



## **TIPS ON SAMPLE SUBMISSION FOR DIAGNOSIS OF NEONATAL CALF DIARRHEA**

*J. Dustin Loy DVM, PhD, Bruce Brodersen, DVM, PhD, Veterinary Diagnostic Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln*

It will soon be that time of year again when the feces are flowing faster and more fluid than anyone likes to see in neonatal calves. This article reviews some points to keep in mind when collecting antemortem or postmortem samples from calves for submission to the Veterinary Diagnostic Center (VDC).

Live untreated calves in the early stages of disease are the best candidates for use in arriving at an etiologic diagnosis. However, owner reluctance to sacrifice a calf usually precludes this option. Frequently, samples received at the VDC consist of sections of small and large intestine in various stages of decomposition which may display virtually a rainbow of colors. Calves from which the samples originated frequently have been treated with one or more antibiotics. When intestinal samples are submitted, the time between death and post mortem examination usually ranges between 3 and 12 hours. Granted, there may be legitimate reasons for these types of samples but conclusive results are not often derived from submission of these samples. Practitioners faced with the decision of what to "send in" might want to reflect on a few points before collecting samples. Given the power of molecular techniques, such as PCR to detect tiny amounts of pathogens, a herd-level diagnosis can frequently be achieved by collection several grams of feces that is representative of the clinical picture. The VDC runs a multiplex PCR panel that enables testing for the most common viruses, bacteria, and parasites in one assay. Fecal specimens can be pooled together or run individually to determine the pathogens present. Additionally, these tests enable quantification of the amounts of each pathogen in the specimens using a cycle threshold, or Ct value, to assist in interpretation of diagnostic findings.

When submitting feces from calves with diarrhea, it is advisable to collect several grams (preferably 10 or more) of feces from at least three calves which have not been treated (especially when bacterial enteritis is suspected). Swabs DO NOT contain adequate material to ensure a uniform sample for PCR testing. Generally, a few days after infection occurs, concentration of the etiologic agent/s in feces may be too low for detection. Considering clinical signs may not appear for at least a day after infection, the window of opportunity for collecting a fecal sample with detectable amounts of the etiologic agent/s is relatively small. Usually about 12 to 18 hours after initiation of clinical signs the value of a fecal sample diminishes rapidly. Feces or distal large intestine contents are superior specimens compared to small intestinal contents. member, most coronavirus infections and coccidia infections occur primarily in the large intestine in cattle.

## Common Pathogens

In calves that are less than 4 - 5 days old *Escherichia coli* would be the primary differential followed by *Clostridium perfringens* type C. Gross lesions are rarely seen in cases of colibacillosis, however, bacterial colonization is observed histologically in the ileum. In Clostridial enteritis, the lesion is that of severe necrotic enteritis and is usually located in the proximal ileum and jejunum. Rotavirus may be implicated any time before 2 - 3 weeks of age but is more commonly seen in calves older than 4 - 5 days. Coronavirus can be encountered at nearly any age of the calf but after a few weeks of age (as with rotavirus) is generally not a severe problem. There is no gross or histologic distinction between the lesions caused in the small intestine by either rotavirus or coronavirus. Coronavirus does cause lesions of mucosal cryptitis in the colon which rotavirus does not. Vaccine strains of rotavirus and coronavirus may be shed in feces but only sporadically and at a low concentration compared to virulent strains. Differentiation between vaccine and field strains cannot be made by conventional methods. Because of the protozoan life cycle, they should be considered in a differential diagnosis after five days of age. The most common protozoan encountered is *Cryptosporidium parvum*. *Eimeria spp.* are not commonly seen in calves less than three weeks old.

## Less Common Pathogens

Some of the less commonly encountered intestinal pathogens are *Salmonella* sp., bovine virus diarrhea (BVD) virus and bovine adenovirus. Salmonellosis is occasionally encountered but usually at an age of 2 - 3 weeks. However, recent outbreaks of *Salmonella*, including *Salmonella* serovar Dublin have been found in high risk or mixed source dairy calves. Often the lesion seen with salmonellosis is that of catarrhal or hemorrhagic enteritis and is usually confined to the ileum in the early stages but can be seen in the jejunum and colon later in the disease. BVD may cause lesions similar to those seen in salmonellosis and coronavirus infection. Attaching and effacing *E. coli* can occasionally cause diarrhea in 2-4 week old calves. These are frequently diagnosed by histopathological observation of the bacteria, combined with culture and PCR testing for the appropriate virulence factors.

While PCR testing of feces has replaced most of the old test methods, it is still important to collect intestinal segments from appropriate locations within the intestinal tract. At a minimum, a segment of ileum and colon should be submitted. Minimum samples for histopathology should include at least two nonadjacent sections each from the ileum and colon. Depending on the suspected disease, fresh and fixed segments from other regions of the intestinal tract are necessary. As noted above, autolytic changes greatly reduce the chances of an accurate diagnosis. Autolysis begins immediately after death and because of this fact it is important that samples be collected as soon as possible. Within minutes, epithelial cells begin to slough off the intestinal mucosa beginning at the tips of villi and are easily washed away during processing. When present, mucosal crypt and deeper lesions may still be visualized but the ability to evaluate villous length and changes in the epithelium at the distal end of villi is lost. Intestine segments should be opened before immersion in formalin and do not need to be any longer than one inch (an unopened segment of intestine immersed in fixative even from a euthanized animal will have autolytic changes in the mucosa). Sloughing of villous epithelium due to autolysis greatly hampers accurate histologic diagnosis of the most common enteric diseases. Lesions are seen grossly in the jejunum. Segments of

Mesenteric lymph node and Peyer's patch are best for BVD virus identification. For bacterial culture, the ileum is preferred over jejunum unless obvious lesions are seen grossly in the jejunum. Segments of fresh small intestine and colon need not be longer than 6 - 8 inches. Diagnosis of *Cryptosporidia parvum* is either by identification of the organism histologically or acid-fast stained smears of intestinal contents. Coccidia are identified either on fecal floatation or by histology.

One of the best methods of prevention of calf diarrhea is by utilizing the Sandhills Calving System. Sometimes even the best management may realize outbreaks of diarrhea but with proper and timely sample submission a quick and accurate diagnosis can be made.

Pathogen	Age	Test	Specimen
<i>E. coli</i> (F5/K99)	2-5 days	PCR Panel/Culture	Feces
Cryptosporidium	≥ 5 days	PCR Panel/Acid Fast	Feces
Rotavirus	≥ 7 days	PCR Panel	Feces
Coronavirus	≥ 7 days	PCR Panel	Feces
<i>C. perfringens</i>		Histopath/Culture	Fixed/Fresh Intestine
<i>Salmonella</i>	2-12 weeks	PCR Panel/Culture	Feces
Eimeria (Coccidia)	≥ 2 weeks	Floatation	Feces
Coronavirus	≥ 7 days	PCR Panel	Feces

Questions about PCR Calf Panel Testing Contact:

Dr. J. Dustin Loy DVM PhD DACVM

Veterinary Microbiologist

Phone: 402-472-1434

Email: jdloy@unl.edu