Cow-Calf Practice

Dr. Robert Miller, Chairman

*Newer Knowledge of Viral Agents in Calf Scours

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At the 1970 meeting of this organization we reported on the clinical signs of the disease, pathology induced, and methods of isolating a reovirus-like neonatal calf diarrheic agent. In this paper the subsequent research on calf diarrhea will be summarized.

First, I would like to briefly review the reovirus-like infection in experimental calves. The incubation period in gnotobiotic calves after oral inoculation with reovirus-like agent was 14-20 hours. The calves became depressed, anorectic, and developed diarrhea. The diarrheic period lasted 6-8 hours. Calves free from *E. coli* during the diarrheic period appeared normal 24 hours after the onset of diarrhea; about 50% of the calves contaminated with *E. coli* died. Calves killed within ½ hour after the onset of diarrhea had morphologically normal small intestinal villous epithelium by light microscopy; however, by immunofluorescent (FA) microscopy all the villous epithelial cells contained viral antigen. Within four hours after the onset of diarrhea, the infected villous epithelial cells were lost and replaced by squamous to cuboidal cells (2).

The reovirus-like agent was adapted to cell culture (1). High fetal bovine kidney (FBK) cell culture passage virus was attenuated by additional passages on FBK cells incubated at 29 to 30 C. The oral calf vaccine used in a previously reported 1971 field experiment was produced on primary or secondary FBK cells and frozen until used. On ranches where the reovirus-like agent had been previously found in diarrheic feces, calves were vaccinated orally shortly after birth. A total of 9,583 calves in 35 herds were vaccinated. The incidence of diarrhea was significantly reduced in 27 of 35 herds (3).

The attenuated reovirus-like agent was then adapted to and propagated on diploid FBK cells and a lyophilized vaccine prepared. Potency tests were performed in colostrum-deprived calves kept in isolation units. The calves were vaccinated orally when 6-7 hours old, observed for 48-72 hours and then challenged orally with 10 ml. of reovirus-like agent from infected, gnotobiotic calf diarrheic feces. Twenty-four out of twenty-four potency test calves remained clinically normal after vaccination. Two of these 24 calves developed a mild diarrhea after challenge; the others remained normal. Five non-vaccinated challenge control calves developed diarrhea. Four calves were vaccinated and not challenged; 30 days after vaccination these calves

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had serum neutralization (SN) titers of 64 to 256 for the reovirus-like agent.

In a 1972 field experiment using the above lyophilized attenuated reovirus-like agent, 10,411 calves in 56 herds in nine states were vaccinated. The incidence of diarrhea and mortality for 1971 and before vaccination in 1972 were obtained from the owner's records. In these herds in 1971 there were 20,350 calves born which had a calf diarrhea morbidity and mortality of 50% and 8.7% respectively. Before vaccination in 1972 there were 5,816 calves born with a morbidity of 50% and mortality of 9.3% After vaccination was started, the morbidity in 10,411 calves was 16.7% and the mortality 1.2%.

During the 1971 and 1972 field experiments there were herds in which the vaccine did not reduce calf diarrhea morbidity. In the majority of the problem herds, calves developed diarrhea when 5-21 days old. However, there were several herds in which the diarrheic calves were 2-3 days old. Diarrheic feces from both age groups were negative by FA for the reovirus-like agent.

A colostrum-deprived calf developed diarrhea after being inoculated via duodenal injection with diarrheic fecal material from one herd in which calves were developing diarrhea when five to 21 days old. Coronavirus-like particles were found in feces by electron microscopic examination. Subsequently, the infection was studied in gnotobiotic calves.

The incubation period after oral inoculation of newborn, colostrum-deprived, bacteria free calves with bacteria free diarrheic feces containing the coronavirus-like agent varied from 19-24 hours. The calves became moderately depressed and had diarrhea, but would usually consume milk. Twenty-four hours after the onset of diarrhea, the calves were more active; the feces were liquid and contained curd-like material. This type of diarrhea continued and by 42-96 hours after the onset of diarrhea the calves were dehydrated, weak and were killed.

