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AGRI-PRACTICE – IMMUNOLOGY

A study was conducted to examine the potential for providing greater protection against morbidity and mortality in newborn calves through oral administration of antiviral as well as antibacterial immunoglobulins. Oral administration of a bivalent hyperimmune bovine antibody preparation (First Defense™ :ImmuCell Corporation, Portland, Me.) was shown to reduce the severity of diarrhea and dehydration in newborn calves against two major enteric pathogens in independent challenge trials This immunoglobulin supplementation also reduced the mortality rate in response to challenge with either K99$^+$ enterotoxigenic *Escherichia coli* (ETEC) or coronavirus These results have important implications in the management of neonatal calf diarrheal disease

Protection of Neonatal Calves Against K99-*E. coli* and Coronavirus Using a Colostrum-Derived Immunoglobulin Preparation

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Introduction

Escherichia coli, coronavirus, rotavirus, and *Cryptosporidium* spp. are the major pathogens which cause acute diarrhea (scours) in newborn calves. These organisms account for the vast majority of enteric infections in neonatal calves worldwide, with individual disease incidences varying, depending on geographical regions and seasons. Among these pathogens, enterotoxigenic *E. coli* (ETEC) and bovine coronaviruses are the most frequent isolates associated with severe diarrhea and death in neonatal calves.[1,2] ETEC strains bearing the K99$^+$ antigen and secreting a heat stable toxin are the most common scours pathogens, with reported incidences of 25-30% among diarrheic calves in the first 3-4 days after birth.[3] Mortality statistics have shown that K99$^+$ ETEC may be responsible for up to 30-50% of scours-related deaths in calves that are newborn.[3] Coronaviruses have been isolated in approximately 30% of scouring calves and generally produce more severe illness than do rotavirus infections.[2] Coronavirus infections, either alone or in combina-

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tion with other scours agents, may account for an additional 15-20% of deaths.[2] The aim of the present study is to examine the potential for providing greater protection against morbidity and mortality in newborn calves through oral administration of antiviral as well as antibacterial immunoglobulins. Such a bivalent preparation of hyperimmune colostral immunoglobulins containing coronavirus-neutralizing and anti-K99 pilus antibodies was evaluated in separate calf challenge studies.

Materials and Methods

COLOSTRAL IMMUNOGLOBULIN PREPARATIONS

The antibody preparation used in these studies was prepared from coronavirus-*E. coli* K99 hyperimmune colostrum by the manufacturer (Bovine Coronavirus, *Escherichia coli* Antibody, First Defense, US Veterinary License #327:ImmuCell Corporation, Portland, Me.). Hyperimmune colostrum is obtained by immunization of pregnant Holstein cows with ImmuCell's proprietary *E. coli* K99 and coronavirus vaccines. Partially purified colostral antibodies are dried and incorporated into gelatin capsules. Each capsule administered in these clinical studies was from a single lot of product and contained approximately 50% IgG on a protein basis, as judged by sodium dodecyl sulfate (SDS) gel electrophoresis. The potency of individual doses against K99-*E. coli* and bovine coronavirus is standardized by the manufacturer through K99 antigen specific enzyme-linked immunosorbent assay (ELISA) and virus neutralization assays, respectively.

ANIMALS

Newborn, colostrum-deprived Holstein dairy calves were obtained within 2 hours of birth. Colostrum deprivation was confirmed on an incoming serum sample, using the Bova-S Test (VMRD:Pullman, Wash.), and Bova-S positive animals (≥ 5 mg/ml Ig) were rejected from the study. A single immunoglobulin-containing capsule was administered to each animal in the treated groups 4-6 hours after birth, using a bolus gun. This was followed by 2 quarts of bottle-fed commercial milk replacer. Control animals received milk replacer alone. Calves were administered the virus or bacterial challenge inoculum in a small volume of saline which was delivered to the back of the throat with the aid of a syringe 10-12 hours after birth. All of the calves were fed 2 quarts of milk replacer every 12 hours over the 5-day study. The animals were euthanized at the end of the study under the supervision of veterinarians. Thirty animals were used for the *E. coli* challenge study and 28 animals were used for the coronavirus study. Assignment of the animals to treated or control groups followed a predetermined protocol. Animals which had mortality of questionable etiology were necropsied to determine the cause of death. Animals which died of causes unrelated to the challenge were eliminated from the study in accordance with standards established at the start of the trials.

