BOVINE ABORTION

Specimens to submit: Tissues are received in best condition if removed at necropsy in the field. Samples should include:

- **Brain**: Formalin-fixed (1/2 of entire tissue)
- **Dam's serum**: 3 - 5 ml from affected cows. Optional, see notes on abortion serology.
- **Heart**: Formalin-fixed (1/2 cm slice)
- **Ileum**: Formalin-fixed
- **Kidney/Liver/Spleen**: Fresh, formalin-fixed (1/2 cm slice)
- **Lung**: Fresh (cranioventral), formalin-fixed (1/2 cm slice)
- **Placenta (very important)**: 3 cotyledons, fresh; 2 cotyledons, formalin-fixed (please submit placenta when possible - this increases the diagnostic success rate)
- **Skeletal Muscle**: Tongue and diaphragm formalin-fixed (1/2 cm slice)
- **Skin (lesions/ear notch)**: Formalin-fixed (1/2 cm slice)
- **Stomach content**: 1-3 ml in sterile syringe or tube, fresh
- **Thoracic fluid**: 1-3 ml in sterile syringe, fresh
- **Thymus/Adrenal gland**: Fresh, formalin-fixed (1/2 cm slice)

*Alternatively, the entire fetus and placenta can be submitted.*

SAMPLING SUGGESTIONS

1. Do NOT freeze fresh tissues; keep them chilled.
2. Always submit placenta if possible! Failure to submit placenta severely diminishes the diagnostic success rate of bovine abortion cases.
3. It may be useful to submit serum from affected and unaffected dams.

COMMON INFECTION AGENTS DETECTED BY ROUTINE EXAMINATION, CULTURE, AND PCR OF FETAL AND PLACENTAL TISSUES

<table>
<thead>
<tr>
<th>Bacteria</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trueperella</em> (Arcanobacterium) pyogenes*, <em>Bacillus</em> spp., <em>Brucella</em> spp., <em>Campylobacter</em> spp., <em>Histophilus somnis</em>, <em>Salmonella</em>, <em>Listeria monocytogenes</em>, <em>Leptospira</em>, <em>Ureaplasma</em>, <em>Mycoplasma</em> (PCR or culture), etc.</td>
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</table>

<table>
<thead>
<tr>
<th>Fungi</th>
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<tbody>
<tr>
<td><em>Aspergillus</em>, <em>Phycomycetes</em></td>
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</table>

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<thead>
<tr>
<th>Protozoa</th>
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<tbody>
<tr>
<td><em>Toxoplasma gondii</em>, <em>Neospora caninum</em>, <em>Tritrichomonas foetus</em></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Viruses</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><em>IBR</em>, <em>BVD</em></td>
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</tr>
</tbody>
</table>

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

- **Eyeball (aqueous)**: Nitrate/nitrite

COMMENTS

- If leptospirosis is suspected, extra effort should be made to deliver freshly aborted, chilled fetuses directly to the lab. PCR testing can be conducted on fetal tissues (kidney). Serology on affected dam sera is very helpful.
- Diagnosis of *Neospora caninum* abortion is based on histopathologic examination of brain, heart, skeletal muscle, liver, lung, and placenta for characteristic lesions. Presence of the organism can be confirmed by immunohistochemistry. Absence of serum antibody in the cow would rule out neosporosis.
- *Tritrichomonas foetus* infection is best diagnosed by placing preputial wash or fetal fluids/stomach contents directly into TF pouch for PCR.
BOVINE CENTRAL NERVOUS SYSTEM DISORDERS

Specimens to submit: Tissues from euthanized or dead animals including:

Blood sample  EDTA tube for lead analysis or cholinesterase inhibition
Eyeball (aqueous)  Cations (calcium); nitrite
Brain (including brain stem)  DO NOT FIX BRAIN IF RABIES TESTING IS DESIRED
                          Non-Rabies: split longitudinally, 1/2 brain fresh; 1/2 brain, formalin-fixed
Colon  Optional, nervous coccidiosis. Several partial loops with contents, fresh. 1 cm pieces, formalin-fixed
Liver  Optional, lead toxicosis. Fresh
Kidney  Optional, lead toxicosis. Fresh
Peripheral Nerves  Depending on clinical signs. Fresh and fixed
Skeletal Muscle  Depending on clinical signs. Fresh and fixed
Spinal cord  Optional, locomotor involvement
                      Entire carcass or vertebral column, fresh OR
                      Dissected cord, fresh w/cross-sections (1/2 cm) of cord from 4-5 levels, formalin-fixed
Rumen content  Fresh/chilled

SAMPLING SUGGESTIONS

1. Entire head can be submitted. Chill all samples before shipment if possible.
2. Do NOT freeze fresh brain or head.
3. Fresh half of brain should be packed carefully to avoid crushing.
4. Fixed half of brain should be incised, at least once, transversely (not longitudinally) into the lateral ventricle to aid fixation if the brain is large.

