SMALL RUMINANT ABORTION DIAGNOSTICS

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1. Triaging your client over the phone
   - Temperature
   - Take the doe/ewe's temperature
   - Is it late?
   - Put the doe/ewe in isolation if possible
   - Save fetus & placenta
   - Collect fetuses and placentas and refrigerate

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2. Gather a history
   - Open ovariectomized?
   - Recent travel to flocks?
   - Other species on premises?
   - Vaccines given?
   - Stage of gestation at time of abortion?
   - Number / total abortions
   - Health status of dams
   - Diet or changes

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Categorizing abortions

- Gestational length: 100 days
- Abortion vs. premature stillbirth
  - "Sheep and goats have a high incidence of abortion compared with other farm animals"
  - This common
  - <5% good
  - <2% excellent
  - "Abortion storm" >20%, >2%

- 5% is common
- <5% good
- <2% excellent

Placenta review

- Cotyledon - fetal side
- Caruncle - maternal side
- Placentitis - inflammation of the placenta
- Inter-cotyledon - between cotyledons
- Chorioallantois: lining
- Amnion: covers fetus

Examine placenta

- Necrosis/infarct/suppurative in both cotyledons and intercotyledon
- Bacterial
- Chlamydia
- Campylobacter
- Toxoplasma gondii

- Multifocal and small necrotic areas on cotyledons
- Coated parts
Samples to collect on site:
- Placenta
- Fetal/fetuses
- EDTA whole blood and serum from dam(s)

Field necropsy can be performed
- Fresh tissue culturing for pathogens
- Formalin fixed tissue histopathology (1:10 ratio)
- Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube
  Histopath interprets the significance of pathogens
  AHDC test $150 per animal

Infectious v noninfectious causes
- Bacterial
- Viral
- Parasitic
- Mineral deficiency
- Genetic
- Toxin/toxic plant

Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals, p79
Anasarca fetuses just for fun

Photos courtesy of Leslie Green – when my genetic testing was allaneous收拾works were not cut from me.

My pathogen list:

- **Viruses**
  - Cache Valley Fever (CVF)
  - Bluetongue virus
  - Rift Valley Fever (BVD)
  - Caprine herpesvirus 1
- **Protozoa**
  - Toxoplasma gondii*
  - Neospora caninum*

*varied sompressions causes
Z = zoonotic

- **Bacteria**
  - *Chlamydia abortus* (enzootic abortion)
  - Goats
  - *Coxiella burnetii* (Q fever)** Z
  - *Campylobacter felis* (Q fever) (Vibrio) Z
  - Sheep
  - *Listeria monocytogenes* Z
  - Other bacteria (E. coli, Salmonella)

What’s known in the literature?

- Study from UC Davis:
  - 211 cases of abortion from 1991-1998
  - 112 (53%) had no diagnosis
  - 79 (37%) had infectious causes
    - *Chlamydia abortus* (14%)
    - *Coxiella burnetii* (9%)
    - *Toxoplasma gondii* (4%)
    - *E. coli* (2%)
What’s known in the literature?

- Study from Netherlands:
  - 453 cases from 2006-2011
  - 356 ovine
  - 97 caprine
  - Infectious agents found in 41% ovine and 43% caprine
  - 350 ovine and 335 caprine
  - Ovine:
    - 1. Chlamydia
    - 2. Campylobacter
    - 3. Toxoplasma gondii
    - 4. Listeria
  - Caprine:
    - 1. Coxiella
    - 2. Chlamydia
    - 3. Listeria
    - 4. Toxoplasma gondii

What’s known in the literature?

- Study from Italy 2003-2005
  - 366 ovine and 35 caprine cases
  - 1. Toxo (13%)
  - 2. Chlamydia
  - 3. Coxiella
  - No HEDO - what significance of Q fever?