Changes induced by the coronavirus-like agent were studied in gnotobiotic calves killed approximately three hours and 42 to 48 hours after onset of diarrhea. The epithelium of the villi in sections from the upper, middle and lower small intestine and in the colon of calves killed three hours after the onset of diarrhea appeared morphologically normal. However, all the small intestinal villous epithelial cells and the surface epithelial cells in the spiral colon fluoresced with conjugate for the coronavirus-like agent. Villi in all three levels of the small intestine from calves killed from 42-48 hours after onset of diarrhea were shortened and had cuboidal epithelial cells. Villi in the lower small intestine were the most severely affected. The average villous to crypt ratio in the lower small intestine of the control calf and two calves killed three hours after onset of diarrhea was 5.6, while the ratio in four calves killed 42 to 48 hours after onset of diarrhea was 1.2. In these calves a few immunofluorescent epithelial cells were present on the ends of the shortened villi and there was extensive colonic epithelial fluorescence.

Three colostrum fed calves inoculated orally when four to five days old with diarrheic feces containing the coronavirus-like agent had SN titers for the coronavirus-like agent ranging from 537 to 646. The calves developed diarrhea and were killed 5, 44 and 48 hours after the onset of diarrhea. These calves when killed had a less profuse diarrhea than the colostrum-deprived calves; the calves were alert and in good condition. Sections of upper and middle small intestine resembled those of a control calf and were immunofluorescent negative. The lower small intestinal villi in all three diarrheic calves were shortened. Immunofluorescent epithelial cells were present on lower small intestinal villi and in the colon. A fourth colostrum fed calf from the same herd inoculated orally when 14 days old also developed diarrhea. In this calf the severity of diarrhea and the intestinal lesions more closely resembled those seen in the colostrum-deprived calves.

The coronavirus-like agent was adapted to and attenuated in fetal bovine kidney cell culture. Twelve colostrum-deprived calves in isolation units were vaccinated orally when six-seven hours old and challenge inoculated orally with diarrheic feces when three to four days old. All the calves remained clinically normal after vaccination. One of 12 vaccinated calves developed a mild diarrhea after challenge. Two challenge control calves developed severe diarrhea. One colostrum-deprived calf was simultaneously inoculated orally with attenuated reovirus-like and coronavirus-like agents. The calf remained normal. Challenge inoculation with the coronavirus-like agent caused a moderate diarrhea. This suggested that the reovirus-like vaccine interfered with induction of resistence by the attenuated coronavirus-like agent. Evidence of an interference effect of the reovirus-like vaccine on virulent coronavirus-like agent was observed in the 1971 and 1972 field experiments of the reovirus-like vaccine. When the oral reovirus-like vaccine was used in several herds in which the coronavirus-like agent was present in

diarrheic feces, the mortality but not the morbidity of calf diarrhea was reduced. Apparently the reovirus-like agent replicating in the intestine decreased the severity of the coronavirus-like agent infection.

An orally administered attenuated coronavirus-like agent was field tested in the spring of 1972. Seven hundred sixty calves were vaccinated on nine ranches from which diarrheic feces from 5-20 day old calves had been found to contain coronavirus-like particles. Four ranchers reported a significant reduction in calf diarrhea after vaccination was started. The other five ranchers reported that incidence of diarrhea was reduced, but some calves did scour.

The attenuated coronavirus-like vaccine was also used on several calves in three herds in which coronavirus-like particles were found by electron microscopy in diarrheic feces from two to four day old calves. These vaccinated calves developed a more severe diarrhea than the non-vaccinated calves. An agent recovered from diarrheic feces collected from these younger calves before vaccine was used has been passed in experimental calves. An SN test on convalescent serum has indicated that the two coronavirus-like agents are serologically unrelated. We are currently attempting to adapt this agent to cell culture.

The onset, severity and duration of diarrhea observed in the reovirus-like and coronavirus-like infections could be correlated with the lesions. At the onset of diarrhea in both infections, the small intestine was lined by histologically normal epithelial cells but by immunofluorescent staining the cells were shown to contain viral antigen. One can speculate that initially the viral infection impaired the normal cell transport systems allowing ingested fluids and gastrointestinal secretions to accumulate in the digestive tract resulting in diarrhea. In reovirus-like agent infections uncomplicated by bacteria, the infected epithelial cells were lost, the villi remained relatively long and the epithelium was restored. Thus these calves appeared to have recovered about 24 hours after onset of diarrhea. The continuing diarrhea in the coronavirus-like infections is believed to result from the extensive loss of villous epithelial cells, shortened villi and delayed repair of the injury. Once the mature villous epithelial cells were lost and milk continued to be ingested, there was a deficiency of enzymes to hydrolyze the nutrients to absorbable molecules plus a reduced ability to absorb nutrients and water. Water retention in the gut lumen was also enhanced by an increased osmotic pressure resulting from milk partially digested by gastric and pancreatic fluids. Evidence of the relationship between the extent of small intestinal injury and the severity of diarrhea was seen when the lesions and severity of illness in the coronavirus-like agent infected colostrum-deprived and colostrum-fed calves were compared. In the colostrum-deprived calves, the upper, middle, and lower small intestine were affected and diarrhea was severe, while when only the lower small intestine was affected there was a reduced volume of diarrheic feces. In bacterial contaminated calves, we believe that the partially digested milk in the intestine both at the onset of diarrhea and later provides a medium for bacterial proliferation. Large numbers of bacteria in the small intestine may then inhibit the restoration of normal epithelium; their metabolic products may be toxic and they may penetrate the tissue, resulting in a bacteremia.

