Foodborne diseases include **food poisoning** and **food infection**.

- Food poisoning results from the action of microbial toxins.
- Food infections result from the growth of microorganisms in the body.

### Annual foodborne disease estimates for the United States

<table>
<thead>
<tr>
<th>Organism/Pathogen</th>
<th>Disease Type</th>
<th>Estimate (cases)</th>
<th>Feed Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>Intestinal campylobacteriosis</td>
<td>300,000</td>
<td>Raw and undercooked meat</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Intestinal campylobacteriosis</td>
<td>200,000</td>
<td>Raw and undercooked meat</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Intestinal campylobacteriosis</td>
<td>50,000</td>
<td>Raw and undercooked meat</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>Intestinal campylobacteriosis</td>
<td>100,000</td>
<td>Shrimp, many other foods</td>
</tr>
</tbody>
</table>

**Total**
- *Campylobacter*: 380,000
- *Salmonella*: 550,000
- *Enteric fever*: 950,000
- *Foodborne disease*: 8,800,000

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### Food Poisoning

- The disease results from infection with ingested *Salmonella* introduced into the food chain from food production animals or food handlers.
- *Enteropathogenic Escherichia coli* can cause serious food infections.
- Specific measures, such as radiation of ground beef, have been implemented to curb the spread of these pathogens.
- Large-scale processing methods for meats and meat products allow contaminants from a small number of individual carcasses to contaminate or infect large numbers of products.

### Campylobacter Infection

- *Campylobacter* infection is by far the most prevalent foodborne bacterial infection. Though usually self-limiting, this disease affects nearly 2 million people per year. **Poultry and dairy products**

### Listeria monocytogenes

- *Listeria monocytogenes* is an environmentally ubiquitous microorganism. In normal individuals, *Listeria* seldom causes infection. However, in immunocompromised individuals, *Listeria* can cause serious disease and even death.
Topics

• *Salmonella typhimurium*
  – Pathogenesis
    • Disease
    • Virulence factors
  – Prevention and Treatment

• *Listeria monocytogenes*
  – Pathogenesis
    • Disease
    • Virulence factors
  – Prevention and Treatment

*Salmonella typhimurium*… Characteristics

• *Salmonellae* are
  – Gram negative,
  – Closely related to E. coli and Shigella
  – Facultative anaerobes
  – Most are motile (peritrichious flagella).
  – They are widespread throughout nature.
  – They are effective commensals, as well as pathogens, and have been isolated from the gastrointestinal tracts of mammals, reptiles, birds and insects.

Transmission and Susceptibility

• Contaminated food
  physically from food and by food handlers
  chicken and eggs

• Contaminated water

• Fecal-oral
  • Anyone consuming foods contaminated with large numbers of Salmonella,

• Particularly children younger than 1 year old, elderly, patients with reduced gastric acids, and patients with AIDS, individuals exposed to carriers

Recent outbreaks

• In 1985, a salmonellosis (S. typhimurium) outbreak involving 16,000 confirmed cases in 6 states by low fat milk and whole milk from one Chicago dairy.

• Largest outbreak of food-borne salmonellosis in the U.S. Investigations discovered that raw and pasteurized milk had been accidentally mixed.
Disease

• There are two main clinical manifestations of Salmonella infections:
  
  
  – **Enteric** ("typhoid") fever (17-20 million cases/yr): 15% mortality in the pre-antibiotic era. Gradual onset (5-21 days) of fever with abdominal tenderness. Very little diarrhea. Self-resolving: 4 weeks.
  
• In some cases, typhoidal *Salmonella* will chronically infect a host (*Typhoid Mary*).

Adherence

• The bacteria adhere to intestinal epithelia via different fimbria.
  
• five different fimbrial operons (fim, lpf, agf, sef and pef).

Identification of Virulence Factors

– Virulent Wild type *Salmonella*

  Transposon mutagenesis
  Test individual colonies for loss of phenotype
  
  loss of invasion
  loss of survival in macrophages
  loss of mouse virulence
  Clone and sequence gene

Selected events in *Salmonella* pathogenesis

Figure 3a: Steps in the progression of the disease caused by *S. enteritidis*. (Left) Factors in host cells. (Right) Pathogen interactions.
• After adherence to the intestinal epithelia, *Salmonella* engages a receptor on the surface of the host cell, leading to massive cytoskeletal rearrangements and bacterial entry into the host cell.

