

### **Research Report**

Nasdaq: ICCC

### *In Vitro* Neutralization of Calf-Guard<sup>®</sup> with Colostral Whey: Using Lab Results to Understand the Outcome at the Calf-Level

Jessica Bonnema, DVM, MS

#### **Quick Read**

- Calf diarrhea (scours), especially that caused by pathogens such as rotavirus and coronavirus, is a major cause of disease in unweaned dairy and beef calves. While most herds strive for good colostrum management, those practices could still fall short of providing full immune protection. For additional scour protection some herds provide an oral scour vaccine at birth. Calf-Guard<sup>®</sup> is a commonly used, modified-live oral scour vaccine against bovine rotavirus and coronavirus.
- Colostrum samples from across the U.S. were analyzed for their ability to inactivate (neutralize) the vaccine (Calf-Guard®) *in vitro*. In all samples from both vaccinated and unvaccinated cows, Calf-Guard® was 100% neutralized by maternal antibodies present in colostrum, when that colostrum was immediately added to the sample. When colostrum was withheld from samples for 30 minutes, then added, Calf-Guard® was neutralized in samples from both vaccinated and non-vaccinated cows by 93.9% and 92.5%, respectively.
- These results demonstrate that a majority of the Calf-Guard<sup>®</sup> vaccine was neutralized, and thereby rendered ineffective as a vaccine, which is a trend likely mirrored in the calf. Therefore, the usage of an oral scour vaccine such as Calf-Guard<sup>®</sup>, is detrimental to a newborn calf's overall immune status, and has a negative economic benefit to the producer.

#### **Background**

Calf diarrhea is a major cause of disease and economic loss among unweaned calves in the United States<sup>1</sup>. The 2007 National Animal Health Monitoring Service (NAHMS) summary reported U.S. dairy calf mortality was estimated to be 57% due to diarrhea, of which coronavirus and rotavirus are major causes<sup>2</sup>. Beef calf mortality, while a slightly different type of system, follows similar trends. In both dairy and beef operations, a variety of techniques are employed to combat the effects and spread of diarrheal disease including vaccination, biosecurity, and colostrum management. Yet, as an industry, significant reductions in scour related illnesses have not been achieved over the years. One of the vaccinations often administered to calves just after birth is Calf-Guard<sup>®</sup>, an oral modified-live rota-coronavirus vaccine. However, the effectiveness of this vaccination is often in question, especially in the face of high antibody levels calves receive from colostrum.

#### **Study Objective**

The goal of this study was to investigate Calf-Guard<sup>®</sup> vaccine viability in the presence of colostral samples from herds using ScourGuard<sup>®</sup> versus no dam-level scour vaccine. This *in vitro* mechanism mirrors the physiological processes occurring in the calf at the time of birth, colostrum administration, and vaccination.

#### Study Design

Colostrum samples were collected from four geographical regions: NY/PA, TX, IN/OH and CA. Each region provided eight colostrum samples; four from individual cows vaccinated with ScourGuard® and four individual cow samples from herds not using dam-level scour vaccines. Experiments were conducted at RTI, LLC (Brookings, SD) using an *in vitro* cell-based assay. All samples were processed via two-step centrifugation with the middle (whey-based) fraction collected and frozen at -20°C until tested.

As an indication of vaccine neutralization, coronavirus titer was determined by serial titration of reconstituted vaccine on MDBK mammalian monolayer of cells. Coronavirus neutralization was assessed by infecting the cells using Calf-Guard® in the absence or presence of colostral whey-based samples. A reduction in the number of foci relative to the control was considered indicative of neutralization. Two experimental designs were utilized *in vitro* to replicate the use of Calf-Guard® in the field. In the first design processed samples at one dilution (17 replicates) were immediately added to vaccine and subsequently plated on the monolayer of cells, mimicking the on-farm delivery of colostrum and Calf-Guard® together. To mimic on-farm colostrum delay, diluted samples were added to the cells 30 minutes after the addition of vaccine. The percent of coronavirus titer reduction was directly compared to the "positive control" wells containing Calf-Guard® only.

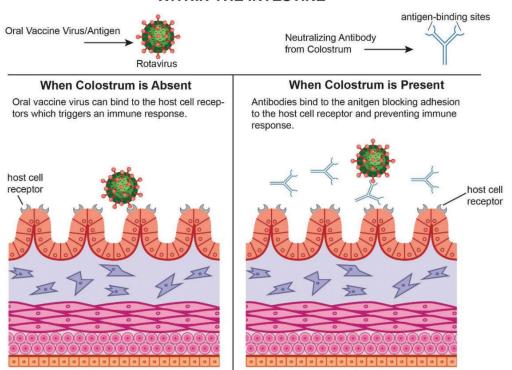
#### **Results**

Sample results were averaged across all regions and sample type. Results are presented in Table 1.

Table 1.	Calf-Guard <sup>®</sup> Neutralization	
	Calf-Guard <sup>®</sup> administered	Calf-Guard <sup>®</sup> administered and
	with Colostrum	colostrum withheld for 30 min*
Vaccinated Dam	100%	93.9%
Non-Vaccinated Dam	100%	92.5%
		*P<0.001

- In samples with immediate colostrum addition, both ScourGuard® vaccinated and non-vaccinated dams had 100% neutralization of coronavirus in Calf-Guard® vaccine, leaving no corona-vaccine antigen left for monolayer infection and rendering the vaccine virus ineffective.
- When colostrum was withheld from samples for 30 minutes, there was a statistically significant difference between vaccinated and non-vaccinated dams. Colostrum from vaccinated dams demonstrated 93.9% vaccine neutralization, consequently rendering only 6.1% Calf-Guard<sup>®</sup> vaccine available for monolayer infection. Colostrum from non-vaccinated dams evidenced 92.5% vaccine neutralization, leaving only 7.5% Calf-Guard<sup>®</sup> vaccine available for monolayer infection. This type of antibody binding is illustrated in Figure 1 below.