General diagnostics - ANTIGEN

- PCR: 90-145
- EIA for "target" antibodies
- Culture: 45-90
- Other: isolation, long-term cultivation
- Serological column tests for fastidious pathogens
- Monoclonal antibodies
General diagnostics - ANTIGEN

- Fluorescent Antibody
  - Positive
  - Fluorescence under microscope = positive

- Virus isolation: S75 – AG
  - Blood
  - EDTA whole blood 5 ml
  - Set on cells

General diagnostics - ANTIBODY

- IFA
  - Serum or fetal fluid
  - Serum neutralization: $15.90 – AB
  - Microscopic Agglutination Test: $20 – AB

- Complement fixation: $20 – AB

- Monoclonal antibody stain applied to fresh tissue
  - Fluorescence under microscope = positive
Cache Valley Fever Virus

- Orthobunyavirus (order Bunyaviridae)
- Enveloped, RNA
- Akabane and Schmallenberg FADs
- Spread by both culicoides and mosquitoes
- Transovarial transmission in mosquitoes
- Bite naïve pregnant doe in the fall
- Deformed fetus born January–March
- Uptick in CFV abortions in the northeast 2021–2022
- Virus can invade the placenta, then fetal brain, spinal cord, and skeletal muscles.

<table>
<thead>
<tr>
<th>Days Gestation Infected</th>
<th>Fetal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-32d</td>
<td>Early embryonic death and mummification</td>
</tr>
<tr>
<td>32-37d</td>
<td>Deformities in brain, spinal cord (hydranencephaly, hydrocephalus, cerebellar and cerebral hypoplasia)</td>
</tr>
<tr>
<td></td>
<td>Musculoskeletal deformities (arthrogryposis, torticollis)</td>
</tr>
<tr>
<td>37-49d</td>
<td>Musculoskeletal deformities only</td>
</tr>
</tbody>
</table>

Cache Valley Fever
Images courtesy of Dr. Mary Smith, Cornell University
Cache Valley Fever diagnosis

- Serum neutralization titers on fetal fluid
- Heart blood: serum
- Fluid in pleura
- Brain fluid: serum
- Brain blood
- Peripheral blood: serum
- Fluid in thorax
- Fluid in abdomen
- Serum from doe: negative: rules it out
- Positive: could be exposure
- Need paired samples
- Virus itself? Long gone!

Cache valleyserology examples

<table>
<thead>
<tr>
<th>Doe/ewe</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doe/ewe 1</td>
<td>128</td>
<td>128</td>
<td>192</td>
<td>384</td>
</tr>
<tr>
<td>Doe/ewe 2</td>
<td>128</td>
<td>512</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Doe/ewe 3</td>
<td>128</td>
<td>128</td>
<td>96</td>
<td>48</td>
</tr>
<tr>
<td>Doe/ewe 4</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

Courtesy of Dr. Mary Smith

The good news

- Doe/ewe immune for years to life
- Consider breeding outside mosquito season
- Climate change
Cache Valley Fever SN titers at the AHDC 2017-2022 for Sheep and Goat

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>Fetus</th>
<th>Total</th>
<th>Positive</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>276</td>
<td>136</td>
<td>412</td>
<td>215</td>
<td>30 (23%)</td>
</tr>
</tbody>
</table>

Bluetongue virus

- Orbivirus (T振奋inovirus family)
- Double-stranded RNA
- Affects sheep, goat
- Culicoides transmission
- Infection 10-60 days post-infection
- Clinical signs: depression, encephalopathy
- Neurologic signs: meningitis, encephalitis, retinal dysplasia, hydrancephaly

Diagnostics available

- BTV-PCR on fresh tissues
- BTV ELISA (antibodies on serum)
The good news

Not "endemic" in the northeast

2 adult sheep were PCR positive on spleen in NY 3/2022

3 WTD died of BTV on Long Island 9/2022

https://www.dec.ny.gov/press/126187.html

Pestiviruses: Border Disease

Infected animal sheds virus directly

Reproductive issues in sheep

Fertility

Abortions/stillbirths

Premature/weak lambs

Hairy Shakers (persistently infected lambs)

Exposed <60-85 days gestation

Hydrocephalus/hydrancephaly or leukomalacia

Hair - like fleece

Rhythmic tremors


Pestiviruses: Bovine Viral Diarrhea

2013 paper from Switzerland: "A goat given as a social companion to a BVDV PI calf gave birth to a PI goat kid"

Goat PI housed with pregnant goats and made 2 PI twins

All died before 2 yrs old

Potential for BVD to be maintained in goat herd

We have seen BVD titers in goats at AHDC

Don’t house small ruminants with cattle
Pestivirus diagnostics
- Virus isolation ETS
- PCR fetal tissues
- RT-PCR whole blood
- Immunoassay enzymes
- Rev A and D PVU 100x5 R0
- Others
- BVDV A and B
- Serum and fetal tissues
- BDV C and D
- Fresh

