Dairy Split Session I

Moderator - Karen Jacobsen

Bovine Neonatal Cryptosporidiosis

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Abstract

The purpose of this paper is to review some of the more significant features of the body of research concerning cryptosporidiosis and discuss some of the opportunities for prevention and therapy which may become available in the future.

Introduction

Cryptosporidiosis is the most widespread etiologic agent of bovine neonatal diarrhea in American dairy calves. Although reports concerning the significance of this pathogen had surfaced throughout the United States for over ten years, a nationwide survey (the National Animal Health Monitoring System Dairy Heifer Evaluation Project) proved the significance of the organism to the American dairy industry. In this study, sixty percent of 1103 dairy farms tested in 28 states had one or more positive calves detected. Of calves aged seven to 21 days, roughly 50% were found to be positive. Herds in the Western states experienced the highest prevalence. Larger dairies (greater than 100 milking cows) and farms with multiple-cow maternity facilities were most likely to have infected calves.[1]

For those of us dealing with infections in beef calves, attack rates are often as severe as in dairy calves. Widespread disease is often a feature of calves born under harsh weather conditions. It is particularly difficult to control where a common calving area is utilized and when calving in any particular group of cows is extended beyond six to eight weeks. Given the man-power and animal handling constraints of many beef operations, severe infections in beef calves can be more difficult to cope with than in dairy calves.

As information concerning cryptosporidiosis as a cause of enteric disease in calves began to unfold in the 1980's, the role of this etiologic agent as a zoonotic pathogen also began to be recognized. At first, it was thought to be a serious cause of zoonotic disease only in immunocompromised humans[2,3], but it was soon found to induce disease in the non-immunologically depressed[4,5], in animal handlers[4,6], and in veterinary students exposed to infected calves.[7-10] *Cryptosporidium parvum* was also associated at this time with diarrheal disease in travelers and as a cause of disease in children in day-care centers.[11,12] The threat of contamination by cryptosporidium oocysts to water supplies or surface water was recognized long before the recent outbreak in Milwaukee that affected 400,000 people.[13,14] As the zoonotic significance of this organism has unfolded, it has become increasingly important for the food animal veterinarian to assume an important role in preventing dissemination of infection and misinformation to the public.

Pathophysiology and Epidemiology

After the host enterocyte becomes infected with *C parvum* a host-plasma membrane surrounds the organism, allowing it to develop as an extracytoplasmic but intracellular parasite. This undoubtedly contributes to this coccidian's ability to resist standard chemotherapeutic agents. Damage to the brush border and absorptive surfaces of the enterocytes of the intestinal villi results in a maldigestive-malabsorptive syndrome and secondary osmotic diarrhea.

The massive numbers of oocysts excreted by an infected calf and the ability of these oocysts to persist in a contaminated environment are the major features

through which infection persists within a facility. It is likely that infection from previously infected calves is far more important than transmission from the dam. Bull calves born in a common maternity stall located far from where older (24 hrs to 21 days) calves were housed, and moved to a research facility within 6 hrs of birth, did not develop clinical disease. Heifer calves born in the same maternity stall and moved into calf barns or crowded hutches developed disease during the second week of life. A similar conclusion has been drawn by another researcher in Idaho.[15] This opinion has not been shared by Scottish investigators, who have documented infection in adult cows at every sampling period over an 18 month period.[16] If the dam does contribute significantly to the infection of the environment of the neonate, then disruption of the disease cycle will continue to be difficult particularly in beef herds.

The significance of aerosol exposure should not be overlooked by the veterinarian, particularly when zoonotic spread is a concern. Aerosolization and respiratory membrane infection can lead to enteric disease in humans and calves.[15]

Resistance to Disinfectants and Antimicrobial Agents

C. parvum oocysts are resistant to most agents used for environmental disinfection including phenols, chloroform, perchlorethylene, and perchloracetic acid. A few agents have been reported to be partially effective against the oocysts including half strength household bleach[15] , peroxide, a two phase product that produced ammonia (Oo-cide) and a chlorine dioxide-based cold sterilant (Exspor).[17] Small articles contaminated with oocysts can be frozen in a standard freezer and the oocysts will be destroyed.

Cryptosporidium oocysts are infective when shed, and under favorable conditions they can survive for several months. Dry conditions, temperatures higher than 65°C or below 0°C will reduce or destroy the infectivity of the oocyst in a few hours/days.[18] The infective agents are particularly difficult to eliminate from calf barns, especially those with dirt floors and wooden pens. Properly spaced calf hutches that are rigorously cleaned between occupants, turned over and allowed to dry in the sun are the most significant means of preventing severe clinical disease. In areas where calf hutches cannot be spaced far apart, using gravel or crushed rock covered by a layer of sand will help keep the ground from becoming heavily infected. Straw bedding used inside hutches should be removed and burned.

