Emerging Infectious Diseases
Erin Goodrich DVM, DACVPM

Scenario 1: Farm History
- Buys heifers from auction to sell as bred heifers
- Range from 1-6 months old currently
- 4/12 died in past 10 days
- Severe respiratory disease

AHDC submission
- 3 mo Holstein calf submitted for necropsy
- Had severe lung consolidation on thoracic U/S
- Received 1 injection of Draxxin, Banamine and Bo-Se
- Previous calves treated with Draxxin or Nuflor
- Housed in 2 groups sharing a fence line
- No vaccine history
Differential Diagnoses

- Mannheimia haemolytica
- Pasteurella multocida
- Histophilus somni
- Trueperella pyogenes
- Bibersteinia trehalosi
- Mycoplasma spp.
- Salmonella Dublin sepsis
- BVD
- BRSV
- BHV1
- Coronavirus
- PI3
- Lungworm
- Selenium deficiency
Samples/Tests

- Lung
- Mycoplasma culture
- PCR or FA for each virus (intestine preferred sample for BVD)
- Fecal float (lungworm)
- Selenium
- Histopathology

Feces
- Liver
- Fixed tissues (esp. lung, heart, liver, spleen, kidney, GI)

Results

1. Cat - Donor, Healthy, FEMALE
   Lung: Aerobic Culture Isolate Result
   - Mycoplasma species, Group D1
   - Salmonella species, Group D1
   Lung: Bovine Herpesvirus 1 PCR
   - Not Detected

2. Intestine: Bovine Viral Diarrhea Virus PCR
   - Not Detected
**Salmonella enterica subsp. enterica Serotype Dublin**

- Gram (-), facultative anaerobe
- Genus: Salmonella
- Species: enterica
- Subspecies: enterica
- **Serogroup (based on cell wall (O) antigens only):** D1
- **Serotype (based on cell wall (O) and flagellar (H) antigens):** Dublin
- Reported as Salmonella group D1 or Salmonella Dublin

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**Cornell AHDC Salmonella isolates 2006-21**

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Serotype</th>
<th>Number of Isolates</th>
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<tbody>
<tr>
<td>A</td>
<td>Typhimurium</td>
<td>2,100</td>
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<tr>
<td>B</td>
<td>Newport</td>
<td>1,000</td>
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<tr>
<td>C</td>
<td>Antwerp</td>
<td>1,200</td>
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<tr>
<td>D</td>
<td>Dublin</td>
<td>1,800</td>
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<tr>
<td>E</td>
<td>Anatum</td>
<td>1,400</td>
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<tr>
<td>F</td>
<td>Muenster</td>
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<tr>
<td>G</td>
<td>Give</td>
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<tr>
<td>H</td>
<td>Enteritidis</td>
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<td>J</td>
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<td>K</td>
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<td>Z</td>
<td>Cerro</td>
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<tr>
<td>Others</td>
<td></td>
<td>1,000</td>
</tr>
</tbody>
</table>

**Number of Isolates 2006-2021**

Courtesy of Missy Aprea
Salmonella Dublin

- Host-adapted strain in cattle
  - Healthy appearing carriers
  - Sustained losses in infected herds
  - Prevention more successful than treatment

- Calves <1yr shed ~9 weeks
- Heifers/cows shed ~3 weeks
- Clinical cases:
  - 18% become carriers
- Subclinical cases:
  - 1.5% become carriers

Salmonella Dublin: Transmission

- Shed in feces, milk, colostrum
- Contaminated feed, water, housing
- Maternity pen
- Group calf housing
- Aerosolization?

