- All sorts of genera -
  *Francisella, Actinobacillus, Moraxella, and Neisseria*

**Francisella tularensis**

- Small non-spore-forming, capsulated gram-negative coccobacilli
- Previously was in the genus *Pasteurella*, but reclassified as a new genus after its discoverer Dr. Edward Francis
- Four subspecies; the subspecies *tularensis* is most virulent
- Fastidious growth requirements in lab, requires cysteine-supplemented media
- Primary isolation from clinical samples can be done in Level 2 diagnostic lab, but identification requires Biosafety Level 3
- "Many laboratories actively avoid opportunities to cultivate it" (S & Post text pg 212)

- Bioterrorism Category A agent
- High potential for lab-acquired infections by aerosol inhalation
- Has been used as a biological warfare agent in WW II, and it has received renewed attention recently owing to concerns about bioterrorism (high infection risk by inhalation, low infection dose, high mortality rate, no licensed vaccine)
Q. Why is this bug *Francisella tularensis* so special?

- *F. tularensis* causes the zoonotic disease *tularemia*, a plague-like disease transmitted by an arthropod reservoir & vector, *Ixodid ticks*
- Deer flies & mosquitoes are mechanical vectors
- Primarily affects wild rodents & lagomorphs, the natural/reservoir hosts
- Also infects many other animal species - mammals & humans, birds, fish, reptiles
- Endemic in North America (subspecies *tularensis*) & Europe (subspecies *holarctica*)
- Survives in environment for months

Q. Why is this bug *F. tularensis* so virulent?

- *Francisella tularensis* is a facultative intracellular pathogen (FIP)
- Invades & multiplies inside macrophages.
- Has an antiphagocytic capsule, and a pathogenicity island (PI) responsible for intracellular growth & survival.
**F. tularensis invasion and survival in host**

Figure 3. *Francisella tularensis* enters the respiratory tract and (2) the lamina propria of the respiratory bronchioles via M cells; (3) Digested antigen is taken up by dendritic cells; the dendritic cells travel to regional lymph nodes and present *F. tularensis* antigens to T-helper 1 cells; (4) T-helper 1 cells proliferate; they may return to site of initial infection; (5) restimulation by local antigen presenting cells results in interferon-γ production and macrophage activation; (6) Failure to clear the *F. tularensis* results in granuloma formation.


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**F. tularensis and disease - tularemia (rabbit fever)**

- Infectious dose is very low (10 bacteria):
  - Can penetrate unbroken skin (Doesn't need any help like *P. aeruginosa* does!)
- Clinical syndromes depend on infection route → plague-like septicemia
  - Contact or infected insect bite → ulceroglandular form, with skin ulcer at point of entry
  - Ingestion → oropharyngeal and typhoidal forms
  - Inhalation → primary pneumonic form (this form has the highest mortality)
  - Eye infection → oculoglandular form
  - Lymph node infection without obvious skin ulcer → glandular form
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*Cat, lung. Numerous small pale foci disseminated throughout all lung lobes. Credit: Dr. J. Niefteld, Kansas State University, College of Veterinary Medicine*

*Cat, spleen and liver. Numerous small pale foci disseminated throughout the spleen; fewer pale foci in the liver lobes. Credit: Dr. J. Niefteld, Kansas State University, College of Veterinary Medicine*

*Beaver liver - disseminated small pale foci of necrotizing hepatitis. Credit: Dr. G. Wobeser Canadian Cooperative Wildlife Health Centre*
Q. What is important for us to know about tularemia?

- **Cats** - clinical illness can be severe, differential diagnosis is *Y. pestis* plague
- **Dogs** - can be infected by eating wild rabbits, & bites by infected ticks illness is milder than cats
- **Humans** - highest risk is from arthropod vector bites and handling infected tissues, examples - vets, farmers, hunters & trappers skinning rabbits and other wild species – ex. squirrels, beavers, muskrats, pheasants
- Humans also can be infected by contact with cats and dogs, mowing lawns, cutting brush in endemic areas
- Treatment - first choice is gentamicin; tetracycline, chloramphenicol

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**Francisella tularensis & disease – tularemia**

Q. How do we prevent tularemia infections?

- Include tularemia as a differential diagnosis of febrile illness in endemic areas
- Cats and dogs - flea and tick control, limiting hunting & other outdoor adventuring in endemic areas
- Use of disinfectants to clean equipment
- Hunters - wear latex gloves when handling carcasses and cook meat thoroughly before eating.

