Coxiella burnetii: The Disease and Clinical Manifestations

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Q-fever - History

- First reported in 1930's almost simultaneously by two different research teams
  - Abattoir workers in Brisbane, Australia
  - Montana Rickettsial laboratory
- "Q" is for "query" fever

- Edward Holbrook Derrick was the director of Microbiology at Queensland Health Laboratory
- Sent samples to Macfarlane Burnet and Mavis Freeman who reproduced disease in guinea pigs in 1937
Q-fever History

- Gordon Davis, at the Rocky Mountain Laboratory in Hamilton, Mont was studying Rocky Mountain Spotted Fever
- Ticks feeding on guinea pigs induced disease unlike RMSF
- In 1938 Cox propagated organism in embryonated eggs

Q-fever - History

- The first reported lab acquired Coxiella infection occurred in 1938 when Rolla Eugene Dyer, Director of the National Institutes of Health went to Montana to confirm findings and was infected
- Blood from Dyer infected guinea pigs

- Following initial description reports increased both domestically and internationally
- World wide distribution with the exception of New Zealand
- Has been isolated from a wide variety of domesticated and wildlife species
Q-fever - History

• Soon described as an important cause of enzootic abortions of small ruminants
• Abortions, infertility and low birth rates reported in cattle
• Human disease was poorly defined and heavily underreported

Q-fever - History

• Human illness associated with Coxiella
• Prolonged febrile illness
• Atypical pneumonia
• Hepatitis
• Chronic endocarditis - especially predisposed individuals

Q-Fever - History

• Traditional perspective of public health community
• Exposure
  • Handling parturient/aborting sheep
  • Processing pregnant sheep in abattoir
  • Consuming unpasteurized milk
Q-fever - History

- Veterinary and Laboratory Animal Perspective
- Very heavy focus on sheep
- Less focus and discussion about goats and cattle
- Lab Animal Concerns about shedding in research environment

Q-fever - Bacteriology

- *Coxiella burnetii*
- Small gram negative coccobacillus
- Similar to *Legionella, Francisella* and *Rickettsiella*
- Obligate Intracellular Organism
- Does not grow on routine bacteriological cultures

Bacteriology

- Undergoes phase variation in the LPS
- Humoral response to both phases is used to determine chronicity of human infections
- Phase II antigen response predominate in acute infections
- Chronic infections have rising phase I
- Minimal data in animals
Growth

- Is considered a Class B Bioterrorism agent
- To grow must be in BSL-3 with full select agent license
- Traditionally required embryonated eggs or cell culture to isolate
- Lives in phagolysosomes in highly acidic environment

Survival

- SCV is highly resistant “spore-like” form
- Not a true spore
- Withstand osmotic, pH, desiccation, disinfection etc
- Can be easily aerosolized
- Concern for bioterrorism

Aerosol

- Organism has been known to easily aerosolize
- Current data out of Netherlands confirms
  - 59% of human cases have occurred in individuals that live within 5 km of infected farm while only 12% of population
  - RR of infection if live within 2km vs greater then 5 km is 31
Transmission

- Other routes
  - Oral ingestion - likely not as big of an issue as once thought to be
  - Tick - not a major route in humans
  - Person-Person is extremely rare
    - Obstetrician performing abortion
    - Blood transfusions, vertical transmission, autopsy

Animal Carriage

- 2003 CDC study demonstrated 92% seropositive bulk tanks (by IFA) from vet school dairy cattle herds
- 2005 study by Cornell demonstrated 94% of dairy cattle bulk tanks positive by PCR - mostly NY but 18 other states represented

Animal Carriage

- Sheep and goat rates in US are unclear
- Sheep NAHMS 2011 has a herd level prevalence of around 24%
- Guelph study showed 69% and 75% of sheep and goat abortions respectively positive by PCR (Hazelton, J VET Diagn Invest 2013 25: 359)
Animal Carriage

- Cats can be a significant vector
  - 8.5% of client owned cats
  - 41.7% of stray cats
  - Human illness has been associated with reproductive failure