Salmonella Dublin: Human health concerns

- Zoonotic (multidrug resistant, high case fatality rate)
- Food safety
- 2019 outbreak in US
  - Linked to ground beef in CA
  - 13 infected, 8 states, 9 hospitalized, 1 died
  - 6 were bacteremic
Salmonella Dublin: Clinical presentation

- Acute respiratory disease in calves (5 days-6 months) most common
- High mortality
- High fever (≥104)
- Septicemia
- Abortion in naïve herds
- Diarrhea RARE in adults or calves

Salmonella Dublin: Antemortem diagnosis

- Blood culture
  - 2-3 ml
- NOT FECES
  - Salmonella Dublin only cultured 6-14% of the time from feces in carriers
  - 25-50% from feces in sick animals

Courtesy of Dr. Kaitlyn Kremer
Salmonella Dublin: Antemortem diagnosis

- Blood culture $34
- Bottle $3, ~1yr shelf life
- Store/ship at room temp
- Overnight delivery to lab
Salmonella Dublin: Post-mortem

Images courtesy of Dr. Michael Capel

Salmonella Dublin: Post-mortem

Images courtesy of Dr. Lila Knowlton-Grallert

Salmonella Dublin: Post-mortem
**Salmonella Dublin: Post-mortem**

- Aerobic culture of tissue
  - Collect 5 cm piece of lung, lymph node, spleen, kidney, or liver
  - Individual sterile containers
  - OR swab, stab, swab transport media
- Histology on formalin fixed tissue aids in diagnosis

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**Salmonella Dublin culture by site 2006-21**

<table>
<thead>
<tr>
<th>Site</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
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<tbody>
<tr>
<td>Liver</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Spleen</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

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**Salmonella Dublin: Multidrug resistant**

- Use of a fluoroquinolone in a food-producing animal that is not in accordance with the label is illegal.
- "Baytril 100 is indicated for the treatment of BRD associated with Mannheimia haemolytica, Pasteurella multocida and Histophilus somni in beef and non-lactating dairy cattle."

**Salmonella: “Why can’t you just do a PCR?”**

- No isolate for SENSITIVITY
- No isolate for SEROTYPING
- Enrichment step required before fecal PCR and fecal culture = PCR not faster

**S. Dublin: Surveillance & Monitoring**

- Salmonella Dublin EUSA antibody titer $12.50
  - Milk, bulk tank milk, or serum
  - ≤ 7 weeks to seroconvert
  - Cross reacts with S. Typhimurium
  - Detects EnterVene®-d & colostral antibodies
  - Does not detect SRP® Salmonella Newport vaccine
  - "Carrier" = + 3 times over 8 month period
5. Dublin: Surveillance & Monitoring

- Surveillance & Monitoring
- Risk Assessment Tool
- Series of housing/management questions:
  - Calving area
  - Calves before weaning
  - Calves after weaning, up to 6mo
  - Rearing heifers >6mo
  - Cows
  - Interactions with other herds

Scenario 2: Herd background

- 1,100 milking dairy herd in NY
- Milking in 2 different barns
- 800 milking with 4 Lely robots
- 300 milking in parlor – this houses the lower producing and older cows
- Diet:
  - Started feeding 2022 1st cut haylage 3 wks ago
  - Parlor cows on low forage and DNB cows
  - Drinking water is town water
  - Waterers scrubbed weekly
  - Herd production steady
  - Sprinklers are well water
  - No rumination collars
Relevant herd history
- Standard vaccine protocol
- Not testing for BVD
- Herd treated with Ultraboss recently
- Shared needles for vaccines and oxytocin administration
- Send heifers to heifer grower that also purchases and raises other calves from other states

Initial clinical findings
- July 2022: late lactation cows from only low group affected
- Affected group is milked in parlor (not by robots)
- 6 cows died from 7/1-7/5/2022
- Approx. 30 cows with similar clinical signs over 2-3 week period
- Signs:
  - Severe weakness
  - Anemia
  - Icterus
  - Death

7/5/22: Two necropsies performed
- Cow 1: 5th LACT, 7 yrs old, 359 DIM, BCS 1/5
- Cow 2: 2nd LACT, 4 yrs old, 281 DIM
- Necropsy findings on both:
  - Severe icterus
  - Enlarged liver and spleen
  - Liver grainy texture
  - Petechial hemorrhages on heart
  - GI tract empty, poor rumen fill
Cow 3: Antemortem serum chemistry

