Present and Future Control of Cryptosporidiosis in Cattle

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Abstract

Cryptosporidiosis continues to be a problem for dairy calves and a perplexing public health risk, despite recent advances in molecular biology that have contributed to our knowledge of the pathobiology of the causative organism. Cryptosporidium infects at least 155 mammals, including humans and dairy cattle, primarily resulting in diarrhea. Unfortunately, the somewhat unique lifecycle of this protozoan and the armor of its oocysts leaves us little defense. This paper reviews aspects of transmission, disinfection, treatment, prophylaxis, nomenclature, and zoonotic concerns of cryptosporidium.

Introduction

Cryptosporidiosis in dairy cattle can be very frustrating to deal with. The agent of this disease, *Cryptosporidium parvum*, is the pathogen most often diagnosed in preweaned scouring calves.[4,33] It does not appear to be as life threatening as, for example, some serotypes of salmonella, but under the right group of conditions can cause severe diarrhea and death in young calves.[29] Clinically affected calves are the most likely animals to shed large numbers of oocysts in feces, but calves with no outward signs of infection may also shed large numbers of oocysts. In fact, during an average infection a calf may excrete oocysts for six to nine days,[9] scour for three days,[35] and shed a total of approximately 40 billion oocysts.[34] Mature cattle have also been shown to shed oocysts, albeit in lower concentrations, in their manure, especially around parturition.[5,10] Thus, this pathogen can be very prevalent in the calf's rearing environment and can be present in the maternity area as well. Various reports have estimated the herd-level prevalence for young calf fecal shedding in North America ranges from 59-89%.[12,44] Furthermore, the within herd calf-level prevalence has been reported to average 40-48%,[12,49] with a range of 0 to 72% among different calf populations.

Calves are primarily infected via the fecal-oral route and it likely takes less than 50 oocysts to infect a healthy calf.[30] The reproductive and infective structure, the oocyst, survives very well in the environment with a portion of the oocysts retaining infectivity after freezing.[7] It is also resistant to many disinfectants at farm-friendly concentrations, e.g. sodium hypochlorite (bleach), peroxygen (Virkon), chlorine and iodophores.[1,3] Six percent hydrogen peroxide and 10% formalin have shown activity against oocysts,[3,50] but hydrogen peroxide is readily deactivated in the presence of organic matter. Thus, for practical purposes, disinfectants have minimal activity against oocysts. The potentially large number of oocysts that survive well in the environment leads to a high likelihood of a susceptible calf being exposed to an infectious dose of oocysts. Once the intestine is colonized, the life cycle of the parasite allows for auto-infection of nearby cells, further decreasing the number of ingested parasites required to initiate infection and possibly leading to chronic disease.

Therapy and Prophylaxis

The almost constant environmental presence of oocysts, the well-adapted life cycle of the parasite, and limited impact of oral vaccines that have been evaluated under field conditions[17] often leave us attempting to treat sick calves. Unfortunately, that remains frustrating as well. Many antimicrobial agents have been investigated for treatment or prophylaxis of calves at risk for cryptosporidiosis. Among them are allicin, ionophores (monesin and lasolocid), amprolium, decoquinate, sulfas, paromomycin, nitazoxanide and halofinone. Most other antimicrobials have limited pharmacologic basis for use against a protozoan pathogen (e.g. ceftiofur) and/or are prohibited from extralabel use in food animals (e.g. metronidazole).

Allicin, a sulfur-containing component of garlic that is available as an additive to milk replacer, was shown in a randomized controlled trial to neither alter the duration of diarrhea due to *C. parvum* nor enhance weight gain in infected calves.[36] Monensin was also found to be ineffective in an oocyst-inoculation trial in calves and rats.[43] Lasalocid has been reported to have some efficacy at relatively high doses—5-15 mg/kg body weight (BW).[13,40] Unfortunately, this cannot be recommended because doses of 5-8 mg/kg BW have been shown to be potentially lethal to neonatal calves.[2] Trimethoprim-sulfa, sulfadimidine, sulfadimethoxine and amprolium have also been demonstrated to be ineffective against

the infection.[28] Nitazoxanide, a nitrothiazole benzamide with a wide spectrum of antibacterial and antiprotozoal activity, has shown some efficacy in early human trials, especially those with intact immune systems. Unfortunately work in calves has been less promising.

