The Chaos Theory

“A very small occurrence can produce unpredictable and sometimes drastic results by triggering a series of increasingly significant events.”
Enterobacteriaceae - The Genus Yersinia
**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)**

- Plague (urban plague cycle) has occurred in three pandemics, killing millions (50% mortality rate) (read history in S & P text, page 138)

- The first pandemic, called The Justinian Plague 540 – 750, from Africa to the Middle East, and then to Mediterranean Europe

- The second pandemic from Asia to Europe, 1347-1351, known as the Black Death, Black Plague or the Plague (killed 30 – 50% population)

- The third pandemic in 1855 from China → North America, and is considered to be re-emerging

“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 have argued that the epidemiology, virulence, and population dynamics of the Black Death second pandemic were too different from modern *Y. pestis* plague disease to have been caused by *Y. pestis*.

- They propose that the Black Death must have been caused by another organism, such as *Bacillus anthracis*, a virus (like Ebola virus), or a now extinct pathogen.

- However......
Digging up the Dead
Confirming that *Y. pestis* is the cause of the Black Death and other plague epidemics

- Previous genetic studies to find *Y. pestis* in medieval human skeletal samples have were viewed with skepticism as being contaminated by modern bacterial DNA.

In 2011, a report from a team of German and Canadian scientists:
- Did targeted enrichment and sequencing of human DNA and the *Y. pestis* pPCP1 virulence plasmid from bones and teeth of 100 skeletons from the East Smithfield plague mass burial site (1348-1352) collection, London, England.
- Found that the Black Death in medieval Europe was caused by a variant of *Y. pestis* that may no longer exist, but contains the pPCP1 plasmid found in modern *Y. pestis* isolates.
More good evidence that *Y. pestis* was the agent, 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.

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The black rat - *Rattus rattus*

The brown rat - *Rattus norvegicus*
Yersinia pestis: Plague transmission cycle - a zoonotic disease
The Genus Yersinia:

Q. Why is *Yersinia pestis* unique?

- *Y. pestis* is a clone that evolved from the soil-dwelling *Y. pseudotuberculosis* 1,500–20,000 years ago.

- *Y. pestis* is different from *Y. pseudotuberculosis* and *Y. enterocolitica*.

- Has two unique virulence plasmids which enabling growth in fleas & transmission from flea to host. The pFra plasmid (also called pMT1) and the pPCP1 plasmid.

- This arthropod-transmission is unique among enteric bacteria.
The Genus Yersinia:

Q. Why is *Yersinia pestis* so virulent?

- Has a common virulence plasmid (low calcium response plasmid) shared with *Y. pseudotuberculosis* and *Y. enterocolitica* (the plasmid has different names in each species *Y. pestis* = pCD1, *Y. enterocolitica* = pYV and *Y. pseudotuberculosis* = pIB1)

- The pCD1 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)
  - Causes cytotoxicity
  - The Yop system confers the ability of the three *Yersinia* species to resist the host’s primary immune response, and confers a tropism for lymphoid tissue. *Yersinia* are both *intracellular* (can grow in MΦ, PMNs) & *extracellular* parasites

- The three *Yersinia* species also have a pathogenicity island (HPI) for iron-binding protein (yersiniabactin)
The Yop Type III Secretion System
Bacterial weapons of host cell invasion and mass destruction

This diagram is NOT intended for memorisation. It's just to illustrate that *Y. pestis* infection is complicated.
Yersina pestis and plague disease in humans

- 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 and their predators, the endangered species black-footed ferret are both at risk

• 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 by bacterial culture and identification is not done by routine diagnostic labs.

- 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

**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.
Y. pestis in the Research Lab - The risks of working with a potential bioterrorism agent

- Molecular genetics professor Malcolm Casadaban died September 13, 2009, from the plague.
- Casadaban, 60, (U of Chicago) was investigating a weakened laboratory strain of Yersinia pestis, as a vaccine to protect against the plague. It had been approved by the Centers for Disease Control and Prevention (CDC) for laboratory studies without the special safety precautions taken with harmful strains. This strain did not have the Yersinia iron-binding protein.
- Casadaban did not know he had hemochromatosis, an inherited condition, (causing too much iron buildup in the liver), with twice normal population frequency in Europeans. The hemochromatosis that contributed to Casadaban’s fate has been credited with protecting people from strains of plague that circulate in the wild. Sharon Moalem, an evolutionary biologist and author of “Survival of the Sickest,” posited that the disorder shifts iron from certain white blood cells, where it is typically sought by the plague bacterium. So Europeans with hemochromatosis are descendants of survivors of medieval Europe plague pandemics.
**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 Yop system plasmid with Yops described for **Y. pestis**
*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*)
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
What about Kids Kissing Pigs?
(zoonosis and reverse zoonosis)
Y. ruckeri - and disease in fish

- Y. ruckeri is a fish pathogen
- Rainbow trout, Atlantic salmon, and recently, Atlantic cod
- 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”
This is the end of the lecture

Nikki, Mother of Champions