

Nasdaq: ICCC

## First Defense Tri-Shield® Reduces Rotavirus Shedding in Newborn Calves

Protect the calf and the environment.

### QUICK READ

- First Defense Tri-Shield® is a single-dose USDA approved veterinary biologic containing guaranteed levels of *E. coli*, Coronavirus and Rotavirus hyper-immunized colostrum antibodies.
- First Defense Tri-Shield® is proven to be effective for passive immunity against scours causing pathogens, specifically; aiding in the reduction of mortality and morbidity from scours caused by K99+ *E. coli* and Coronavirus while also reducing the severity and duration of scours caused by Rotavirus.
- Rotavirus disease amplifies within a herd when infected calves shed the pathogen into the environment thereby exposing and potentially infecting herd mates.
- Limiting shedding will limit environmental pathogen load, reduce level of disease challenges herd mates are exposed to, and reduce the frequency and severity of rotavirus scour outbreaks.
- First Defense Tri-Shield® significantly reduced rotavirus shedding during the height of the disease (day 2 post challenge) in calves who achieved successful passive transfer as a result of a quality colostrum feeding (1 gallon, pooled and pasteurized) after birth.

### Risk factors associated with Rotavirus disease outbreaks

Disease amplification or a significant disease outbreak can occur in a population of calves when pathogens propagate within infected calves and are broadcast/shed by these calves into the environment where all calves are housed. This environmental pathogen load builds-up to a level which can overwhelm the immune status of herd mates, creating significant illness and death loss. A core driver of new rotavirus infections is large exposure to the pathogen shed into the environment over a short span of time, particularly under conditions of stress. It has been suggested that the major mode of rotavirus spread is from actively infected calves to susceptible ones. Increasing environmental contamination will amplify diarrheal disease in calves (Holland, 1990). Calves will excrete rotavirus in feces starting the second day of infection. Infected feces may contain as many as  $10^{10}$  virus particles/gram (Radostits et al 2007).

Decreased shedding and the accumulation of virus in the calf environment are very important risk factors in rotavirus morbidity and diarrheal disease amplification throughout the calf herd. The virus survives in feces for several months and is resistant to many disinfectants (Radostits et al 2007). A product that reduces the number of intestinal cells infected and the quantity of virus shed should decrease disease amplification and morbidity in a herd of calves. This product could not only impact individual calf health but also would provide partial herd immunity in controlling the lateral spread of disease through less environmental contamination.

## Study Design

The objective of this trial was to evaluate if First Defense Tri-Shield® would reduce rotavirus shedding in calves receiving First Defense Tri-Shield® at birth when rotavirus exposure mimicked typical on-farm conditions (day 6 challenge) and with all calves receiving quality colostrum aiming for successful passive transfer.

### The treatments were

- First Defense Tri-Shield®: calves received one 30-cc tube of First Defense Tri-Shield® after birth followed by 4 liters of pooled colostrum.
- Control: calves received 4 liters of pooled colostrum and a placebo tube containing everything within the First Defense Tri-Shield® formula except the active ingredient (specific antibodies for *E. coli*, coronavirus, and rotavirus).

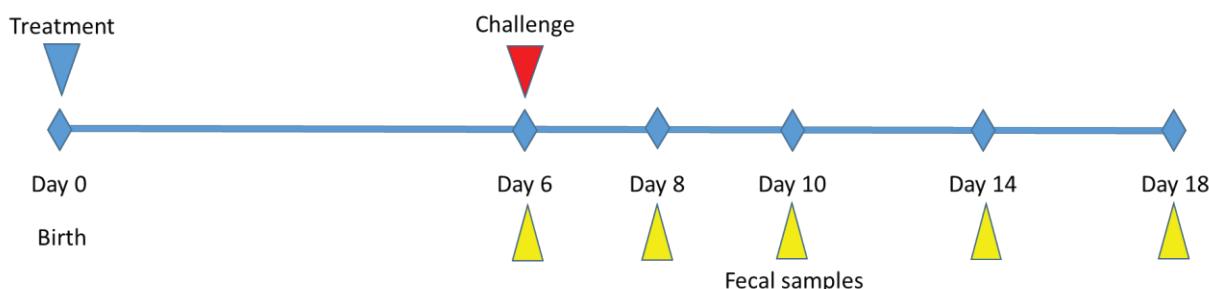
Twenty calves were enrolled. All calves were processed according to “clean sand” collection protocol at birth with navels dipped immediately. This “clean sand” model is highly controlled yet conducted within a commercial facility. Calves were then moved to the processing area where the naval was again dipped with a tincture of iodine and the following prophylactics administered: vitamins, intranasal vaccine, and Excede®. Immediately after processing, and within 30 minutes of birth, calves received either First Defense Tri-Shield® or Placebo randomly administered. Veterinary staff administering treatments and recording data were blinded to the treatment. Four liters of clean, quality, pooled colostrum was administered 2 hours after initial processing. Initial processing also included unique individual animal identification, body weight, blood samples for FTP and BVD PI status were taken.

