Bovine abortion: diagnostic methods
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Bovine abortion continues to result in severe economic losses for beef and dairy producers throughout the world. The average herd abortion rate reported in various studies in the literature varies from 2% to 12%, with much of the variation due to differences in case definition (what constitutes an abortion), methods of data recording, management styles, geography and prevalence of bovine abortifacients. It is interesting to note that in spite of an abundance of vaccine products for abortion prevention, these figures remain virtually unchanged. The endless battle for prevention of abortion losses continues. This presentation will discuss the methods of diagnosis for the common causes of bovine abortion. Possible ruleouts for bovine abortion will also be covered.

Success rates for abortion diagnosis are often very low averaging around 30% in most laboratories. Many cases of non-infectious abortion will go undiagnosed. The Animal Disease Research and Diagnostic Laboratory (ADRDL) at South Dakota State University has adopted a uniform approach for dealing with cases of bovine reproductive failure that is unique in the field of veterinary diagnostic medicine. Unique does not necessarily mean the only way or the best way. All cases of reproductive failure submitted to ADRDL are assigned to one pathologist (i.e. a reproductive disease specialist) who acts as case coordinator until the case is completed. The most obvious advantages of this system are consistency and accountability. As the reproductive disease specialist, I encourage practitioners to feel free to contact me with questions and special needs on difficult cases. I will not always have an answer, but I will always try to lend as much assistance as is possible.

Submission of case material for abortion diagnosis is a straightforward process. However, a high percentage of submissions every year are incomplete, reducing the already limited diagnostic success rates. Start every abortion investigation by obtaining a complete history including a description of on-farm management practices, vaccination protocols and an evaluation of recent on farm events that may have predisposed the cow to abortion disease. Previous disease problems, new purchases and nutritional programs should be recorded. A complete history is received with probably less than 10% of submitted cases.

An intact fetus and placenta are desirable for diagnostic evaluation. If the fetus is large and shipping costs become prohibitive, a complete necropsy should be performed on the farm or at the veterinary clinic. A necropsy is not a difficult task; however, diagnostic success will often hinge on the submission of appropriate samples. Encourage producers to attempt to recover placenta. Often the placenta is retained and must be retrieved from the cow, or is left lying on the ground or lost to scavengers. The bottom line is that the placenta is the most important tissue for abortion diagnosis and without it, the odds of an etiologic diagnosis are reduced. Educate the client as to the importance of the placenta for diagnostic success in abortion cases.

The procedure for a necropsy on bovine fetuses relatively easy and requires no specialized equipment. A sharp knife (Chicago cutlery 62S), pruning shears, meat saw or back saw, scissors, forceps, sterile sample bags (Whirl-Pacs®, Nasco, Ft. Atkinson, WI) and a leakproof container of 10% buffered neutral formalin is all that is required. The pruning shears are excellent for removing the rib cage. The saw is useful for removal of the brain. The secret to success in performing necropsies is repetition and observation. Collect appropriate samples the same way every time and you will not leave out something that may be useful for diagnosis. Record your observations. Package samples so that cross-contamination between tissues will be minimized. If you package lung and placenta in the same bag, and the placenta was laying in the mud for hours, we probably will culture organisms from the mud instead of the more slowly growing abortion pathogens. Fetal stomach content and thoracic fluid are collected with a sterile 18-gauge needle and syringe. Ideally, you should transfer the fluid to a sterile tube and avoid the risks associated with sending syringes with attached needles. Whirl-Pac® bags that are properly sealed will not routinely leak during transit. Postal authorities are becoming increasingly alarmed at the possible risks associated with leaking packages in the mail system. Use common sense and good packing materials to
avoid these risks. An insulated container system is available for purchase from many laboratories and will aid in the shipment of fresh samples to the diagnostic laboratory. Take into consideration the ambient temperature when submitting samples. Avoid weekend stays in the post office if possible. Temperatures over 100°F tend to adversely affect an already autolysed fetus. Conversely, histologic examination of frozen fetuses is also less than ideal.

When a case submission is received at the diagnostic laboratory, samples are processed and submitted to the appropriate laboratories for further diagnostic investigation. For bacteriology, fetal lung and stomach content are our tissues of choice. Occasionally, liver and placenta will be cultured. Routine samples are cultured on Columbia agar with 5% sheep blood. The plates are incubated at 37°C in an atmosphere containing 10% CO₂ and routinely examined at 24 hour intervals. When numerous similar colony types are observed, the organism is isolated and characterized by biochemical reactions. Stomach content is often examined by darkfield microscopy for preliminary identification of *Trichomonas fetus*, *Campylobacter sp.* and leptospirosis. For *Campylobacter sp.*, special media with added antibiotics are used for primary isolation. Specific atmospheric conditions are also required. *Campylobacter jejuni* is the most common species isolated from bovine abortions.

Mycotic abortions are best diagnosed by examination of affected placentas. Gross examination of the placenta will often lead to a presumptive diagnosis based on the characteristic thickening in intracotyledonary spaces. The placenta should be spread out flat and extra debris should be removed for a complete examination. A fluorescent dye called calcofluor white M2R is routinely used to detect fungal elements in placental scrapings and impression smears. Routine fungal cultures are plated on appropriate media including Sabouraud’s and mycobiotic agar, and incubated at 25°C for seven days.

