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Outline

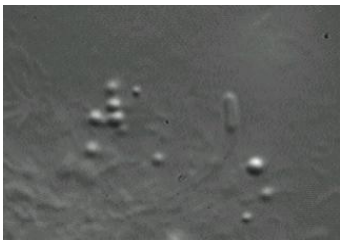
- Introduction
- Significance of *Listeria monocytogenes*
- Foodborne listeriosis
- Regulations
- Control

What is Listeria?

- Listeria monocytogenes is a type of bacteria that is found in water and soil
- Vegetables can become contaminated from the soil, and animals can also be carriers
- Listeria has been found in uncooked meats, uncooked vegetables, unpasteurized milk, foods made from unpasteurized milk, and processed foods
- Listeria can be killed by pasteurization and cooking

The *Listeria* genus

- Gram(+), non-sporeforming bacillus
- Six identified species
  - *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii*, and *L. grayi*
- Primary human pathogen
  - *L. monocytogenes*



Listeria monocytogenes is a rod-shaped aerobic and gram positive pathogenic bacterium that invades the cytoplasm of living cells. It develops a distinctive rocket tail structure to help push through the cytoplasm. Eventually, these "rockets" push bacteria into neighboring cells, propagating the infection

Significance of *L. monocytogenes*

- Ubiquitous presence
- Foodborne pathogen that causes listeriosis
  - 25,000 cases/year
  - exceptionally high mortality rate of 30%

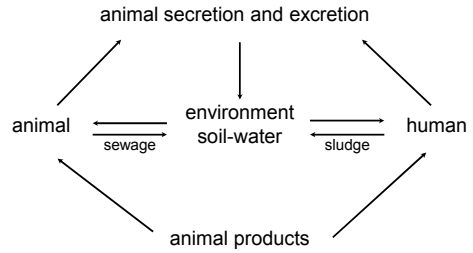
Epidemiology in US

- About 2,500 people in the U.S develop *Listeriosis* each year.
- 5 out of every 100 people carry *Listeria Monocytogenes* in their intestines.
- About 20% of people die from the infection.
  - In 1989, there were 1,965 cases of Listeriosis with 481 deaths.
  - In 1993, there were 1,092 cases of Listeriosis with 248 deaths.
  - *Listeria Monocytogenes* reached the blood and cerebrospinal fluid in 89% of cases.
  - *Listeriosis* results is a higher number of hospitalizations than any other food-born illness.
- Pregnant women account for 27% of cases, people with immunodeficiency disorders account for 70% of cases.
- AIDS patients are 280 time more likely to contract *Listeriosis* than others.

Distribution of *L. monocytogenes*

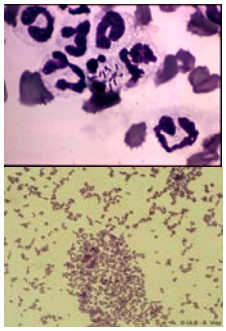
- The environment
  - soil, water, animal feces, sewage, decaying vegetation
- Food
  - fresh food products of animal or plant origin
  - Prevalence
    - lightly preserved fish products up to 75%

Circulation of *L. monocytogenes*



How do you get *Listeriosis*?

- Eating food contaminated with *Listeria.*
- Babies can be born with *Listeriosis* if the mother eats contaminated food during pregnancy.
- *Listeriosis* is generally not contagious from person to person.



Factors affecting growth and survival: I

- Temperature
  - -0.4-45ºC, optimum 30-35ºC
- pH
  - pH 4.4-9.4, optimum pH 6.5-7.5
  - pH tolerance is temperature and acid dependent
    - antimicrobial activity
    - acetic acid > lactic acid > citric acid > malic acid > HCl

Factors affecting growth and survival: II

- Water activity
  - temperature and humectant dependent

temperature (ºC)	humectant		
	glycerol	sucrose	NaCl
4ºC	0.92	0.93-0.96	0.94
21 ºC	0.91	0.925	0.924
30 ºC	0.90	0.92-0.96	0.92

  - *L. monocytogenes* is second only to staphyococci as a foodborne pathogen capable of growing at a_w<0.93

