Update on Campylobacter and Chlamydia Infections

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Campylobacter

Initial Description

- First reported by McFadyean et al. in 1913 during a study of epizootic abortions in sheep of Great Britain
- Coined term “vibronic abortion” with the isolation of Vibrio fetus in pure culture from a aborted lamb

Bacteriology

- Non spore forming, curved or spiral gram-negative rod
- Limited ability to survive in high oxygen environments
- Motility mediated by a single or double polar non-sheathed flagella
- Relatively biochemically inert
Campylobacter fetus

- Two subspecies
  - C. fetus subspecies fetus
    - Previously known as Vibrio fetus subsp. intestinalis
    - Cause of abortions in sheep
  - C. fetus subsp. venerealis
    - Previously known as Vibrio fetus subsp. venerealis
    - Infectious infertility in cattle

Thermophilic Campylobacters

- Demonstrate improved growth at 42 C compared to 37 C
- C. jejuni and C. coli are the most common
- These are the leading cause of bacterial associated food borne enteritis in the US
- Typically not associated with enteritis in animal host
- Becoming increasingly important in abortions

Campylobacter in sheep

- Traditionally the “vibronic abortion” associated with C. fetus was the primary entity observed
- Currently in some parts of the US we are seeing an increased prevalence of thermophilic strains
  - Does not seem to be the case in New Zealand

Epidemiology

- Australia and New Zealand
  - Predominantly C. fetus with heterogeneity in the population
  - Small number of C. jejuni
- Previous studies in the US
  - 1993 from SD
    - C. fetus predominant but increasing C. jejuni
  - Idaho
    - 15 samples, mostly C. jejuni but heterogeneous
Shedding

- *C. jejuni* and *C. coli* can be readily isolated from the intestinal samples and feces of sheep and cattle
  - 91.7% of intestinal samples of lambs
  - 24-50% shed in feces
    - Almost 100% around lambing
  - Lambs are born negative but 100% colonized and shedding by 5 days

Introduction of new Strains

- Sheep abortion outbreaks are often associated with introduction of new breeding animals
  - Exposure to new strains
  - Particular strains more virulent (?)
- Also must consider migratory birds, ground water and tomites

Abortion storm

- Often see abortion of a single ewe as the sentinel case
  - Then see a sharp rise in the abortion rate 2-3 weeks later
- Abortion rates can exceed 70% in some cases

Pathogenesis

- Oral ingestion of contaminated feed stuffs or aborted material
- Proceeds to a bacteremia
- Seeding of the uterus and fetus
- Associated with late-term abortions, premature births and weak full term lambs
Pathology

• Fetus
  – Many will have no gross lesions however some will have serosanguineous edema, discrete necrotic liver lesions or bronchopneumonia
• Placental cotyledons
  – Enlarged, yellow in color with intercotyledonary edema

Case Confirmation

• Culture of organism
  – Placenta, fetal stomach contents and liver
• Dark field microscopy with observation of small bacteria with appropriate morphology
  (McBride 1993)
• Immunohistochemistry for detection of C. fetus antigens
  – Works for both subsp fetus and subsp venerealis
Immunity

- Clinical experience and literature suggest some degree of immunity
  - Commonly see outbreak in one year followed by several years of no abortions
  - Introduction of these animals into other flocks can lead to abortions in that flock
- Unclear degree of cross protection between *C. fetus* and *C. jejuni*

Vaccination during an outbreak

- Gilmour et al. 1975 evaluated the ability to vaccinate following the first abortion in a flock
  - *C. fetus subsp. fetus* bacterin and challenge

Vaccination

- Two commercially available vaccines
- Colorado Serum
  - Bivalent, includes *C. fetus subsp. fetus* and *C. jejuni*
- Hygieia
  - Bivalent, conditionally licensed in most states

Prevention

- Currently there is a feed-grade chlortetracycline approved for use at 80 mg/head/day for “reducing the incidence of (vibrionic) abortion caused by *C. fetus* infection susceptible to chlortetracycline”
- 100% of abortion isolates are resistant to tetracycline in sheep
Management during outbreak