There are many anecdotal reports from practitioners in the field attesting to the utility of a high dose (e.g. 5x) of decoquinate in the prophylaxis and treatment of cryptosporidiosis in calves (AABP-L). In addition, one trial with five Holstein bull calves suggested it may reduce the number of days of oocyst shedding and improve fecal scores, but did not prevent shedding of the organism when given prophylactically.[42] Unfortunately, in another trial, decoquinate showed little-to-no activity against the parasite in either cell culture or mice.[23] The authors of this trial postulated that any apparent clinical improvement of calves with cryptosporidiosis following treatment with decoquinate was due to effects other than on *C. parvum*. The most recent trial that examined the effect of decoquinate[30] used a dose of 2.5 mg/kg BW (5x label dose) in dairy calves and failed to show any effect of treatment on oocyst shedding or clinical signs associated with cryptosporidiosis. This trial did, however, show that the lower the dose of oocysts received by the calves, the shorter the duration of shedding.

Paromomycin, a human-labeled aminoglycoside, has been shown to have utility in ameliorating signs of cryptosporidiosis in cell and rodent models when used prophylactically, and is often used as adjunct therapy in human AIDS patients with cryptosporidiosis. A suggested and researched preventive dose in calves is 100 mg/kg BW for 10 days, which has been shown to decrease days with diarrhea, severity of diarrhea and the number of oocysts shed compared to untreated controls.[6] Unfortunately, this comes with the vagaries of using an aminoglycoside in food-producing animals as well as a price tag of about $60/day for a 40 kg calf, i.e. $600 USD. In addition, weight gains did not differ between treated and control calves in this trial.

Halofuginone lactate is one antimicrobial that has shown promise prophylactically to mitigate cryptosporidiosis in dairy calves. In at least three European trials with calves it has decreased oocyst shedding and improved fecal consistency scores when administered orally to calves for the first seven days of life.[20,21,37] In a trial performed in Canada, calves were administered either 5mg of halofuginone lactate (Halocur) orally one time per day after morning milk for the first seven days of life or a placebo.[19] This work did not demonstrate a difference in average daily gain, starter intake, water intake, or milk intake between the two groups of calves, confirming the safety of halofuginone at the recommended dose. In addition, it showed a significant delay in the onset of shedding of oocysts and a significant decrease in the total number of oocysts shed by calves treated with halofuginone. Unfortunately, to the authors' knowledge halofuginone is currently not available in the US but is available in Canada with an Emergency Drug Release. In the future, the drug may become fully licensed in North America, but this is likely to take substantial time.[45]

Finally, work is under way on a recombinant protein vaccine against *C. parvum* that is administered to dry cows in a similar manner to an *Escherichia coli* K99 scours vaccine to produce hyperimmune bovine colostrum.[39] At this point in time it is not commercially available, but it has moved from the research laboratory to testing in the pharmaceutical industry.

So now what? The bugs and drugs paradigm often does not work with this pathogen, or most others for that matter, causing scours in dairy calves. Ask yourself, "Can a pathogen that is usually present on a farm be the cause of an increase in disease incidence?" The answer is usually "No". While cryptosporidium can cause diarrheal disease in the absence of other pathogens,[18] usually some other factor in the host (in this case calf), pathogen (in this case cryptosporidium) and environment triad is usually also altered. An example of a host factor is immune competence, examples of environmental factors include poorly cleaned milk and grain buckets, and examples of pathogen factors include co-colonization with other more virulent enteropathogens or different *Cryptosprodium* sub-genotypes.

So what can we do? Fortunately, most clinically ill calves respond to fluid therapy and supportive care. Remember to watch for metabolic acidosis associated with cryptosporidium-induced diarrhea. Consider supplementing intravenous fluids with sodium bicarbonate.[a] Be persistent and intervene early with oral electrolyte solutions, while continuing to feed milk or milk replacer at the normal daily rate (divide it into more frequent, smaller feedings if necessary and feasible). Recall the ability of *C. parvum* to auto-infect adjacent cells and the calf's slow immune response to the parasite that can lead to protracted disease and necessitates vigilance in care of these calves.

