

The Usefulness of Antemortem Fecal Samples as an Alternative Diagnostic Specimen for the Etiological Diagnosis of Neonatal Calf Scours

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Abstract

Practitioners commonly face a "diagnostic dilemma" when dealing with neonatal calf scours outbreaks. Most are aware that an ideal specimen for investigating an enteritis problem is an acutely ill, untreated, sacrificed, and immediately necropsied animal. Unfortunately in a diagnostic sense but understandably, the cattle producer does not see this as a very palatable approach. This reluctance is in part due to the fact that one calf represents a significant economic unit to the producer and because many times the animal can be saved with treatment. Therefore, many times the diagnostic lab does not receive acute cases to perform diagnostic tests upon and primary pathogens may be long gone due to chronicity. Postmortem autolysis is another important hindrance to diagnostic success. When calves are treated until they die, the window of opportunity to collect "fresh" tissue is no longer under the veterinarians control when compared to sacrificing the animal and immediately doing a necropsy and properly collecting specimens.

In contrast, swine producers are ready and willing to sacrifice live acutely ill piglets to determine a specific etiological diagnosis and subsequently enjoy a much higher diagnostic success rate (90% or greater). However, when traditional neonatal calf scour submissions (bodies and tissues) are utilized, the diagnostic success rate suffers greatly due to the submission of chronic and/or autolyzed specimens (success less than 70%). One solution to this "diagnostic dilemma" was to analyze and evaluate alternative diagnostic specimens. This South Dakota Animal Disease Research and Diagnostic Laboratory of SDSU conducted a 6 month study in 1993 with two purposes: 1. to determine the diagnostic success rate of antemortem fecal specimens from calves in the acute stages of diarrhea, relative to the diagnostic success of shipped tissue specimens and to whole bodies brought to the laboratory; and 2. to encourage the use of antemortem fecal specimens as an alternative diagnostic tool, if the study indicated usefulness.

From January 1 through June 30, 1993 the laboratory received 721 antemortem fecal specimens, of which 313 came with completed supplemental antemortem fecal survey forms. We found 71.5% of the antemortem fecal samples to be positive for one or more of the four major enteric pathogens for calves under 30 days old (ie rotavirus, coronavirus, K99 *E. coli* or cryptosporidia). Testing for the same pathogens in other submissions types during the same time period resulted in the following diagnostic success rates: whole bodies = 75.8%; shipped tissues = 67.1%. This suggests that antemortem fecal specimens can be a useful alternative diagnostic tool under the right conditions, when investigating calf scours. Further details and pitfalls to be aware of will be discussed during the presentation.

Veterinary practitioners commonly face a "diagnostic dilemma" when investigating outbreaks of neonatal calf scours. Most practitioners are aware from their veterinary training that an ideal specimen for determining the etiology of an enteritis problem in any species is an acutely ill, untreated, sacrificed and immediately necropsied animal. This ideal is dictated by the two facts: a) the opportunity to detect primary infectious agents in the gut can be quite short; and b) the intestinal tract, rich in metabolic enzymes and abundant in normal flora, rapidly decomposes after death. The dilemma occurs because the cattle producer does not view sacrificing a live, 'not too sick' calf as a very palatable situation. This reluctance is somewhat understandable since one calf often represents a significant economic unit to the cattle producer and because many times the animal can be saved with treatment.

Therefore, many times the diagnostic lab does not receive acute cases to perform diagnostic tests upon and primary pathogens may be long gone due to chronicity. Postmortem autolysis is another important hindrance to diagnostic success. When calves are treated until they die, the window of opportunity to collect "fresh" tissue is no longer under the veterinarians control when compared to sacrificing the animal and immediately doing a necropsy and properly collecting specimens.

In contrast, swine producers are much more willing to sacrifice live acutely ill piglets to determine a specific etiological diagnosis and subsequently enjoy a much higher diagnostic success rate in the investigation of neonatal enteritis in piglets (greater than 90%). The willingness of the swine producer to bring in one or two live piglets for diagnostic procedures is in part due to the fact that a couple of sick piglets out of a barn of 100 to 300 or more piglets, represents a much smaller economic unit for the producer.

Because of these factors, the typical neonatal calf scour submission to veterinary diagnostic laboratories include a high percentage of decomposed and chronic cases. The diagnostic success rate suffers greatly because of autolysis and chronicity, and is typically in the range

of between 50 and 70%. One solution to this "diagnostic dilemma" was to analyze and evaluate alternative diagnostic specimens.

The South Dakota Animal Disease Research and Diagnostic Laboratory of SDSU conducted a 6 month study (January through June) in 1993 with two purposes: 1. to determine what diagnostic success rate could be achieved utilizing antemortem fecal specimens and stressing the importance of sending acute specimens. 2. to encourage the use of antemortem fecal specimens as an alternative diagnostic tool, if the study indicated a useful role for such specimens.

