Lecture 15: Pathogen Exit Strategies (continued)

Listeriosis

- Gram positive, facultative, motile, rod
- Opportunistic intracellular pathogen
- *L. monocytogenes* is naturally found in:
  - Soil and vegetation (wet and dry)
  - Freshly harvested grass
  - Grass with higher moisture content
  - Fecal material (human and animal)
  - Isolated in healthy livestock (2-16%), wild animals, human sewage
  - Polluted water
  - Animal feed (silage and straw)

Human Listeriosis

- *L. monocytogenes* is a category B biodefense select agent pathogen.
- Has been recognized as a foodborne pathogen since the 1980s
- Shown to be a pathogen in over 50 mammals
- The only *Listeria* species pathogenic to humans
- Intracellular pathogen - causes the food-borne illness, listeriosis, in susceptible persons
Human Listeriosis

Susceptible Populations
- Elderly and infants
- Pregnant women
- Neonates
- Impaired immune system (AIDS)
- Immunosuppressive therapy for malignancy or organ transplant
- Predisposing illness (alcoholism, diabetes, cirrhosis of the liver)

Infectious doses
- Minimum infectious dose (MID) not determined
- Consumption of < 1000 organisms in milk may cause disease
- 10^8 cells were required to cause disease in healthy primates

Why is it such a problem?

What do these images have in common?

Growth Conditions
- LM is a facultative organism
  - Can grow under aerobic (oxygenated conditions)
  - Can grow under reduced oxygenated conditions (semi-anaerobic)
  - Vacuum packaging provides a facultative environment → growth during long term refrigerated storage
- Salt concentration
  - Growth at 10%
  - Survival at 25.5%
Growth Conditions

- Growth range = 30 to 113°F (-1 to 45°C)
  - Optimum = 86 to 98.6°F (30 to 37°C)
- Psychrotrophic (refrigeration temperature; <40°F)
- Mesophilic (room to body temperature; 65-100°F)
- Temperatures <32°F moderately inactivate LM
- LM can survive freezing!!! (ice cream anyone?)

Biofilm Formation

Even on stainless steel surfaces: great majority of food Industry materials

On a rubberized surface
**Listeria Actin Polymerization**

I added this after the lecture - thought this would be helpful.

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**Extrusion Mechanism**

<table>
<thead>
<tr>
<th>Cellular Exit</th>
<th>Intrusive</th>
<th>Excluding Chlamydospore</th>
<th>Exit of membrane-bound bacteria</th>
</tr>
</thead>
</table>

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**Chlamydia Cell Exit Strategies**

Two mutually exclusive exit strategies

- Cell lysis
- Local spreading
- Extrusion dissemination to new locations
**Plasmodium species**

- Causitive agent of Malaria
- ~155 species named
  - Infect birds, reptiles, rodents, primates, humans
- Species is specific for host and vector
- 4 species cause human disease
- No zoonoses or animal reservoirs
- Transmission by Anopheles mosquito

- *P. falciparum*
- *P. vivax*
- *P. malariae*
- *P. ovale*

**Distribution of Malarial Parasites**

- **P. vivax**
  - Most widespread, found in most endemic areas including some temperate zones
- **P. falciparum**
  - Primarily tropics and sub-tropics
- **P. malariae**
  - Similar range as *P. falciparum*, but less common and patchy distribution
- **P. ovale**
  - Occurs primarily in tropical west Africa

**Distribution of Malaria**
40% of world’s population is at risk
300 - 500 million cases per year
1.5 to 2.0 million deaths per year
#1 cause of infant mortality in Africa!

Malaria in the United States

- Malaria was quite prevalent in the rural South
- It was eradicated after World War II in an aggressive campaign using, treatment, vector control and exposure control (along with overall improvement of living conditions)
- This was the CDC’s initial mission

History of Malaria

- Considered to be the most important infectious disease on a worldwide scale
- Mal aria - “bad air”
- Swamp disease
- Periodic fever episodes
- Egyptian records
- Roman empire
- World War II

http://www.malaria-site.com/MALARIA/History.htm
The Disease

- **Sweating stage**
  - Profuse sweating
  - Declining temperature
  - Exhausted, weak
  - Lasts 2-4 hours

- **Cold stage**
  - Feeling of intense cold
  - Vigorous shivering, rigor
  - Lasts 15-60 min

- **Hot stage**
  - Intense heat
  - Dry burning skin
  - Throbbing headache
  - Lasts 2-6 hours

Malaria Paroxysm

- Paroxysms are associated with the synchrony of merozoite release - rupture of RBCs - release of toxins
- Rapid climb in body temp.
  - Mild delirium
- Between paroxysms patient feels well with normal temps
- *P. falciparum* may not exhibit classic paroxysms
  - Continuous fever
  - Less synchronous
- Concurrent infections with more than 1 species is common

Plasmodium Life Cycle
Mosquito bite
Sporozoites deposited into dermis
Girling motility
Circulatory system
Hepatic cells
Exoerythrocytic Forms (EEF)

Sporozoite Arrest and Maturation in Liver

Methods to Study Merosome Dynamics

Previous studies were in vitro fixed samples

Intravital Microscopy
Can visualize for up to 6 hrs

Ex vivo Microscopy

Plasmodium Merosome Budding?
Dynamics in vessels

Model of Merosome Dissemination and Merozoite Liberation

Budding mechanism

1. Sporozoite infects hepatocyte
2. PV containing merozoites - schizont
3. Remodels hepatocyte - no apoptosis
4. Compression due to EEF expansion
5. Merosomes bud and subdivide
6. Enter hepatic circulation
7. Pass through right ventricle
8. Accumulate in lungs
9. Merosome membrane disintegrates
10. Merozoites invade RBCs

Little is known about this mechanism
An important vector-borne disease, first described in 1899 in Japan. During World War II, this disease killed thousands of soldiers who were stationed in rural or jungle areas of the Pacific theatre.

