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# Viral Calf Enteritis[1]

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## INTRODUCTION

The problem of calf diarrhea in a herd may be continuous and affect all calves, may affect only calves from first calf heifers, or may occur after introduction of new animals. Calf diarrhea can be devastating when a newly assembled herd calves.

In the past, most cases of calf diarrhea have been diagnosed as colibacillosis. Strains of *E. coli* isolated from diarrheic calves have usually been resistant to available antibiotics. Other less frequent diagnoses have been salmonellosis, clostridial enteritis, and coccidiosis. Control and treatment methods based on bacteriological findings generally have not been satisfactory.

At the University of Nebraska we have worked with a reovirus-like agent (13, 18) and a coronavirus (15, 22) isolated from diarrheic calves and developed diagnostic methods (14) for detecting these agents in outbreaks of calf diarrhea. Detection of these viruses assists in making a specific diagnosis and, more importantly, helps locate those herds where unknown agents are involved.

Animals in research on calf diarrhea have been naturally-born colostrum-fed calves, colostrum-deprived calves in isolation rooms, and gnotobiotic calves. In the search for new pathogens, colostrum-derived calves are a valuable aid because they normally lack antibodies to the common bovine diseases. However, deprivation of colostrum does not make a calf susceptible to agents not normally pathogenic for the bovine. If a colostrum-deprived calf is susceptible and a colostrum-fed calf resistant, this means the colostrum-fed calf has obtained passive protection from colostrum. In the study of intestinal diseases, the use of gnotobiotic calves is the most satisfactory way of proving the pathogenicity of an agent because the flora in these calves can be determined.

Results from using the reovirus-like and coronavirus calf diarrheal agents in colostrum-deprived and gnotobiotic calves have helped to understand the disease as it occurs in the field.

## REOVIRUS-LIKE AGENT

REO is an acronym for respiratory, enteric orphan. The first viruses of this group were isolated from the respiratory and enteric tracts of animals. However, because they could not be associated with disease, they were called orphans.

The calf diarrhea reovirus-like agent belongs to the family REOVIRIDAE (5, 23, 24, 25) on the basis of morphology, cytopathogenicity, resistance to lipid solvents, stability at $pH_3$, and presence of double straned RNA. An important practical property of the calf reovirus-like agent is its stability. Feces kept at room temperature for 7 mo still contained viable virus (7).

The recent association of a reovirus-like agent with acute nonbacterial gastroenteritis of infants and young children in many parts of the world has stimulated extensive studies (1, 2, 3, 4, 6, 10, 11). As a result of these studies, we now recognize a group of morphologically similar enteric viruses: reovirus-like agent of calf diarrhea, human reovirus-like agent of infantile gastroenteritis, reovirus-like agent of porcine diarrhea (26). epizootic diarrhea of infant mice (EDIM) (21), simian SA 11 virus, and O agent from sheep and cattle (12). The calf reovirus-like agent is related antigenically to the human reovirus-like agent (8, 9, 10), porcine reovirus-like agent, and epizootic diarrhea of infant mice (8, 9, 10) and serologically unrelated to reoviruses 1, 2, and 3 (9). These viruses are called reovirus-like agents.

### Clinical Signs

The incubation period after oral inoculation of a gnotobiotic calf with 10 ml of feces containing virulent calf diarrheal reovirus-like agent was as short as 12.5 to 13 h. When a low

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viral titer inoculum was used, the incubation period was longer. The animal became depressed, anorectic, had a few strings of thick saliva hanging from its lips and developed a diarrhea. The diarrheic period lasted 5 to 6 h, and the animal passed about 300 ml of liquid yellow feces. The volume of feces depended on the amount of milk consumed. Twenty-four hours after the onset of diarrhea, the gnotobiotic calf appeared fairly normal, suckled, and had pasty feces (17).

If colostrum deprived calves were contaminated with *E. coli* before or when inoculated with the virus, up to 50% died. If calves were inoculated with whole feces from field cases, some died before diarrhea began. Most colostrum-deprived calves contaminated with *E. coli* after the diarrheic period lived. Feces from a diarrheic gnotobiotic calf were liquid but not watery. Watery feces, feces which after collection quickly separated into watery fluid and small amount of solids, were characteristic of a severe secondary bacteria, viz. *E. coli* infection. These calves dehydrated rapidly.

