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. 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 INFECTIOUS AGENTS DETECTED BY ROUTINE EXAMINATION, CULTURE, AND PCR OF FETAL AND PLACENTAL TISSUES

Bacteria	*Trueperella (Arcanobacterium) pyogenes, Bacillus* spp., *Brucella* spp., *Campylobacter* spp., *Histophilus somnus, Salmonella, Listeria monocytogenes*, *Leptospira*, *Ureaplasma*, *Mycoplasma* (PCR or culture), etc.
Fungi	*Aspergillus*, Phycomycetes
Protozoa	*Toxoplasma gondii, Neospora caninum*, *Tritrichomonas foetus*
Viruses	IBR, BVD

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.

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:

Abomasum	Fresh and formalin-fixed
Cecal contents	10 ml fluid contents, fresh
Colon	Several partial loops, fresh
	2-3 1cm pieces, formalin-fixed
Ear notch	Formalin-fixed
Ileum	Two or three 10-15 cm segments, fresh
	Three 1 cm pieces, formalin-fixed
Jejunum	Two or three 10-15 cm segments, fresh
	Three 1 cm pieces, formalin-fixed
Kidney/Liver/Lung	Fresh and formalin-fixed
Mesenteric lymph node	Fresh and formalin-fixed
Rumen	Fresh content and formalin-fixed tissue
Spleen/Thymus	Fresh and formalin-fixed

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

Bacteria	*E. coli, Salmonella* spp., *Clostridium* spp.
Parasites	Cryptosporidia, Coccidia
Viruses	Rotavirus, Bovine coronavirus, BVD virus: IHC on lymphoid tissue and skin, PCR on fresh lymphoid tissue/lung

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:

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.

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.

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BOVINE PNEUMONIA

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

Nasal swabs, Deep naso-pharyngeal 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.
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 a euthanized or dead calf should include:

Ear notch	Formalin-fixed
Heart/Liver	Fresh and formalin-fixed
Lung	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	Fresh and formalin-fixed
Trachea	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.