• Invasion is important in *Salmonella* pathogenesis because bacteria that cannot invade epithelial cells are attenuated in their ability to cause disease after oral inoculation.

### Regulation of Invasion.

- The invasiveness of *Salmonella* is regulated by growth state, high osmolarity, low oxygen tension and pH. In the gut, the level of oxygen is low, but nutrients are high.

- Many genes that are regulated by these environmental stimuli have been shown to be important for bacterial invasion of host cells.

- Most of these invasion genes are clustered together in a region of the chromosome in what has been termed *Salmonella* Pathogenicity Island 1 (SPI-1). SPI-1 genes encode the components of a Type III secretion system.

### Invasion

- Type III Secretion System, a needle-like multi-protein complex that is associated with transferring toxic proteins to host cells.
Salmonella survival within cells

- The ability of Salmonella to survive inside cells is essential for pathogenesis. Salmonella mutants that are unable to replicate within mammalian cells are completely avirulent.

- The second type III secretion system (SPI-2 Type III secretion system) is encoded within a region of the chromosome termed Salmonella Pathogenicity Island-2 (SPI-2).

- The expression of SPI-2 genes and the secretion of SPI-2 effector proteins require an acidic pH.

- SPI-2 acts by inhibiting macrophage oxidative burst and the deposition of reactive oxygen intermediates to the Salmonella vacuole.

Salmonella Pathogenesis

How did Salmonella become a pathogen?

- Despite the many similarities between the Salmonella and E. coli, there are large spans of chromosomal DNA that are present in Salmonella but not E. coli.

- These discreet regions of DNA, which are sometimes referred to as "pathogenicity islands", encode many of the virulence properties of Salmonella.

- Many of these virulence factors were probable acquired through bacteriophages which inserted at tRNA loci.

Prevention and Treatment

Proper Food handling

Normal gut flora

The vast amount of gut flora actually can prevent invasion of Salmonella.

- the normal flora simply out-competes Salmonella for available space in the gut and may for nutrients.

- This is supported by data from people who were taking antibiotics before going to areas of high Salmonella got the disease at a higher rate than those who did not take the drug.

- Also, studies with animals have shown that if they are fed cultures of normal flora then challenged with Salmonella, they were protected from disease.

Vaccines

- Live vaccine against S. typhi provides short-lived immunity. There is almost no cross-serotype protection. A Vi antigen preparation is currently used for S. typhi.

Antibiotics
Summary_Salmonella

- *Salmonella* sp. cause both gastritis and systemic disease.
- Host range specificity is determined by adhesins and invasins which are present in mobile genetic elements.
- A type III secretion system (SPI-1) and its secreted proteins are required for cell invasion and inflammation.
- The assemble of the type III secretion system and gene expression are coordinated.
- *Salmonella* secretes proteins into the cytosol of host cells that modify the host cytoskeleton.
- *Salmonella* survives and replicates within phagocytic cells.
- A type III secretion system (SPI-2) and its secreted proteins are expressed within mammalian cells and are required to survive within vacuole.
- The acquisition of "Pathogenicity Islands" have expanded the repertoire of Type III secreted virulence factors available to *Salmonella* to possibly increase host range and immunomodulatory capacity.

Listeria monocytogenes

Characteristics of *Listeria monocytogenes*

- Low G+C gram positive, facultative anaerobe.
- Growth temperature range is unusually broad, 4C-44C.
- Motile by flagella at lower temperature
- Unusually broad ecological niche; isolated from over 50 different warm-blooded animals and readily isolated from soil, water and vegetation, and dairy products.

Listeriosis

- Listeriosis, a serious infection caused by eating food contaminated with the bacterium *Listeria monocytogenes*, has recently been recognized as an important public health problem in the United States.
- A person with listeriosis has fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions can occur.
- Infected pregnant women may experience only a mild, flu-like illness; however, infections during pregnancy can lead to miscarriage or stillbirth, premature delivery, or infection of the newborn.
- In the United States, an estimated 2,500 persons become seriously ill with listeriosis each year. Of these, 500 die.
- At increased risk are: Persons with weakened immune systems and pregnant women
- Healthy adults and children occasionally get infected with Listeria, but they rarely become seriously ill.
How does *Listeria* get into food?