Black-tailed prairie dogs are highly sociable, a trait that facilitates the spread of infectious diseases like plague and tularemia. NPS photo.
**Actinobacillus species:**

- Taxonomy of the Genus *Actinobacillus* has been reviewed* with a definition of the nine "true" actinobacilli species associated with animals

- Habitat is mucosal membranes of upper respiratory tract, GIT, & genital tract; carrier animals are needed for disease transmission

- Non-motile gram-negative bacilli
- Are pleomorphic, a mix of rod & coccoid shapes, giving a "Morse code" (dot & dash) appearance on Gram stain
- Most have capsules
- Urease positive
- Some species can grow on MacConkey agar as tiny LF colonies


"Actino" means "rays" referring to the radiating structures (sulfur granules) formed by *A. lignieresii* in tissue

*Actinobacillus* species of veterinary importance:

1. *A. pleuropneumoniae* - pigs
2. *A. suis* (pigs only)
3. *A. equuli* subspecies *equuli* - horses & pigs
4. *A. equuli* subspecies *haemolyticus* - horses only
5. *A. lignieresii* - ruminants and others
Actinobacillus pleuropneumoniae

- The only Actinobacillus species considered a primary pathogen
- **Causes contagious porcine pleuropneumonia**
- Necrotizing haemorrhagic pneumonia with pleurisy
- Highly infectious
- Worldwide, 15 serotypes (1,5,7 in N.A); & 2 biotypes
- Usually young pigs affected (<6 months age)
- High morbidity & mortality (30-50%)

**Acute signs** - sudden death, pyrexia (shivering), coughing, expiratory dyspnea in young pigs, bloody froth from nose or mouth
- Necrotizing hemorrhagic pneumonia of the caudodorsal aspect of caudal lung lobe + fibrinous pleuritis

**Survivors** - chronic lung lesions (lung scarring, abscesses, pleural adhesions, necrosis, sequestra) → poor doers
- Outbreaks (fall/winter) preceded by introduction of carrier into “clean herd”; or stresses in a low carrier herd (ventilation/temp problems, viral or mycoplasma infections)

A. pleuropneumoniae - acute respiratory distress

Q. How does *A. pleuropneumoniae* cause severe necrotizing pneumonia?

- *A. pleuropneumoniae* virulence is associated with having RTX toxins (pore-forming cytolytic toxins; RTX = “repeats in toxins”), these kills host immune cells

- There are several types of RTX genes found in *A. pleuropneumoniae*, *A. suis*, *A. lignieresii*, and *A. equuli* subsp. *haemolytica*
- *A. pleuropneumoniae* has RTX toxins Apxl, Apx II, Apx III & Apx IVA
- Certain serotypes are more virulent

- **Urease** (important for acquiring ammonia as a nitrogen source)
- **Capsule**, LPS, transferrin binding proteins, hemoglobin-binding OMPs, iron binding proteins, proteases

- Sustained inflammatory response → tissue necrosis

Actinobacillus pneumoniae pneumonia - necrosis, hemorrhage, infiltration of neutrophils.
Laboratory for Genomics & Bioinformatics, University of Oklahoma Health Sciences Center
A. pleuropneumoniae - disease control

- **Disease control by vaccination:**
  - Vaccines available → passive protection from sow
  - Bacterins reduce mortality but have variable efficacy
  - PLEUROSTAR APP™ Novartis - a subunit vaccine (against RTX toxins & OM proteins)

- **Disease control by management procedures to prevent direct contact or aerosol exposure in intensive pig production environment:**
  - Quarantine
  - Segregated early weaning
  - All-in/all-out production
  - Cleaning & disinfection
  - Serological monitoring for and culling of carriers

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How do we identify *A. pleuropneumoniae* in the lab?

- Culture on BA, also on BA with Staph streak, or on chocolate agar because *A. pleuropneumoniae* Biotype 1 requires NAD = Factor V (NAD = nicotinamide adenine dinucleotide)

- Look for tiny hemolytic colonies that "satellite" around the Staph streak
- Urease-positive

Satellitism: bacterial colonies cluster around a *Staphylococcus* streak line which provides NAD (V-factor)

- **Also is CAMP- positive** (synergistic hemolytic action of RTX with *Staph. aureus* beta toxin)

*Actinobacillus pleuropneumoniae* cross streaked with a feeder colony of *Staphylococcus aureus* demonstrating a CAMP reaction, hemolytic activity, & dependence on NAD (V-factor) for growth.

- We have to distinguish *A. pleuropneumoniae* from *Haemophilus parasuis*, which also satellites and also from nonpathogenic commensal *Haemophilus* and *Actinobacillus* species in respiratory tract
**Actinobacillus suis**

- Genuine *Actinobacillus suis* has only been isolated from pigs
- Resides in nostrils, tonsils, vaginal mucosal membranes of healthy pigs

Note: Because it is hard to distinguish from *A. equuli* subsp. *haemolyticus* using only biochemical tests (phenotypically) it has previously been reported from horses (Lab identification errors).