The oocysts are as resistant to standard antimicrobial agents as they are to disinfectants. Decoquinate® delayed onset of oocyst shedding, decreased days of oocyst shedding and improved stool fluidity, but had to be administered at very high concentrations in order to obtain the desired response (1750 mg/feeding delivered in a gelatin capsule — 10 times the recommended dose).[19]

Calves challenged with oocysts while receiving paromomycin in their milk at 25, 50 or 100 mg/kg bwt for 11 consecutive days levels experienced fewer total days of diarrhea, less severe diarrhea, and shed oocysts for fewer total days than unmedicated calves. Calves receiving 100 mg/kg shed significantly fewer oocytes (none) than unmedicated calves. Surprisingly, weight gains did not vary significantly between the unmedicated and medicated groups. Presently, the cost of this drug precludes it from becoming a common therapy for calves although it has been useful in human infections.[20] Another antimicrobial (Azithromycin) has been effective in eliminating infection in immunesuppressed humans. In our experience, the manufacturer has refused to sell this antimicrobial to veterinarians and the drug is cost prohibitive (in 1995, cost for 18 tablets — $117). One report in the French literature suggests that treatment of calves with a combination of sulfadimethoxine, diaveridine and toltrazuril is usually effective in controlling cryptosporidium infection, but sulfadimethoxine by itself has failed to protect calves against infection.[21,22]

One European drug - Halofuginone lactate - has been reported to have some success against cryptosporidial infection.[23] Early controlled challenges in lambs showed promising results. A study that involved about 300 calves with natural and often mixed infections showed calves treated with 0.5 mg/kg of halofuginone lactate solution *per os* for 3 consecutive days resulted in relatively rapid resolution of diarrhea. Treatment was associated with a reduction in appetite.[24] Calves in another study received halofuginone lactate at 60 and 120 µg/kg/day, respectively, for 7 days following cryptosporidium oral challenge. The calves receiving halofuginone experienced a marked reduction in severity of clinical cryptosporidiosis. This efficacy was dose-dependent; a lower dose did not prevent clinical disease and mortality but clinical signs were absent with the 60 and 120 µg/kg/day doses. Calves did shed oocysts after drug withdrawal, however.[25]

Other Aspects of Therapy

It is important to remember that the most consistent necropsy finding in fatal cryptosporidial infection of calves is emaciation rather than dehydration. Because of the protracted nature of the disease process, infected calves are particularly prone to fatal hypoglycemic episodes. It is unwise to remove milk from the diet of affected calves; small, frequent feedings (with whole milk wherever possible) and free choice electrolytes are most successful in restoring dairy calves to

health. Calves with immediate access to oral electrolyte solutions often appear to maintain their hydration status without requiring supplemental i.v. fluids. Use of glucose-rich electrolyte solutions is controversial, however. The value of isosmotic glucose in cryptosporidial infection has been questioned since glucose-sodium cotransport is diminished in the gut proportionally to the severity of gut damage and loss of absorptive surface caused by *C. parvum*.[26] This implies that commercial oral electrolyte products that are hyperosmotic from elevated glucose concentrations may contribute to the severity of the ongoing osmotic diarrhea that is secondary to cryptosporidial infection.

Some electrolyte solutions containing pectin or pectin-like substances claim to bind oocytes within the matrix formed by these products within the gut, decreasing the auto-infection rate of enterocytes. These products have not been evaluated in the US.

Vitamin A deficiency has been documented in calves infected with *C. parvum*. This study concluded that vitamin A should be provided parenterally to young calves with enteric cryptosporidiosis in an attempt to avoid depletion of concurrent low liver vitamin A reserves.[27] Addition of certain amino acids to electrolyte solutions or supplementations with other natural factors that stimulate brush border healing are currently being evaluated in cryptosporidial infection at NCSU and may ultimately enhance repair and recovery in infected calves.

Future Prophylaxis Through Vaccination

Several groups are presently concentrating on immunoprophylaxis against cryptosporidium. These groups are attempting to develop an effective vaccine for use in the dam which would provide the calf with colostral antibody of the specificity and magnitude capable of minimizing or delaying enteric *C. parvum* infection. Hyperimmune bovine colostrum has been shown to protect mice and calves against oocyst challenge but sensitization of the dam required intramammary injections which were not practical in a field situation.[28-31] Careful selection of appropriate immunogens common to all stages of the infective organism (oocyst, sporozoite, merozoite) have brought many of these groups closer to producing an effective vaccine for the neonatal ruminant.[32] Another vaccine, derived from lyophilized oocysts, did seem protective against experimental challenge but the number of infective oocysts administered in this study was of much lower magnitude than is routinely used by other research groups.[33]

Although these vaccines continue to show great promise, it appears that daily supplementation of hyperimmune colostrum may be necessary to prevent clinical cryptosporidiosis; systemic antibody absorbed from colostrum appears to have little effect against luminal infection.[34] A novel means of producing large quantities of specific antibody against enteric pathogens has been suggested by hyperimmunizing hens to produce large quantities of specific anti-Cryptosporidium antibody. This technology might result in unlimited and inexpensive quantities of specific antibody which could be administered on a daily basis to housed calves. Such suggestions must be evaluated carefully since the full function of the abomasum is reached at about 24 hrs of age and normal digestive processes may destroy the egg derived specific antibody before it ever has an opportunity to reach the small intestine.[35]

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Abstract

Use of composite milk samples for diagnosis of *Staphylococcus aureus* mastitis in dairy cattle

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Reliability of use of composite milk samples for diagnosis of intramammary *Staphylococcus aureus* infections in dairy cattle was compared with that of individual gland samples. Microbiologic culturing of composite and individual gland milk samples collected from cows suspected of having subclinical mastitis revealed a relative sensitivity of 0.63 for composite samples, with relative specificity of 0.98. Factors influencing the relative sensitivity of composite samples were the number of infected glands per cow, the amount of *S aureus* shedding from infected glands, and the proportion of the composite sample obtained from each gland. If 3 consecutive samples had been collected in 95% of the cows with at least 1 infected gland, *S aureus* would have been found in at least 1 of the 3 composite samples. Increasing the inoculum volume from composite samples from 0.01 to 0.05 ml increased the relative sensitivity.