Fresh fetal tissues
- EDTA whole blood 5 ml
- Typing and sequencing available
- BDV and BVDV SN $15.90
- Serum
- BVD ACE $6
- Fetal or adult skin, serum
- BVD PCR $41
- Fetal lung

Caprine herpesvirus 1
diagnostic
- Adults asymptomatic
-Abort at end of last trimester
- Autolyzed fetus
- 1-2 mm white foci in fetal lung, liver, kidney
- Intranuclear inclusion bodies in adrenal, liver, kidney, testis, liver, and placenta
- Case report from Czech in 2010:
- BVDV and PVU antigens
- 80% mortality of newborns

Caprine herpesvirus 1 diagnostics
- Panherpes PCR $41
- Sequencing: FastTrack 820
- Virus isolation ETS
- Positive sample
Toxoplasma gondii

- Protozoa
- Cat is the definitive host
- Ingests infected placenta/fetus (intermediate host)
- Shed millions of oocysts in feces for 7-12 days
- Goats > Sheep infected by ingesting feces or oocysts in environment
- Tachyzoites go to CNS and muscles, encyst into bradyzoites, travel to placenta in pregnant cats

"Cats are EVERYWHERE"

30% adults in US have antibodies

- <40 days = embryo resorbed
- 40-120 days = mummy or abortion
- >120 days = premature, stillborn or weak
Necrotic and calcified cotyledons

- Diagnose with HISTOPATH of placenta, fetal brain and heart
- Serology: TOXO MAT antibody titer on fetus and dam

Photo by Neumann Salen

The good news

- Don't kill the barn cat! Study showed dead infected cats
- Keep cat population stable
- "Only 1-2% of kittens born transplacentally infected" Kirkbride
- "T. gondii positive flocks were observed in successive pregnancies in 25% of lambs during study period, 2000-2003. Morley et al 2007.

T. gondii titers tested at the AHDC 2017-2022

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>229</td>
<td>42</td>
</tr>
<tr>
<td>Goat</td>
<td>205</td>
<td>48</td>
</tr>
</tbody>
</table>

Sheep
- 1/2 229 - Donor flocks (T. gondii positive) 1000
- 2/2 1673 - Donor flocks (T. gondii positive) 1000
- Fold of 500/1673 - Donor flocks (T. gondii positive) 1000

Goat
- 1/2 205 - Donor flocks (T. gondii positive) 1000
- 2/2 1673 - Donor flocks (T. gondii positive) 1000
- Fold of 500/1673 - Donor flocks (T. gondii positive) 1000
Neospora caninum

- Typically, Bovine/Canidae transmission cycle
- Sheep abortion storms described in Italy (González-Warleta et al. 2014)
- Hydrocephalus, cerebellar hypoplasia
- CNS presentations:
  - Ataxia
  - Opisthotonos

Diagnosing *N. caninum*

- Histo lesions like Toxoplasma
- Encephalitis/Meningitis
- Myocarditis
- *Neospora* IFA antibody titer $\geq 18$
- Have not yet confirmed any small ruminant cases

*Chlamyphila abortus "Enzootic abortion"

- Gram-negative, intranuclear bacteria
- Lives in gut
- Shed in feces
- Transmitted via nasal discharges
- Zoonotic - pregnant women can abort!
**Chlamydomphila abortus diagnosis**
- Chlamydomphila abortus PCR: $41 or FA: $27.50
- Placenta and fetal lung
- Histopath of placenta and fetus: Coagulative necrosis of fetal lung
- Placental necrosis, trophoblasts with intracytoplasmic organisms
- Fluorescent antibody stain

**Chlamydomphila abortus 2017-2022**

<table>
<thead>
<tr>
<th>Year</th>
<th>Sheep</th>
<th>Goat</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>221</td>
<td>0</td>
<td>221</td>
</tr>
<tr>
<td>2018</td>
<td>233</td>
<td>3</td>
<td>236</td>
</tr>
<tr>
<td>2019</td>
<td>245</td>
<td>5</td>
<td>250</td>
</tr>
<tr>
<td>2020</td>
<td>250</td>
<td>10</td>
<td>260</td>
</tr>
<tr>
<td>2021</td>
<td>265</td>
<td>0</td>
<td>265</td>
</tr>
<tr>
<td>2022</td>
<td>270</td>
<td>3</td>
<td>273</td>
</tr>
</tbody>
</table>