Human Exposure

- Recent CDC seroepidemiologic survey of blood banks showed 3.1% seropositive
- 508 veterinarians tested at AVMA in Hawaii showed 22.2% seropositive
- Generally less then 200 cases in humans per year in US although reporting rates have increased over 8X in last decade

Animal Reservoirs

- All the animal kingdoms have been demonstrated
- Ruminants are the primary reservoir
- Shed in many wildlife species, including mice and rats
- Can survive in amoeba for up to 6 months
Animal Shedding

- The organism can be shed in milk, feces, fetal fluids and tissues, blood, urine
- Some general trends are evident in shedding patterns of ruminants
  - Cattle shed more in milk and for longer periods
  - SR shed more in vaginal and more periparturiently

Animal shedding

- It is clear that animals do not have to be seropositive to shed organism

Table 3
Assessment of goats that experienced Q fever-related abortions and shed C. burnetii at two successive kidding seasons in both vaginal swab and milk samples

<table>
<thead>
<tr>
<th></th>
<th>Kidding seasons 1</th>
<th>Kidding seasons 2</th>
<th>Both kidding seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats with reproductive failures</td>
<td>18 (30%)</td>
<td>5 (9%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>PCR (+) vaginal swab</td>
<td>15 (25%)</td>
<td>51 (94%)</td>
<td>14 (26%)</td>
</tr>
<tr>
<td>PCR (+) milk</td>
<td>17 (25%)</td>
<td>14 (26%)</td>
<td>12 (22%)</td>
</tr>
</tbody>
</table>

Goats that experienced reproductive failures included females that either aborted or had stillbirth.
Animal shedding

Table 1. Determination of the presence of C. burnetii DNA in environmental samples from six states

<table>
<thead>
<tr>
<th>State geographic region</th>
<th>No. of samples</th>
<th>No. of positive results</th>
<th>% Positive</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rocky Mountains</td>
<td>271</td>
<td>124</td>
<td>44.6</td>
<td>November 2006</td>
</tr>
<tr>
<td>South-central</td>
<td>285</td>
<td>103</td>
<td>36.1</td>
<td>December 2006</td>
</tr>
<tr>
<td>Upper Midwest</td>
<td>271</td>
<td>87</td>
<td>34.7</td>
<td>November 2007</td>
</tr>
<tr>
<td>West Coast</td>
<td>271</td>
<td>37</td>
<td>13.7</td>
<td>April 2008</td>
</tr>
<tr>
<td>East Coast</td>
<td>266</td>
<td>16</td>
<td>6.0</td>
<td>April 2008</td>
</tr>
<tr>
<td>Deep South</td>
<td>258</td>
<td>42</td>
<td>16.3</td>
<td>June 2008</td>
</tr>
<tr>
<td>Total</td>
<td>1,622</td>
<td>386</td>
<td>23.8</td>
<td></td>
</tr>
</tbody>
</table>
Diagnostics

• Histopathology and IHC
  • Visualized most commonly with Gimenez Stain
  • Certainly seen in placentas of severe abortion storms but not seen in all shedding animals
• Species differences
  • Bovine - few organisms in few cells
  • Caprine - cells swollen with organism

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A prospective study of sheep and goat abortion using real-time polymerase chain reaction and cell pellet extraction tissues
demonstrated the presence of 'swollen' cells and bodies concurrently with other sugar pathogens

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Table 1. Diagnosis of case of sheep abortion by pathogens with associated real-time polymerase chain reaction results.