COMMON AGENTS DETECTED BY ROUTINE EXAM AND/OR CULTURE

Bacteria  Histophilus somnus, Listeria monocytogenes, Trueperella (Arcanobacterium) pyogenes, etc.
Non-infectious  Polioencephalomalacia

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Deficiencies  Magnesium (serum, entire eyeball, fresh/chilled), calcium (serum, fresh/chilled)
Parasites  Coccidia (flotation; feces, fresh/chilled) - NO lesions in brain
Toxicoses  Lead (whole blood in EDTA, liver, stomach contents, fresh/chilled), organophosphate (whole blood in EDTA, brain, rumen, fresh/chilled)
                      Sodium (whole blood in EDTA, brain, rumen, fresh/chilled)
Viruses  Rabies (FA - requires entire brain to be submitted fresh/chilled), herpesviruses: IBR, pseudorabies (PCR - brain, fresh/chilled)

COMMENTS

- Cerebellum and brain stem are affected by most infectious causes of CNS disease and should always be included in submitted samples.
- Many toxic, nutritional, and metabolic causes of CNS disease do not induce lesions in the brain and must be diagnosed by analysis of other tissues. For most toxicoses, submission of rumen contents, complete feed, water and feed components, liver, kidney, serum, and whole blood (in EDTA) as well as brain would include the tissues necessary for diagnosis.

Veterinary Diagnostic Laboratory • Iowa State University • Ames, IA 50011
www.vetmed.iastate.edu/vdl
BOVINE ENTERITIS – CALVES < 2 MONTHS OF AGE

Specimens to submit: Antemortem fecal samples are of value if collected on the first day of diarrhea. Tissues collected from a euthanized or dead calf should include:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fresh and formalin-fixed</th>
<th>10 ml fluid contents, fresh</th>
<th>Several partial loops, fresh</th>
<th>2-3 1cm pieces, formalin-fixed</th>
<th>Formalin-fixed</th>
<th>Two or three 10-15 cm segments, fresh</th>
<th>Three 1 cm pieces, formalin-fixed</th>
<th>Fresh and formalin-fixed</th>
<th>Fresh content and formalin-fixed tissue</th>
<th>Fresh and formalin-fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
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<td>Cecal contents</td>
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<td>Colon</td>
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<td>Ileum</td>
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<tr>
<td>Jejunum</td>
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<tr>
<td>Kidney/Liver/Lung</td>
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<tr>
<td>Mesenteric lymph node</td>
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<td>Rumen</td>
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<tr>
<td>Spleen/Thymus</td>
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</table>

Because autolysis occurs very quickly in bovine intestines, samples removed at necropsy in the field and properly preserved soon after death are usually better than a whole dead calf submitted to the lab.

SAMPLING SUGGESTIONS

1. Samples must be taken as soon after death as possible.
2. Fresh samples should be chilled quickly. DO NOT FREEZE.
3. Intestines do not need to be tied off at the ends.
4. Flush intestinal segments for histopathologic examination with formalin and drop in fixative. Or, gently open ends of 1 cm segments with scissors or forceps to expose mucosa as immersed. Do not split open.
5. Pool all formalin-fixed tissues from each calf in one bag; individual calves can be pooled or kept separate as desired. Package fresh intestines separately from other tissues and each calf in a separate bag.

COMMON AGENTS DETECTED BY ROUTINE EXAM, CULTURE, PCR, AND/OR FECAL FLOTATION

<table>
<thead>
<tr>
<th>Category</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>E. coli, Salmonella spp., Clostridium spp.</td>
</tr>
<tr>
<td>Parasites</td>
<td>Cryptosporidia, Coccidia</td>
</tr>
<tr>
<td>Viruses</td>
<td>Rotavirus, Bovine coronavirus, BVD virus: IHC on lymphoid tissue and skin, PCR on fresh lymphoid tissue/lung</td>
</tr>
</tbody>
</table>

COMMENTS

- In cases of necrotic enteritis, submit both necrotic and adjacent non-necrotic segments fresh and fixed.
- In-house quick tests (acid-fast stained impression smears) may be of value for detection of cryptosporidia. The preferred site for impression smears/mucosal scrapings for cryptosporidia is ileum. As such, it is helpful if fresh ileum is submitted in a separate container.
- Colon is the preferred tissue in which to identify lesions of coronavirus enteritis, for laboratory confirmation with BCV IHC, and for observation of coccidia. Colon should be submitted with all calf diarrhea cases.
### Bovine Enteritis - Calves > 2 Months of Age, Feedlot Cattle, Adults

