POSSIBLE VACCINATION FAILURE IN BEEF COW HERDS CAUSED BY INFECTION WITH ROTAVIRUS DISTINCT FROM THE VACCINE VIRUS: CLINICAL OBSERVATIONS

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Neonatal enteritis in beef calves has long plagued the world cattle industry. The National Animal Health Monitoring System (NAHMS), in a recent assessment of disease incidence in the State of Colorado, found a mean incidence per 100 cows of 14.4 and 14.7 respectively of bovine enteric disease, from surveys involving two consecutive years (1). The incidence of enteric disease ranged as high as 95 cases per 100 cows in herds in the study, which included over 24,000 cows. Costs of enteric disease were determined to be $4.23 and $4.34, respectively, on a per cow basis, during the two-year study (2).

Rotaviruses frequently are found in cases of calf diarrheas submitted to the veterinary diagnostic laboratories in the State of Nebraska. This report involves case investigations of two ranches located in Western Nebraska, USA, that experienced morbidity and mortality associated with severe enteritis in neonatal calves. The investigations and results presented here reflect the procedures performed to reach applicable diagnostic conclusions. Investigations of field cases, such as these, do not always allow unlimited study, yet still provide valuable scientific data which can shed some light on a complex syndrome such as neonatal calf diarrhea.

Literature Review

Bovine rotavirus (BRV) is one of the most common etiological agents associated with neonatal calf diarrhea (3,4). Since its first isolation by Mebus et al. in Nebraska USA, rotavirus has been associated with neonatal calf diarrhea throughout the world (5). Economic losses for the dairy and beef industry caused by rotavirus are important not only in terms of clinical disease and animal death, but also because of retarded growth, increased medical costs and special care (6).

Bovine rotavirus is a member of Genus Rotavirus in the family of Reoviridae (7). The name rotavirus was coined by Flewett et al., based on the virus' characteristic wheel-like appearance by electron microscopy (8). Each virion contains eleven distinct genome segments of double-stranded RNA which are packaged into a double-shelled capsid composed of three major structural proteins - VP4, VP6 and VP7 (7,9). VP6, which is encoded by gene segment 6, is located in the inner capsid and contains rotavirus group specific antigens. VP4 and VP7 are encoded by gene segments 4, 8 or 9, respectively, and are located on the outer capsid. They are involved in neutralization of the virus by antibodies.

Based on antigenic differences of the group antigens rotaviruses have been assigned to at least six distinct groups (Gp), A through F (7) Rotaviruses from different groups are morphologically identical yet have distinct antigens that can be demonstrated by serology. The different groups of rotaviruses can also be distinguished from one another based

on the migration pattern of their individual genome segments by gel electrophoresis and the sequence of terminal nucleic acids of each genome segment (10). To date Gp A and Gp B rotaviruses have been shown to naturally infect calves, but Gp A rotaviruses are by far the most prevalent (11).

Group A rotaviruses have been serotyped according to their antigenic differences on VP7 or G protein. Using serology, at least three G serotypes of BRVs, 6, 8, and 10 have been reported (12). Limited studies on the prevalence of individual serotypes associated with neonatal calf diarrhea in the USA and UK indicate that G serotype 6 is most widespread while G serotype 10 accounts for a minor percentage of cases (13,14). More recently, serotypic characterization of rotaviruses has been expanded to include VP4 or P serotypes, and at least three different P serotypes have been identified in cattle (9,15). Studies on immunity and prevention of calf diarrhea caused by BRV have concentrated on the role of passive transfer of specific colostral antibodies to the suckling neonate. The goal with this approach is to prevent rotaviral diarrhea in the calf by vaccinating the dam before parturition. The mechanism of protection against rotavirus induced diarrhea using colostrum is attributed to the continuing presence of a sufficient amount of specific antibodies in the gut lumen, so called "lactogenic immunity." Although circulating antibodies were originally thought to be unable to reach the intestinal mucosa in sufficient concentrations to prevent infection, recent evidence suggests a role for serum antibodies in long term protection of the neonate (16,17). Passive protection against infection by BRV in the conventional calf is relatively inefficient since a sufficiently high titer of rotavirus specific antibodies in cattle is present only in the first day colostrum (18).

Scourguard 3-K, manufactured by Smith-Kline-Beecham Animal Health, is the only commercially available vaccine for prevention of virally induced calf diarrhea. It contains killed G serotype 6-NCDV-Lincoln strain of rotavirus, coronavirus, and Escherichia coli K99 antigenic fractions. Although a vaccine against calf diarrhea caused by rotavirus is available commercially, rotavirus continues to be routinely detected in stool samples of calves from vaccinated herds.

Current information on immunity and protection against challenge inoculation with homotypic (same serotype) and heterotypic (different serotype) of BRVs is incomplete. However, indications that active or passive immunity directed at one serotype is ineffective against sequential challenge by heterologous serotypes in experimental calves has been generated (19,20). Infection of calves by a strain different from the vaccine BRV (heterotypic stain) and failure of cross protection between the two strains may explain apparent vaccination failure in some herds. This suggests that herd immunity and protection against serotypes of GpA rotaviruses and Gp B rotaviruses can only be obtained through natural exposure or vaccination with vaccines prepared from the autologous strain.