- 2nd LACT, 3 yrs old, 140 DIM, 100 lbs/day
- Chemistry:
  - Elevated:
    - Alkaline phosphatase (ALP)
    - Aspartate aminotransferase (AST)
    - Calcium
    - GGT
    - BUN
  - 1st \textit{r}

Differential Diagnoses (hepatopathy/death)

- **Infectious**
  - **Bacterial**
    - Clostridial—always a dx for sudden death
    - Anaplasma marginale/other tick-borne
  - **Viral**

- **Toxins**
  - Blue-green algae
  - Cyanogenic plants (Sorghum, Sudan grass, etc.)
  - Urea/Fertilizers
  - Nitrites/nitrates
  - Dicoumarol (Rodenticide)
  - Iron
  - Heavy metals (lead, arsenic, cadmium, etc.)

Necropsy samples: formalin fixed

- **FIX EVERYTHING**
- Fix $150 for field necropsy regardless of # of tissues
- Always fix the lesion / organ system involved
- Fixed tissue set:
  - Lung (section from each lobe)
  - Liver (section from each lobe)
  - Spleen
  - Kidney
  - Pancreas, spleen, ovaries and colon
  - Skin
  - Skeletal muscle
  - Heart
  - Brain
  - Uterus
  - Mammary tissue
Necropsy samples: fresh (chilled or frozen)

- Fresh tissue set in individual containers
- Freeze until shipment

Lung
Liver
Kidney
Spleen
Bile
Heart
Intestine (ligated)
Lymph node
Brain
Kidney
Skeletal muscle
Feces/colon contents
Aqueous humor
Rumen contents
Heart blood (serology!)
Urine
Brain

Diagnostic Tests

- Heavy metal testing on liver
- Clostridial testing liver, kidney
- Bacterial cultures multiple organs
- Lepto PCRs

Liver: 07/05/2022

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>&lt;0.025 ppm</td>
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<tr>
<td>Cadmium</td>
<td>&lt;0.03 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.025 ppm</td>
</tr>
<tr>
<td>Thallium</td>
<td>&lt;0.005 ppm</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.06 ppm</td>
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<tr>
<td>Selenium</td>
<td>0.1 ppm</td>
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</table>

Histopathology: Massive hepatic necrosis

Liver

- Normal liver
- Massive hepatic necrosis

Kidney

- Normal kidney
- Hepatic necrosis

Bacterial cultures:

<table>
<thead>
<tr>
<th>Organ</th>
<th>Culture Type</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Escherichia coli</td>
<td>No growth</td>
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<tr>
<td>Kidney</td>
<td>Escherichia coli</td>
<td>Negative</td>
</tr>
<tr>
<td>Spleen</td>
<td>Escherichia coli</td>
<td>Negative</td>
</tr>
<tr>
<td>Intestine</td>
<td>Escherichia coli</td>
<td>Negative</td>
</tr>
</tbody>
</table>
More diagnostics…all negative

- Mycotoxin testing on feed negative
- Blue green algae?
  - Tranquil water, waterers cleaned weekly
- Sent rumen contents to K State for microcystin ELISA — negative

7/11/22: Cow 4 (3rd necropsy)

- 5th LACT, 6 yr old, 90 DIM, 120 lbs, BCS 1/5
  - No rumen movement
  - Tachycaolic, pale mm
  - Collected EDTA whole blood and serum antemortem
  - Blood watery and dark
  - Necropsy findings:
    - Icteric
    - Spleen large and friable
    - Liver enlarged
    - Dilated heart with large ventricles

CBC results from cow 4

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Result</th>
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<tbody>
<tr>
<td>RBC, Hemoglobin</td>
<td>8 x 10⁶</td>
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<tr>
<td>HCT</td>
<td>38</td>
</tr>
<tr>
<td>MCH</td>
<td>32</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.5</td>
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<tr>
<td>Platelets</td>
<td>310 x 10⁴</td>
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<tr>
<td>MPV</td>
<td>9.5</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>230</td>
</tr>
<tr>
<td>Total White Blood Cells</td>
<td>12 x 10⁶</td>
</tr>
</tbody>
</table>

Many Anaplasma marginale on blood smear!
PCRs for hemoproteozoa

- Cows 1 and 2 were Anaplasma marginale PCR positive on spleen.
- Cow 4 was coinfected with Anaplasma marginale and Theileria orientalis (both PCR positive on spleen).

