The World of Veterinary Microbiology

Objectives

- Identify the role of the diagnostic laboratory in public health/ONE Health
- Describe the characteristics of *Staphylococcus pseudintermedius* and *Bacillus anthracis*
- Discuss the role of *Staphylococcus pseudintermedius* and *B.anthracis* in animal disease

NDSU Veterinary Diagnostic Lab

North Dakota Century Code

Who keeps us in check?

- Accreditation: American Association of Veterinary Laboratory Diagnosticians (AAVLD)
- Clinical Laboratory Improvement Amendments (CLIA) certification

Who we serve

- Veterinarians
- Animal owners
- Ranchers
- Producers
- Public Health Sector
- Zoos

NDSU Veterinary Diagnostic Lab

- Who we are
  - Toxicology*
  - Virology
  - Serology
  - Urinalysis
  - Molecular Diagnostics
  - Bacteriology
  - Mycology
  - Parasitology
  - Biosafety Level 3 Laboratory
  - Histology
  - Pathologists

ONE Health

- The recognition of the relationship between animal health, human health and environmental health
  - We all have a role in this initiative
    - Local, regional, national and global collaboration
    - FDA
    - USDA-APHIS
    - CDC
    - State Health Lab
    - Many more

ONE Health

Veterinary Diagnostic Lab's Role-Microbiology

- National Animal Health Laboratory Network (NAHLN) through the USDA
- Vet-LIRN through the FDA
- Report select organisms to state health officials, CDC, USDA or FDA

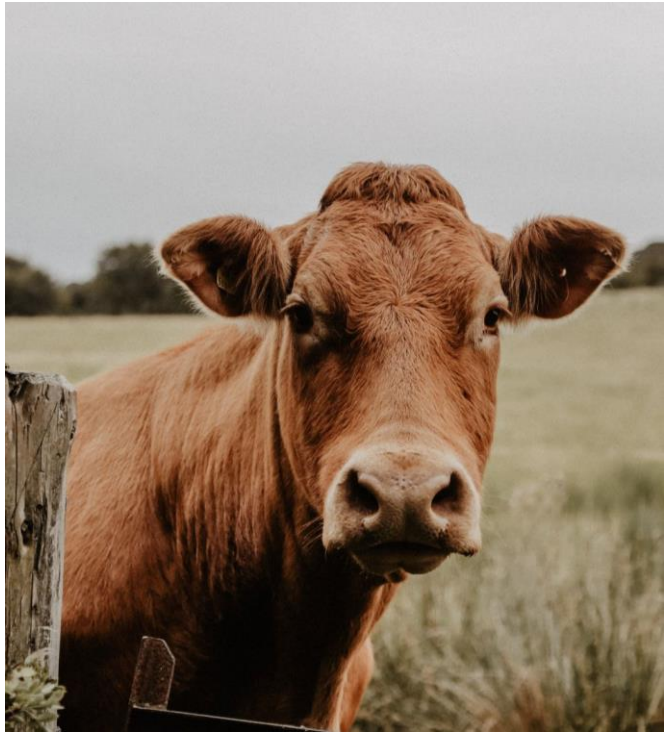
What can be monitored?

- Zoonosis tracking
- Antimicrobial resistance
- Chemicals in food animals
- Toxins in plants
- Others

Case Study #1

A rancher was out checking his cows in the pasture. The cows appeared healthy last night, but after coming outside this morning, the rancher noticed that one of the cows looked weak and was “breathing hard.” The cow died shortly after. Blood was noted to be seeping from the nose, rectum and eyes. The rancher called the veterinarian, who noted that there were not any other significant findings. The veterinarian collected a vial of blood from the jugular vein to send to NDSU Veterinary Diagnostic Lab for testing. The owner was concerned about nitrate toxicity and mycotoxin presence in the feed, so the veterinarian also collected appropriate water and feed samples.