- *Listeria monocytogenes* is found in soil and water. Vegetables can become contaminated from the soil or from manure used as fertilizer.
- Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products.
- The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing, such as soft cheeses and cold cuts at the deli counter.
- Unpasteurized (raw) milk or foods made from unpasteurized milk may contain the bacterium.

*Listeria* is killed by pasteurization and cooking; however, in certain ready-to-eat foods such as hot dogs and deli meats, contamination may occur after cooking but before packaging.

Outbreaks

- Outbreak in Nova Scotia - 1981
  - 34 perinatal cases
    - 9 stillbirths
    - 23 live births of ill infants with 27% mortality rate
    - 2 live births of healthy infants
  - Adult mortality rate - 28.6%
  - Coleslaw determined to be cause
- Outbreak - Northeastern United States - Fall 2002
  - 46 ill - 7 deaths & caused 3 miscarriages
  - Resulted in largest recall due to *Listeria* in history - 27.4 million pounds of poultry and chicken products
  - The strain causing illness was found in processing environment using DNA strain-typing

Stages of infection

- **Adherence**
  - *Listeria* can attach to and enter mammalian cells. The bacterium is thought to attach to epithelial cells of the GI tract by means of D-galactose residues on the bacterial surface which adhere to D-galactose receptors on the host cells.
- **Invasion**
  - The bacteria are then taken up by induced phagocytosis. An 80 kDa membrane protein called internalin probably mediates invasion
**Stages of infection**

- **Escape from primary vacuole:**
  - After engulfment, the bacterium escape from the phagosome before phagolysosome fusion occurs mediated by a toxin, *listeriolysin* O (LLO) and enzyme phospholipase-C (PI-PLC), that removes charged head groups from phospholipids.
  - Growth in the cytosol: Rapid growth with a doubling time of approx. 1 h.

- **Movement through cytoplasm, cell to cell spread**
  - ActA-interacts with host cell proteins to stimulate actin polymerization.
  - Actin tails forms one end of cell, propels bacterium through cytoplasm.
  - Projections from the host cell surface containing living *L. monocytogenes* are formed and engulfed by adjacent cells.
  - Direct cell-to-cell spread of *Listeria* in an infected tissue may occur without an extracellular stage.
  - Bacteria inside double membrane vacuole.

- **Escape from secondary vacuole:**
  - enzyme phospholipase-C (PC-PLC), that removes charged head groups from phospholipids.

- **Disruption of signal transduction pathways of host cells**

- **Regulation of virulence factors**
  - PrfA, positive regulator of virulence genes, may respond to temperature.
  - Many virulence genes are clustered in the same location on the chromosome.

**Identification of virulence genes**

- A screen designed to identify genes necessary for growth and cell-to-cell spread.

- Can look for small plaque mutants or no-plaque mutants.

**Treatment and Prevention**

- If diagnosed early enough, antibiotic treatment of pregnant women or immunocompromised individuals can prevent serious consequences of the disease.

- Avoid products made with unpasteurized milk.

- Avoid coleslaw and deli meats.

- It must also be constantly recognized that *L. monocytogenes* is able to grow at low temperatures.
Summary

- Listeriosis, a serious infection caused by eating food contaminated with the bacterium *Listeria monocytogenes*.
- *L. monocytogenes* is able to grow at low temperatures.
- Stages of infections include: invasion, escape from primary vacuole, movement through cytoplasm, cell to cell spread, Escape from secondary vacuole.
- Actin based motility

Study Questions

1) Define type III secretion system.
2) What is the importance of type III secretion system in Salmonella virulence?
3) What are pathogenicity islands?
4) The bacterium "*S. lysis*" is a human pathogen that you can grow in culture in the lab. *S. lysis* is able to efficiently lyse any mammalian cell in culture.
   a) Describe an experimental approach that you would use to identify genes in *S. lysis* that are required for lysis of mammalian cells. Be specific and explain what you expect at each step of your approach. Note: your approach should allow you to identify one or more genes required for lysis.
5) Describe steps involved in motility of *Listeria monocytogenes* inside host cells.