Enterobacteriaceae - The Genus Yersinia

Course: VPM 201 Fall, 2010
Lecturer: C. Anne Muckle
**Enterobacteriaceae - The Genus Yersinia**

- What is the bug *Yersinia*?
- Gram-negative coccobacilli, can show bipolar “safety-pin” staining
- Most are NLFs, oxidase negative (they are members of *Enterobacteriaceae*).
- Optimal growth at 25-28°C, slow growing, small colonies on MAC.
- Four *Yersinia* species cause disease:
  - *Y. pestis* – plague
  - *Y. pseudotuberculosis* – enteritis, lymphadenitis
  - *Y. enterocolitica* – enteritis, lymphadenitis
  - *Y. ruckeri* (fish pathogen) – causes “red-mouth” in salmon and trout.
Bacteriological microscopy trivia - Bi-polar staining:
“The effect of the two ends of a bacillus staining while the centre of the rod remains unstained” ex. Pasteurella, Yersinia, Burkholderia

“closed safety pin”

See S&P text, photo, page 138

Bi-polar morphology, -“safety-pin” phrase used for Wright-Giemsa stained blood smears
Yersinia pestis
Yersinia pestis: Plague (The Black Death)

- Bubonic and pneumonic plague, became known as the Black Death or Black Plague during the second pandemic in Europe (1300s);
- (read history in S & P text, page 138)

- Plague (urban plague cycle) has occurred in several pandemics, killing millions (50% mortality rate)

- Third pandemic in 1955 from Central Asia → North America

“Bring out your dead!”
– Monty Python’s Holy Grail
Is *Yersinia pestis* the cause of the Black Death and other plague epidemics?

- Some historians and epidemiologists contest that *Y. pestis* was the cause, but there is good evidence that it did, including this bacterial genotyping study:

  *Y. pestis* is a clone that evolved from *Y. pseudotuberculosis* 1,500–20,000 years ago, shortly before the first known pandemics of human plague.

- Three biovars (Antiqua, Medievalis, and Orientalis) have been distinguished by microbiologists within the *Y. pestis* clone. These biovars form distinct branches of a phylogenetic tree based on restriction fragment length polymorphisms of the locations of the IS100 insertion element.

- These data are consistent with previous inferences that:
  - Biovar Antiqua caused a plague pandemic in the sixth century,
  - Biovar Medievalis caused the Black Death and subsequent epidemics during the second pandemic wave, and
  - Biovar Orientalis caused the current plague pandemic.

Yersinia pestis:

- Potential bioterrorism agent because of high mortality following aerosol delivery;
- Classified as a Category A Critical Biological Agent by USA.

Was *Y. pestis* the bacterial plague agent in this star-studded 1976 movie?
**Yersinia pestis**: Plague transmission cycle

- **Transmission Cycle is flea-rodent-flea**, accidentally infecting other animal hosts

- The rat flea (and the human flea) are the arthropod vector.

- Fleas (several types possibly involved) ingest *Y. pestis* in blood meals from bacteremic humans & animals and transmit to next host

- Also can be transmitted by aerosols and through skin lesions
Yersinia pestis: Plague transmission cycle - a zoonotic disease
The Genus Yersinia:
Q. Why is *Yersinia pestis* so virulent?

- *Y. pestis* is an FIP and has a tropism for lymphoid tissue, it is both an intracellular (can grow in MΦ, PMNs) & extracellular parasite
  
- Has two unique plasmids enabling growth in fleas & transmission from flea to host;
  
- this arthropod-transmission is unique among enteric bacteria
  
- **pYV virulence plasmid encodes translocator & effector proteins called Yops** (*Yop = Yersinia outer protein*) injected through a Type III secretion system
  
  - Translocator Yops – pore in host cell membranes (including the V antigen)
  
  - Effector Yops – inhibit phagocytosis, local inflammation, induce apoptosis
  
  - The pYV plasmid is turned off when inside flea environment (26°C) & turned on when inside host MΦ (37°C, low Ca levels)
  
- Other virulence plasmids: the pFra plasmid encodes a *capsule* which prevents phagocytosis, promotes serum resistance (*capsular protein is called fraction1 = F1 Antigen*); flea-required plasmids encode toxins, & plasminogen activator
  