- Clean environment
- Minimize environmental contamination with aborted fluids
- Vaccination after first abortion
- Maintain segregation of animals

Zoonotic Exposure

- Has been linked to clinical cases
- Environmental contamination may be high in some areas due to high fecal shedding by asymptomatic animals
- One case in the literature of farmer contracting enteritis following assistance during an abortion
- Involved in some human abortions

New Advances

- Our lab has been collecting sheep abortion isolates for genotyping by Pulsed Field Gel Electrophoresis (PFGE)
- To date we have >120 isolates
  - Iowa
  - Idaho-21
  - California - 9
  - South Dakota - 11

Lane 1 and 15: Lambda ladder, Lanes 2-4: isolates from Iowa; Lanes 5-7: isolates from Idaho; Lanes 8-10: isolates from South Dakota; Lane 15: sheep isolate from unknown origin; Lanes 12-13: C. jejuni laboratory and sequence strains.
Strain Homogeneity

• Appears that 96% of the strains collected from abortions in four states during the last three years are genotypically identical
• This is in contrast to studies published over the last 10 years in the US where they observed genotypic diversity
• This PFGE pattern is dissimilar to the strains isolated from feces at this time

Implications

• This suggest that there is a widely distributed single genotype that is growing in significance with regards to ovine abortions
• At this point it is unclear what molecular characteristics are responsible for the emergence of this strain

Tetracycline Resistance

• Antimicrobial resistance of these isolates
  – Currently have tested 50+ isolates
    • 90%+ were resistant to tetracyclines
    • Most are sensitive to ciprofloxacin, erythromycin and gentamicin

Other Questions

• Role of feed grade tetracycline in the emergence of C. jejuni as the predominant abortion strain?
• Reservoir for maintenance of this genotype
• Molecular aspects of virulence
• Incorporation of this strain in vaccines
Chlamydothila

- Previously referred to as *Chlamydia*
  - Serotype 1 - abortions in small ruminants
  - Serotype 2 - kerato-conjunctivitis (pink-eye)
- Have been descriptions of abortion cases where serotype 2 organisms were isolated from the animal

New Nomenclature

- *Chlamydothila abortus*
  - Old serotype 1
- *Chlamydothila pecorum*
  - Old serotype 2

Microbiology

- These organisms are obligate intracellular pathogens
  - Isolation and culture require
    - Embryonated chick eggs
    - Cell culture media
- The organism will not grow on routine microbiology media
- Complement fixation test used for years is now known to be poor test

New Diagnostics

- ELISAs have higher sensitivity then CF
- Quantitative real-time PCR test
  - Currently available only for research
  - Can detect as low as one DNA copy
  - Has been demonstrated to be 300 times more sensitive than culture methods
Epidemiology

• The sporadic nature of clinical disease has led to uncertainty regarding the mechanisms of transmission
  – This has been complicated by the lack of a good diagnostic test
• Some studies demonstrate complete clearance
• Others demonstrate maintenance in subclinical carriers

Recent Research on Bovines

• Using new RT-PCR
  – 51 virgin Holstein heifers - 53% shedding
    • Not associated with sexual transmission
  – Calves are all born negative but start shedding between 2-6 weeks of age
    • 20% of dams were positive at time of calving
  – Prospective clinical study in 140 cows
    • Cows with positive PCR had higher SCC
    • Vaccination against Chlamydia resulted in a decrease in SCC (P=0.007)

Interpretation

• Most animals are infected after birth and shed the organism for some time
• A percentage of animals remain infected and continue to shed
• Outbreaks are more likely associated with stress invoking a relapse
• The organism may play a role in elevating SCC

Current Research

• Our group currently has grants to evaluate the shedding of the organism in vaginal and ocular secretions as well as milk
• Plan to evaluate for associations between SCC and PCR positive animals
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