Fluorescent antibody (FA) techniques and polymerase chain reaction (PCR) test are routinely used to detect infectious bovine rhinotracheitis virus (IBR), bovine viral diarrhea virus (BVD) and leptospirosis. Virus isolation procedures are routinely performed on all submitted cases that are considered suitable for examination. Fetal tissues including lung, spleen, kidney, heart and liver are pooled and inoculated on susceptible fetal bovine cell lines. Samples of placenta are usually cultured separately. Cultures are evaluated for cytopathic effect (CPE) after seven days. Cultures free of CPE are passed to fresh cells and observed for an additional seven days. Isolates are identified by FA techniques. Infectious bovine rhinotracheitis virus tends to grow rapidly in cell culture and is usually easy to diagnose on FA. In contrast, BVD is much more difficult to culture and may not be isolated until after the first passage.

Tissues for histologic examination are submitted in 10% buffered neutral formalin. Adequate formalin and thin slices of tissue (<0.5 cm) will insure adequate fixation of fetal tissue. Formalin-fixed tissues are sectioned at 5 μm and stained with hematoxylin and eosin (H&E) and examined microscopically. Tissues including placenta that may be involved in a mycotic infection are stained with Gomori’s methenamine-silver nitrate. Keep in mind that autolysis of fetal tissue is often advanced if the fetus was retained in-utero for a prolonged period.

Immunocytochemistry procedures on formalin fixed tissues are routinely available for BVD and *Neospora caninum*-like protozoal agent. This technique may prove to be of considerable value for cases in which fresh tissue is unavailable for examination. Additional tests are currently being added for common abortion-causing agents.

Serology testing is often of little value in individual animal cases; however, serologic profiling for common abortion agents may be of value in a herd situation. Serologic tests are available for IBR, BVD, leptospirosis, brucellosis, and neosporosis.
Common causes of bovine abortion

Infectious

Epizootic (>20%)

- Neospora caninum-like protozoa
- Infectious bovine rhinotracheitis*
- Leptospirosis*
- Brucellosis**
- Epizootic bovine abortion

Sporadic

- High Incidence
- Neospora caninum
- Salmonella sp.
- IBR
- BVD
- T. pyogenes

- Low Incidence
- Campylobacter
- Listeriosis
- Chlamydia
- BVD
- T. pyogenes

Mycotic

- Trichomoniasis
- Bacillus sp.
- E. coli
- Pasteurella sp.
- DN 599

*Uncommon in vaccinated herds
**Eradication program

Epizootic abortion is relatively uncommon - most epizootics investigated by our laboratory are associated with *Neospora* in dairy herds; historically epizootics have been most common with IBR. Sporadic abortions are most often associated with *Trueperella pyogenes* and mycotic abortion with *Aspergillus fumigatus*.

Non-infectious

Very little information is available concerning the causes and incidence of non-infectious abortion.

A. Genetic
   a. lethal genes
   b. chromosomal abnormalities

B. Nutritional—commonly associated with infertility and neonatal mortality
   a. chronic starvation
   b. vitamin A deficiency
   c. iodine deficiency
   d. manganese deficiency
   e. vitamin E/selenium deficiency

C. Toxic plants
   a. broomweed (*Gutierrezia* spp.)
   b. locoweed (*Astragalus* and *Oxytropis* spp.)
   c. pine needles (*Pinus ponderosa*) incidence may vary within a herd depending on exposure and stage of gestation
   d. ergot (*Claviceps purpurea*)
   e. narrowleaf sumpweed (*Iva angustifolia*)

D. Toxins
   a. nitrates/nitrites
   b. mycotoxins
   c. endotoxins
E. Hormonal
   a. progesterone deficiency
   b. estrogens
   c. corticosteroids

F. Physical
   a. trauma
   b. umbilical cord torsion
   c. stress, surgery (xylazine), transportation, systemic disease

G. Miscellaneous
   a. twinning (common cause in dairy herds)
   b. hyperthermia
   c. allergies and anaphylactic reactions

**Bovine abortion-sample submission**

Fetus and placenta:
The entire fetus and placenta, chilled, not frozen, are the preferred specimens when transportation can be arranged. When the entire fetus cannot be submitted to the laboratory, the following specimens are the minimum if a complete examination is to be done:

<table>
<thead>
<tr>
<th>Formalin fixed</th>
<th>Fresh (chilled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung</td>
<td>lung *</td>
</tr>
<tr>
<td>liver</td>
<td>liver</td>
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<tr>
<td>kidney</td>
<td>kidney</td>
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<tr>
<td>spleen</td>
<td>spleen</td>
</tr>
<tr>
<td>heart</td>
<td>heart</td>
</tr>
<tr>
<td>brain (1/2)</td>
<td>brain*</td>
</tr>
<tr>
<td>skeletal muscle (tongue, diaphragm)</td>
<td>placenta*</td>
</tr>
<tr>
<td>placenta (grossly examine for focal lesions)</td>
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</tr>
</tbody>
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Also collect:

- stomach content-1-3 ml in sterile disposable syringe**
- thoracic fluid or heart blood from fetus-3-5 ml in sterile disposable syringe**

Maternal blood should be collected and 3-5 ml of serum should be separated from the clot. Serology on individual animals is often unrewarding. Samples should be saved for further evaluation in a whole herd profile at a later date, if not submitted with the initial case.

Put the fresh tissues in sterile bags, and chill or freeze if delivery to the laboratory will be prolonged. Put formalin-fixed tissue in an unbreakable, leak-proof container. Label samples accordingly. Ship in an insulated container with enough ice packs to maintain refrigerated conditions until arrival at the laboratory.

* package these tissues in separate Whirl-Pacs®
**transfer to sterile tube if possible

Do not hesitate to contact the laboratory for assistance in sample collection or submissions procedures! Procedures will vary from laboratory to laboratory.