<table>
<thead>
<tr>
<th>Pathogen diagnosed in case of abortion</th>
<th>No. of submissions</th>
<th>Presence for C. burnetii</th>
<th>Presence for C. abortus</th>
<th>Mean DNA copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. burnetii</td>
<td>12</td>
<td>11 (92%)</td>
<td>1 (8%)</td>
<td>4.57 × 10^10</td>
</tr>
<tr>
<td>Chlamydiophila abortus</td>
<td>19</td>
<td>17 (80%)</td>
<td>1 (5%)</td>
<td>1.60 × 10^10</td>
</tr>
<tr>
<td>Camplobacter jejuni</td>
<td>21</td>
<td>16 (76%)</td>
<td>5 (24%)</td>
<td>1.75 × 10^10</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>31</td>
<td>28 (90%)</td>
<td>3 (10%)</td>
<td>3.65 × 10^10</td>
</tr>
<tr>
<td>Other infectious agents</td>
<td>11</td>
<td>8 (73%)</td>
<td>3 (27%)</td>
<td>1.87 × 10^8</td>
</tr>
<tr>
<td>Mosaic</td>
<td>73</td>
<td>46 (63%)</td>
<td>27 (37%)</td>
<td>1.61 × 10^8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percentages.

**Data were analyzed using the paired t-test.**
- PCR
  - Both standard and quantitative PCR
  - Generally use IS1111 transposon-like repetitive unit
  - Increases sensitivity due to copy number
  - 10-60 copies depending on strain
  - Can detect as few as 10 organisms

- ISU PCR
  - Additional of Bacillus spore internal positive control
  - Test for both lysis of spore (SCV)
  - Inhibition of PCR
  - Manuscript in prep

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**Table 2.** Detection of canine abortion by pathogen with conventional real-time polymerase chain reaction results.

<table>
<thead>
<tr>
<th>Pathogen Diagnosis</th>
<th>No. of isolations</th>
<th>Mean DNA copies</th>
<th>No. of isolations</th>
<th>Mean DNA copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaniClostridium abortus</td>
<td>15</td>
<td>1.35 x 10^7</td>
<td>9</td>
<td>1.35 x 10^7</td>
</tr>
<tr>
<td>Haemophilus parainfluenzae</td>
<td>30</td>
<td>3.12 x 10^7</td>
<td>20</td>
<td>3.12 x 10^7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20</td>
<td>9.58 x 10^7</td>
<td>15</td>
<td>9.58 x 10^7</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>30</td>
<td>0.98 x 10^7</td>
<td>20</td>
<td>0.98 x 10^7</td>
</tr>
<tr>
<td>Others (see below)</td>
<td>6</td>
<td>3.12 x 10^7</td>
<td>3</td>
<td>3.12 x 10^7</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percentages.
* Data for conventional PCR with an additional Bacillus spore internal positive control.
* The adenovirus diagnosis was for a canine C. abortus, C. saprophyticus, C. sordellii, and C. perfringens abortus, as well as the C. parvum and C. sordellii.
Diagnostics

• Serology
  • CF test has been the traditional test
    • Only one currently offered by NVSL
    • Anti-complementary substances commonly present in samples
    • Restricted to certain subclasses of IgG
      • IgG1 works but IgG2 and IgM can suppress

Diagnostics

• CF (continued)
  • Phase I response can confound
    • Does not work well for chronic humans

Diagnostics

• ELISA
  • 3 Commercial kits - Overseas
    • Idexx Chekit Q-fever
      • Phase I and II IgG, Nile Mile Strain
    • LSI ELISA COX Kit
      • Phase I and II IgG, clinical ovine abortion strain
    • Pourquier ELISA Q fever kit
Diagnostics

• ELISA
  • Benefits
    • Technically easy and automated
    • Numeric output
  • Disadvantages
    • Either only test phase I or does not differentiate phase I and II

Diagnostics

• ELISA vs CF
  • ELISA much more sensitive
    • Discordant results (primarily ELISA + and CF -)
      • 41% in non-aborting
      • 47% in aborting
      • Kappa = 0.17 (0.08-0.34)

Diagnostics

• IFA
  • Reference technique for human laboratories
    • Can be used for IgM and IgG
    • Allows differentiation of phase I and II
  • We have developed and validated a phase I and II IFA for cattle, sheep and goats at ISU and TAMU has one
Diagnostic Approaches

• Dairy Herds
  • Bulk tank PCR testing is a good option
    • Dairy Cattle vs Goats
    • Seasonality
    • Combine with serology for sheep

• Non-dairy herds
  • PCR of individual animals
  • Serology of individual animals
  • Helpful for determining herd status but single time-point testing is not useful on individual animal basis

Sample collection

• Vaginal swabs
  • Comparison of cotton-tipped swabs to histobrushes suggest higher sensitivity of histobrushes (manuscript in prep)
  • Place swab/brush in PCR
What do you do if you get a positive?