Specimens to submit: Fecal samples may be of value if collected on the first day of diarrhea. Tissues collected from a euthanized or dead calf should include:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
<td>Fresh/chilled and formalin-fixed</td>
</tr>
<tr>
<td>Any other gross lesions</td>
<td>Fresh/chilled and formalin-fixed</td>
</tr>
</tbody>
</table>
| Colon                   | Several partial loops, fresh/chilled  
                        | Three 1 cm pieces, formalin-fixed |
| Colon contents          | 10 ml fluid contents, fresh/chilled |
| Ileum                   | Two or three 10-15 cm segments, fresh/chilled  
                        | Three 1 cm pieces, formalin-fixed |
| Jejunum                 | Two or three 10-15 cm segments, fresh/chilled  
                        | Three 1 cm pieces, formalin-fixed |
| Liver                   | Fresh/chilled and formalin-fixed |
| Mesenteric lymph node   | Fresh/chilled and formalin-fixed |
| Rumen                   | Fresh/chilled and formalin-fixed |
| Rumen contents          | Fresh/chilled for pH |
| Spleen                  | Fresh/chilled and formalin-fixed |

Samples removed in the field are better than a whole dead animal submitted to the lab.

### Sampling Techniques

1. Samples must be taken as soon after death as possible (within minutes).
2. Intestines do not need to be tied off at the ends.
3. Flush intestinal segments for histopathologic examination with formalin and drop in fixative. Or, gently open ends of 1 cm segments with scissors or forceps to expose mucosa as immersed. Do not split open.
4. Pool all formalin-fixed tissues from each calf in one bag; individual calves can be pooled or kept separate as desired. Package fresh intestines separately from other tissues and each calf represented in a separate bag. Chill fresh tissues before mailing. Do NOT freeze.

### Common Agents Detected by Routine Exam, Culture, PCR, and/or Fecal Flotation

**Bacteria**

- *Salmonella* spp., *Clostridium perfringens*; *Mycobacterium avium* subsp. *paratuberculosis* (Johne’s disease) - PCR on fresh chilled feces; histopathology and acid fast-stains on intestines and mesenteric lymph nodes

**Parasites**

- Coccidia, GI nematodes

**Viruses**

- BVD virus, Bovine coronavirus: IHC on fixed ileum/colon, PCR on feces

### Comments

- BVD mucosal disease diagnosis: Fixed ileum, spleen, mesenteric lymph nodes, skin, heart, lung, and ANY GROSS LESIONS for immunohistochemistry. Fresh/chilled spleen, lung, thymus, and mesenteric lymph node for PCR.
- Coccidiosis is a common cause of diarrhea in this age group. It is necessary to submit feces and/or colon to diagnose coccidiosis.
BOVINE PNEUMONIA

Specimens to submit: Ante mortem samples from acutely affected calves should include:

Nasal swabs, Deep nasopharyngeal swabs, Tracheal wash/lavage

Use a long, Dacron-tipped swab that reaches deep into the nasal cavity
Swabs to be used for virus PCR should penetrate the mucous layer to retrieve epithelial cells.
Submit separate swabs for bacterial culture and virus isolation in saline or transport media. Do not freeze.
Swabs and/or lavage material can be submitted for PCR respiratory panel (bacteria and viruses) as antemortem samples.
See video on sampling techniques.

Serum samples
Acute and convalescent serum from 5-10 affected and 5-10 normal calves. Hold acute samples and submit with convalescent.

Tissues collected from an euthanized or dead calf should include:

Ear notch Heart/Liver Lung
Formalin-fixed Fresh and formalin-fixed Sample 3-4 areas of lung, generous portions of lesions and adjacent unaffected lung, fresh
Four or more thin slices (1 cm) through affected and adjacent unaffected lung, formalin-fixed

Lymph node/Thymus Trachea
Fresh and formalin-fixed Optional, if lesions are observed. Affected portion (10 cm) with larynx, fresh. Several rings at edge of lesion, formalin-fixed.

SAMPLING SUGGESTIONS
1. Fresh tissues should be chilled before shipping. DO NOT FREEZE.
2. Samples for virus detection need to be taken from ACUTE animals at the onset of respiratory signs.
3. Swabs must be kept moist and cold before and during shipment.

COMMON AGENTS DETECTED BY ROUTINE EXAMINATION, CULTURE, AND/OR PCR

Bacteria
*Histophilus somni, Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis, Trueperella (Arcanobacterium) pyogenes*

Viruses
*IBR, BRSV, BVD, BRCV, PI-3*

COMMENTS
- Acute lesions are most likely to hold active causative agents and are usually most prevalent at the interface of diseased/normal tissue. Chronic lesions in dependent tips or lobes may no longer contain primary pathogens.
- Nasal swabs may be of value to identify viruses if sampled in the early stages (exhibiting serous nasal discharge). Nasal swabs may also pick up resident bacterial flora but may be of value in certain acute cases. Ante mortem swabs from several affected calves can be pooled for PCR testing; and results can be compared with testing of swabs from unaffected calves.
- Tracheal washes submitted on ice may be used for both virus and bacteria identification.