**The good news**
- Vaccine available
- WHO stock
- Antibiotic options tetracycline or penicillin
- Tetracycline (injectable or oral): 1.5-3.0 g/day
- Once in the herd, only the yearlings abort
**Chlamydia CF titers at the AHDC 2017-2022 for Sheep and Goat**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Positive</th>
<th>Negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>146</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Goat</td>
<td>206</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>452</td>
<td>99</td>
<td>51</td>
</tr>
</tbody>
</table>

*Note: this serology test includes all types of Chlamydia, including non-pathogenic intestinal strains.*
*All samples tested with recent abortion.*

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**Coxiella burnetii: "Q fever"**

- Gram neg coccobacilli, intracellular
- 2 forms: phase I (virulent) and phase II
- All animals (domestic and wildlife) can shed
- Spread by inhalation, ingestion, ticks
- Can float 2 miles
- Selected agent with CDC
- Reportable disease with USDA
- Shed mainly during parturition
- May be incidental

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**Coxiella burnetii "Q fever"**

- 3rd trimester abortions
- Stillborn, premature, weakly neonates
- Placentitis - **NEED PLACENTA!**
- Disseminated infection
- Only 99% of humans/bacteria
- DNA/antibody ELISA and Q-animal test for plasma
- Disease in 2019
- No vaccine yet for farm and wild animals
- Cost for 1 test $160
- Cost for 50 tests $4100
Coxiella burnetii "Q fever"

- Zoonotic
- Canine
- Feline
- Rodent
- Porcine
- Avian
- Avian
- Ruminant
- Can be shed in milk
- Can stay in environment
- This pathogen is a giant pain

2009 study monitoring Q fever shedding by Rousset et al:

<table>
<thead>
<tr>
<th>Type</th>
<th>Vaginal Mucous</th>
<th>Feces</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aborted</td>
<td>44%</td>
<td>21%</td>
<td>38%</td>
</tr>
<tr>
<td>Normal</td>
<td>27%</td>
<td>20%</td>
<td>31%</td>
</tr>
</tbody>
</table>

"Serological analysis... showed that at least 24% of the seronegative goats shed bacteria."

The good news

- Animals can asymptptomatically shed
- Animals can be live born and not abort
- You might already be immune
Coxiella burnetii ELISA titers tested at the AHDC 2017-2022

<table>
<thead>
<tr>
<th></th>
<th>Tested</th>
<th>Positive</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>2958</td>
<td>70</td>
<td>2%</td>
</tr>
<tr>
<td>Goat</td>
<td>1623</td>
<td>178</td>
<td>11%</td>
</tr>
</tbody>
</table>

Research facilities and pathogen-free sheep monitoring skew this data!

Campylobacter fetus subsp. fetus (Vibrio) and C. jejuni subsp. jejuni

- Late gestation abortion, stillbirths, weak offspring
- More common in sheep
- Shed in feces in sheep, dogs, birds
- Spreads to other animals via direct contact or infected placenta/fetus (guard dog)
- Zoonotic - diarrhea in people
- By the time abortion starts, entire flock likely exposed

Campylobacter

- Bacitracin-resistant, normal sensitivity
- Hepatic necrosis - white circular lesions
- Pathogenesis:
  - Campylobacter jejuni culture of feces
  - Campylobacter jejuni from placenta and fetus
  - Will not reliably grow in aerobic culture
The good news

- Antibiotic options to control an abortion storm:
  - Tetracycline
  - Injectable (LA 200)
    - Milk withhold concerns
  - Feed (need VFD)
  - Sulfamethazine and Tylosin in the textbooks

- Once in the herd, only the yearlings abort

Listeria monocytogenes

- Gram positive coccobacilli
- Found in soil, feces, silage (pH > 5.0)
- Likes "refrigeration temperatures"
- Presents as:
  - Circling disease
  - Menopausal and abortion in goats
  - Death two blocks of does seven days
  - Placentitis, white fuzzy on fetal organs

Listeria monocytogenes

- Diagnose by aerobic culture of placentae, fetal lung and alimental contents
- Feeding silage, silage pH
- Try to clean up environment to decrease consumption of listeria
Brucella – not a concern

- **Brucella abortus**
  - Sheep, only, some protected herd
  - Orchitis and epididymitis
  - Can cause SDE and abortions
  - AHDC has never cultured it...

