The assorted genera
*Francisella, Actinobacillus, Moraxella,* and *Neisseria*

All sorts of bacteria

Course: VPM 201 Fall, 2010
Lecturer: C. Anne Muckle
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” (Songer & 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)
Francisella tularensis

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*) and Europe (subspecies *holarctica*)*
- Survives in environment for months
Francisella tularensis | Q. Why is this bug *F. tularensis* so virulent?

- *Francisella tularensis* is a facultative intracellular parasite (FIP)
- Invades & multiplies inside macrophages, but its virulence factors are ??

*F. tularensis* invades macrophages

![Diagram of *F. tularensis* invasion process](https://example.com/diagram.png)
Francisella tularensis & 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

Credit:
- Dr. J. Niefeld, Kansas State University, College of Veterinary Medicine
- Dr. G. Wobeser, Canadian Cooperative Wildlife Health Centre

Cat, spleen and liver. Numerous small pale foci disseminated throughout the spleen; fewer pale foci in the liver lobe. Credit: Dr. J. Niefeld, 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

Cat, lung. Numerous small pale foci disseminated throughout all lung lobes. Credit: Dr. J. Niefeld, Kansas State University, College of Veterinary Medicine
Francisella tularensis & disease – tularemia
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
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 → 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 Mac agar as tiny LF colonies

**Actinobacillus species**

“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, 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 –

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”)

- 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 ll, Apx lll & Apx lVA

- Certain biotypes 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
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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
A. pleuropneumoniae

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

- Culture on BA, also on BA with Staph streak, or on chocolate agar because Biotype 1 requires NAD = Factor V

- Look for tiny *hemolytic* colonies that “satellite” around Staph streak

- Urease–positive

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

![Image of Actinobacillus pleuropneumoniae cross streaked with a feeder colony of Beta-hemolytic staphylococcus demonstrating a CAMP reaction, hemolytic activity, & dependence on NAD (V-factor) for growth.](image)

- We have to distinguish *A. pleuropneumoniae* from *H. parasuis*, and also from nonpathogenic commensal *Haemophilus* and *Actinobacillus* species in respiratory tract
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 similar to hemorrhagic pneumoniae caused by *A. pleuropneumoniae*.

**Adult pigs** - metritis, abortion, meningitis, red skin lesions similar to those caused by *Erysipelothrix rhusiopathiae*.

No commercial vaccines.
**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 actimycosis = lumpy jaw, caused by *Actinomyces bovis*, which affects bone, usually the jaw bone of cattle
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

![Image of cattle eating straw and wooden tongue](image1)

![Image of wooden tongue lesions](image2)

*Actinobacillus lignieresii* – wooden tongue lesions in tongue and retropharyngeal lymph nodes
**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-Hoeplli 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.)
A. lignieresii – wooden tongue sulfur granules
Actinobacillus equuli

Two subspecies now recognised:

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

- *A. equuli* subsp. *haemolyticus*, normal mucous membrane flora of 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 pigs
A. equuli ssp. equuli septicemia of neonatal foals

Actinobacillus equuli septicemia - embolic nephritis.
A. equuli ssp. haemolyticus

Q. 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
Actinobacillus species and zoonoses

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
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”
- 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

Pink Eye (Conjunctivitis)
**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
**Moraxella bovis**

Q – What predisposes to IBK by *Moraxella bovis*?

- *M. bovis* is transmitted from asymptomatic adult cattle carriers by 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
Moraxella bovis

Q – How does Moraxella bovis cause disease?

- *Moraxella bovis* has these important virulence factors:
  - Type IV pili (Q and I) → attachment and maintenance of attachment
  - Hemolysin/cytolysin (pore-forming RTX toxin)
  - → damages conjunctival, corneal epithelial cells and PMNs; leakage of lysosomal enzymes from PMNs into cornea → liquefaction and ulceration (a very clever tactic, get the enemy to do the damage!)
  - *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
**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.

- 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*
Is this the end?
Riding away into the sunset...