- Don’t panic
  - Remember that this organism is present on a large number of animal operations
  - Report illness to state veterinarian if required - most states require
  - Prepare for everyone else to get excited

What do you do if you get a positive?

- Response by state varies
  - Ultimately state animal health bureau drives response
  - Currently limited standardization and minimal sources to look to for accurate information

What do you do if you get a positive?

- National panel for the development of Guidance of joint animal health and public health response to Coxiella
  - 11 person panel convened
  - In process of approving recommendations through
    - National Association of State Public Health Veterinarians
    - National Assembly of State Animal Health Officials
NASPHV Guidance

- Should be available online by fall
- Is not binding but provides key information to make a evidence based response
- Provides extensive handouts for education of stakeholders, physicians, veterinarians and public health officials

First Task

- Notify state animal health officials
- Follow their instructions
- Proposed guidelines will be discussed

Full Joint Response

- A joint public health and animal health epidemiologic investigation is warranted under the following situations:
  - A human C. burnetii case is reported to public health officials where the initial interview identifies a potential animal source of infection.
  - Coxiella is identified in animals where there are reports of a febrile, influenza-like illness in exposed persons.
  - Multiple human Q fever cases occur in a geographic or temporal cluster.
  - Other situations at the discretion of public health and animal health authorities.
Key Recommendations

- Educate animal care-takers and owners on clinical signs of animal illness and human illness
- Involve public health department in education of human health issues
- Tell them to inform their doctor that they may have been exposed to Coxiella if they develop clinical signs

Key Recommendations

- Train animal care-takers in the use of PPE
  - Ideally N95 mask
  - How to handle fit testing????
- Dedicated barn clothing (including hats, coats, boots etc)
- Removed prior to going in house

Key Recommendations

- Prompt and appropriate removal of placentas, fetuses, dead newborns and fetal fluids
  - Seek state input on acceptable methods
  - Dispose of carcasses onsite whenever possible, because transporting carcasses and contaminated materials can present a transmission risk. Composting or deep burial is recommended.
  - Prevent scavenging.
  - If carcasses are temporarily stored prior to disposal, use closed containers with absorbent materials to prevent splashing and spilling.
  - If you must send animals suspected to be infected with Coxiella burnetii to a landfill, inform landfill staff so that they can take proper precautions to protect themselves.
Key Recommendations

• Animal movement

  Proposed wording "To control spread of coxiellosis between premises, producers should refrain from moving or selling animals, particularly periparturient animals, while abortions are ongoing."

Key Recommendations

• Control access to animals, especially birthing areas

  Proposed wording "Recommend that visitors be given limited access to animal birthing areas, particularly when abortions are occurring. Limited access is especially important for persons with conditions that put them at higher risk for complications (e.g., heart valve disease, prosthetic heart valves, pregnancy, and immunosuppressive conditions)."

Key Recommendations

• Should the farm be quarantined?

  Proposed wording "Official animal quarantine is generally not recommended, and mass euthanasia of infected herds is never recommended for several reasons:

  • Coxiella burnetii infection is endemic in animals in the U.S.
  • Coxiella burnetii is ubiquitous and persistent in the environment.
  • With the tools currently available, it is nearly impossible to eliminate infection from a herd and to completely decontaminate the environment.
  • There is no known effective treatment to eliminate shedding of bacteria in infected animals.
  • It would be extremely difficult to repopulate a herd with Coxiella burnetii negative animals.
  • Defining the terms for release of quarantine would be almost impossible because of the factors listed above."
Key Recommendations

• Address issue of raw milk
  • Recommend no consumption and stop all commercial sale of raw milk, soaps, cheese etc
  • Pasteurization of milk according to PMO is effective in controlling Coxiella so sale of dairy products marketed after pasteurization can continue