- Also a pathogenicity island (HPI) for iron-binding protein (*yersiniabactin*)
Y. pestis – a complicated & virulent pathogen

This diagram is NOT intended for memorisation. It’s just to illustrate that Y. pestis infection is complicated.
Y. pestis and plague disease

- Following subcutaneous inoculation by flea bite, bacteria reach & grow well in regional lymph nodes causing lymphadenopathy = “buboes” of **bubonic plague**; these can rupture & are infectious

- Rapid development of septicaemia with high fever, pneumonia, allowing airborne spread and primary **pneumonic plague**

- Crowded cities with house rats, poor sanitation, & human fleas, allowed urban plague cycle
**Y. pestis** and sylvatic plague cycle -

- Sylvatic plague cycle is sporadic
- Enzootic foci are found on every continent except Australia, rare in Canada
- Risk factor = residence in a rural endemic area.

- **Classified by WHO as a re-emerging infectious disease**
- In North America, fleas transmit *Y. pestis* among mice, voles, ground squirrels, prairie dogs, wood & kangaroo rats, chipmunks
- black-tailed prairie dogs are at risk which impacts on the endangered species black-footed ferret
- Humans and cats can be infected by exposure to fleas, handling or eating dead rodents, or by aerosols, from dust in rodent burrows
- Dogs are relatively resistant but can become infected; coyotes are used as sentinel species in areas of sylvatic plague
- Cattle, sheep, horses are not susceptible
Y. pestis and feline plague

Q. Why is important to diagnose plague in cats & humans?

It is easily misdiagnosed and the mortality rate is very high if untreated or if treatment is delayed

- Direct transmission from felines to humans occurs (~ 10% of 300 cases reported between 1977-1998)

- Important if you work in endemic areas = south western United States (Arizona, New Mexico, Nevada, Utah, Colorado, California, several others)

- Wild, feral and domestic felids are susceptible and show similar signs as seen in humans.

- Bubonic form: from flea bite or ingestion of infected rodents
  - submandibular lymph node involvement relatively common
  - Lymph node inflammation, necrosis, haemorrhage, edema

- Systemic form: septicemia, spleen, liver and heart involvement (high fever (40°C), vomiting, tachycardia, DIC)

- Pneumonic form: diffuse interstitial pneumonia with coalescing areas of necrosis
**Y. pestis** – sylvatic plague:

Q. – How do you handle a feline plague case?

- **Isolation procedures are required & public health should be notified**

- Owners and vets at risk from cats by aerosol/flea bites & scratches; most human cases from cats are from scratches/bites, fleabites

- Pneumonic animals are particularly infectious, use mask, face shield

- **Diagnosis** (not done by routine diagnostic labs):
  - History, Gram stain of any bubo/abscess exudate (see gram-negative “safety-pin” = bipolar coccobacilli)
  - Serology: Fluorescent antibody test for of *Y. pestis* F1 capsular antigen better than serological tests for *Y. pestis*-specific antibody response
  - Blood culture, BA and MAC (bubo/L.N. aspirates, oral cavity pharynx swabs, liver, spleen, lung, L.N. Post-mortem, PCR

- Treatment: Cats – parenteral gentamicin
- Humans: fluoroquinolones, chloramphenicol, tetracycline, streptomycin
**Y. pestis** – sylvatic plague:

**Q.** – How do you prevent sylvatic plague?

- **Use intervention Strategies:**
  - **FLEA Control:** Cat and dog fleas are poor vectors (*Ctenocephalides* spp), but.....
  - Fleas from wild rodents can stay briefly on non-rodents, including humans – take precautions to keep pets flea-free
  - **Pet Control:** Keep cats indoors in endemic areas; don’t let dogs roam/hunt
  - Killed vaccine available (high risk humans)– not effective against pneumonic or septicemic forms
  - Research with subunit vaccine: contains F1 antigen = capsule & V antigen; are effective against bubonic and pneumonic forms
**Y. pestis** – zoonosis

**Stories of feline plague in wild cats & humans**

- Plague was confirmed as the cause of death of **Eric York**, a 37-year-old National Park Service (NPS) wildlife biologist who was found dead Nov. 2, 2007, in his residence on the South Rim of Grand Canyon National Park.

- Tests conducted by the Centers of Disease Control and Prevention (CDC) determined that the strain of plague that infected York was the same strain of plague that infected a mountain lion with whom York had had direct and recent contact.

