


**SMALL RUMINANT ABORTION DIAGNOSTICS**

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Triaging your client over the phone

**WEAR GLOVES!!!**

Personal Protective Equipment	Wear gloves and rubber boots
Sample Types & Collection	Collect fetuses and placentas and refrigerate
Isolate	Put the doe/ewe in isolation if possible
Temperature	Take the doe/ewe's temperature



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**Gather a history**

- Open or closed?
- Recent travel to shows?
- Other species on premises?
- Vaccines given?
- Stage of gestation at time of abortion?
- Number / % of abortions
- Health status of dams
- Diet- any changes



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### Categorizing abortions

- Gestation length: 150 days
- Abortion v premature v stillborn
- "Sheep and goats have a high incidence of abortion compared with other farm animals"
- 5% is common
- <5% good
- <2% excellent
- "Abortion storm" >20%, >2%



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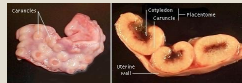
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### Placenta review

- Cotyledon- fetal side
- Caruncle- maternal side= placenta attaches at 17 days
- Placentome- cotyledon and caruncle together =90 days gestation
- Intercotyledon - btw cotyledons
- Chorionallantois- lining
- Amnion - covers fetus



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### Examine placenta

- Necrosis/infarct/supuration in both cotyledons and intercotyledon
  - Bacterial
    - Chlamydia
    - Campylobacter
    - Coxiella
- Multifocal and small necrotic areas on cotyledons
  - Toxoplasma gondii



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### Samples to collect on site:

- Placenta
- Fetus/ fetuses
- EDTA whole blood and serum from dam(s)



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### Field necropsy can be performed

Placenta (maternal and fetal membranes)	Liver	Lung	Heart
Brain	Spleen	Colon	Thymus
GI tract	Kidney	Spine	Reproductive system
Abomasum (ruminant stomach)	Fetal Heart Blood (if present, collect 100 ml)		

- Fresh tissue =testing 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 histo \$160 per animal

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### Infectious v noninfectious causes

- Bacterial
- Viral
- Parasitic
- Mineral deficiency
- Genetic
- Toxin / toxic plant



Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals p79

Vetrum californicum

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### Anasarca fetuses just for fun



Photos courtesy of Leslie Green-Wilham – pursuing genetic testing as all infectious agents were ruled out here

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### My pathogen list:

- Viruses
  - **Cache Valley Fever virus\***
  - Bluetongue virus
  - Pestiviruses (Border Disease and BVD)
  - Caprine herpesvirus 1
- Protozoa:
  - *Toxoplasma gondii*\* **Z**
  - *Neospora caninum*
- Bacteria
  - *Chlamydia abortus*\* (Enzootic abortion) **Z**
    - Goats
  - *Coxiella burnetii* (Q fever)\* **Z**
  - *Campylobacter fetus* and *jejuni* (*Vibrio*) **Z**
    - Sheep
  - *Listeria monocytogenes* **Z**
  - Other bacteria (*E. coli*, *Salmonella*) **Z**

\*=most common causes  
**Z**= zoonotic

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### What's known in the literature?

- Study from UC Davis:
  - 211 cases of caprine abortion from 1991-1998
  - 112 (53%) had no diagnosis
  - 79 (37%) had infectious causes
    1. *Chlamydia* 30 (14%)
    2. *Coxiella* 19 (9%)
    3. *Toxoplasma gondii* 9 (4%)
    4. *E. coli* 5 (2%)

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### What's known in the literature?

- Study from Netherlands:
  - 453 cases from 2006-2011
  - 282 ovine
  - 171 caprine
- Infectious agents found in 48% ovine and 34% caprine
- 42% ovine and 59% caprine had Q fever diagnosis
- Ovine:
  1. *Chlamydia*
  2. *Campylobacter*
  3. *Toxoplasma gondii*
  4. *Listeria spp*
- Caprine:
  1. *Coxiella*
  2. *Chlamydia*
  3. *Listeria*
  4. *Toxoplasma gondii*

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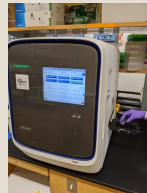
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### What's known in the literature?