- Hemolytic and urease-positive
- Virulence: Has *RTX* toxins, capsule, urease, transferrin-binding proteins
- Disease = septicaemia and localised infections
  
  **Three Syndromes:**
  - Piglets (< 1 month) - septicaemia (50% mortality)
  
  *Grow-Finish pigs* - septicaemia with bronchopneumonia which looks just like hemorrhagic pneumonias caused by *A. pleuropneumoniae*
  
  *Adult pigs* - metritis, abortion, meningitis, red skin lesions similar to those caused by *Erysipelothrix rhusiopathiae*

- No commercial vaccines

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**Actinobacillus lignieresii**

*What does this bug *A. lignieresii* do?***

- *A. lignieresii* causes pyogranulomatous lesions in soft tissues of ruminants (tongue, head and neck, lungs, mammary glands, lymph nodes)
- Called “Wooden tongue” also called “actinobacillosis” in cattle
- Can have sporadic individual cases or can have outbreaks in cattle herds
- Cutaneous form in ruminants: skin lesions and related lymphatics only (ex-outbreak in beef herd near Moncton, NB in 2008)
- Granulomatous abscesses in sheep and cattle, humans, horses, dogs, rats, udder of cows and sows
- Must distinguish “actinobacillosis” from actinomycosis = lumpy jaw, caused by *Actinomyces bovis*, which affects bone, usually the jaw bone of cattle

Lumpy jaw is caused by *Actinomyces bovis*. 
A. lignieresii

Q. What predisposes to wooden tongue by A. lignieresii?

- A. lignieresii is a commensal in oropharynx and rumen of cattle and sheep
- TRAUMA is the predisposing factor — penetrates mucosal or skin barrier — underlying submucosal soft tissues — pyogranulomatous infections (hard, tumorous masses) in tongue and soft tissues of neck and around jaw, can spread by lymphatics to lungs, stomachs & other organs
- Examples of predisposing trauma - coarse feeds, hay, straw

Q. - How do we diagnose wooden tongue?

Clinical Signs:
- drooling, salivating, protruding tongue, dysphagia, weight loss
- Abscesses contain odourless purulent material

Lab Diagnosis:
- "Sulphur granules" in wooden tongue lesions, seen either on direct Gram-stain or histology
- Central masses of gram-negative bacteria surrounded by spicules of calcium phosphate, inflammatory debris (described as radiating, club-like filaments, pathologist’s term = Splendore-Hoeppli reaction)
- In contrast, Actinomyces bovis granules in lumpy jaw lesions contain gram-positive bacteria, are yellow, and are larger than Actinobacillus lignieresii granules

Treatment requires surgical drainage + antibiotics (tetracyclines), potassium iodide (oral or i.v.)
**Actinobacillus equuli**

Two subspecies now recognised:

- *A. equuli* subsp. *equuli*, normal mucous membrane flora of horses and pigs
  - Causes disease in horses but also pigs
  - Is nonhemolytic & CAMP-negative

- *A. equuli* subsp. *haemolyticus*, normal mucous membrane flora of horses only
  - Causes disease in horses only
  - Is hemolytic & CAMP-positive
**Q- Why is *A. equuli* ssp. *equuli* important?**

*A. equuli* ssp. *equuli* causes septicemia of neonatal foals

- Disease is called “sleepy foal disease”, also called “joint ill”
- Sporadic infections largely affecting neonatal & young foals (causes 1/3 of neonatal mortalities)
- Mare is source of infection, with transmission either *in utero*, at birth, or via umbilicus, ingestion, inhalation

- Failure of foal to ingest adequate colostrum is important predisposing cause = failure of passive transfer (FPT)
- Possibly carried by migrating larvae of *Strongylus vulgaris* from GIT to bloodstream

- Septic emboli → microabscesses, particularly in kidney and joints
- Acute clinical signs due to neonatal septicaemia, frequently fatal
- Survivors show signs of chronic infections: purulent nephritis, pneumonia and septic polyarthritis

**Diagnosis:**
Blood culture of septic foal, kidneys, joints, & other organs at PM,

- *A. equuli* ssp. *equuli* can also cause septicemia in piglets and adult pigs
- 2010 Report in CVJ - peracute outbreak of *A. equuli* ssp. *equuli* septicemia killing 300 sows in a two month period.

