Goals for this presentation

- Understand the pathogenesis of pestiviruses
- Identify clinical presentations for each disease
- Confidently diagnose and manage BVD or Border disease in a herd/flock setting

Outline:

- Review pestiviruses: BVD, BDV
  - BVD
    - Typical disease presentations
    - Diagnostics - test on this, vary
    - Management - Evaluation / Prevention
    - Herd immunity - vaccination
    - Find and remove PI
    - Biosecurity
- Border Disease
  - Case example
  - Available diagnostics
- Classical Swine Fever
  - Global ally
  - Eradicated from US in 1976
Nomenclature review

- Family: Flaviridae
- Genus: Pestivirus
- 11 recognized species
  - BVD1 = Pestivirus A
  - BVD2 = Pestivirus B
  - Classical Swine Fever = Pestivirus C
  - Border disease = Pestivirus D
  - Pestivirus E-K
- Enveloped
- Single-stranded RNA virus
- Infect even-toed ungulates

Break into groups of 2-4 to answer:

Part 1:
- Long-term client milking 150 cows with 30 dry cows and 75 youngstock (25 calves, 50 heifers)
- Has a calf born 1 week ago that looks like a BVD PI.
- What sample(s) do you collect?
- What test(s) do you order?

Part 2:
- 1 week later the result comes back positive. The farmer is very motivated to eradicate BVD from his herd.
- What samples do you collect on each age group/lactation group?
- What test(s) do you request?
- Approximately how much will this cost?
- What recommendations do you make in the meantime waiting for results to come?
BVD history

- First described by Dr. Fox (age 23!) at Cornell in 1946

BVD

- 2 Biotypes:
  - Cytopathic: CP BVD
  - Noncytopathic: NCP BVD
- Strains or Genotypes:
  - 1 (1a, 1b)
  - 2 (2a, 2b)
- Currently 1b is circulating in US

The veterinarian is called because cows are off feed, depressed, and scouring. At this stage of the disease, the temperatures, which may have been slightly normal, may have temperatures of 102° to 108° F. After animals have moaned a day or two, the severely affected develop chills in the mouth, ears, and on the muzzle. In the initial outbreak one of us (Fox), found several animals with a leukopenia. Salivation may occur early in the disease and again after chills appear in the mouth. Stiffness may hang from the muzzle to the ground. Nasal or a thick discharges may be present. Some animals may cough through the mouth, and others may sneeze. Mucous membranes are often inflamed. Eyes hang down, and they may have difficulty in flushing their plexus in the head. There may be marked disturbances in the distribution of body heat. Ears, horns, teats, and extremities are cool. Parts of the body may feel too cool while other parts are warm. The nasal and oral mucous membranes vary from a pink to a bright red color. The conjunctiva may show marked injection. Eye indices are not present.
Transmission

- Horizontal
  - Any secretions (nasal, ocular, oral, milk, seminal)
- Vertical
  - DCC abortions
  - PI 40-125 days gestation
  - Congenital defects
  - Normal, seropositive
  - Dam will be viremic but clear virus

Fig. 1. Potential clinical reproductive outcomes following infection with bovine viral diarrhea virus.

Fig. 2. Potential clinical reproductive outcomes following infection with bovine viral diarrhea virus. Source: Reproductive consequences of infection with bovine viral diarrhea virus. Daniel L. Grooms, DVM, PhD. Vet Clinics Food Animal 2004.
Diagnostics for BVD Abortions

- Fetus, placenta, serum and EDTA whole blood
- BVD PCR on fresh fetal lung tissue
- BVD ACE on fetal skin
- BVD SN on fetal heart blood and dam
- Virus isolation from fetal tissues

Trouble with PIs (40-125 days gestation)

- Shed tons of virus
- Reservoir for herd
- PI cow will always give birth to a PI calf
- PI animals have no BVD antibody titer
- Can look normal or weak and die early
- Or die before 2 yrs of age (their own NCP converts to CP or they are vaccinated or exposed to wild type CP BVD)
- Don't always have congenital defects (75-150 days gestation)
- Immunosuppressed

Trouble with PIs (40-125 days gestation)

- "PI animals may shed so much virus that the finite immunity in herdmates can be overwhelmed, resulting in infection of non-PI, immunocompetent and previously exposed and/or immunized herdmates"
Congenital defects 75-150 days gestation

- Cataracts
- Retinal degeneration
- Cerebellar hypoplasia
- Hydranencephaly
- Brachygnathism
- Hairless
- May or may not be a PI

BVD PI example

- 2 yr old heifer came from an auction
- Draining tracts over hips and shoulders
- Shared a field with boarding heifers running with a bull
Diagnostics for a PI

- BVD Antigen capture ELISA (ACE)
  - Can detect any age
- DAR NOTCH any age
- BVD-PCR on EDTA whole blood (does not differentiate PI from acute infection)