- Study from Italy 2003-2005
- 366 ovine and 31 caprine cases
  1. Toxo (13%)
  2. *Chlamydia*
  3. *Coxiella*
- NO HISTO-what is significance of Q fever?



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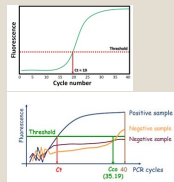
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### General diagnostics -ANTIGEN

- PCR \$41- AG
  - test for "target" (nucleicacids)
- Culture \$46- AG
  - Fetus - placenta, lung, abomasal contents
  - Specific cultures for fastidious pathogens
  - *Campylobacter*
  - *Mycoplasma*



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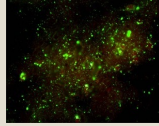
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### General diagnostics -ANTIGEN

- Fluorescent Antibody \$27.50 - AG
- Monoclonal antibody stain applied to fresh tissue
- Fluorescence under microscope = positive



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### General diagnostics -ANTIGEN

- Virus Isolation \$75 - AG
- Tissues
- EDTA whole blood 5 ml
- Set on cells



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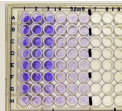
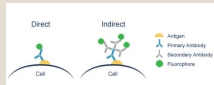
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17

### General diagnostics -ANTIBODY

- IFA \$18 - AB
- Serum or fetal fluid
- Serum neutralization \$15.90 - AB
- Serum or fetal fluid
- Complement Fixation \$20 - AB
- Serum or fetal fluid
- Microscopic Agglutination Test \$20 AB
- Serum or fetal fluid



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18

## Cache Valley Fever Virus

- Orthobunyavirus (order Bunyaviridae)
- Enveloped, RNA
- Akabane and Schmallenberg - FADS
- Spread by both culicoides and mosquitoes
- Transovarial transmission in mosquitoes
- Bite naive 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



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## Cache Valley Fever

Days Gestation	Fetal outcome
Infected	
28-32d	<ul style="list-style-type: none"> <li>• Early embryonic death and mummification</li> </ul>
32-37d	<ul style="list-style-type: none"> <li>• Deformities in brain, spinal cord (hydrancephaly, hydrocephalus, cerebellar and cerebral hypoplasia)</li> <li>• Musculoskeletal deformities (arthrogryposis, torticollis)</li> </ul>
37-49d	<ul style="list-style-type: none"> <li>• Musculoskeletal deformities only</li> </ul>

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## Cache Valley Fever Virus



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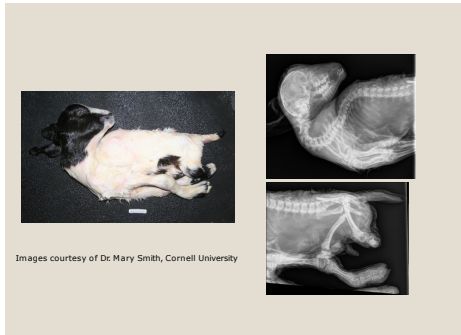
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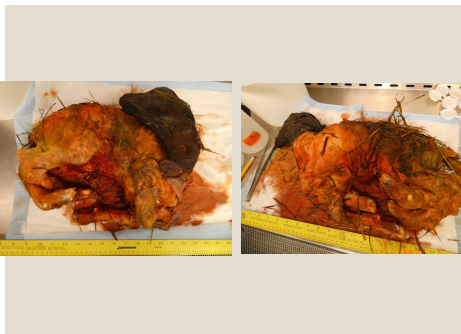
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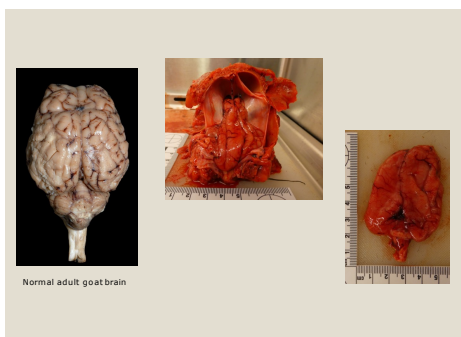
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## Cache Valley Fever diagnosis

- Serum neutralization titer on fetal fluid
  - Heart blood -goosey
  - Fluid in thorax
  - Fluid in abdomen
- Serum from doe
  - Negative: rules Rout
  - Positive: could be exposure
- Need paired samples
- Virus itself? Long gone!