During the first six months of 1993, the South Dakota diagnostic lab received 1,437 neonatal bovine enteritis cases requesting full diagnostic services. This included 728 antemortem fecal cases, 571 tissue cases from field necropsies, and 138 wholebody cases necropsied by the laboratory (89% were dead calves). The overall diagnostic success rate for these three submission categories is listed below. Diagnostic success rate was defined as the ability to identify one or more known enteric pathogens of neonatal calves from the specimens submitted.

Overall Diagnostic Success Rate (%) by Submission Type

Whole Bodies = 75.8%
Tissues = 67.1%
Antemortem Fecals = 71.5%

In our service area, we have found for the past several years a relatively high incidence of *Cryptosporidium* sp. infections in our neonatal calf population. Typically, we see this parasite present in between 30 and 50% of our neonatal calf enteritis submissions. I believe that cryptosporidiosis is an important factor in the calf scour problems we see in the northern Great Plains.¹ But since the exact role cryptosporidia plays in the baby calf scours complex is still uncertain and somewhat controversial.^{2,3,4,5} I have also presented the diagnostic success rate for the same cases excluding *Cryptosporidium* below:

Diagnostic Success Rate (%) By Submission Type Excluding Crypto

Whole Bodies = 66.7%
Tissues = 46.6%
Antemortem Fecals = 51.5%

As the two tables show above, the diagnostic success rate for antemortem fecal specimens was less than that for whole bodies, but surpassed that of mailed in tissue specimens. It appears that by comparison to a previous study at this lab, that the diagnostic success

rate of antemortem fecal specimens was improved by stressing the importance of submitting acute cases. The previous study (1985) indicated an overall diagnostic success rate of less than 50% for fecal samples, compared to an overall success rate of 71.5% for this study.⁶ Although 89% of the whole bodies were submitted dead, the better diagnostic success rate for this category likely reflected two things. Optimal tissue specimens were consistently collected by professional diagnosticians at the laboratory and most of the whole bodies were probably fresher than the average mailed in tissue specimen.

Pathogens were identified in 521 of the 728 antemortem fecal specimens submitted. The standard testing protocol for antemortem fecal samples at our laboratory includes bacterial culture with enrichment media for K99 *E. coli* and *Salmonella*; electronic microscopic examination for rotavirus, coronavirus, adenovirus, Bredavirus or others; and acid fast staining of fecal smears for light microscopic examination to detect *Cryptosporidium*. The 521 positive samples contained the following pathogens listed by percentage:

Pathogens Present in 521 Positive Antemortem Fecal Specimens (%)

<i>Cryptosporidium</i> only	35.7%
Rotavirus only	19.2%
Rotavirus and <i>Cryptosporidium</i>	14.0%
Coronavirus only	8.1%
K99 <i>E. coli</i> only	6.3%
Coronavirus and <i>Cryptosporidium</i>	5.6%
Rotavirus and Coronavirus	4.4%
K99 <i>E. coli</i> and <i>Cryptosporidium</i>	2.3%
<i>Salmonella</i> only	2.3%
Miscellaneous	2.1%
	<hr/> 100%

To obtain more information about the usefulness of antemortem specimens and to alert the lab about potential pitfalls in interpretation of the results, the practitioner could send in their antemortem fecal specimens with a completed survey form and obtain a price break (see appendix). During this time period, we received 313 completed survey forms with the antemortem fecal specimens. Beef calves contributed 82.7% of the specimens and dairy 5.1%. The rest were mixed beef and dairy breeds (8.3%), primarily from beef operations. The calves averaged 9.95 days of age \pm 6.24 days. There were 73 \pm 89 other neonatal calves on the farm at the time of fecal collection and 15 \pm 26 other sick calves at home. The number of other calves dead at the time of submission was 2 \pm 3 at the time of collection. This suggests that antemortem fecal samples were largely used before the death loss from scours was high in the herd.

SDSU ADRDL SUPPLEMENTAL SUBMISSION FORM
NEONATAL BOVINE FECAL SAMPLES ONLY

(One form for each calf)

All samples should be from calves ≤ 30 days old. Send chilled, fresh samples to ADRDL, SDSU, Box 2175, Brookings, SD 57007-1396. If you have questions, call Dr. D.H. Zeman at (605) 688-5171.

VETERINARIAN _____
AND ADDRESS: _____

OWNER AND _____
COMPLETE ADDRESS: _____

BREED(S): _____ EXACT AGE IN DAYS: _____ CALF ID: _____
TOTAL NO. OF CALVES ON FARM ≤ 30 DAYS OLD: _____
NO. OF CALVES ≤ 30 DAYS OLD WITH DIARRHEA: _____
NO. OF CALVES ≤ 30 DAYS OLD ASSUMED DEAD FROM DIARRHEA: _____
WAS THIS CALF TREATED? _____ WITH WHAT? _____

Answer the following questions relative to time of specimen collection and to the nearest 12 hours

DID THIS CALF RECEIVE AN ORAL ROTA/CORONA VACCINE? _____
IF YES, WHEN? _____ hours ago or _____ days ago. WHAT PRODUCT? _____

DID THIS CALF RECEIVE AN E. COLI VACCINE? _____
IF YES, WHEN? _____ hours ago or _____ days ago. WHAT PRODUCT? _____
ROUTE? _____

TO THE NEAREST 12 HOURS, WHEN DID SCOURS START IN THE HERD?
_____ hours ago or _____ days ago.