BVD Clinical Disease: 3 presentations

1. PI can get both CP and NCP strains and then die
2. Non-PI, immunocompetent cows and calves can be subclinical and develop BVD SN
3. Non-PI Clinical disease:
   - B symptoms
   - Tachypnea from fever
   - Diarrhea
   - Oral erosions, GI ulcers
   - Immunosuppressed
   - Die from opportunistic pathogens
   - Thrombocytopenia
   - Can bleed at injection sites
   - Clinical signs can vary by strain
   - Same farm will have same signs

Case example

- 5 mo old Holstein show heifer
- BVD ACE negative at birth = not a PI
- Presented to CUPA for oral and nasal ulcers
- Temp 105-108 few days
- Did not respond to NSAIDs
- Positive for Parapox virus
CBC- Anemia of inflamm dz, neutropenia, lymphopenia = severe acute inflammatory
leukogram
Clinical case diagnostics

- BVD PCR on GI and spleen
- BVD IHC on tissue with lesions
- BVD FA
- ACUTE BVD!

Diagnoses

- BVD Antigen Capture ELISA (ACE)

Sample types:
- Ear notch - any age
- Serum - >61 days

This is what you need for fair papers:
- $6.00 at AHDC
- AHDC does not pool ear notches
- Ear notch baby calves is easy - do it!
Ear notch is good evidence of BVD testing!

BVD PCR
- Detecting either acute infection OR PI
- EDTA whole blood at any age
- Serum if <61 days of age
- Pooling available
  - up to 10
    - $41 per PCR = $4.10/animal
    - If pool is +, individually test each animal with ACE ($6.00)
- MLV vaccine can be detected by PCR within 3 weeks of administration

BVD Bulk Tank
- BVD Bulk Tank testing
  - BVD PCR and Virus isolation
  - $68
  - 400 cows per sample
  - >200 ml
  - NOT CURDLED
  - NOT FROZEN
  - Clean container ok!
  - Record who was in the tank the day of sampling!!
  - No dry cows
  - No treated cows
  - Any cows culled after sampling?
BVD Bulk Tank

- If BT is positive
  - Need to start looking for PI
  - Serum for pooled BT PCR
  - 14+ days of age
  - Then BVD ACE on serum sample if pool is positive

- Notch any cow that is culled while waiting for BT milk results
- Positive BT + negative pooled serum samples = frustrating!

BVD serum neutralization (SN)

- Antibody titer on serum $15.90
- Vaccinated animals will have a detectable titer
- Consider acute and convalescent SN (3-4 wks apart)
- Not overly helpful diagnostic test
- If no titer and herd vaccinated, get nervous
  - BVD PI has no titer

Diagnostics: Find PI animals vs acutely infected animals

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<th>Component</th>
<th>Cost</th>
<th>Notes</th>
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<tr>
<td>BVD ACE</td>
<td>Ear notch any age Serum +40 days</td>
<td>$6.00</td>
<td>PI vs nonPI</td>
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</table>
| BVD PCR  | EDTA whole blood Serum | 5 x $4 | Acute or PI
  - Pool up to 10 animals = $40/10 animal
  - Will detect recent MLV |
| BVD SN   | Serum     | $15.90 | Serum neutralization (antibody titer) reflects vaccine or exposure |
| BVD Bulk Tank | 40 cows $56 | Combination of PCR and virus |
### AHDC BVD ACE data 1/20-4/22

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<td>ME</td>
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Inconclusive: 1
Total: 7

*BVD PI are 2% of population*

### AHDC BVD BT data 1/20-4/22

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Inconclusive: 1
Total: 7

### Management: You must slaughter PI calves

- It is not ethical to send them to market
- It is not ethical to keep them alive
- Pestiviruses can spread to Artiodactyla (bearing wt on 2 toes)
- Experimental infection in pregnant white-tailed deer in 2008
Recent NY submission: Sounds like BVD... what’s wrong?

Herd History

- Purchase animals and they board out their heifers
- 3rd and greater lactation animals had at least seen Triangle 10 at some point in their lives
- 1st LACT animals all got a vaccine at 600 day of age
- 2nd LACT had not ever been vaccinated and those are most severely affected

Pearls of wisdom from Dr. Ed Dubovi

Vaccines

Vaccine “failures” are usually caused by an inadequate vaccine strategy along with a large dose of non-compliance. “No heifer should be bred without receiving at least one dose of modified live BVD vaccine.”
Management: Vaccination

- MLV = killed
- "A Fetal Protection Guarantee program given producers confidence that cattle properly vaccinated with BOVI-SHIELD® will not be persistently infected with BVDV."
- Immunosuppressive
- Don’t give near breeding
- Follow label: Give to pregnant animal within 12 mo