Differential Diagnosis: *B.anthracis*, nitrate poisoning, mycotoxins



Case Study #1

Laboratory Tests Ordered

1. Bacillus anthracis PCR- whole blood
   - Select agent requiring BSL 3 precautions
2. Water testing for nitrates-requested by owner
3. Feed testing for mycotoxins-requested by owner

Case Study #1

PCR testing for *B.anthracis*:

- Performed ONLY in BSL 3 laboratory on designated equipment
- Multiplex assay that detects the presence of:
  - Protective antigen (PA)-a portion of the three-part toxin
  - Capsular antigen (capC)-component of the capsule
  - rpoB gene-codes the beta region of RNA polymerase

- *B.anthracis* is considered virulent only if it has both a capsule and the toxins
  - It is possible to only detect rpoB and PA but not the capC. This means that Bacillus anthracis is present but is avirulent

Case Study #1

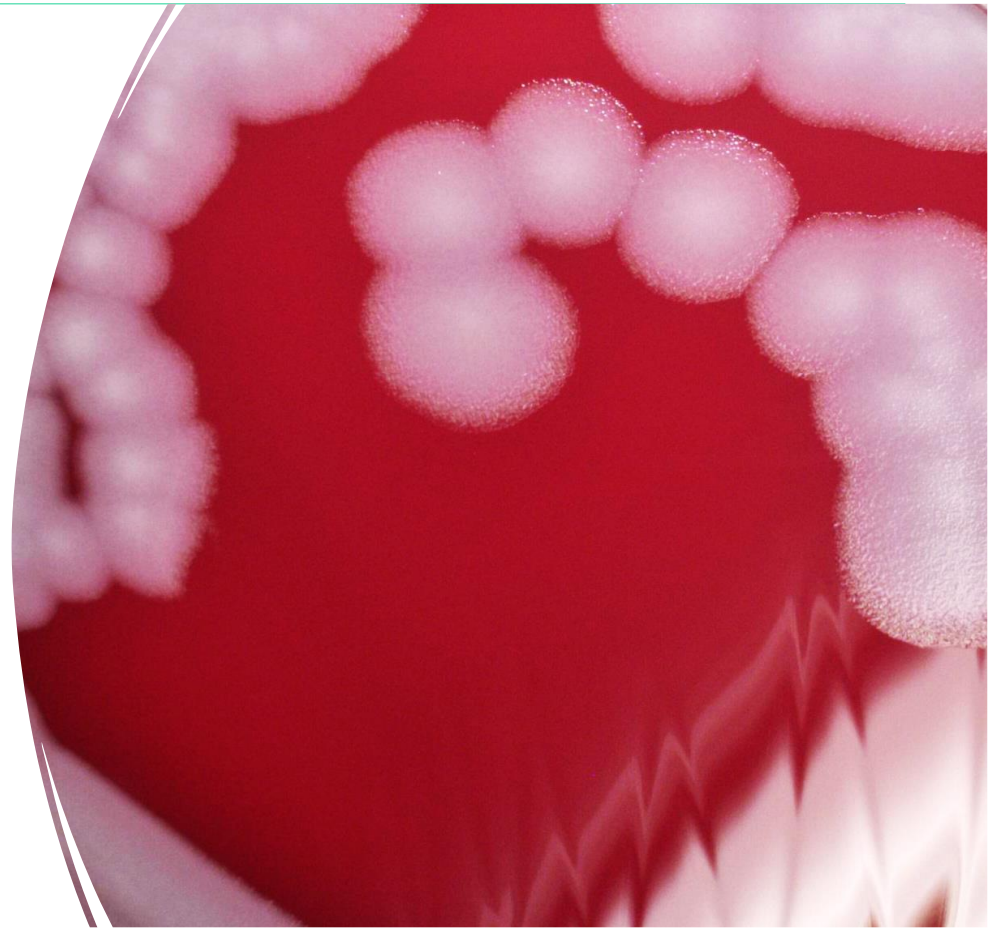
PCR test result=POSITIVE for *B.anthracis*