Outcome

- 15 cows died
  - Theileria orientalis → VA Tech for genotyping = Ikeda
- Pathogenicity: Ikeda > Chitose > Buffeli
- Many reactive lymphocytes on blood smears
- 17/18 additional herdmates BLV positive (ELISA)

Anaplasma marginale

- Intracellular rickettsial bacteria
- Transmission:
  - Ticks (Ixodes, Dermacentor, Rhipicephalus spp.)
  - Mechanical: Needle sharing, blood contaminated equipment
  - Vertical (rare)
- Incubation periods: 7-60 days
- Clinical signs:
  - Fever, regenerative anemia, icterus, weakness, mortality up to 50%
Anaplasma marginale
- Unlikely to cause clinical disease in cattle <1yr of age
  - Persistent infection — recovered animals can become carriers
  - Older cattle = severe disease
- Diagnosis
  - Blood smear — during acute phase
  - PCR — EDTA spleen
  - ELISA — serum; can detect carriers, herd screening
- Treatment
  - Oxytetracycline (extended therapy for clearance)
  - Cleared cattle = susceptible

Theileria orientalis
- Bovine piroplasmosis
- Protozoan erythoparasite
- Transmission:
  - Haemophysalis longicornis (Asian Longhorned Tick)
  - Mechanical: Needle sharing, blood contaminated equipment
  - Vertical may be possible
- Australia, NZ, Japan...then LSin 2017 (VA)
  - New endemic in southeast (90% Boicks)
  - This is first incursion in NY (found Chitose in VT in 2020)
Asian Longhorned Tick (ALT)

- ALT found on sheep in NJ in Aug 2017
- Detected in 17 more states (Feb 2023)
  - Backdated to 2010
  - Parthenogenic
  - Explosive populations/exanguination
  - Confirmed that VA T. orientalis strain was transmitted by ALT in 2021

States with confirmed ALT T. orientalis:

- Incubation period: 1-8 weeks
- Clinical signs: Calves are susceptible
  - Weakness
  - Regenerative anemia
  - Fever
  - Late term abortion
  - ‘Banana cows’

What caused the icterus and deaths?

- *T. orientalis* on RBC - massive hemolysis - severe anemia - macrophages phagocytose infected RBC and inflammatory cytokines - go to liver.
- Severe anemia causes oxygen deprivation affecting liver
- Hepatotoxin we never found?
- Other...?

A. marginale vs. T. orientalis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Therapy</th>
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<tbody>
<tr>
<td>Biopsy</td>
<td>Biorelated (tissue): Peritoneal</td>
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<tr>
<td>Clinical signs</td>
<td>Transient fever: Continuous fever; disease may be less aggressive, spleen less enlarged; severe anemia, anisocytosis, macrocytosis</td>
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<tr>
<td>Presentation</td>
<td>Cattle usually 1-2 weeks later:</td>
</tr>
<tr>
<td>Lick vector</td>
<td>NIM ALT: Dermatocinetes, taurinae and rhadinocinetes: Anisocytosis, smears in long slides (pic)</td>
</tr>
<tr>
<td>Diagnose</td>
<td>Blood smear, PCR, ELISA</td>
</tr>
<tr>
<td>Treatment</td>
<td>Echinate therapy with taurinae</td>
</tr>
</tbody>
</table>
Scenario 3 (Oct 2021): Herd history