Vaccination = MLV

- Heifers at 5-6 months of age
- Again 60 days before breeding
- Adults: yearly 2-4 weeks prebreeding
- If long calving interval: make sure within a year!
  - If using Killed - make sure heifers get a booster

Management: Biosecurity

- Test any new animal
- Test any calf born to a cow that was not from the home farm
- Cluster clean
- Cattle get exposed to BVD at the fair!
Pearls of wisdom from Ed Dubovi

- HERD SCREENING STRATEGY
  - BULK TANK ON LACTATING ANIMALS
  - POOLED SERUM for PCR ON NON-LACTATING COWS
  - EAR NOTCH ON any age animal for BVD ACE

- ON-GOING HERD SCREENING STRATEGY FOR DAIRY HERDS
  - Ear Notch All Calves as they are born
  - Single test defines the status of two animals - calf and dam
  - Negative calf can only come from negative dam

Discussion

- Ear notch BVD ACE all calves under 60 days of age:
  - $6 x 25 = $150
  - Or EDTA whole blood for BVD-PCR on calves under 60 days of age:
    - 25 calves, groups of 10 pooled = $41 x 3 = $123
  - Bulk Tank milk BVD PCR and VI on all lactating cattle
    - $68
  - Pooled BVD PCR on serum from nonlactating cattle
    - 30 dry cows, groups of 10 pooled = 3 x $41 = $123
  - Pooled BVD PCR on serum from youngstock < 60 days
    - 50 heifers, groups of 10 pooled = 5 x $41 = $205
  - TOTAL initial testing: $546

43

44

45
New in the literature: “First gene-edited calf with reduced susceptibility to a major viral pathogen”

- Workmen et al 9 May 2023
- Used skin fibroblasts
- Substituted 6 amino acids in the BVD binding domain CD46
- Put 8 unedited wild type CD46 and 8 CD46 substitution embryos into Gir cattle

Outcomes

- In vitro, primary cells, fibroblasts and immune cells from gene edited calf had up to 96% reduction in BVD susceptibility
- Ex vivo fetal kidney lung SI liver and heart cells reduction in BVD susceptibility

Outcomes

- 1 wild type and 1 edited calf were housed w a PI at 10 mo of age
- Both fed TMR. One MR
  - Wild type calf was BVD PCR pos for 28 days, cough, rhinitis, mononuclear cells infected 12 days
  - Edited calf BVD PCR pos 3 days, mononuclear cells neg
  - Goal would be decreased viremia during gestation = no PI
Association of Border Disease Virus with a high mortality outbreak amongst 3-month-old feeder lambs shipped from Colorado to New York State

Outline:
- Case history
- Clinical presentation
- Diagnostics
- Review of Border Disease
- I will publish this . . . someday!

Lambs arrive in NY Summer 2019
- Experienced sheep farmer in CO
- Born on open range 9,000-11,000 ft elevation
- Closed flock
- No vaccines
- Breeds: Suffolk, Dorset, Rambouillet crosses
- Lamb crop split NY and SD
- Weaned when loaded on trailer
- Healthy approximately 100-115 lb lambs

Transport
- 1,708 feeder lambs were transported from CO to NY
- NY lambs arrived in 4 loads
- Hauler brings sheep out on 4 deck trailer, pine shavings
- 17 hr trip
- Returns with cattle as a 2 deck
- Clean with citrus acid wash between animals
NY Housing / Management
- 9/25/19 lambs arrived in NY
- Housed in new barn w/ center feed alley
- 4 pens
- 5 Wagyu/Holstein crosses had been on property 60 days prior
- 9/27/19 CDT vaccine and injectable Ivermectin dewormer

Fed low quality hay 1st 3 wks
- No grain
- No supplementation

Case History
- 10/16/19 started feeding oats
- 10/30/19 CDT booster
- Early Nov: Flock shorn
- Cold snap the following week
Deaths began shortly after

- Very thin, BCS 1-2/5
- Found dead in am, producer attributed it to poor nutrition and exposure
- Field veterinarian performed 20 autopsies
  - Emaciation
  - Pneumonia
  - Ruminal acidosis

Approx 382 animals died or were euthanized over 3 wks (22.4%)
Histology

- Lamb 1:
  - Bronchopneumonia
  - Pasteurella multocida cultured from lung
  - Sepsis

- Lamb 2:
  - Bronchopneumonia
  - Pasteurella multocida cultured from lung
  - Sepsis

- Lamb 3:
  - Bronchopneumonia
  - Necroulcerative esophagitis
  - Necroulcerative ileitis with lymphoid depletion of Peyer’s patches
  - Bibersteinia trehalosi cultured from lymph node