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25

## Cache valley serology examples

Doe	CVF SN	KLd	CFV SN
1	384	1a	48
2	512	2a	64
3	192	3a	96
4	384	4a	48

Courtesy of Dr. Mary Smith

Panel Fluid	Result
Bovine Disease Virus SN	NEG 15
Cache Valley Fever Virus SN	Pos 22
Neospora FA	Negative

1998 Doe - Owe	Result
Serum	
Bovine Viral Diarrhea Virus SN, Type 1	NEG 6
Bovine Viral Diarrhea Virus SN, Type 2	NEG 6
Cache Valley Fever Virus SN	Pos 4996

Cache Valley Fever Virus SN	Result
1	NEG 1
2	Pos 210
3	Pos 4996
4	Pos 4996
5	Pos 248
6	Pos 248
7	Pos 248
8	Pos 248
9	Pos 248
10	Pos 248
11	Pos 248
12	Pos 248
13	Pos 248
14	Pos 248
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26

## The good news

- Doe/ewe immune for years to life
- Consider breeding outside mosquito season
- ...Climate change




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27

### Cache Valley Fever SN titers at the AHDC 2017-2022 for Sheep and Goat

	#tested	#positive	%positive	#toxic
Adult	296	171	58%	4
Fetus	136	42	31%	30 (22%)
Total	432	213	49%	34 (7.9%)

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### Bluetongue virus

- Orbivirus (Reoviridae family)
- Double stranded RNA
- Affects sheep >goat
- Culicoides transmission
- Infect 40-60 days gest - fetal death
- Infect later - stillborn or weak lambs
  - Hydrancephaly
  - Retinal dysplasia
  - meningoencephalitis



29

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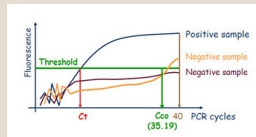
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### Diagnostics available

- BTV PCR on fresh tissues
- BTV ELISA (antibody on serum)



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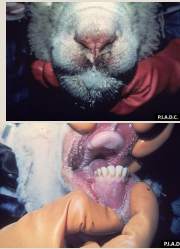
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### The good news

- 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.dcr.ny.gov/press/126187.html>



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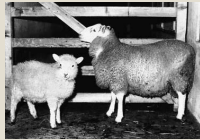
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### Pestiviruses: Border Disease

- Infected animal sheds virus directly
- Reproductive issues in sheep
  - Fertility
  - Abortions/ stillborn
  - Premature/ weak lambs
  - Hairy Shakers (persistently infected lambs)
    - Exposed <math>40\text{D}</math> days gestation
    - Hydrocephalus/hydrocephaly or telencephalic
    - Hair - like fleece
    - Rhythmic tremors



Settlerson, P.F. and Wiloughby K. Diseases of Sheep, Fourth Edition, Chapter 18 Border Disease, 91.9-120

32

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### Pestiviruses: Bovine Viral Diarrhea

- 2013 paper from Switzerland: "A goat given as a social companion to a BVDV f1 calf gave birth to a f1 goat kid"
- Goat f1 housed with pregnant goats and made 2 f1 twins
  - All died before 2 yrs old
- Potential for BVD to be maintained in goat herd
- We have seen BVD titers in goats at AHCC
- Don't house small ruminants with cattle



33

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### Pestivirus diagnostics

- Virus Isolation \$75
  - Fresh fetal tissues
  - EDTA whole blood 5 ml
  - Typing and sequencing available
- BVD AND BVDV SN \$15.90
  - Serum
- BVD ACE \$6
  - Fetal or adult skin/serum
- BVD PCR \$41
  - Fetal lung



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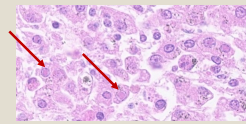
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### Caprine herpesvirus 1

- Adults asymptomatic
  - Abort end of last trimester
    - Autolyzed fetus
  - 1-2 mm white foci in fetal lung liver kidney
  - Intranuclear inclusion bodies in adrenal, liver, lung, kidney
  - No histo lesions in placenta
- Case report from Quebec in 2002:  
 • 80 head herd of goats  
 • 50% aborted or had stillborns