E. COLI CHALLENGE STUDY

This study was conducted at the University of Wisconsin, Madison, Wisconsin, and was performed in two parts. The two parts differed in the size and source of the bacterial inoculum (enterotoxigenic *E. coli* B44 09:K30:K99:F41:ST$^+$) given to challenge animals. Animals in the first half of the study received a challenge consisting of approximately 1.8×10^{10} organisms, and calves in the second half of the study received approximately 9×10^{10} organisms per challenge dose. The strain used in the second half of the study was a fecal isolate from a moribund control animal in the first half of the study. This "calf-passed" isolate of the original B44 challenge strain was characterized as K99$^+$ and used in an effort to increase the virulence of the challenge inoculum. Challenge inocula were administered orally, via syringe, at 10 hours after birth, followed by 60 ml of milk replacer. Fecal samples and clinical observations were obtained at 12-hour intervals for a total of 5 days post-challenge.

CLINICAL SCORING

The diarrhea and dehydration scoring system used in both studies is as follows:

- Fecal Score
  - 0 = normal; manure firm and well formed;
  - 1 = abnormal but not diarrheic (softer than normal);
  - 2 = mild diarrhea; and
  - 3 = severe, watery diarrhea.
- Dehydration Score
  - 0 = eyes bright and skin pliable;
  - 1 = mild dehydration; and
  - 2 = severe dehydration.

Comparisons of the clinical responses between control and treated groups were made by chi-squared analysis (mortality) and One Tailed t-test for differences between mean cumulative scores (morbidity). Mean cumulative clinical scores are reported as the mean of fecal scores plus dehydration scores per individual summed over the entire 5-day study interval. Mean cumulative dehydration

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TABLE 1
Clinical Outcome of *E. coli* Calf Challenge Study

Study	Treatment Group	Mortality	(%)	Cumulative Clinical Score (Mean ± SD)	Cumulative Dehydration (Mean ± SD)
1[a]	Control	3/5	(60)	27 ± 8.4	8.8 ± 5.7
	Treated	0/8	(0)	17.9 ± 5.2	3.0 ± 2.6[f]
2[b]	Control	3/5	(60)	32.6 ± 7.3	10.6 ± 5.2
	Treated	2/12	(17)	19.7 ± 8.5	5.0 ± 4.4[e]
Combined	Control	6/10	(60)	29.8 ± 8.3	9.7 ± 5.2
	Treated	2/20	(10)[c]	19.7 ± 7.4[d]	4.2 ± 3.9[d]

[a] 1.8×10^{10} ETEC (Strain B44) challenge
[b] 9×10^{10} ETEC (Strain B44) challenge
[c] $p < 0.001$ vs. control by chi-squared analysis
[d] $p < 0.01$ vs. control by one tailed t-test
[e] $p < 0.025$ vs. control by one tailed t-test
[f] $p < 0.005$ vs. control by one tailed t-test
SD = Standard Deviation

scores are reported as the mean of dehydration scores per individual summed over the entire 5-day study interval. Animals which died before the end of the study interval were assigned the maximum score in each category for the remaining observation periods.

CORONAVIRUS STUDY

This study was conducted at Cornell University, New York State College of Veterinary Medicine, Ithaca, New York. The challenge strain was a virulent bovine coronavirus that was expanded in a newborn calf. Determination of the optimal challenge dose followed several pilot infections. Fecal samples, clinical scoring, and data analysis followed the methods described previously for the *E. coli* study.

Results and Discussion

Calves which received the capsule containing hyperimmune colostral immunoglobulins showed less mortality and a reduced severity of clinical disease when compared to the control groups in the coronavirus and the *E.coli* studies. K99$^+$ *E. coli*-induced mortality was 60% in the control calves in both halves of the trial (Table 1). Scours-related deaths were significantly reduced in each of the antibody-treated groups which had 0% and 17% mortality in the first and second halves of the study, respectively. The second half of the study used a more severe challenge (approximately 9×10^{10} organisms compared with 1.8×10^{10} in the first half). The increased mortality in the treated group receiving the higher challenge inoculum (17%) possibly reflected saturation of the prophylactic antibody preparation with excess K99 sites in the bacterial challenge. However, statistically significant protection was still achieved under these challenge conditions. Interestingly, the mortality rate among control group calves did not increase in response to the higher inoculum. The reasons for this unexpected result are not clear, since higher mortality rates have been observed for K99$^+$ ETEC in other challenge studies.[4] Differences in the challenge protocol could also have been responsible for higher mortality rates observed in other studies. Overall mortality rates among K99$^+$ ETEC-infected calves observed in the field are estimated at 25-30%, which is much lower than the 60% mortality observed for control calves in this study.[3] Exposure to K99$^+$ ETEC in the field might involve ingestion of concentrations of *E. coli* as high as those used in this study, and, therefore, it is expected that this passive antibody preparation could also be efficacious in field situations.