Field Investigation

Data were collected from two adjacent ranches where occurrence of diarrhea had resulted in submission of samples to the Diagnostic Laboratory (Table 1).

Table 1 Herd History

Ranch	# Breeding females	Calving season	Outbreak time	Age at onset (days)	Morbidity (%)
A	500	2-4/91	2-4/91	3-15	80
B	172	3-6/91	4-6/91	21	11.6

Ranch A consisted of a total of 500 breeding animals, of which 80 were two-year-old, first calf heifers. Twenty-five of the heifers had been purchased during the fall, 1990 with the remaining being ranch raised. Calving season in the Hereford-Angus cross herd began on February 20, 1991 for the heifers and on March 1, 1991 for the cows. Severe diarrhea, weakness, dehydration, and death of calves began on March 5 with about 50 calves born in the herd. New cases were encountered until about April 15. The average age at onset of clinical signs was 12 days with calves as young as 3 days or as old as 15 days of age affected. Approximately 80% of the heifer's calves and 25% of the cows' calves developed diarrhea. Nearly all affected calves required treatment with all affected heifers' calves and 20% of the cows' calves treated. Mortality records were unavailable. Treatment consisted of oral electrolytes, kaopectin, and antibiotics.

The heifers were kept separate from the cow herd during the entire previous summer and during the winter and spring of 1990-91. As calving approached, heifers were moved to a 60-acre calving pasture and cows were moved to a 120-acre calving pasture about one week prior to calving. Shortly after calving, cow/calf pairs were moved to one of three pastures ranging from 160 to 540 acres in size. Dystocia cases were brought to the ranch calving facility and the herd was brought to the building area during severe winter weather. Nutritional requirements during the winter feeding period were met by keeping cattle on winter range, feeding of additional prairie hay, including 2 pounds per day of 20% all natural protein range supplement. The water source for the herd was well water supplied in tanks.

Vaccination consisted of a combination of rotavirus, coronavirus, and E. coli K99 product which had been used for the past six years with the exception that a killed E. coli K99 product was used for one year five years previous. This vaccine was originally a modified live product but since has been converted to a killed product. Additionally, an oral rotavirus-coronavirus product had been administered to newborn calves during the 1989 calving season. The vaccine was administered to heifers during mid September and again on about February 10 according to manufacturer's instructions. The cow herd was given a yearly booster on March 1.

Ranch B consisted of 179 breeding females with 59 coming two-year-old first calf heifers. Although this ranch had diarrhea problems in previous years, the owner reported incidence of diarrhea was less during this calving season then in previous years. Both cows and heifers began calving on March 20 and finished on June 20. The herd was moved to calving pastures different from the wintering areas prior to calving. Heifers and cows were calved in separate areas. Space allocation in calving pastures was approximately five acres per animal. All cow/calf pairs were removed from the calving pasture at less than 48 hours of age and taken to other larger pastures. Approximately 140 of the calves (81.44%) were born prior to onset of the diarrhea problem.

Morbidity due to calf diarrhea was estimated at 20 head (11.6%) with 11 animals receiving treatment consisting of oral electrolytes, kaopectin, and antibiotics. Average age at onset was estimated to be 21 days with recovery at about 28 days. Mortality in this herd included five diarrhea associated losses (5.9%) and four calves lost at calving for a total of 9 head (5.2%).

Nutrition in the herd consisted of about 25 pounds per head per day of prairie hay and alfalfa hay. Cows were given one pound of supplemental cottonseed cake and heifers were fed two pounds of cottonseed cake per day. Both cows and heifers were given a killed rotavirus-coronavirus-E. coli K99 Clostridium perfringens Type C vaccine (Scourguard 3-K-C) according to manufacturer's directions on January 16 and February 26, 1991.

Laboratory Investigation

Ranch A submitted fecal materials from four calves. An E. coli was isolated by routine bacteriological examination and serotyping of the isolate yielded negative results for K99, K88, and 987P antigens. Examination for presence of cryptosporidia was negative. ELISA test for rotavirus was positive as were transmission electron microscopy (TEM) results.

Ranch B submitted two calves and fecal materials from four animals. An E. coli was isolated by bacterial culture, but was negative for presence of K99, K88, and 987P antigens. Fecal samples were positive for the presence of rotavirus by ELISA and TEM. Histo-pathological examination revealed a mild purulent cryptitis and lymphoid depletion of Peyer's patches. A fluorescent antibody stain on frozen sections of intestine was positive for bovine viral diarrhea virus although virus isolation attempts were negative. Examinations for presence of cryptosporidia were negative on all samples submitted.