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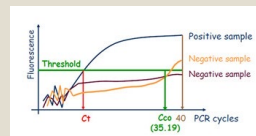
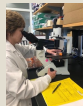
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### Caprine herpesvirus 1 diagnostics

- Panherpes PCR \$41
- Sequencing if positive \$15
- Virus Isolation \$75



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## Toxoplasma gondii

- Protozoa
- Cat is the definitive host
- Cat ingests infected placenta/fetus (intermediate host)
- Shed millions of oocysts in feces for 7-12 days
- Goats > Sheep infected by ingesting feces w sporulated oocysts
- Tachyzoites go to CNS and muscle, encyst into bradyzoites, travel to placenta when preg



"Cats are EVERYWHERE"

37

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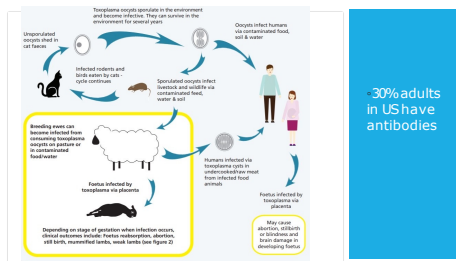
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## Toxoplasma gondii

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



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### Necrotic and calcified cotyledons

- Diagnose with HISTOPATH of placenta, fetal brain and heart
  - Encephalitis / Meningitis
  - Myocarditis
- TOXO MAT antibody titer on fetus and dam supporting evidence








Photo by Jitender P. Dubey

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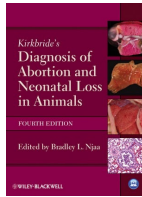
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### The good news

- Don't kill the barn cat! Only shed when first infected.
- Keep cat population stable
- "~4% of animals transmit parasite vertically through transplacental transmission" Kirkbride
- "*T. gondii*-positive lambs were aborted in successive pregnancies in 21% of lambings during study period, 2000-2003" Morley et al 2007.



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### *T. gondii* titers tested at the AHDC 2017-2022

	#tested	#positive	%positive
Sheep	229	42	18%
Goat	208	48	23%

Toxoplasma MAT		
Item		Result
1	1524 - Ovine Katahdin Female Serum - 04/16/2023	1:1600
2	1343 - Ovine Katahdin Female Serum - 04/16/2023	1:800
4	Fetus of 1343 Larger - Ovine Katahdin Female Peritoneal Fluid	1:25



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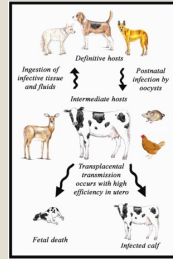
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## Neospora caninum

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



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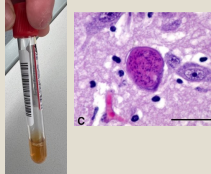
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## Diagnosing *N. caninum*

- Histo lesions like Toxoplasma
  - Encephalitis / Meningitis
  - Myocarditis
- Neospora IFA antibody titer \$180n fetal fluid and dam serum
- Have not yet confirmed any small ruminant cases



Test	Result
SDS - Cow, sheep, Ferret	
Seren 1416/2023	
Bovine Viral Diarrhea Virus RN Type 1	Neg B
Bovine Viral Diarrhea Virus RN Type 2	Neg B
Canine Valley Fever Virus DN	Pos 12R
Chlamydia CF	Neg 1.16
Neospora IFA	POSITIVE

Disclaimer: This test has not been validated for use on this species.

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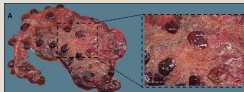
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## *Chlamydia abortus* "Enzootic abortion"

- Gram negative, intracellular bacteria
- Lives in goat GI tract
  - Shed in feces
- Travels to blood stream then placenta
- Fetus dies, is aborted last 2 months gestation
- Placenta looks thickened and necrotic
- Transmitted via sniffing vaginal discharges
- Zoonotic- pregnant women can abort!