#### Figure 1.



WITHIN THE INTESTINE

Despite these differences, whether colostrum was withheld for thirty minutes or not, the overall effect is that an **oral scour vaccine (Calf-Guard®) is inactivated and rendered ineffective in the presence of colostrum.** 

#### **Conclusions**

- The importance of excellent colostral management cannot be overemphasized. In the bulk of U.S. beef and dairy herds, colostrum is administered as soon as possible after birth to ensure optimal antibody transfer. In particular, most beef calves have access, and do indeed nurse colostrum immediately. If calves are receiving proper antibody transfer through colostrum, an oral scour vaccine such as Calf-Guard<sup>®</sup> is neutralized and made worthless in the face of those antibodies.
- The Calf-Guard<sup>®</sup> vaccine label does not indicate the importance of delaying colostrum to ensure the modified-live vaccine is not inactivated, and producers should not omit or delay colostrum administration as a general practice. Although it is possible Calf-Guard<sup>®</sup> could be helpful in those calves with inadequate colostrum and/or diminished immune status, careful consideration should be taken for its use. For the most part, Calf-Guard<sup>®</sup> is being largely neutralized when calves are born into herds with typical common-sense colostrum management programs, providing the \$3.50-\$4.50 per calf product cost investment little, if any, return.
- Additionally, as maternal antibodies from colostrum are taken up with binding to the vaccine virus, they are less available to combat naturally occurring pathogens that calves will surely encounter. Consequently, there is an overall lower circulating level of antibodies, making the calf's immune system function at a suboptimal level.

Therefore, it is logical to maximize calf health and economic benefits by NOT using an oral calf vaccine, such as Calf-Guard<sup>®</sup>. Producers should look to enhance colostrum administration, herd health, and sanitation in an effort to ward against newborn calf disease, rather than place their faith in a one-time vaccination. Alternatives to oral modified-live scour vaccines, such as the First Defense<sup>®</sup> line of USDA approved antibody products, should be considered to enhance immunity for newborn calves, especially for those at high risk for infection.

#### For more information: mail@immucell.com or 800-466-8235

#### Sources:

Calf-Guard® and ScourGuard® are registered trademark of Zoetis Services LLC.

- 1. USDA. Dairy 2007 Part II: Changes in the U.S. Dairy Cattle industry, 1991-2007. Fort Collins: USDA-APHIS-VS, CEAH; 2008. pp. 57–61.
- 2. Yong-il Cho, Kyoung-Jin Yoon. An overview of calf diarrhea—infectious etiology, diagnosis, and intervention. J Vet Sci. 2014 Mar; 15(1): 1-17)



# **RESEARCH PROVEN**



"You can just compare calves that you have on First Defense<sup>®</sup> compared to calves that aren't. There's definitely a big difference. Maybe not day one, but as they're in their first three weeks when they're in the calf hutches and they're on milk. Once they start grain, we noticed a big difference on their milk and grain intakes, as well as just being healthier all around."

- Zach Damrow, Seagull Bay Dairy , American Falls, ID



"Prior to adding First Defense<sup>®</sup> to our regimen, our calves would always run into problems with salmonella after having scours seven to 10 days after birth. We have been using First Defense® at Cal Poly State University's dairy unit and have seen calves getting through the first two weeks of life stage really successfully. We would recommend First Defense<sup>®</sup> to anyone. This product is simple to administer and produces results."

- Rich Silacci, Cal Poly State University, San Luis Obispo, CA



"The thing that I like about First Defense® is that you give to the calf as soon as it's born. There are some products on the market today that you have to give to the calf at birth and the recommendation is to wait 30 minutes or longer before you can feed colostrum. It's so hard to tell a dairyman or a calf raiser to delay feeding colostrum when that's the most important thing to give the calf."

- Steve Hayes, DVM, Day 1 Technology, Winona, MN



"The First Defense<sup>®</sup> product is a hyperimmunized colostrum. The antibodies are condensed down into a bolus. Once it gets into the gut, those antibodies are absorbed and help protect the calf. Calving areas can get used over and over. That second half of that calving season, there is probably a lot antigens built up - First Defense<sup>®</sup> is a great supplement to provide some added protection."

- Lance Kurtz, DVM, Countryside Vet Clinic, **Fullerton, NE** 



"I recommend producers implement First Defense<sup>®</sup> in their calf protocols even when using cow scour vaccines. First Defense<sup>®</sup> provides calves with consistent protection by delivering a timely and accurate dose of antibodies they need to avoid scour outbreaks."

- Joe Strahm, DVM, Pender Vet Clinic, Pender, NE



"We have been using First Defense® for the last 3-4 years and are really happy with the product. For us it's important, it's a big investment and a huge return. The product is convenient and versatile. Knowing we have protection as soon as the calf hits the ground is satisfying and comforting. This product gives assurance."

- Dan Kullot, DVM, Syracuse Dairy, Syracuse, KS

## **ImmuCell**<sup>°</sup> FirstDefenseCalfHealth.com 1-800-IMMUCELL