The disease occurred and threatened people throughout Asia & Australia. The range stretches from the Far-east to the Middle-east (from Japan and Korea, Southeast Asia, Pakistan, India, to Arab countries and Turkey). There are approx. 1 million cases each year world-wide, & over 1 billion people at risk.

**Orientsia (Rickettsia) tsutsugamushi**

- Scrub typhus
- Japanese “tsutsuga” = small and dangerous and “mushi” = creature
- “Scrub” - associated with terrain with scrub vegetation
- Vector - chiggers (mite larva)
- Reservoir - chiggers and rats
  - Transovarian transmission
  - Normal cycle - rat to mite to rat
- Humans are accidentally infected

**Scrub Typhus - A Rickettsial Disease**

An acute febrile, rickettsial disease caused by a gram-negative, rod-shaped (cocco-bacillus) bacterium, known as *Orientia tsutsugamushi* (Rickettsia).

*O. tsutsugamushi* is transmitted to vertebrate hosts (rodents-primary host & humans-secondary or accidental host) by the bite of larval mites (chiggers) of the genus Leptotrombidium, e.g. *L. deliens*, *L. dimphalum*, etc.
Humans acquire the disease when infected chiggers bite them and transmit *O. tsutsugamushi*. Bacteria multiply at the inoculation site and frequently form a papule that ulcerates & becomes necrotic. This pathognomonic focal lesion is called an eschar. Regional lymphadenopathy develops & progresses to generalized lymphadenopathy in a few days. In the severe cases, it can lead to:

- Pneumonia with adult respiratory distress syndrome
- Circulatory failure resulting in death.

Mortality rates in untreated patients normally range from 0-30% but rates as high as 60% have been reported. Significant morbidity and mortality can be prevented in patients who receive timely, appropriate treatment with antibiotic drugs.

The term scrub of scrub typhus came from the type of vegetation (terrain between woods & clearings) that harbor the vectors.

Significant morbidity and mortality can be prevented in patients who receive timely, appropriate treatment with antibiotic drugs.

Chiggers Life Cycle
Reservoir Host: *Rattus rattus*

*O. tsutsugamushi* bacteria is transmitted:
- **trans-ovarially** (from adult female to eggs) &
- **trans-stadially** (from egg to all immature & adult stages)

*O. tsutsugamushi* bacteria are found throughout the mite’s body, but the highest number are present in the salivary glands. When the mite feeds on rodents & humans, the parasites/bacteria are transmitted to the host.

Only larval *Leptotrombidium* mites (chiggers) transmit the disease; however, the bacteria can be found in all mite developmental stages (from egg to adult).

**Scrub Typhus Transmission**

**Replication of *Rickettsia* and *Orientia***

- Infect endothelial cells in small blood vessels - Induce phagocytosis
- Lysis of phagosome and entry into cytoplasm via Phospholipase
- Intracellular Replication
- Release - three mechanisms - *Orientia tsutsugamushi* - budding

**Expulsion Mechanism**

Cryptococcus as a genus contains approximately 37 species and only *C. neoformans* is pathogenic.
Cryptococcus neoformans

Cryptococcal meningitis
- Most common fungal infection of CNS
- 3rd most frequent neurological complication for AIDS
- Up to 30% mortality rates for AIDS patients in Africa
- Incidence has increased in last 10 years.
- Infection acquired by inhalation of basidiospores
- Initial pulmonary infection
- Most people probably have already been infected, but have cleared the infection or remains dormant.
- If immunocompromised, disease progresses and reaches CNS

Cryptococcus neoformans

- Encapsulated yeast
- Polysaccharide capsule that excludes India ink
- Melanin producing organism - aids in protection against oxidative killing
- Brown pigmented colonies are Cryptococcus
- White pigmented colonies are Candida

Multiplication in Host cell

- Infected lung from AIDS patient
- Encapsulated yeast
- Red stained = Cryptococcus
- Division in host cell - asexual haploid stage
Environmental Replication

A distasteful type of mushroom? Basidiomycetes - most are mushrooms and toadstools.

Similar life cycles:
- Cycling between sexual stages (Meiosis) and asexual stages.
- In response to nutrient limitations - development of mating type and fusion.
- Spores are eventually produced that can be carried to new sites with nutrient supplies.

Capsule Production

Expulsion Mechanism

J774 = mouse macrophage cell line

Time lapse movie 1 frame/minute
Expulsion Mechanism

human primary macrophages

Time lapse movie 1 frame/1.5 minute