Key Recommendations

• Management of manure
  • Ideally composting
    • Minimum of 90 days and requires appropriate monitoring for temperature etc
  • Consult appropriate state officials for acceptable methods

Key Recommendations

• Management of manure
  • If composting is not an option
    • Cover manure during transport
    • Store where run-off and water contamination is minimal
    • Transport and spread manure only on non-windy days, ideally with dew
    • Land apply promptly after stall clean-out and plow in immediately
    • Only sell manure that has been properly composted
Key Recommendations

• Control rodents, wildlife and domestic pet access
• Rodents can carry organism easily and have been shown to play a role in transmission
• Cats and dogs can both abort due to agent and act as a means of spread

What about treatment?

• Oral tetracycline has been used
• No evidence of efficacy
• Bioavailability of these formulations is limited in cattle
• Anecdotally, use has not shown benefit in abortion storms

What about treatment?

<table>
<thead>
<tr>
<th>Route</th>
<th>Treated</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td>82%</td>
<td>72%</td>
</tr>
<tr>
<td>Feces</td>
<td>61%</td>
<td>77%</td>
</tr>
<tr>
<td>Milk</td>
<td>57%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Kinetics of Coxiella burnetii excretion in a commercial dairy sheep flock after treatment with oxytetracycline:

75% of flock treated 25% controls

At lambing
What about treatment?

- During abortion storm - 13 ewes aborted prior to treatment
- 20 mg/kg OTC +100 and +120 of gestation
- 7 additional abortions (5 in OTC group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cases</th>
<th>Age (weeks)</th>
<th>Body wt (kg)</th>
<th>No. of Cases</th>
<th>Age (weeks)</th>
<th>Body wt (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>OTC</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
</tbody>
</table>

What about treatment?

Effectiveness of vaccination and antibiotics to control Coxiella burnetii shedding around calving in dairy cows

- OTC 20 mg/kg administered at dry-off and dry-off+ second dose 15 days later
What about vaccination?

- No *Coxiella* vaccines available in the US
- *Coxevac* is a phase I vaccine available in Europe
- Showed benefit in Netherlands
- Limited supply
- Not available for import to US
- Canada can import

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*Coxiella burnetii*: Lessons from the Netherlands

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I have the following disclosures* related to my presentation:

- None relevant to this presentation

*Disclosures include spouse and immediate family where relevant.
Q-Fever
Zoonosis

Classic Context
- Food safety emphasis
  - Coxiella set new PHO pasteurization standard
- Sporadic epidemics
  - Several people directly ill
- Animal "smoking gun"
  - Lambing sheep or slaughterhouse associated
  - Herd abortion storm

Case Example
- 2003 Germany – Farmer’s Market (Porten et al. 2006, BMC Inf Dis)
  - Single lambing ewe on exhibit
  - Ewe and home flock positive
  - 299 reported human cases
  - 25% adult human clinical attack rate
  - 25% adult human hospitalization rate

Netherlands

Netherlands Context
- High population density of both animals and humans
  - Noord-Brabant in south
    - Poultry 5042/sq km
    - Swine 1009/sq km
    - Cattle 125/sq km
    - Goats 23/sq km
    - Sheep 20/sq km
Netherlands Context

- Milk quota for bovine milk resulted in rapid growth of the dairy goat industry.

Netherlands Dairy Goat

- Rapid expansion of goat dairies in early 2000s
- Mainly internal growth - high biosecurity
- Large Farms - avg 1500 does, large farms in the 6000-7000 range
- Close proximity to residential areas - often within stone throw

Netherlands Dairy Goat

- Predominantly housed inside on bedded pack
- Managed to minimize kids - often only bred 2-3 times in lifetime
Abortion Storms

- In 2005 the first abortion storm on a dairy goat operation was identified. A second followed that year.
- 2006=6, 2007=7, 2008=7 predominantly in SE
- 2 dairy sheep operations
- Average goat herd size 900, 20% avg abortion rate (10%-60%)

