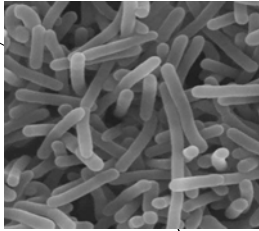


## *Listeria monocytogenes*



<http://Biology.kenyon.edu>

Adapted from Dr. Harris's notes

## Outline

- Introduction
- General characteristics
- Growth of Lm
- Taxonomy and serology
- Reservoirs
- Listeriosis, virulence factors, and infections
- Outbreaks of listeriosis
- Risk assessment and “zero tolerance” policy
- Control of Lm

## Introduction

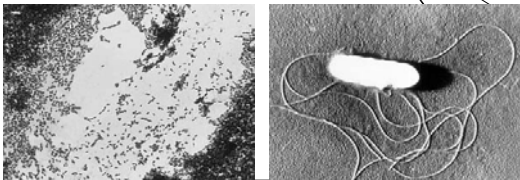
- Pathogen first described in 1923
- Before 1982,
  - recognized as a cause of abortions and encephalitis in many animals (particularly cattle and sheep)
  - thought to be associated with contaminated animal feed or silage

## Introduction

- In 1929, Lm was recognized as a cause of human illness
- It was 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.

## General Characteristics

- Gram-positive
- Ovoid to rod-shaped



<http://textbookofbacteriology.net/Listeria.html>

## General Characteristics

- Ubiquitous in the environment
- Facultative anaerobe
- Acid but not gas producing from glucose
- Can multiply at temperatures between ~0 and 45°C – a psychrotroph

## General Characteristics

- Relatively resistant to NaCl (growth at 10%; survival at 20 to 30%)
- Can tolerate low pH
- Not inhibited by carbon dioxide
- Can survive many processing techniques such as freezing and drying.

## Growth Characteristics of *Lm*

Limit-range	Temp. (°C)	pH	NaCl	Water activity
<b>Minimum</b>	-0.4	4.39		0.92
<b>Optimum</b>	37	7.0		
<b>Maximum</b>	45	9.4	Growth at 10%	

## Canadian Policy on RTE Foods (*L. monocytogenes*)

- RTE foods NOT supporting the growth of *L. monocytogenes* include:
  - pH 5.0 - 5.5 and water activity <0.95
  - pH < 5.0 regardless of water activity
  - water activity of  $\leq 0.92$  regardless of pH
  - frozen foods

(Farber and Harwig, 1996)

## Lag and generation times for *Lm* using USDA-PMP

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

Version 6.0; pH of 6.0 and water activity of 0.98

## Taxonomy

- Genus *Listeria*
  1. *monocytogenes*
  2. *innocua*,
  3. *welshimeri*
  4. *seeligeri*
  5. *grayi* ↔ *murrayi*
  6. *ivanovii*
- *L. monocytogenes* — only species considered to be of public health significance
- *L. seeligeri* and *L. ivanovii* — implicated in human infections on rare occasions

## Serology

- 13 serotypes of *L. monocytogenes*
- All serotypes may cause human listeriosis
- 95% of human isolates
  - 1/2a, 1/2b, or 4b
  - 4b accounts for 33–50% of human listeriosis worldwide, and has been responsible for most recorded foodborne outbreaks.

## Reservoirs

- *L. monocytogenes* is ubiquitous.
  - decaying vegetation
  - soils
  - animal and human feces
  - sewage
  - silage
  - water
- It has been isolated from a wide range of retail foods.

## Reservoirs

- Numbers of Lm are often very low in retail products, however, multiplication of Lm can potentially occur during refrigerated retail and home storage.
- Psychrotrophic nature of m.o. raises concern in refrigerated foods with extended shelf life.

## Reservoirs

- Outbreaks of listeriosis have been associated with vegetable, dairy, and meat products.
- ~80 to 90% of listeriosis cases are linked to ingestion of contaminated food – primarily foodborne.
- Implicated products include soft cheeses, deli meats, and hot dogs – products that are refrigerated, may have long shelf life, and permit growth.

## Listeriosis

- Infectious dose:
  - Unknown, but thought to be highly strain and host dependent.
  - It is likely that <1000 CFU is of no concern to healthy adults.
  - In susceptible persons, this level is assumed to cause illness.

## Listeriosis

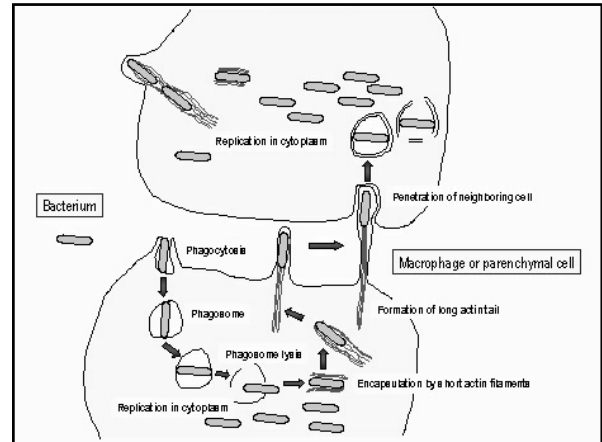
- Incubation period : 24 hr to 91 days
- Symptoms:
  - Range from flu-like to septicemia and meningitis.
  - Listeriosis refers to the more serious life-threatening illnesses while gastroenteritis is the relatively mild illness experienced by healthy adults.
  - Pregnant women, newborns, elderly and immunocompromised individuals are most susceptible and experience a more severe illness.
  - Case fatality rates for these groups range from 13–34%.

## Virulence Factors

- Intracellular parasite
- Abnormalities in T-cell (lymphocyte) 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.
- Eight genes clustered on the chromosome are associated with virulence.