Therefore, the current recommendation of some practitioners of not feeding milk once diarrhea starts and supporting the calf on fluids has merit. Because, by withholding milk, the nutrients for bacterial growth are reduced and the digestive tract is allowed to rest and heal.

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QUESTIONS AND ANSWERS

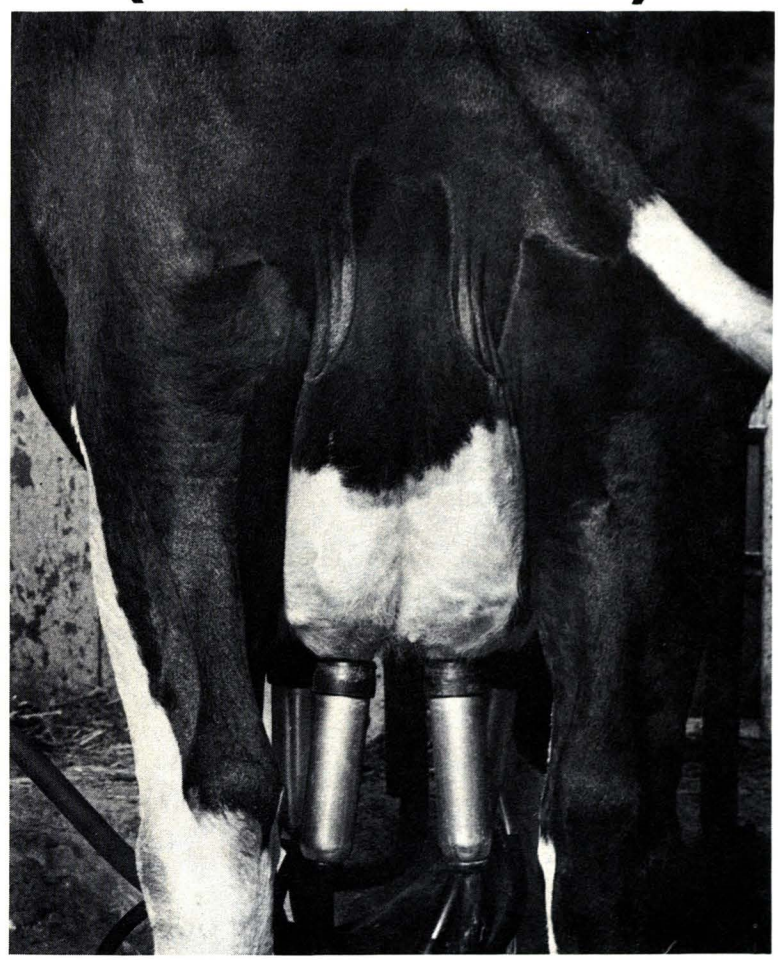
Question: How widespread is this?

Dr. Mebus: Essentially, the rheo virus has been found as far east as Pennsylvania, and I think in Utah and from New Mexico and Arizona up to the Dakotas. It also has been found in several herds in Canada and the distribution of the corona virus I would say has been found in eight or nine states and Canada. Will the antibacterial antiserums protect any? They won't protect against the viral infection but they will help protect and inhibit the secondary bacterial infection and with the rheo virus infection, really the secondary bacterial infection is the critical one. That ends up killing the calf.

Question: Is there any diagnostic laboratory checking on these?

Dr. Mebus: We have sent out material to other laboratories. The success by these laboratories have been variable. Norden Laboratories are planning on conducting a seminar in February for any diagnostic personnel who want to attend to try to get the procedures that we are using more uniform.