Small Ruminant Epi

- 2008 serologic assay of sheep and goats
- Sheep 2.4% (95% CI 2.1-2.7%)
- Goats 7.8% (95% CI 6.9-8.7%)
- 26% of BMT positive by PCR

Historic Human Data

- Q-fever became reportable in Netherlands in 1978
- Period of 78-2006 human cases avg 17 (1-32)
- Most were occupationally associated
- During 94-2001 a total of 49 people were hospitalized
Initial Human Cases

- May 2007, several atypical pneumonias reported in Noord-Brabant, not responsive to antibiotics
- Same time a physician in Herpen reported increase in atypical pneumonia
- 2 weeks later another physician reported atypical pneumonias

- In total in 2007, 182 cases were identified
- Week 18-24 had highest reporting
- Age 7-87
- Female: Male = 1:1.7
- Hospitalization rate = 43%
- Persistent Fatigue in many patients

Human Epidemic

- Graph showing number of cases and deaths from 2007 to 2013
- 2007: 5 cases, 2 deaths
- 2008: 5 cases, 0 deaths
- 2009: 6 cases, 1 death
- 2010: 7 cases, 1 death
- 2011: 11 cases, 1 death
- 2012: 66 cases, 2 deaths
- 2013: 2354 cases, 1000 deaths
- Graph showing number of cases and deaths from 2007 to 2013
Response

- 2008 a voluntary vaccination program for small ruminants was initiated
- Coxevac - inactivated phase 1
- 35,000 goats vaccinated
- Goal was to reduce shedding and decrease environmental contamination

Response

- Spring 2009 - vaccination made mandatory in impacted areas of country for farms >50 animals and farms with intense human contact
- In Feb 2009 the government also initiated strict hygiene measures
- Farmers obliged to fight vermin
- Aborted fetuses and placenta must be rendered
Response

- Hygiene Plan
- Cannot clean manure for 1 month following kidding
- Cover manure when hauling
- Plow under immediately
- Compost for min 3 months

Netherlands

- 2009 - Mandatory vaccination between April and Nov - 250,000 vaccinated
- Oct 1 2009 - bulk tank monitoring mandatory
- Slaughter of 50,000 dairy goats on 55 farms

Mass Culling
What can we learn?

- Animal and human proximity
- Goats confined and in very close proximity to dairies
- Estimated that 12.6% of the local population lived within 5 km of goat dairy
What can we learn?

Q Fever: Single-Point Source Outbreak With High Attack Rates and Massive Numbers of Undetected Infections Across an Entire Region

- Smaller cluster in outbreak
- Serologic attack rate
  - 92% in farm residents/employees
  - 56% in farm visitors (3 for less than 1 hour)
  - 50% in household contacts
What can we learn?

- Environmental Loads from 2009
  - In 2009 100% of aerosol samples and surface samples positive
  - Radial sampling of 1000m from farm - 92% positive
  - Higher loads in stable then surrounding
  - All samples but one in "high-load"

What can we learn?

- 2010 aerosol sampling
  - 33% positive at 2 km
  - Lower load levels
Inhalation is most productive means of induction

Organism localizes to trophoblast by 28 dpi

Fetus infected as early as 28 dpi and stays infected throughout gestation
What can we learn?

<table>
<thead>
<tr>
<th>Feces</th>
<th>Vaginal Mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

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What can we learn?

- Summary
- Abortion storms can lead to human illness
  - High risk for exposed workers but also downwind aerosol
- Vaccination can be useful but none currently available

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What can we learn?

- US risk is likely much lower than Netherlands
- Proximity of population to dairy goats
- Biosecurity of dairy goat operations
- Size of operations
- However seroprevalence in animals mirrors that of Netherlands

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What can we learn?

- Vaccination and hygiene management likely sufficient to control
- Do not need to rely on culling in most circumstances
- Aerosol from abortions is important - not just manure
- Human cases can be temporally distant to abortions

Environmental loads can be very high and persistent for long periods of time
- Shedding may be delayed until parturition
- Normal kids may have high bacterial loads
- Risk to pregnant women may not be as great as previously believed however prudent control of exposure is still warranted