## Virulence Factors

- Lm cells cross the intestinal barrier via intestinal epithelial cells or the M cells of Peyer's patches
- Internalized by phagosomes
  - Surface proteins internalin and p60 thought to aid internalization
- Once internalized, vacuole membrane lysed and m.o. released to cytoplasm where multiplication occurs.



## Virulence Factors

- Lm has the ability to spread cell to cell – an essential component of virulence.
- To accomplish this:
  - Lm uses the host cell actin machinery continuously assembling an actin tail at a pole of the bacterial cell surface.
  - The tail serves to propel the bacterium across the cytoplasm pushing the organism against the host cell membrane.
  - A protrusion is formed which can be ingested by an adjacent cell.
  - Three genes *mpl*, *actA*, and *plcB* have been linked with this process.

## Virulence Factors

- Phagosomes are transported via blood to lymph nodes, liver and spleen.
- Dissemination to brain, placenta gives various forms of illness.
- Lm is able to cross the gastrointestinal, materno-fetal, and blood-brain protective barriers.

## Listeria foodborne illness

- Well-defined high-risk groups
  - Pregnant women, neonates, immunocompromised adults



## Illness caused by Listeria

- Types:
  - Zoonotic infection
  - Infection during pregnancy (listeriosis)
  - Neonatal infection (listeriosis)
  - Infection of non-pregnant adults and children >1 month (listeriosis)
  - Gastroenteritis

## Zoonotic Infection

- Local infection of the skin lesions
- Mild and self-resolving
- 1–2 days

## Listeriosis during pregnancy

- Mother
  - Acquired following ingestion of contaminated food
  - Asymptomatic or mild flu-like illness
  - Serious complications for unborn infant (preterm delivery, spontaneous abortion, fetal death or stillbirth)
- Infection more common in 3rd trimester
- Onset from 1 to several months
- Pregnant women Not more susceptible to most foodborne pathogens.
- Pregnancy leads to down-regulation of cellular immunity.

## Neonatal (Newborn) Infection

- Infection of babies from mother during birth, or cross-infection from other neonates.
- Neonatal listeriosis can be highly severe.
  - Sepsis, pneumonia (less than 7 days old)
  - Meningitis, sepsis (greater than 7 days)
  - Death

## Infection of non-pregnant adults and children >1 month

- Acquired following ingestion of contaminated food
  - Asymptomatic or mild illness
  - Illness may progress to central nervous system infections such as meningitis.
  - Most common in immunocompromised or elderly
  - Onset with 1 day or up to several months

## Gastroenteritis

- Eating contaminated food with exceptionally high levels of Lm ( $>10^7$ /ml).
  - Vomiting and diarrhea
  - Can progress to bacteremia, but usually self-limiting
  - Onset <24 hr after consumption
  - Duration 1-2 days

## Immunocompromised adults

- 98% some kind of underlying illness
- AIDS, immunosuppressive medication, diabetes, age, kidney disease
- Alcoholism, cirrhosis, conditions associated with iron overload
- Mild illness which can progress to nervous system infections (meningitis)

## Outbreaks of Listeriosis

### Outbreak #1

- Coleslaw, 1981 Maritime Provinces of Canada
- Levels unknown
- Serotype: 4b
- 41 cases, 18 deaths, 2 adults and 16 fetal/newborn
- Contributing factors: Cabbage suspect contamination from uncomposted sheep manure. Cabbaged stored before shredding
- Control options: apply good agricultural practices, storage  $<1^{\circ}\text{C}$  or  $<10$  days

### Outbreak #2

- Mexican-style white cheese, pH 6.6, 1985, L.A.
- Levels unknown
- Serotype 4b
- 145 cases, 64 deaths
- Contributing factors: environment and equipment grossly contaminated, raw milk deliveries exceeded pasteurization capacity
- Control options: Adequate pasteurization, sanitation

### Outbreak #3

- Chocolate milk, 1995, Illinois
- Levels  $10^7$ – $10^9$  CFU/ml
- 45 persons (gastroenteritis)
- Outbreak strain found in tank drain and unopened pack of milk. Milk pasteurized then held. Jacket of holding tank poor repair, sanitizing spray “ball” blocked. Cartons transported to picnic and held un-refrigerated several hours.
- Control options: Equipment in good repair, adequate sanitation, temperature control.

### Outbreak #4

- Hot dogs and deli meats, 8/98–01/99, multi-state (22)
- Levels unknown
- 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, post-packaging pasteurization.

### Sporadic Cases

- 1986/87 CDC Case control study
  - Hotdogs not reheated, undercooked chicken
- 1988/90 CDC Case control study
  - Soft cheese, food from delicatessen counters, undercooked poultry

## Risk Assessment (FDA/USDA, 2003)

- Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods
  - <http://www.cfsan.fda.gov/~dms/lmr2-su.html>

## Impact of Foodborne Illness

- The population susceptible to foodborne listeriosis continues to rise.
- Numbers and types of foods in which Lm is able to survive and grow continue to increase.
- The economic impact of Lm 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 at 1,526–1,767; estimated deaths at 378–485; and annual estimated medical costs and productivity losses are 0.2 to 0.3 billion dollars

## Impact of Foodborne Illness

“Zero Tolerance” in Ready-to-Eat (RTE) food

## Controlling *Listeria*

- Barriers
  - Organism is widespread in environment
  - Grows under refrigerated conditions
- Sanitation important, and killed by heat

## Control in the Processing Environment

- Separation of raw product and processing areas.
- Processing controls monitored, records maintained
- Dry processing areas
- Cleaning and sanitizing
- Human hygiene
- Environmental and product sampling