Preventing infection requires following sound management practices and meticulous attention to hygiene for calves. Be aware that a number of studies have indicated that the lower the infectious dose (i.e. the amount of organism in the environment) the shorter the duration of shedding. Thus, removing the calf from the maternity area as soon as possible, and putting it in an environment that has been cleaned from previous calf use, are both beneficial. Cleaning should include removing bedding and the base (e.g. geotextile fabric or large gravel), and steam disinfection of the pens. Exposure to pasteurization conditions (160°F [72°C] for 15 seconds) has been shown to kill oocysts in water or milk.[16] Re-

member though, that water can spread other pathogens around if it is not used judiciously and the area not allowed to dry between calves. As always, wear clean clothes and boots when working with calves. Reducing fly populations can also help to decrease cryptosporidium transmission, as filth flies have been shown to transmit oocysts.

Zoonotic Concerns

Cryptosporidium spp also cause diarrhea and its sequelae in a wide range of other hosts, including humans, by infecting the microvillus border of the gastrointestinal epithelium.[8] In people there have been outbreaks associated with contaminated drinking water,[25] food,[41] and recreational exposure to water,[22] as well as multiple sporadic cases.[27] The severity and persistence of cryptosporidiosis is related to the immunocompetence of the host, with the disease usually being self-limiting in people with functional immune systems and life threatening in those that are immuno-compromised.[14] An oddity of human infection is that adults seem to be highly susceptible, whereas most other species acquire some age-related resistance. Note that 50% of human volunteers became infected after ingesting 132 oocysts of a bovine isolate of cryptosporidium, and one of five humans became infected with only 30 oocysts. The infective dose is therefore very low.

When *C. parvum* was first found to be a human pathogen in the early 1970s it was thought to be only transmitted via a zoonotic cycle, i.e. only between animals and humans. Thus, by virtue of their presence in watersheds, and the common occurrence of *C. parvum* in calves, cattle have often been implicated as one of the sources of *C. parvum* oocysts that have led to drinking-water borne outbreaks in people.[46] For example, among many factors hypothesized to have been associated with the infamous Milwaukee outbreak of 1993, where an estimated 400,000 human cases occurred,[24] cattle manure in the watershed was originally advanced as a source.[b] Recently, numerous investigators have determined that there are at least two genotypes of *C. parvum* that infect humans and at least two transmission cycles by which it persists in nature.[31,38,48] These genotypes were designated H or 1, and C or 2. More recently these genotypes have been described as separate species: *C. hominis* (formerly genotype 1) and *C. parvum* (formerly genotype 2).[32] Apparently, the H (or 1) designation was chosen because it was found predominately in isolates from humans, whereas the C (or 2) genotype has a wide host range that includes cattle, humans and at least 10 other mammals. A recent review[11] indicated that of all the drinking water-associated outbreaks in North America that have had genotype analysis performed of parasite isolates, none in the United States and only one in Canada have been associated with the zoonotic (C) genotype. In contrast, all the others were associated with the H (or 1) genotype.[2] This genotype (or *C. hominis* species) had been only found in humans until recently, when it was identified in two out of 411 fecal samples collected from cattle in northeastern Scotland. Similarly, a study from the UK concluded that the likely source of contamination of water associated with five outbreaks in people was human feces.[26] However, it should be noted that the C genotype has been associated with a significant proportion of sporadic human cases.[27]

Conclusions

Oocysts from cryptosporidium are immediately infective after being shed from the host. Once in the environment, they may persist viably through many physical insults, including freezing and moderate heat. In addition, oocysts are not susceptible to most disinfectants at farm-friendly concentrations. They are also small enough to evade physical capture as well. Infected hosts shed many times the infective dose for young calves (and most people too). Unfortunately, there are few to no treatment or prophylactic methods to deal with cryptosporidiosis in ruminants, though halofuginone lactate presents a potential bright spot. There are no commercial vaccines available as of yet, though there is research making progress in the area of hyperimmune colostrum. Whether or not these tools become available, control will rely on an integrated approach to reduce flies, curb transmission and decrease environmental loading. Diligence in proper calf care and animal husbandry (i.e. clean, dry, isolated, well-ventilated calves that have received suitable colostrum) will serve calves well until age-related resistance associated with the normal development of the rumen and intestinal flora begins.

Footnotes

[a] Bicarbonate Needed = (base deficit) * (body weight (kg)) * 0.5;
For example, mEq of Bicarb for a calf with a very weak suckle and barely able to stand:
10mmol/L x 50kg x 0.5 = 250 mEq HCO_3^- ;
8.4% NaHCO3 = 1mEq/ml; 5% $NaHCO_3$ = 0.6mEq/ml

[b] Isolates from the Milwaukee outbreak later were determined to be the type I genotype suggesting human sewage was the source of infection.

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