- Jersey dairy, milking 25
- Recently turned out to pasture
- 7 affected
- Not eating hay, still grazing & eating grain
- Drooling; oral erosions (5 of 7)
- Lame; swollen coronary bands (all 7)
Differential Diagnoses

- Bovine viral diarrhea virus (BVD)
- Epizootic hemorrhagic disease virus (EHD)
- Bluetongue (BT)
- Malignant catarrhal fever (MCF)
- Bovine papular stomatitis virus (BPS)
- Vesicular stomatitis virus (VSV)
- Foot and Mouth disease (FMD)
- Trauma
- Exposure to caustic substance

Oral erosions…Stop!

- Don’t leave the farm
- Call your SAHO or AVIC
- In this case, an FAD investigation was initiated
- Samples to Foreign Animal Disease Diagnostic Lab (FADDL) on Plum Island
- Typically, duplicate samples also sent to NAHLN lab (AHDC)
Samples/Tests on 4 cows
- Swab of oral erosion
- EDTA blood
- Serum
  - FMD PCR
  - VSV PCR
  - MCF PCR
  - EHD PCR
  - BT PCR
  - FMD ELSA
  - VSV ELSA

Results
- All negative for FMD, VSV, BT, MCF
- All 4 EHD PCR positive on EDTA blood at NVSL/FADDL
- All recovered
- 2 were pregnant and carried to term

Epizootic Hemorrhagic Disease (EHD)
- Orbivirus from the Reoviridae family, Culicoides vector
- Other Orbivirus diseases: African horse sickness, Bluetongue
- 7 serotypes worldwide (3 in US)
- White tailed deer most common host in North America (severe dz)
  - Mule deer, moose, reindeer, elk, other ruminants
**EHD: Deer**

- Late summer/fall
- High mortality
- Incubation: 36 hours
- Found near water
- Acute:
  - Sudden death
  - Bruising
  - Edema of head/neck
  - Swollen tongue
  - Respiratory distress
  - Bleeding from nose/mouth
- Chronic:
  - Hoof lesions
  - Ruminal erosions
  - Emaciation

**Pathology**
- Tissue hemorrhage
- Epistaxis
- Scleral hemorrhage
- Histology:
  - Vascular necrosis
  - Inflammation

**EHD: Cattle**

- Clinical signs in cattle
  - Low morbidity/mortality
  - Mostly subclinical or transient fevers
  - Occasional cases of inappetence, oral erosions, dysphagia (esophagitis, upper GI erosions)
  - Often triggers investigation for FMD/VSV
  - Direct contact transmission possible, high-density herds

**Diagnosis**
- PCR (EDTA blood, spleen, liver, hoof; serology)

EHD in captive bison (Oct/Nov 2021 and Jan/Feb 2022)
- Multiple animals affected
- Various clinical signs noted:
  - Facial swelling
  - Bloody or mucopurulent nasal d/c
  - Conjunctivitis
  - Ocular swelling
  - Lameness/hoof sloughing in one

EHD in VT cow (Nov 2021)

EHD in NJ Yak (Sept 2021)
EHD

- Treatment/Control
- Supportive care
- Limit need for walking
- Clean/dry/well-bedded to rest
- Insect control

Scenario 4: Sudden death in 2 western NY dairies

Farm A:
- Sudden deaths calves 3-4 months old
- Late summer 2017 and 2018
- 2 week period each summer (mortality 33% and 17%, respectively)
- 1 had ↑ HR, ↑ RR, vocalization, convulsions for minutes preceding death
- In 2018, all dead and 20% cohorts had mammary gland enlargement

Farm B:
- Sudden deaths calves 4 months old
- Late summer 2019
- 3% mortality
- Mammary gland enlargement

Farm A:
- Milking 1500 Holsteins/1500 youngstock
- Fed raw colostrum, then pasteurized whole milk
- Housed in hutches, then group housing on wood shavings (at capacity)
- Pens stripped/replaced once weekly
- High temps, high humidity, increased rainfall preceded outbreak
**Farm B**