- Also of note:
  - Several Canadian Lynx transplanted into Colorado (6 out of 129) died from *Y. pestis* infection
**Y. pestis** in the Research Lab –
**The risks of working with a potential bioterrorism agent**

- Molecular genetics professor Malcolm Casadaban died September 13, 2009, possibly from the plague.

- **Casadaban, 60, was investigating a weakened laboratory strain of *Yersinia pestis***, the bacteria that causes the bubonic plague, in hopes of finding a better vaccine.

- An initial autopsy revealed no other clear cause of death besides the weakened strain of *Y. pestis*, which grew in routine cultures of Casadaban's blood. More studies are being conducted to determine the potential role of the bacteria in Casadaban's death.

- The strain Casadaban studied is used as a vaccine to protect against plague, and has been approved by the Centers for Disease Control and Prevention (CDC) for laboratory studies without the special safety precautions taken with harmful strains.

(By Ella Christoph, published: September 21st, 2009)
**Y. pseudotuberculosis & Y. enterocolitica -**

*Q* - What do veterinary clinicians and diagnosticians need to know about these bugs?

- *Y. pseudotuberculosis* and *Y. enterocolitica* are invasive enteric pathogens and FIPs*
- Both cause enterocolitis & mesenteric lymphadenopathy in animals and humans
  - **Invade** Peyer’s patches - erosive suppurative enterocolitis
  - → micro-abscesses, mesenteric lymphadenitis (caseous abscess)
    - pain mimics acute appendicitis in humans

- Both can disseminate → liver and splenic caseous abscesses, septicaemia, reproductive disease (abortion, stillbirths, orchitis, epididymitis) and mastitis

- Both have several virulence factors:
  - Including pYv (70 kb) plasmid with **YOPs** described for *Y. pestis*
  - YadA adhesin (on pYv) and inhibitor of complement fixation
  - Invasin (Inv) facilitates adhesion to host cells
Y. pseudotuberculosis – and disease “pseudotuberculosis”

- **Is primarily** a rodent pathogen, causing diarrhoea, micro-abscesses, caseous abscesses, and septicaemia
- Causes disease in guinea pigs, cats, turkeys, pigs, wild ruminants
- Reproductive problems in cattle and goats
- Disease in humans – mesenteric lymphadenopathy
- *Wild birds, such as starlings, grackles are reservoirs* – predation of birds by cats, dogs can cause transmission
  - Contamination of pig feed with wild bird feces, dead birds has → ulcerative colitis outbreaks in pigs
**Y. enterocolitica:**

Q. Why is **Y. enterocolitica** important?

- **Y. enterocolitica** is an important food-borne zoonotic pathogen
- **Y. enterocolitica** is **psychrophilic** (can grow at 4°C) and survive in water, sewage, in the fridge

**Pigs are reservoirs of infection for infections in humans:** (serotypes 0:3, 0:9, 0:5, and 0:8, which are also prevalent in human infections)

- Carry serotypes causing human disease in their tonsils, tongues, and feces, contamination of food by feces ("chitterlings" AKA "chitlins" in southern USA)
- Human disease can be mild diarrhoea +/- systemic infections depending on the serotype
- Enteritis, with mesenteric adenitis
- Humans with HLA B-27 MHC gene can develop ReA from **Y. enterocolitica** infection (like *Salmonella*)
What about Kids Kissing Pigs?
(zoonosis and reverse zoonosis)
Y. enterocolitica:

- Lab diagnosis:

- Best isolated at 25°C on CIN agar

(CIN agar is selective/differential medium used for Yersinia; contains Cefsulodin, Irgasan, Novobiocin and Mannitol)

- Y. enterocolitica on CIN agar forms “bull’s eye” colonies with bright red/burgundy centres and transparent borders
**Y. ruckeri** – and disease in fish

- *Y. ruckeri* is a fish pathogen
- Causes a septicemia with exophthalmos and hemorrhages/blood spots in the eye, mouth and throat
- Disease in rainbow trout is acute = “enteric red mouth”
- Disease in Atlantic salmon is milder = “yersiniosis”

*Ermogen* bacterin vaccine:
formalin-inactivated cultures of *Yersinia ruckeri*
Is this the end?