- rpoB, PA, and the cap genes detected
- Sample reflexed to culture

Case Study #1

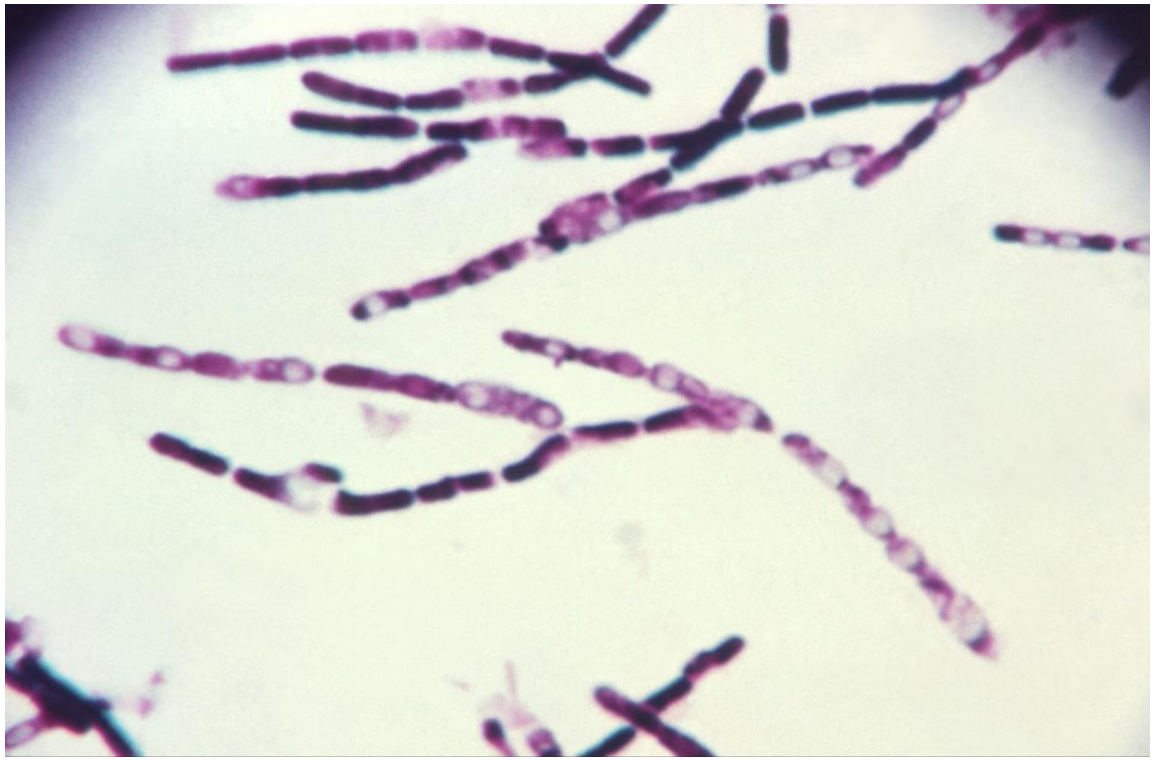
B.anthracis Identification

- Growth on BAP, CHOC
  - White, opaque, ground glass, sticky
- No growth on MacConkey's
- Gram stain=large, gram-positive bacilli
  - "Boxcar"
- Catalase+
- Nonmotile
- PG=S, Lecithinase production+
- DO NOT USE MALDI-TOF



Next Steps:

- Sent to State PHL for confirmation
- Report to the CDC/APHIS(Form 4a), veterinarian, animal/herd owner



Case Study #1

Where does *B.anthracis* come from?

- Endospores in the soil are ingested or inhaled by animals during grazing
  - Improper disposal of carcasses from animals with *B.anthracis*
  - Geographical patterns
  - Increased moisture
- Eating contaminated food
- Skin abrasions
- Biting insects

Case Study #1

B.anthracis disease

Ruminants

- Acute form: Sepsis, rapid death (1-2 days)
- Subacute form: fever, weakness, low appetite, death (Approximately 4 days)

Equine

- Ingestion/inhalation: Septicemia with colic and enteritis
- Wound infections: edema and lymphadenitis

Porcine

- Pharyngeal swelling, regional lymphadenitis
- Enteritis

Dogs and Cats

- Susceptible if in contact with contaminated blood

Case Study #1

Treatment and Control

- Bovine: Nearly 100% fatal due to the rapid onset of illness
- Horses and pigs: Penicillin if needed
- All other animals should be moved to another location
- Vaccinate susceptible animals
- Insect control
- Proper disposal of animal carcasses

Case Study #1

Public Health: how does this scenario factor in?

