

IOWA STATE UNIVERSITY

Veterinary Diagnostic Laboratory

Pathology Submission Guide

BOVINE ABORTION

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

Brain	Formalin-fixed (1/2 cm slice)
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	Fresh/chilled (1 entire kidney), formalin-fixed (1/2 cm slice)
Liver	Fresh/chilled (1/8 - 1/4 of organ), formalin-fixed (1/2 cm slice)
Lung	Fresh/chilled (1/8 - 1/4 of organ), formalin-fixed (1/2 cm slice)
Placenta (very important)	3 cotyledons, fresh/chilled; 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)
Spleen	Fresh/chilled (1/2 of organ), formalin-fixed (1/2 cm slice)
Stomach contents	1-3 ml in sterile syringe or tube, fresh/chilled
Thoracic fluid	1-3 ml in sterile syringe, fresh/chilled
Thymus	Fresh/chilled, formalin-fixed (1/2 cm slice)

Alternatively, the entire fetus and placenta can be submitted.

SAMPLING TECHNIQUES

1. Do NOT freeze fresh tissues.
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.

AGENTS DETECTED BY ROUTINE EXAMINATION OF FETAL AND PLACENTAL TISSUES

Bacteria	<i>Trueperella (Arcanobacterium) pyogenes</i> , <i>Bacillus spp.</i> , <i>Brucella spp.</i> , <i>Campylobacter spp.</i> , <i>Histophilus somnus</i> , <i>Salmonella</i> , <i>Listeria monocytogenes</i> , etc.
Fungi	Aspergillus, Phycomycetes
Protozoa	<i>Neospora caninum</i> (see comments), <i>Toxoplasma gondii</i>
Viruses	IBR, BVD

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Bacteria	Leptospira (see comments below), Ureaplasma, Mycoplasma (culture)
Protozoa	<i>Tritrichomonas fetus</i> infection is best diagnosed by placing preputial wash or fetal fluids/stomach contents directly into TF pouch for culture
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 and FA tests can be conducted on kidney. Serology on 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.

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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)	1/2 brain divided longitudinally, fresh/chilled 1/2 brain, formalin-fixed
Colon	Optional, nervous coccidiosis. Several partial loops with contents, fresh/chilled. 1 cm pieces of several loops, formalin-fixed
Liver	Optional, lead toxicosis, fresh/chilled
Kidney	Optional, lead toxicosis, fresh/chilled
Spinal cord	Entire carcass or vertebral column, fresh/chilled Dissected cord, fresh/chilled Cross-sections (1/2 cm) of cord from 4-5 levels, formalin-fixed
Rumen contents	Fresh/chilled
SAMPLING TECHNIQUES	
<ol style="list-style-type: none">1. Entire head can be submitted. Chill 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.	
AGENTS DETECTED BY ROUTINE EXAM	
Bacteria	<i>Histophilus somnus</i> , <i>Listeria monocytogenes</i> , <i>Arcanobacterium pyogenes</i> , 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), pseudorabies virus (FA, VI); bovine herpesvirus (brain, fresh/chilled)
COMMENTS	
<ul style="list-style-type: none">• 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, and whole blood (in EDTA) as well as brain would include the tissues necessary for diagnosis.	

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BOVINE ENTERITIS – CALVES < 2 MONTHS OF AGE

Specimens to submit: Antemortem fecal samples are of value if collected on the first day of diarrhea. Alternatively, tissues should be removed from a euthanized calf.

Abomasum	Fresh/chilled and formalin-fixed
Cecal 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
Spiral colon	Several partial loops, fresh/chilled Several 1 cm pieces, formalin-fixed
Spleen	Fresh/chilled and formalin-fixed
Ear notch	Formalin-fixed

Because autolysis occurs very quickly in bovine intestines, samples removed at necropsy in the field are usually better than a whole dead calf 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 in a separate bag. Chill fresh tissues before mailing. Do NOT freeze.

AGENTS DETECTED BY ROUTINE EXAM

Bacteria	<i>E. coli</i> , <i>Salmonella spp.</i> , <i>Clostridium spp.</i> , <i>Enterococcus durans</i>
Parasites	<i>Cryptosporidia</i> , Coccidia
Viruses	Rotavirus, Bovine coronavirus (BCV)

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

BVD virus	IHC on fixed ileum, colon, mesenteric lymph node, spleen, skin, and any gross lesions; VI on chilled mesenteric lymph node, spleen, kidney, thymus, and lung
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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 and for laboratory confirmation with BCV IHC. **Colon should be submitted with all calf diarrhea cases.**

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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. From euthanized or dead animals, tissues should include:

Abomasum	Fresh/chilled and formalin-fixed
Any other gross lesions	Fresh/chilled and formalin-fixed
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.

AGENTS DETECTED BY ROUTINE EXAM

Bacteria	<i>Salmonella</i> spp., <i>Clostridium perfringens</i>
Parasites	Coccidia
Viruses	BVD virus (see comments below)

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Bacteria	Culture of feces, mesenteric lymph nodes, and intestine; histopath and acid fast-stains on intestines and mesenteric lymph nodes
Parasites	Coccidia and GI nematodes; (feces, fresh/chilled for fecal flotation)
Viruses	Bovine coronavirus (feces for ELISA, fixed ileum and colon for histopath and IHC)

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 mesenteric lymph node, and kidney for virus isolation.
- Coccidiosis is a common cause of diarrhea in this age group. It is necessary to submit feces and/or colon to diagnose coccidiosis.