- Lamb 4:
  - Severe bilateral pneumonia
  - VI negative on lung tissue

- Lamb 5:
  - Pneumonia and neck abscess
  - VI negative on lung tissue

- Lamb 6:
  - Neck abscess
  - VI+ on lung tissue

Gross necropsies on 3 others
Ancillary Diagnostics

- Liver Seleniums WNL
  - 1.60 ppm, 1.57 ppm, 1.46 ppm (RI 1-7.5 ppm)
- Liver mineral panels mostly WNL
  - Lamb 1 molybdenum 3.25 ppm (RI 9-30 ppm)
- BRSV PCR: not detected in 3 lambs
- BVD PCR: Moderate positive for Lamb 3

BVD Monoclonal antibody FA of lymph nodes

- Cultured a pestivirus in both ovine and bovine cell lines from lambs 1, 3 and 6, and on 11/23 on 2 more ram lambs.
- Presumed it was Bovine Viral Diarrhea initially, and had BVD PCR positive
- Whole Genome Sequencing from Lamb 1 isolate:
  - Border Disease Virus Ranging 100% - 92% match with isolates

Virus Isolation

- Virus Isolation
### Made a Border Disease serum neutralizing antibody test

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- 11/15/19: Sampled 100 lambs. All had very low titers (≤4).
- 12/15/19: Sampled 100 lambs. All had very low titers (≤4).
- Included 29 from the initial sampling.
- 9 more had seroconverted.

### Number of Deaths Sept 23 - Nov 23 2019

<table>
<thead>
<tr>
<th>Date</th>
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### Border Disease Review

- Typically causes reproductive issues:
  - Fertility
  - Abortions
  - Premature/Abortions
  - Stillborn
- Hairy Shakers: Persistently infected lambs
- Tremors
- Hair loss

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Diseases of Sheep: I

March 2007

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MRCVS, FRAgS, Fppe

Nettleton, Chapter 18

Reviewed and published 119 and 126 Border Disease: 15. March 2007

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Border disease infection and gestation


• Less than 60-85 days gestation:
  • Fetal death or mummy OR hairy shaker
  • No NA titer
• More than 60-85 days:
  • Fetal death or weak lambs or normal lambs
  • Detectable NA titer
• Ewes 60-85 days <?

Other high mortality BDV outbreaks

• 1984: French outbreak w 50% mortality, enteritis and leukopenia in 3-5 week-old lambs
  • 1,500 ewes and 24,000 lambs
  • 67 from 3-5 week-old lambs "AV strain"
  • Experimentally infected lambs, 15/31 died with leukopenia

Previous BDV Report

• 1988: Netherlands report of CSFV contaminated by infected lamb kidney cells (Kissens et al.)
  • Sows vaccinated
  • Caused congenital infections in piglets
  • Experimentally infected pigs with contaminated vaccine strain
  • Autopsies revealed pleuropneumonia and hemorrhagic enteritis

• 2000: Tunisian sheep pox vaccine contaminated by BD (Thabti et al.)
  • Repro issues in sheep
  • Experimentally inoculated lambs with contaminated vaccine strain of BD: developed leukopenia and fever and NA to each strain
  • 2004: 21 Pyrenean chamois sheep in Spain died of BD, BD detected (Arnal et al.)
“Identification of border disease virus in naturally infected pigs in Mexico” Navarro-Lopez et al 2021

- 8/2021: 15 45-day-old piglets died on a semi-intensive farm in Mexico
- Piglets had fever, anorexia, prostration and died
- 2 necropsies:
  - Hemorrhage in lungs, intestine, bladder, kidney
  - Pleuritis
  - BDV PCR pos spleen, kidney, tonsil
  - Sanger sequencing clustered with BDV-1
- From 2011-2021, BDV seroprevalence in pigs in Mexico was 41.7%

“Detection of border disease virus in Mexican cattle” Romero et al, 2016

- Unique presentation of BDV
  - High mortality rate
  - Unsure where virus came from
  - Did not test all sheep for BD
  - Did management factors predispose?
  - Long transport
  - Going from open range to intensively housed
  - Poor nutrition / underweight
  - Shearing and then unseasonably cold weather

Current diagnostics available:

- BDV Sn $15.90
  - Serum
  - Plotted back titers
- Virus Isolation $75
  - Fresh tissues (intestine, lung etc)
  - EDTA whole blood 5 ml
- Sequencing available
- BVD ACE $6 (maybe...
Example from 2021:

- A large farm in PA doing AI and ET, bought in recepts recently
- Had lambs with “hairy wool”, thought on purpose
- 5/6 died, tested a 6 wk old
- “Dams carrying PI have very high antibody titer because of continual antigenic challenge”

References

- Aspen M Workman and others, First gene-edited calf with reduced susceptibility to a major viral pathogen, PNAS Nexus, Volume 2, Issue 5, May 2023, pgad125, https://doi.org/10.1093/pnasnexus/pgad125