TO THE NEAREST 12 HOURS, WHEN DID SCOURS START IN THIS CALF ONLY?
_____ hours ago or _____ days ago.

LIST KNOWN LABORATORY CONFIRMED SCOURS PATHOGENS ON THE FARM IN THE LAST TWO YEARS _____

The purpose of this study is to gain information that will be helpful to increase the diagnostic success rate for bovine neonatal scours investigations. Our hypothesis is that fecal specimens from calves in the ACUTE stages of clinical diarrhea will increase the diagnostic success rate when compared to calves or tissues submitted several days after the onset of the disease and treatment.

To qualify for the HALF-PRICE break: 1)The above information must be supplied in entirety. 2)Your permission is given for us to send a follow-up herd survey to your client. Thank you for your cooperation. Results will be shared after our first full cycle of calf scours has taken place.

FOR LAB USE ONLY

LAB NOTES

DATE RECEIVED _____

ESTIMATED VOLUME OF FECES: _____ ml

EM RESULTS: _____

BACTERIA CULTURED: _____

CRYPTOSPORIDIA RESULT: _____

The surveyed cases were largely from calves in the acute stages of diarrhea. Of the surveyed cases, 70% came from calves that had been scouring less than 48 hours. The volume of feces collected did not appear to be a major factor in the detection of pathogens. An ideal fecal sample to work with was around 10 to 20 ml, collected fresh, chilled and sent to the lab on ice packs. Producers could also collect samples in clean containers (jars or plastic bags) and take the samples to their local veterinarian for subsequent submission to the lab.

It is important to keep in mind that a diagnosis made from antemortem fecal specimens is presumptive, since there is no way of correlating pathologic lesions with the pathogens identified from such specimens. However, the presumption appears relatively safe if the animal is clinically ill and if the signalment and history are compatible. The study suggests that antemortem fecal samples are sensitive in detecting cryptosporidia infections and may be under-sensitive in detecting coronavirus infections when compared to the other specimen types. Approximately equal sensitivities were demonstrated for rotavirus and K99 *E. Coli* detection. Another pitfall to be aware of is that false positive EM results for viruses can occur up to 5 days after a calf is given an oral vaccine containing rotavirus or coronavirus. If other per os biologicals that contain live organisms come on the market, the same pitfall could occur. Such information should be provided on the history form to aid the diagnostician in interpretation.

Some clinicians state that an etiological diagnosis **IS NOT** necessary to **institute treatment** of baby calf scours. There is some truth to that statement, however I firmly believe that an etiological diagnosis **IS** necessary to **correctly evaluate and modify treatment regimens**. Also, an etiological diagnosis **IS** necessary to **accurately institute and evaluate various preventative measures including the success or failure of vaccination programs**. Informed clients want

to know and the veterinarian is going to be asked, "What do they got Doc, and are we doing all that we can to get them through it?" An etiological diagnosis will help the veterinarian answer that question in a professional manner.

In summary, the antemortem fecal specimen is a useful diagnostic tool for the investigation of the etiological diagnosis of neonatal bovine scours. It is useful on farms where calves have not yet died but have clinical disease. It is useful and palatable on farms where the client is unwilling (as often is the case) to sacrifice a live acutely ill calf. This especially happens when the disease incidence is low, but still some diagnostic information is desired. It is also a useful alternative on farms where previously submitted tissues or dead calves yielded no results due to either chronicity of disease or postmortem autolysis. The diagnostic success rate of antemortem fecal samples surpassed that of mailed in tissue specimens, when it was stressed to submit samples from acute cases (calves scouring less than 48 hours). The interpretation of results from antemortem fecal samples requires some assumptions and there are some pitfalls to be aware of as discussed. However, the assumptions and pitfalls do not differ markedly from the same problems faced when evaluating the results from autolyzed tissue specimens.

References

1. DA Moore and DH Zeman. Cryptosporidiosis in neonatal calves: 277 cases (1986-1987). *J Am Vet Med Assn* 198;1969-1971, 1991.
2. S Tzipori. Cryptosporidiosis in perspective. *Advances in Parasitology* 27:63-129, 1988.
3. L Xiao, RP Herd and DM Rings. Concurrent infections of *Giardia* and *Cryptosporidium* on two Ohio farms with calf diarrhea. *Vet Parasitol* 51:41-48, 1993.
4. DA Moore. Minimizing morbidity and mortality from cryptosporidiosis. *Vet Med* August, p. 811-815, 1989.
5. CE Kirkpatrick. *Cryptosporidium* infections as a cause of calf diarrhea. *Vet Clin of No Am* Vol. 1(3):515-528, 1985.
6. JE Collins. Enteritis in young calves. Presented at the Western States Veterinary Conference, Las Vegas, NV, 1985.