lm.htm>

A government website devoted to *L. monocytogenes*. See recent risk assessment.

<http://www.foodsafety.gov/~dms/lmrisk.html> Also check [www.foodsafety.gov](http://www.foodsafety.gov)

**Economics of listeriosis**

<http://www.ers.usda.gov/briefing/FoodborneDisease/listeria/index.htm>

For consumers: **Listeria and Food Safety Tips (May 1999)**. PDF Brochure.

For the meat industry: **Listeria Guidelines for Industry (May 1999)**.

FDA BAM Manual

<http://www.cfsan.fda.gov/~ebam/bam-toc.html>