(for treatment)



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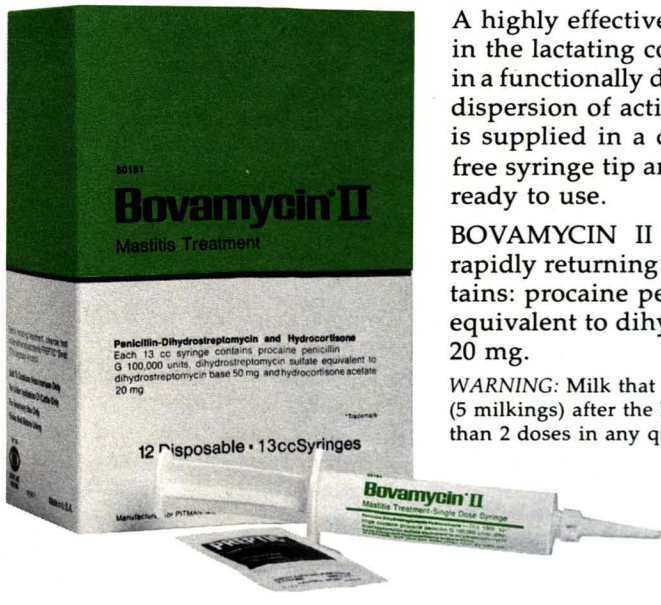

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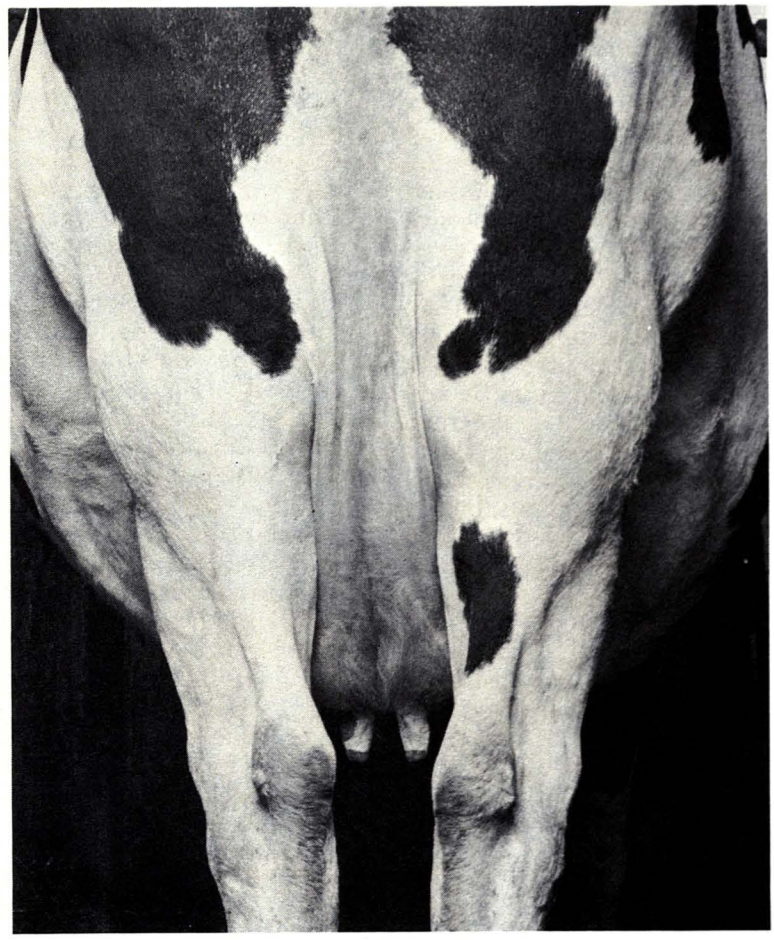

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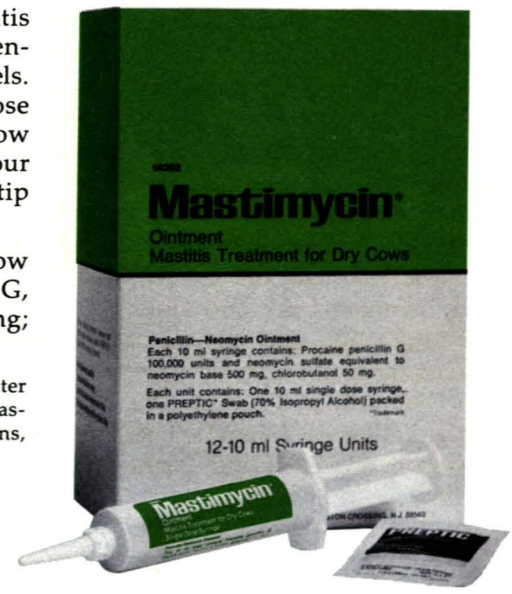

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Question: Is there any advantage in vaccinating cows for colostral antibody?