水分活性之定義

- 以水蒸氣壓，平衡相對濕度，可導出如下之 Aw 的關係式
  - Aw＝P／Po …… (1)
  - ∴ 0. 0 ≦ Aw ≦ 1. 0
- 式 (1) 為 Aw 之定義式。此地 P 為在某溫度下，物體所含水之蒸氣壓，Po 為在同溫度之純水的蒸氣壓。
  - Aw＝ERH (%)／100 …… (2)
- Aw 亦可以平衡相對濕度 (ERH) 之 (2) 式表示關係。

Factors affecting growth and survival: III

- Nutritional requirements
  - Vitamins
    - biotin (B_7), riboflavin (B_2), thiamine (B_1), and thioctic acid
  - amino acids
    - cysteine, glutamine, isoleucine, leucine, and valine

Foodborne listeriosis: I

- Foodborne illness caused by *L. monocytogenes*
  - 14 serotypes of *L. monocytogenes* have been designated
    - 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a,4b, 4bX, 4c, 4d, 5, 6a, 6b
  - Serotype 1/2a, 1/2b, and 4b account for 96% of human infections in the United States

Foodborne listeriosis: II

- At risk individuals
  - immunocompromised patients, pregnant women, the elderly, neonates, patients with diabetes
- Two forms of listeriosis
  - Invasive
  - non-invasive

Invasive listeriosis

- Incubation period
  - 30 days
- Minimum infectious dose
  - 10^3-10^9, depending on host and strain
- Symptoms
  - flu-like illness, meningitis, septicemia, non-meningetic central nervous system infection, spontaneous abortion*, stillbirth*, perinatal septicemia*

Non-invasive listeriosis

- Incubation period
  - 18-20 h
- Minimum infectious dose
  - unclear, but greater than invasive listeriosis
- Symptoms
  - febrile gastroenteritis, fever, fatigue, headache, nausea, cramps, vomiting, diarrhea

What makes *L. monocytogenes* so deadly?

- Listeriolysin O
  - the most significant virulence factor
  - responsible for β-hemolysis on erythrocytes and the destruction of phagocytic cells
  - present in all strains of *L. monocytogenes*
  - encoded by gene *hly*

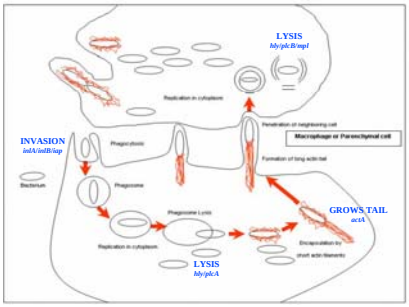
Molecular determinants of pathogenesis

- 9 genes responsible for *L. monocytogenes* pathogenesis
  - *hly*:SH-activated hemolysin
  - *plcA*: phosphatidyl inositol specific phospholipase
  - *plcB*: lecithinase
  - *mpl*: lecithinase-specific metalloprotease
  - *actA*: surface protein (actin assembly)
  - *inlA*: internalin
  - *prfA*: positive regulatory factor of *hly*, *plcA*, *plcB*, and *mpl*
  - *inlB*: surface bound protein
  - *iap*: invasion associated protein

Mode of infection

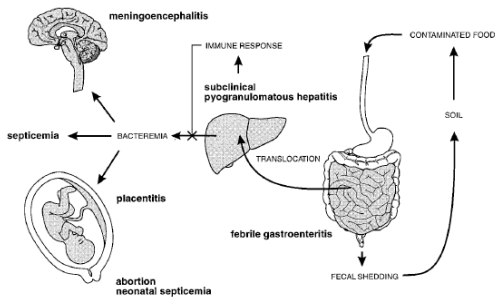
- Enter via oral route, attachment and invasion of intestinal mucosa
- Enters lamina propria
- Engulfed by phagocytes
- Produces listeriolysin O to break phagosome and enter the cytoplasm of the phagocyte
- multiplies in the phagocyte and invades other tissues

Mode of infection



Listeria monocytogenes

The Disease Entity



Listeria during pregnancy

Risks for pregnant woman getting listeriosis

- According to the Center of Disease Control (CDC), an estimated 2,500 people became seriously ill each year in the U.S. and among these, 500 will die.
- Pregnant women account for 27% of these cases
- The CDC claims that pregnant women are 20 times more likely to become infected than non-pregnant healthy adults.