- Animal disposal/Environmental contamination
- Herd health
- Food source
- Potential exposures for animal owner, veterinarian and laboratory staff
- Monitor Foreign Animal Diseases (FAD)

Case Study #2

A cocker spaniel (Kibble) was taken to the veterinarian to have his front leg examined. Kibble's owner states that the dog seemed to be licking and chewing the area, and that it was red and inflamed. On occasion, it also oozed yellow/cream fluid. Kibble is also known to have a long history of eczema that is poorly controlled.

Case Study #2

- Tests ordered: Aerobic culture with susceptibility testing if appropriate
- Sample collected: Swab of exudate on lower leg
- The swab was plated to BAP, CNA and MacConkey’s

Case Study #2

Culture presentation:

- Growth after overnight incubation on BAP and CNA
- No Growth on MAC
- Opaque white/cream colonies with beta hemolysis
- Gram stain=Gram positive cocci, clusters

Identification

- MALDI-TOF=*Staphylococcus pseudintermedius*

Case Study #2

Test	*Staph pseudintermedius*
Catalase	+
Coagulase	+*
Novobiocin disc	S
Polymyxin B	**S**
Mannitol Salt Agar	**-/weak +**

Case Study #2

Staphylococcus pseudintermedius disease

- Dogs and cats are the primary host
- Pyometra
- Dermatitis
- Pyoderma
- Otitis externa
- Others: respiratory illness, osteomyelitis, joint infections, wounds and conjunctivitis
- Other animal species affected: Equine and bovine
- Human infection is documented with significant links to animal interaction

Case Study #2

Staphylococcus pseudintermedius risk factors

Companion animals

- Immunodeficiency
- Allergies
- Preexisting dermatosis with antibiotic treatment
- Exposure to *Staphylococcus pseudintermedius*

Bovine

- Contaminated milk equipment

Case Study #2

AST testing of *Staphylococcus pseudintermedius*

- AST testing is always performed in cases of infection
- Generally considered to be beta-lactamase positive, although there are isolates susceptible to Penicillin
- Inducible resistance to clindamycin is encountered and tested for via the D-test
- Methicillin resistance is screened for using the Oxacillin MIC

Antibiotic testing/treatment in Veterinary Microbiology

- More than drug-bug combinations
- CLSI guidelines and limitations
- Access to antibiotic options
- Treatment compliance

Case Study #2

Drug	MIC	Interpretation	Guidelines Available
Penicillin	>8	Resistant	Yes
Erythromycin	>4	Resistant	No
Clindamycin	<0.12	Resistant	Yes
Enrofloxacin	>4	Resistant	Yes
Chloramphenicol	<=8	Susceptible	No
Tetracycline	>1	Resistant	Yes
Trimethoprim-Sulfa	>4	Resistant	No
Gentamycin	8	Intermediate	No
Oxacillin**	>0.5	Resistant	Yes
Rifampin	0.5	Susceptible	No

**MRSP-all cephalosporins and carbapenems reported as Resistant

Case Study #2

Treatment

- In this case, Rifampin was prescribed
- Taken as directed!!

Prevention

- Control allergies
- Address any other underlying concerns i.e. endocrinology issues
- Groom properly

Case Study #2

Public Health: how does this scenario factor in?

- ONE health: Antimicrobial resistance is actively being monitored by NAHLN and Vet LiRN laboratories
- Animal-human transmission is known

- Address obstacles in the veterinary world
  - Promote stewardship
  - Review proper treatment options and stress a tiered approach to treatments
  - Educate
  - Get involved

Summary

- Medical laboratory science professionals play a significant role in animal, environmental and human health.
- Knowledge of both automated bacterial identification systems and traditional methodologies is important for microbiology staff.
- *Bacillus anthracis* poses a serious risk for animals. As a Category A agent, it is important to handle the sample utilizing the appropriate precautions. Molecular methodologies are particularly useful for identifying these types of pathogens.
- *Staphylococcus pseudintermedius* is the most common cause of pyoderma in small animals. Developing resistance is concerning and being followed by closely.
- There are great opportunities for MLS professionals to advance the goals of veterinary laboratory efforts. Education, development and research all can be impacted by laboratorians in the veterinary community.

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Thanks!

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Questions