- **Brucella melitensis**
  - Sheep and goats are the reservoir hosts
  - **B. melitensis** is not present in the US
  - Endemic in Asia, the Middle East, South America, and Africa
  - Responsible for most of the global human brucellosis cases
  - 500,000 cases per year worldwide

Leptospirosis

- Very rarely found in literature
  - Documented in Spain and Ireland
  - L. hardjo, pomona, castellonis, etc

- We offer PCR $41 on placenta and fetal kidney
- *Leptospira interrogans* MST $20 per-soman

- **We offer PCR** $41 on placenta and fetal kidney
- **L. interrogans** MST $20 per-soman
- **Pomona**
- **Hardjo-prajitno**
- **Icterohemorrhagiae/copenhageni**
- **Grippotyphosa**
- **Canicola**

Mineral deficiencies:

- Selenium
  - Test on EDTA whole blood $31
  - Or liver $60

- Copper
  - Test liver, mineral panel $51.65
  - Includes calcium, phosphorus, manganese, magnesium, sodium, potassium, copper, iron, zinc, and molybdenum

- Iodine $31 at Michigan DL

- Enzootic ataxia/swayback in offspring
- Demelination of brain and cord

- Goiter $31 at Michigan DL
- Saline
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Sample and diagnostic test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Viral Diarrhea</td>
<td>Fetal lung for FCR and Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing</td>
</tr>
<tr>
<td>Border Disease Virus</td>
<td>Fetal tissues for viral isolation and Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing</td>
</tr>
<tr>
<td>Cache Valley Fever</td>
<td>Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing</td>
</tr>
</tbody>
</table>

**Pathogen**

<table>
<thead>
<tr>
<th>Sample and diagnostic test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma gondii (Fresh tissue: liver, placenta, adrenal, intestine, spleen, kidney, lung for fluorescent antibody (antigen)) and Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing</td>
</tr>
<tr>
<td>Neospora caninum (Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing)</td>
</tr>
</tbody>
</table>

**Pathogen**

<table>
<thead>
<tr>
<th>Sample and diagnostic test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter fetus (&quot;Vibrio&quot;) or C. jejuni (Fetal liver for culture)(antigen)</td>
</tr>
<tr>
<td>Chlamydia abortus (&quot;Dioovioabres&quot;) Placenta or fetal lung for FCR (antigen)</td>
</tr>
<tr>
<td>Coxiella burnetii (&quot;Q fever&quot;) Placenta or stomach contents for PCR (antigen)</td>
</tr>
<tr>
<td>Listeria monocytogenes Placenta, fetal lung or stomach contents for culture (antigen)</td>
</tr>
<tr>
<td>Other bacterial/fungal Placenta, fetal lung or stomach contents for culture (antigen)</td>
</tr>
</tbody>
</table>
Practice abortion workup:

- Goat herd of 20 pregnant does
  - 2 sets of twins, 1 singleton
  - 3 abortions within 7 days, all within 2 weeks of due date

- Goat also had dome shaped head, arthrogryposis, and torticollis
  - Singleton also had dome-shaped head, arthrogryposis, and torticollis
  - Guard dog on premise

- Barn cat just had a litter of kittens 1 month ago

- Purchased a buck in September of previous year

Practice abortion work-up

- The placenta looks grossly normal
  - List top 3 differentials
  - List sample type and test requested to find diagnosis
  - Limited financial resources
  - Owner has no financial limitations

Questions?
Farm 2: calf recumbent at birth, then ok at day 5, then at 1 month:

Extensive histo slides read:
- Rabies neg
- Listeria culture neg
- Full cerebral panel EEG on brain and liver
- BVD ACE negative
- Viral isolation negative
Options for genetic testing:

- STgenetics
  $10, Hair?

- Feanixbio
  https://www.feanixbio.com/cattle-tests/p/calf-recumbency
  $10, Hair, TSU, DNA

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