#### Pathology

The changes caused by the reovirus-like calf diarrhea agent were studied by inoculating gnotobiotic calves orally and examining them at necropsy, about .5 h and 4 h after the onset of diarrhea. Grossly, all tissues appeared normal. The stomach usually contained curdled milk and thick saliva. The contents of the small intestine and colon were liquid. At the onset of diarrhea, most of the small intestine was lined by immunofluorescent tall columnar villous epithelial cells. Within 4 h after the onset of diarrhea, the immunofluorescent positive tall columnar intestinal villous epithelial cells were lost and replaced by low cuboidal cells.

On the basis of the light, immunofluorescent, and electron microscopic findings in this series of gnotobiotic calves, the following pathogenesis of diarrhea was proposed (17). The reovirus-like agent replicates primarily in the mature villous epithelial cells. At the onset of diarrhea, the intestine is lined by morphologically normal epithelium, but the cells contain a large quantity of viral antigen. The viral infection of the villous epithelial cells redirects the cell function from absorption to virus production, and the digestive fluids and partially digested milk accumulate in the intestinal lumen. The infected epithelial cells have an accelerated migration toward the tip of the villi, are shed from the ends of the villi, and are replaced by an immature epithelium.

### CORONAVIRUS

The prefix corona is used to designate a group of viruses with a particular morphology. Viruses in this group viewed by electron microscopy have a central round core surrounded by projecting pear-shaped petals. When this type of particle was first observed, the radiating projections were thought to resemble the corona of the sun, hence, the name coronavirus.

#### Clinical Signs

The incubation period after oral inoculation of 10 ml of coronavirus-infected feces is about 20 h. The calf usually is not as depressed as with the reovirus-like infection. Initially, the feces are liquid and yellowish, with the volume depending on the amount of milk fed. After the initial diarrhea, the liquid feces contain curds and mucus. The main clinical difference between the reovirus-like and coronavirus infections is that the coronavirus infected calf continues to have diarrhea for 5 to 6 days (16). Bacteriologically sterile calves have become moribund or died 48 to 62 h after the onset of diarrhea. These calves had blood packed cell volumes of 45 to 50%, indicating severe dehydration.

#### Pathology

Grossly there was no lesion except liquid intestinal contents in calves necropsied 4 h after onset of diarrhea. At 48 h after the onset of diarrhea, there was submicroscopic villous atrophy.

The histologic lesions in calf diarrhea coronavirus infection were studied with gnotobiotic calves. Lesions occurred in the small and large intestines. At the onset of diarrhea, the small and large intestines were lined by morphologically normal epithelium which contained a large quantity of viral antigen. Calves killed 48 to 96 h after the onset of diarrhea had severely shortened small intestinal villi covered by a cuboidal epithelium. In the large intestine, the colonic ridges were atrophied and were covered by a cuboidal epithelium; scattered colonic crypts were dilated and lined by squamous to

cuboidal epithelial cells. These calves had extensive immunofluorescence in the colonic epithelium (16).

The mechanism for initial diarrhea in coronavirus infection is believed to be similar to that proposed for the reovirus-like agent, with the continuous diarrhea resulting from severe villous atrophy and prolonged damage to the intestinal epithelium.

## ROLE OF BACTERIA IN ENTERIC VIRAL INFECTIONS

Bacteria viz. *E. coli* complicate and frequently increase the severity of these viral infections. As noted above, the viral infections alter normal digestion and absorption from the intestine. Therefore, I believe that it is the altered physiology and resultant increase in partially digested milk in the intestine that enables the bacterial proliferations to occur. The damaged intestinal epithelium then allows bacteria to enter the body. Although the primary infection may be viral, rapid dehydration and death within 12 to 24 h after the onset of diarrhea are due to bacteria.

## BIOLOGICAL CONTROL OF REOVIRUS-LIKE AND CORONAVIRUS DIARRHEA

Both viruses have been adapted to grow in cell culture and have been attenuated. The reovirus-like agent is commercially available as an oral vaccine. The coronavirus is currently being field tested as an oral vaccine. Initial protection by these vaccines is apparently by an interference phenomenon, and at 5 to 7 days post-vaccination protection results from locally produced antibody (15, 20).

In both the reovirus-like and coronavirus infections, circulating antibody does not seem to be protective.

## FUTURE RESEARCH

The diagnostic techniques developed during investigation of the reovirus-like agent and coronavirus are being adapted for use in other outbreaks of calf diarrhea as an aid in detection of new agents.

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