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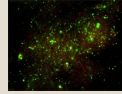
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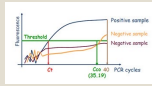
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### Chlamydomphila abortus diagnosis

- Chlamydia PCR \$41 or FA \$27.50
- Placenta and fetal lung
- Histopath of placenta and fetus
  - Placental necrosis, trophoblasts with intracytoplasmic organisms
  - Coagulative hepatocellular necrosis



Fluorescent antibody stain




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### Chlamydomphila abortus 2017-2022

PCR	#tested	#positive	%positive
Sheep	221	0	0
Goat	213	3	1.4

FA	#tested	#positive	%positive
Sheep	230	0	0
Goat	391	0	0

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### The good news

- Vaccine available
  - Who uses it?
- Antibiotic options to control an abortion storm:
  - Tetracycline
    - Injectable (LA 200) (milk withdrawal concerns)
    - Feed (need VET)
- Once in the herd, only the yearlings abort




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48



### *Chlamydophila* CF titers at the AHDC 2017-2022 for Sheep and Goat



	#tested	#positive	%positive
Sheep	144	30	21%
Goat	286	69	24%
Total	430	99	23%

- Note: this serology test includes all types of *Chlamydia*, including non-pathogenic intestinal strains.
- ≥1:80 consistent 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
- Select Agent with CDC
- Reportable disease with USDA
- Shed mainly during parturition
- Main mode of transmission
- May be incidental



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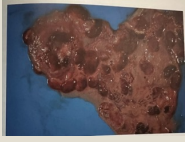
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### *Coxiella burnetii* "Q fever"

- 3<sup>rd</sup> trimester abortions
- Stillborn, premature, weak neonates
- Placentitis - **WE NEED PLACENTA!**
  - Cotyledons thickened
  - Gray/white to brown/redundate
- DX is histopath \$160 and PCR \$41 of placenta
  - Need BOTH for causation
  - Coxiella* may be there and not cause abortion
  - Histo of fetus WNL



Courtesy of Sheeha, Geak and Cervid Medicine

51

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### *Coxiella burnetii* "Q fever"

- Zoonotic
  - Inhale 1-10 organisms
  - 50% are asymptomatic
  - Mild fevers
  - Endocarditis
  - Encephalitis
  - Chronic Pneumonia
  - Abortion
- Can be shed in milk
  - Goat >> sheep
- Slays in environment
- THE PATHOGEN IS A GIANT PAIN



52

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### 2009 study monitoring Q fever shedding by Rousset et al.

Path loading per site:	Vaginal mucous	Feces	Milk
Aborted	44%	21%	38%
Normal	22%	20%	31%

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

53

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### The good news

- Animals can asymptotically shed
- Animals can have titer and not abort
- You might already be immune



54

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*Coxiella burnetii* ELISA titers tested at the AHDC 2017-2022



	#tested	#positive	%positive
Sheep	2958	70	2%
Goat	1633	178	11%

Research facilities and pathogen free sheep monitoring skew this data!

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

- Late gestation abortion, still births, weak offspring
- More common in sheep
- Shed in feces in sheep, dogs, birds
- Spreads when animal ingests feces or infected placenta/fetus (guard dog)
- Zoonotic- diarrhea in people
- By the time abortions start, entire flock likely exposed



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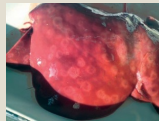
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*Campylobacter*

- Bacteremia – placentitis – fetal septicemia
- Hepatic necrosis – white circular lesions
- Diagnostics:
  - *Campylobacter jejuni* culture of fetal liver
  - *Campylobacter fetus* FA of placenta and fetal liver
- Will not reliably grow on aerobic culture



57

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**The good news**

- Antibiotic options to control an abortion storm:
  - Tetracycline injectable (LA 200) (milk withhold concerns)
  - Feed (head VFD)
  - Sulfamethazine and Tylosin in the feedstuffs
- Dice in the herd, only the yearlings abort




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58

**Listeria monocytogenes**

- Gram positive coccobacilli
- Found in soil, feces, silage (pH > 5.0)
- Likes "refrigeration temperatures"
- Presents as
  - Circling disease
  - Or septicemia and abortion in goats
  - Don't see both at the same time
  - Abort in 2<sup>nd</sup> or 3<sup>rd</sup> trimester
  - Placentitis, white foci on fetal organs