Samples positive for the presence of rotavirus particles by TEM were adapted to cell culture in an established cell line of fetal Rhesus monkey kidney cells (MA-104) using standard procedures (7,21). The cell culture adapted virus isolate from ranch A, NS-1 was further characterized by virus neutralization using hyperimmune guinea pig antisera to reference G serotype 6 BRV, strain Nebraska calf diarrhea virus (NCDV)-Lincoln, B641, and G serotype 10 BRV, strain B223 (22,23). RNA extracted from cell culture adapted NS-1 and NS-2 (ranch B) was further analyzed by polyacrylamide gel electrophoresis, and Northern blot cross-hybridization assays using whole-genome probes prepared form each reference BRV isolates (24).

Results

Both NS-1 and NS-2 were cytopathic for MA-104 cells and had identical Gp A electropherotypes characterized by 11 distinct segments of dsRNA. Virus neutralization assays clearly indicated that NS-1 virus was distinct from the vaccine strain of BRV, G serotype 6, strain NCDV-Lincoln (Table 2). NS-1 appeared as related to B641 as NCDV-Lincoln, but was clearly different from BRV G serotype 10, strain B223 (Table 2). Results of cross-hybridization experiments indicated that the two field isolates were identical to each other at all gene segments and hybridized at gene segment 4 with strain B641 but not with strain NCDV-Lincoln and strain B223. Also, both isolates did not hybridize with gene segment 5 of both NCDV-Lincoln and B641, but did hybridize with B223. RNA-RNA hybridization experiments confirmed partial homology

between gene segment 9 of NS-1 and NS-2 and strain B641, and no homology with strain NCDV-Lincoln and B223 (data not shown).

Table 2 Serological Relationships Between NS-1 and Reference Strains of Group A Bovine Rotaviruses.

Test Virus	Test Antibody		
	NCDV-Lincoln	B641	B223
NCDV-Lincoln	2,263*	475	400
B641	475	800	<168
B223	<168	<168	7,611
NS-1	336	475	<168

* Neutralization titer expressed as the reciprocal of the highest dilution giving 50% neutralization of cytopathic effect.

Discussion

Rotaviruses have 11 distinct gene segments that can be exchanged when animals are infected by more than one virus at the same time. Exchange of gene segments results in progeny viruses that have a genetic make-up derived from both parents. Gene exchange or reassortment is an important adaptation mechanism of rotaviruses which allows them to evade the host immune response and persist in a susceptible population. Because the genes coding for the two viral outercoat proteins (proteins P and G) can be exchanged between rotaviruses during reassortment and because at least three different P proteins and at least two different G proteins are present in BRVs, at least six surface protein combinations could potentially result from infection of a calf by more than one rotavirus strain.

Based on serological and genotypic analyses, the field isolates obtained from each ranch most likely represent naturally-occurring reassortant viruses that contain a gene segment 4 homologous to strain B641, a gene segment 5 homologous to strain B223, and a gene segment 9 with partial homology to strain B641 and distinct from strain NCDV-Lincoln. Since immunity to rotavirus is specific to the P/G protein combination present in the vaccine, infection of calves by a BRV differing from the vaccine at the level of both genes coding for the neutralization determinants is likely to account for calf morbidity and mortality associated with rotavirus infection on both ranches.

Although virus neutralization antibody titers were not determined on sera from vaccinated dams, it is unlikely that outbreaks of neonatal calf diarrhea occurred as a result of a lack of antibody response or passive transfer of colostral antibodies in all cases. It has been shown that when the vaccine is used according to the manufacturer's recommendations, it is highly immunogenic for rotavirus, providing high titers of BRV specific antibodies in colostrum and milk for at least 28 days post-calving (25). Additionally, failure of passive transfer of maternal antibodies alone cannot account for all cases of neonatal diarrhea occurring on these ranches (26,27).

These are the first documented field outbreaks of vaccine failure associated with BRVs with gene segments distinct from vaccine virus. Although the extent of losses directly attributed to this virus cannot

be ascertained beyond question, this report does document the existence of rotavirus differing from vaccine virus in calf diarrhea outbreaks. These findings must be considered by practitioners when diagnostic laboratory results are indicative of rotavirus involvement in cases of neonatal bovine diarrhea.

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Summary

Vaccination of beef cow herds as part of preventive programs for viral calf diarrheas has become a common practice in the United States. Vaccine used contains rotavirus, coronavirus, and Escherichia coli K99 antigens. Varying degrees of success and failure have been associated with the use of vaccine against calf scours and these have been attributed to aspects of nutrition, management, and other causes of calf diarrheas.

This report includes two ranches which experienced rotaviral diarrhea in spite of use of preventive vaccination programs and good management procedures. In these two operations, rotaviruses differing from the vaccine at the level of the two genes coding for neutralization determinant of the virus were able to cause disease. Field isolates were obtained from feces positive for rotavirus by electron microscopy. Results of cross hybridization assays indicated the isolates were identical to each other at all gene segments and hybridized at gene segment 4 with strain B641 but not with NCDV-Lincoln, the vaccine virus, or with the B223 virus. Differences at gene segment 4 and 9 will result in differences in surface proteins. This report provides the first documentation of a strain of rotavirus distinct from the vaccine virus associated with vaccination failure in Nebraska beef cow herds.