Examination of the morbidity data provides a clue as to the extent of passive antibody protection from K99$^+$ ETEC (Table 1). The treated groups showed reduced overall morbidity compared to the controls, as judged by the differences in both clinical and dehydration scores. While animals in all groups showed scouring and dehydration, the reduced dehydration scores in the treated groups indicate that the treated calves had a higher resistance to the debilitating effects of the challenge and were more likely to recover in the absence of any other supportive therapy. Thus, consideration of the results of both halves

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of the *E. coli* study together shows the mortality rate in the treated calves to be 10%, as compared with 60% in the controls, a highly significant difference ($p < 0.001$). Logically, this result is a consequence of the improved clinical outcome observed in the treated group with respect to overall scouring and dehydration.

The effects of antibody treatment in the coronavirus trial are similar to results in the *E.coli* study. The notable exception is that the overall coronavirus mortality (40%) was lower than that observed in the *E. coli* study (60%), reflecting the rank of severity observed in the field. Coronavirus-related mortality in the treated group was 0% compared to 40% in the control group, a statistically significant difference ($p < 0.01$) by chi-squared analysis (Table 2). The treated group suffered much less morbidity than the control group, as judged by mean cumulative clinical (fecal scores plus dehydration scores) and dehydration scores (Table 2). As observed in the *E. coli* study, no difference was seen in either onset or duration of scours between treated and control groups. Thus, the reduced mortality observed among antibody-treated calves in both the coronavirus and *E. coli* studies appears to be due to increased protection against systemic illness and accompanying diarrhea provided by the administration of the capsule containing colostral immunoglobulins.

The timing of the *E. coli* challenge in these studies (10-12 hours after birth) spans the most likely time for acquisition of K99$^+$ ETEC in the field, since calves have been shown to be most sensitive to K99$^+$ ETEC in the first 24 hours of life.[5] However, viral diseases, including rotaviral and coronaviral infections, appear to predominate in the 5- to 15-day age interval.[6] The current challenge protocol is not intended to reproduce this late-onset syndrome. It would be very difficult to control all the health-related variables in order to demonstrate a reproducible viral diarrheal syndrome in calves of that age. Moreover, rotaviral and coronaviral illnesses in calves older than 4 days tend to be associated with lower overall morbidity and mortality, in part because the animal is larger and somewhat more resistant to the systemic effects of diarrhea. It is the early-onset disease, including mixed pathogen infections, which is more life threatening and more amenable to colostral or supplemental antibody prophylaxis.[2,7] Besser and associates suggest that the protective effects of antibodies administered in the first few hours of life could afford protection for a period of 5-15 days due to the fact that immunoglobulins absorbed in the first 24 hours are transported back into the gut over the ensuing 2 weeks.[8]

TABLE 2
Clinical Outcome of Coronavirus Calf Challenge Study

	Control Group	Treated Group
Mortality (%)	4/10 (40)	0/18 (0)[a]
Cumulative Clinical Score (Mean ± SD)	24.7 ± 1.84	20.5 ± 4.3[b]
Cumulative Dehydration Score (Mean ± SD)	5.1 ± 3.1	1.2 ± 1.6[b]

[a] $p < 0.01$ vs. control by chi-squared analysis
[b] $p < 0.005$ vs. control by one tailed t-test
SD = Standard Deviation

Conclusion

Oral administration of a hyperimmune bovine antibody preparation was shown in these studies to reduce the severity of diarrhea and dehydration in newborn calves against two major enteric pathogens in independent challenge trials. This immunoglobulin supplementation also reduced the mortality rate in response to challenge with either K99$^+$ ETEC or coronavirus. These results have important implications in the management of neonatal calf diarrheal disease, as these two pathogens are those most commonly implicated in the etiology of neonatal scours-related mortality. The use of passive antibody preparations combined with good colostrum feeding practices could significantly improve the outcome of neonatal calf diarrhea on dairy and beef farms. This may be particularly true when the quantity and quality of available colostrum are less than adequate or when lapses of active herd vaccination have occurred in the face of a scours outbreak. ■

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