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59

**Listeria monocytogenes**

- Diagnose by aerobic culture of placenta, fetal lung and abomasal contents
- If feeding silage, check pH
- Try to clean up environment to decrease consumption of listeria

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### Brucella – not a concern

- *Brucella ovis*:
  - Sheep only, rams persistently shed
  - Orchitis and epididymitis
  - Can cause EED and abortions
  - AHDChas 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

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61

### Leptospirosis

- Very rarely found in literature
  - Documented in Spain and Ireland
  - L hardjo, pomona, castellanis, icterio
- We offer PCR \$41 on placenta and fetal kidney
  - Lepto interrogans MAT \$20 on serum
    - 5 serovars
      - Pomona
      - Hardjo-prajitno
      - Icterohaemorrhagiae/copenhageni
      - Grippityphosa
      - Canicola




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62

### Mineral deficiencies:

- Selenium
  - test on EDTA whole blood \$31
  - Or liver \$60
- Iodine \$31 at Michigan DL
  - Goller
- Test liver mineral panel \$51.65
  - Includes calcium, phosphorus, manganese, magnesium, sodium,




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63

Pathogen	Sample and diagnostic test
Bovine Viral Diarrhea	Fetal lung for PCR (antigen) and Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing
Border Disease Virus	Fetal tissues for viral isolation (antigen) and Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing
Cache Valley Fever	Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing

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Pathogen	Sample and diagnostic test
<i>Toxoplasma gondii</i>	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
<i>Neospora caninum</i>	Fetal heart blood or pleural, pericardial or peritoneal effusion in a red top tube for antibody testing

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Pathogen	Sample and diagnostic test
<i>Campylobacter fetus</i> ("Vibrio") or <i>C. jejuni</i>	Fetal liver for culture (antigen)
<i>Chlamydia abortus</i> ("Enzootic abortion")	Placenta or fetal lung for PCR (antigen)
<i>Coxiella burnetii</i> ("Q fever")	Placenta or stomach contents for PCR (antigen)
<i>Listeria monocytogenes</i>	Placenta, fetal lung or stomach contents for culture (antigen)
Other bacterial/fungal	Placenta, fetal lung or stomach contents for culture (antigen)

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### Practice abortion workup:

- Goat herd of 20 pregnant does experiences 3 abortions within 7 days, all within 2 weeks of due date
- 2 sets of twins, 1 singleton
- 1 each from the sets of twins and the singleton have arthrogryposis and torticollis
- The singleton also has a dome shaped head, and you suspect hydrocephalus
- Guard dog on premise
- Barn cat just had a litter of kittens 1 month ago
- Purchased a buck in September of previous year

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67

### Practice abortion work-up

- The placentas look grossly normal
- List top 3 differentials
- List sample type and test requested to find diagnosis
  - Unlimited tissue number
  - Owner has no financial limitations ☹

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68

### Questions?



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



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### Extensive histo slides read

- Slide 6: Skeletal muscle - triceps
- Slide 7: Skeletal muscle - triceps
- Slide 8: Skeletal muscle - neck/limbs
- Slide 9: Skeletal muscle - semimembranosus
- Slide 10: Skeletal muscle - nuchal muscles
- Slide 11: Frontal cortex, histiocyte
- Slide 12: Frontal cortex, histiocyte
- Slide 13: Mesencephalon
- Slide 14: Cerebellum, granular
- Slide 15: Brainstem
- Slide 16: Cervical spinal cord
- Slide 17: Thoracic spinal cord
- Slide 18: Lumbar spinal cord
- Rabies neg
- Listeria culture neg
- Full mineral panel WNL on brain and liver
- BVD ACE negative
- Virus isolation negative

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### Options for genetic testing:

- STgenetics
  - <https://www.stgen.com/articles/etec-ss>
  - <https://www.stgen.com/articles/etec-ss>
  - \$10, Hair?
- Feanixbio
  - <https://www.feanixbio.com/etec-ss>
  - <https://www.feanixbio.com/etec-ss>
  - \$10
  - Hair, TSU, DNA



STgenetics  
Leading Genetics  
**100%**  
RECUMBENCY FREE



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