- ~60 miles from Farm A
- Milking 3600/2000 youngstock
- Also fed raw colostrum, then pasteurized whole milk
- Weaned calves in groups of 4-6 per pen, 3 barns, bedded with wood shavings
- High temps, high humidity preceded outbreak

**Diagnostics on Farm A: 2017**

- CBC/Chemistries
- Ionophore quantification of feed
- Selenium on EDTA and liver; Vit E on serum
- Heavy metal screening/liver mineral panels
- Serum troponin
- 9 necropsies → 6 with precocious udders (histo=vascular congestion, edema, hemorrhage, ductular hyperplasia)
- Cardiomyocyte degeneration in 5

**Diagnostics on Farm A: 2018**

- 5 more necropsies → similar findings to 2017
- Fecal sample collected from 1 calf at post-mortem → 18,800 eggs/g of the nematode parasite Strongyloides papillosus
- Confirmed with PCR of larval cultures
- Subsequently found in feces from 2 other calves
Presumptive *Strongyloides papillosus* cardiotoxicity as cause of calf mortality

Diagnostics on Farm B: 2019

- 2 necropsies with similar results
  - No mammary tissue submitted for histol
  - Mild pulmonary hemorrhage in 1
  - Enteritis: neutrophilic cryptitis with intraluminal nematodes in 1
  - Cultures/ viral testing/ selenium within normal limits
  - *S. papillosus* larvae and eggs present in feces (25 larvae/g in 1; 13,300 and 12,705 eggs per gram in each)
  - Subsequent fecal float: 53% prevalence in age group

*Strongyloides papillosus*

- Nematode parasite with worldwide distribution
- Predilection for small intestine of ruminants (cattle, goats, sheep, deer)
- Juvenile ruminants most susceptible
- Known to cause ill thrift/diarrhea in young ruminants
- Associated with sudden death in weaned calves and lambs
  - Fatal arrhythmias
  - May rarely see tachyphnea, vocalization, collapse, convulsions prior to death
**Strongyloides papillosus**

- Hypothesis: sudden death associated with S. papillosus hyperinfection, secondary to parasite-associated cardiac toxin from adult female nematodes in proximal small intestine (experimental infections)
- No mammary enlargement noted in experimental studies
- No consistent bloodwork abnormalities

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![Diagram of Strongyloides papillosus lifecycle](image)

1. L3 
2. L3 intra-host (Calf) 
3. Larvated egg 
4. 2 days 
5. L3 
6. Larvated egg shed in milk 
7. Prepatent period 14 days 
8. Migration to duodenum, matures to adult male and female 
9. Sexual reproduction to produce larvated eggs 
10. Larvae shed in milk 
11. Prepatent period 14 days 
12. Migrates to duodenum, matures to adult female 
13. Female nematodes is to produce larvated eggs

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**Citation:** Journal of the American Veterinary Medical Association 260, 2; 10.2460/javma.21.09.0424
### Strongyloides papillosus: Transmission

- Generally, via percutaneous or oral mucosal penetration by L3
- Transmammary transmission of Strongyloides spp. in some hosts
- Pre-natal infections demonstrated in pigs, cervids
- Why the congested mammary glands? Larval migration? (none seen in tissues)
- Wood shavings provide humid substrate for development
- Standing water in pens; promoted percutaneous migration of L3

### Strongyloides papillosus

- Consider as differential for sudden death (mammary enlargement) in weaned calves and lambs during late summer/fall
- Diagnosis:
  - Fecal egg counts for fecal floatation (not 100% sensitive; consider testing in body)
- Prevention:
  - Clean, dry bedding
  - Monitor fecal egg counts in weaned calves during summer/fall
  - Monitor for mammary enlargement
- Treatment:
  - Effective anthelmintics: doramectin (deaths stopped in 24 hours in NY outbreak), fenbendazole, moxidectin, thiabendazole

### References

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Questions?
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