Calves were housed in individual hutches with wire fence in the front representing individually housed set-ups typical for the industry. Hutches were placed in naïve environment without a history of housing calves or cows.

On Day 6 all calves were challenged with live rotavirus. Health scores, including fecal scores, were recorded twice daily. Fecal samples from all calves were collected for fecal shedding evaluation on days 6 (before challenge), 8, 10, 14, and 18. Experimental design is illustrated in Figure 1.

Quantitative PCR was used to quantify amount of rotavirus DNA particles present. Threshold cycle frequency was used to compare rotavirus shedding between treatments. The higher threshold cycle frequency the less rotavirus particles being shed into the environment.

**FIG 1 Experimental Design**



## Results

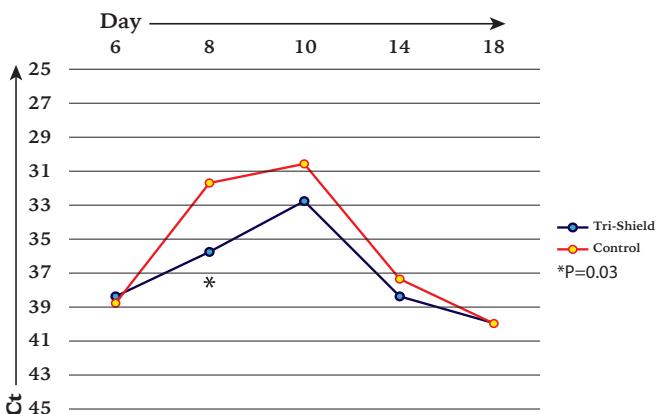
There were no calves that had failure of passive transfer, defined as a TP < 5.0 g/dL. There was a trend ( $P = 0.07$ ) for the Control group to have a higher total protein levels (6.5 vs 6.2 g/dL).

There were no differences observed in clinical symptoms (scour scores, scour days, treatments) between First Defense Tri-Shield® and Control calves. The live rotavirus pathogen dose was not high enough to manifest clinical signs but was high enough to inducing shedding.

Control calves tended to have higher shedding of rotavirus with day 8 being significantly higher than in First Defense Tri-Shield® calves. The threshold cycle frequency was 4.52 cycles lower which would correspond to approximately 1.5 log greater shedding of rotavirus in calves not receiving First Defense Tri-Shield®.

See Figure 2.

**FIG 2 Threshold Cycle Frequency**  
indication of pathogen shedding



## Conclusion

First Defense Tri-Shield® provided an additive effect to a quality colostrum program by significantly reducing rotavirus shedding in feces of calves even when they achieved successful passive transfer. Disease amplification and transmission to herd mates can be minimized by limiting the amount of rotavirus in the environment. Quantitative PCR (qPCR<sup>3</sup>) measures the amount of rotavirus DNA in a fecal sample. DNA can still be detected even when the virus is no longer viable, pathogenic or able to infect herdmates. Antibodies in Tri-Shield bind to the pathogen making it no longer infectious, but the rotavirus DNA would still be present in this case. The reduction in shedding and impact on the spread of disease could be much greater than this methodology was able to detect.

## SOURCES

1. Holland R: Some infectious causes of diarrhea in young farm animals. Clinical Microbiology Reviews, 1990; 345-375.
2. Radostits O.M, Gay C.C, Hinchcliff K.W, Constable P.D: Veterinary medicine-A textbook of the diseases of cattle, horses, sheep, pigs and goats. USA: Saunders 10th edition; 2007. p: 1286-1296.
3. qPCR Definition: measures the number of DNA amplification cycles that are required for a sample to reach a given threshold. The more amplification cycles required, the lower the amount of target DNA in the sample. After 40 amplification cycles the sample is considered to be negative for the target DNA.

### Research Team

Trial Coordinator: Andrew Skidmore DVM, PhD

Site Investigator: Jan Gawthrop DVM, Calf Care, North Manchester, IN

#### Collaborators:

David Hurley BA, PhD University of Georgia College of Veterinary Medicine  
Scott Sturgeon DVM, Sturgeon Veterinary Services, Hydro, OK  
Steve Hayes DVM, Day1 Technology, Winona, MN

Lab Analysis: University of Wisconsin Diagnostic Lab

Statistics: ACER Consulting, Guelph, ON