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**A. equuli** ssp. *equuli* septicemia of neonatal foals

- Actinobacillus *equuli* septicemia - embolic nephritis.
What is important for us to know about this equine pathogen?

- Found in horses only
- Sporadic cases of wound and joint infections, metritis, abortion, endocarditis and meningitis in adult horses
- Not as common opportunistic pathogen as *Streptococcus zooepidemicus*, but an important pathogen of horses

Possible zoonotic risks of horse and pig bites:

- Human wounds and bite wound infections caused by *A. suis* from pigs and *A. equuli* from horses (and other bacteria, such as *Streptococcus zooepidemicus*, & other normal oral flora)
Genus *Moraxella*

*Moraxella bovis* and *Moraxella ovis*

- *Genus Moraxella* are short, plump, gram-negative bacilli/coccobacilli, frequently in pairs “diplobacilli” or chains, can stain "gram-variable" (see gram-positive & gram-negative cells together on a smear of a bacterial colony, which can be confusing)
- Nonmotile, fastidious, aerobic, do not grow on MacConkey agar

- Several commensal strains of *Moraxella* and *Neisseria* on skin, mucous membranes and conjunctivae of animals

- The important *Moraxella* species in animals are:
  - *Moraxella bovis*, hemolytic → conjunctivitis in cattle
  - *Moraxella ovis*, hemolytic/ nonhemolytic → conjunctivitis in sheep & goats, cattle
  - *Moraxella equi*, a non-hemolytic variant of *M. bovis* → conjunctivitis in horses

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*Moraxella bovis* - why is this bug important?

- *M. bovis* causes infectious bovine keratoconjunctivitis (IBK or IBKC)
  - Also called “pinkeye”, “New Forest disease”, or “New Forest eye” (UK)
  - A highly contagious and painful eye disease common in cattle worldwide with significant economic losses ~ $ 200 million annually
  - Most common eye condition seen in cattle in UK, important in Australia & USA
  - Outbreaks in grazing cattle in summer, high morbidity, young cattle most susceptible
  - Herefords and cattle without pigmented ocular area more prone

Clinical Signs:

- Early → conjunctivitis, chemosis ( = conjunctival edema), lacrimation/serous discharge, photophobia, blepharospasm
- Late → purulent discharge, corneal edema, opacity, ulceration, scarring or rupture of cornea, panophthalmitis, blindness
- PAINFUL!: cattle become temporarily or permanently blind; go off-feed

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Pink Eye (Conjunctivitis)
Q - What predisposes to IBK by *Moraxella bovis*?

- *M. bovis* is transmitted from asymptomatic adult cattle carriers by ocular/ nasal exudates, fomites, cows licking calves
- Face fly transmission is also important
  - (look at Figure 21-2, page 170, S&P text - what are those flies doing?)

- Predisposing factors: ocular irritants - UV light, dust, wind, grasses, ammonia, other eye infections (mycoplasma, viral), also relationship to vaccination with modified live IBR vaccine

Q - How does *Moraxella bovis* cause disease?

- *Moraxella bovis* has these important virulence factors:
  - **Type IV pili** (Q and I) which allow attachment and maintenance of infection
  - **Hemolysin/cytolysin** (pore-forming RTX toxin)
    - damages conjunctival, corneal epithelial cells and PMNs; leakage of lysosomal enzymes from PMNs into cornea → liquefaction and ulceration (clever tactic: get the enemy help you invade the castle!)
  - Only *piliated and hemolytic* *M. bovis* bacteria can cause IBK
  - **Capsule**
**Moraxella bovis** and IBK

**IBK Management/Control:**
- Provide shade (dark stall), third-eyelid flaps, eye patches, tarsorrhaphy (sew eyelids partially together) to protect eye and reduce pain
- Topical corticosteroids, topical &/or systemic oxytetracycline
- Vaccines available, but aren’t effective, do not increase ocular IgA, do not give protection against the different pilus serotypes
- Fly control, pasture management (mowing weeds), vitamin A supplements, shade

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**Moraxella ovis**

**Q - What is important for us to know about Moraxella ovis?**

- *Moraxella ovis* causes infectious keratoconjunctivitis (IBK) in sheep and goats, and likely has a role in IBK of cattle
- One report of *M. ovis* IBK in deer and moose in Wyoming, USA, 2000
- Has pili and a hemolysin/cytotoxin similar to *M. bovis* hemolysin
The Genus Neisseria

- Q - What is important for us to know about animal Neisseria species?

- Are normal flora on mucous membranes
- Can be significant in animal bite infections - ex. Neisseria canis & N. weaveri, also Moraxella canis

This is the end of my lectures

Good luck Class of 2014!