Dr. Mebus: For about three years now we have vaccinated in several herds with an inactivated rheo virus vaccine. The cows have been vaccinated approximately 60-90 days prior to calving. In these herds we have had pretty good control of the rheo virus infection. Actually, we have not found a rheo virus infection in these herds in which the cows have been vaccinated. One disadvantage of the cow vaccine that we are only getting passive protection and how long this antibody is being secreted in the milk, I do not know. This has not been looked into. You have to remember with the passive antibody infection though, the calf does have to go through an active infection at some time in order to become immune. This spring, actually, we are starting to vaccinate right now approximately 16,000 cows that will be vaccinated with a combined and activated rheo-corona virus. It will be interesting to see what happens. On the rheo vaccine, Norden Laboratories are handling it, and you would have to go through Norden Laboratories. On the inactivated rheo corona virus, due to government regulations, we are pretty much restricted to using it only in the state of Nebraska.

Question: Where do these calves develop or get the rheo infection?

Dr. Mebus: We only have circumstantial evidence on the source of the infection and that is the evidence indicates that there must be carrier animals in these herds in which periodically they shed virus and then there are susceptible calves around to start the infection. This evidence comes from the fact that some herds that have been sold essentially as groups of animals and have gone through to another premise 40 or 50 miles away had the same problem in calving as they did on the home ranch. Apparently the cows took it with them. Once it starts, these calves will have a virus titer in their feces, meaning that you can dilute this stuff a million times and still have infectivity. So, once you get the first calf scouring, there is plenty of virus! The initial infection apparently must, we suspect, come from a carrier animal. Is this limited to beef cattle? The dairy industry does have it—we had worked primarily with beef calf operations. Rheo vaccine last year was used in some dairy herds and the results from the rheo vaccine was only 15% in the dairy herd. What does the laboratory need for diagnosis? We have been asking for fecal material collected from calves shortly after the onset of diarrhea. The fluorescent antibody (FA) method that we use for the rheo virus agent is only a herd diagnosis and, therefore, we ask that six, eight or ten samples be submitted and these must be collected during the first five or six hours after the onset of the diarrhea into small jars and frozen and shipped frozen to us.

Question: Should you start fluid therapy right after the onset of diarrhea?

Dr. Mebus: I would say right after the first couple of calves and you see how the thing is going. If they were mine—beef cows it is hard to do—I would take them off of milk but if you could take them off milk and support them with fluid therapy until the diarrhea stops and then introduce them back to milk. That would probably be the desirable way of doing it.

Panel Discussion

Introduction: Dr. Robert Miller, Moderator

The members of this panel are Dr. Herb Lloyd of Belle Glade, Florida, who is a mixed practitioner and an Auburn graduate; Dr. Barry Allen, Rotan, Texas, who has a very mixed practice of cow-calf, dogs, cats, feedlot and everything else; and Dr. Robert Jackson, Lancaster, Wisconsin, who we had on the program here yesterday so I know you're all well acquainted with him. We will start this by having each man say a few words about his practice so that you will all get in your own mind what their problems are and then we will try to get a good question and answer session going. I would like to start out with Dr. Allen.

Dr. Allen: I think you would probably call my practice as diversified as any practice can be. We have the backyard farmer with one milk cow and on the top end we have one rancher with 200 sections of land and 6,000 cows, plus everything in between. So, we will attack this problem from all angles and maybe we can give you some pointers on how we handle it in our part of the country.

Dr. Lloyd: My practice in Florida is relatively new and I purchased it about two years ago so I cannot say for the practice that it is an old one. It is an old practice but I have not been in it very long! I worked five years full-time as resident veterinarian prior to that so I had a little insight into that type of practice. In our area, we are going to larger and larger ranch operations and less and less of them, yet we still have some small ranch operations. Currently, we have a full-time small animal practice except that I have an associate that takes care of that part of it right now. He has, this