Symptoms of Listeriosis

- Shows up 2-30 days after exposure
- Mild flu-like symptoms, headaches, muscle aches, fever, nausea, and vomiting
- If the infection spreads to the nervous system it can cause stiff neck, disorientation, or convulsions
- Even though the infection can occur at any time during pregnancy, it is most common during the third trimester when your immune system is somewhat suppressed.

Effects on the Fetus

- Miscarriage
- Premature delivery
- Infection of the newborn
- Death to the newborn
- Treatment
  - Antibiotics
  - Antibiotics are also given to newborns with listeriosis

Neonatal Disease: I

- Early onset:
  - Results from intrauterine infection, which can cause clinical illness in the newborn at birth or shortly after.
  - Is characterized by widely disseminated granulomas in the liver and placenta as well as other organs
  - May be associated with aspiration of infected amniotic fluid, which can lead to respiratory distress.
  - Signs of meningitis are rare in early-onset infection

Neonatal Disease: II

- Late onset:
  - Occurs several days to weeks after birth
  - Infants are generally born full term, healthy at birth, and delivered to mothers who have uncomplicated pregnancies.
  - Is more likely to present as meningitis
  - Case fatality rates are lower in late-onset disease than in early-onset infection (Britain study)

Infants with Listeria

- Infants who become infected with listeria monocytogenes during the birth process develop symptoms, usually of meningitis, in the 8-12 day period following delivery.
- The incidence of human listeriosis is highest in the first month of life (Nichols and Woolley, 1962).

Meningitis in Children

- Symptoms:
  - In most cases, meningitis develops over 1-4 days, however in some cases, a child who looks healthy can rapidly become seriously ill within 1 day (death is possible)
  - Depending on the child's age signs and symptoms are not always obvious, because the child cannot communicate symptoms the same way an adult can
- Symptoms in infants <3 months"
  - decreased liquid intake
  - Vomiting
  - increased irritability

Symptoms Cont…

-Increased lethargy
-fever
-bulging fontanelle
-seizure activity
Symptoms in children >1 year
-nausea and vomiting
-headaches
-increased sensitivity to light
-fever
-lethargy
-altered mental status
-seizure activity
-neck stiffness or pain
-knees automatically brought up toward the body when the neck is bent forward or pain in the legs
-inability to straighten the lower legs after the hips have already been flexed

Listeria monocytogenes

- Human stillborn -- Granulomaosis infantiseptica

Exams and Tests

- Upon arrival at emergency room
- Temperature
- Blood pressure
- Respiratory rate
- Pulse
- Oxygen

If necessary:
-a spinal tap or lumbar puncture (this is an essential procedure in which cerebrospinal fluid is obtained from the child and then analyzed in a laboratory)
-the fluid is used to check for white and red blood cells, protein, glucose, and organisms

Regulations on *L. monocytogenes*

- FDA/FSIS zero tolerance in cooked and ready to eat foods
  - absence of *L. monocytogenes*/25g food sample
- 9 CFR Part 430
  - a ready to eat product is adulterated if it contains *L. monocytogenes,* or if it comes into direct contact with a food contact surface that is contaminated with *L. monocytogenes*
  - product can be subjected to recall or seizure

Control of *L. monocytogenes* in food

- Products at high risk
  - raw material or product exposed to contamination
  - product manufactured with no processing stage capable of destroying *L. monocytogenes*
  - product with little or no preservation factors
    - e.g. neutral pH, low salt, high moisture
  - product exposed to post-process contamination
  - product sold with long shelf-life under chilled conditions
  - product sold as ready-to-eat

Control of *L. monocytogenes* in food

- Dependent on four key factors
  - preventing contamination of raw materials or growth in raw materials, if present
  - destroy or reduce if present in raw material
  - prevent recontamination after a reduction or destruction stage
  - minimize growth during the shelf-life or the final product

Control of *L. monocytogenes* in raw material

- Ubiquitous nature of *L. monocytogenes* them impossible to eliminate from raw materials
- Goal
  - reduce contamination levels entering the process
    - improve sanitation at raw material producer facility
    - maintain and monitor hygiene practices

Control of *L. monocytogenes* in raw material

- Raw milk
  - hygienic milking practices involving udder cleaning
  - cleaning and sanitation regime applied to the milking and milk storage equipment
    - all elements from farm to processing factory must be accounted for
  - provide incentive payment schemes for rewarding consistent high-quality milk

Control of *L. monocytogenes* in raw material

- Raw meat and fish
  - control of hygiene during slaughter and preparation stages critical for minimizing contamination
  - routine monitoring of incoming material to ensure high quality

Control of *L. monocytogenes* in raw material

- Fruit, vegetables, and other raw material
  - use of animal waste must be carefully controlled
    - must be properly composted to ensure sufficient heat generation for microbial reduction prior to application
    - artificial fertilizers should be employed when possible
  - good storage conditions
    - low temperatures or short periods, minimize moisture
    - maintain intactness of produce

Control of *L. monocytogenes* in processing

- Proper application of processing can significantly reduce or achieve the destruction of *L. monocytogenes*
  - validation is critical
    - assists in identifying where to apply the controls and the parameters necessary to ensure reduction or destruction

Control of *L. monocytogenes* in processing

- Cooking
  - primary process to control *L. moncytogenes* in cooked meats, ready meals, dairy desserts
  - important control factors
    - minimum in-going temperature of material
    - product size
    - cold spots/temperature distribution
    - container fill load/oven load
    - minimum time/temperature setting per load

Control of *L. monocytogenes* in processing

- Fermentation processes
  - use of fermentation and dry process to reduce levels of microbial contaminants present in raw material
  - Control
    - effective pasteurization if applicable
    - avoidance of post-process contamination
      - ripening and slicing stages

Control of *L. monocytogenes* in processing

- Washing processes
  - double ended sword
    - can spread contamination as well as reduce contamination
  - ensure that the active ingredient is present in its active form on a continuous basis
  - regularly change wash water

Control of *L. monocytogenes* in processing

- Post-process contamination
  - contamination sources
    - environment
    - direct product contact surfaces
    - cross contamination from raw materials
  - Control
    - reduce or eliminate *L. monocytogenes* from the post-processing environment

Control of *L. monocytogenes* in processing

- Post-processing control
  - segregation of raw material processing (low risk area)and finished product areas (high risk area)
    - e.g. dividing walls
  - flow control
    - positive air pressure maintained in high-risk area ensures air flows from the high- to the low-risk area
    - drainage from high- to low-risk area

Control of *L. monocytogenes* in processing

- Post processing control
  - effective cleaning and disinfection
    - Equipment
      - parts should be routinely dismantled and thoroughly cleaned
    - non-contact surface reservoirs
      - often overlooked
      - i.e. table ledges, door handles
    - floors, walls, cracks, drains
  - routine monitoring of cleaning efficacy

Control of *L. monocytogenes* in the final product

- Packaged final products sold as units are generally protected from further contamination
- Exceptions
  - bulk products sold to retailers for slicing or open display on the deli counter
- Cleaning and disinfection of utensils, equipment, and retail surfaces used for slicing and preparation is crucial

Control of *L. monocytogenes* in the final product

- Shelf-life control
  - important for non-cooked products with occasional contaminants
  - shelf life estimation
    - predictive models of *L. monocytogenes* in foods under varying physico-chemical conditions
    - supplement with challenge tests in actual product

Control of *L. monocytogenes* in the final product

- Product labeling
  - consumer actions are important in maintaining the safety of products following purchase
  - temperature control labels
    - "Keep Refrigerated" labels
  - shelf-life indicator
    - "use-by" date

Control of *L. monocytogenes* in the final product

- Product labeling
  - advise vulnerable populations of the need to avoid certain foods where *L. monocytogenes* may be an occasional contaminant
  - cooking guidance
    - should be correct and clearly printed on the package

Conclusion

- Main challenge of controlling *L. monocytogenes*
  - consistent implementation of known control strategies
- Effective application of HACCP principles the only way ensure the safety of minimally processed foods with increased shelf-life

懇請賜教