Cryptosporidiosis: Cattle Production and Zoonotic Concerns

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Introduction

National Cattlemen's Association recently set policy. Under **Animal Health and Inspection,** the first phase reads, "Support research to reduce the presence of crypto."

Cattle practitioners know cryptosporidiosis from several standpoints. First, the finding of cryptosporidial occysts is frequent in stool specimens submitted for scours diagnosis. Second, diagnosticians report "Enteritis due to *Cryptosoridium*" after necropsy of calves which died with dehydration and diarrhea. Third, recent graduates have been witness to cryptosporidiosis with severe clinical signs in themselves or some classmates.

Cryptosporidiosis is in fact a zoonotic disease. Therefore, veterinarians should be aware of their responsibilities in that regard. Also, we should know that regulation of cattle businesses will take into account the widely-held belief that human cryptosporidiosis is the result of watershed contamination by cattle and other livestock feces.

What follows is up-to-date information on all aspects of cryptosporidiosis, as it impacts the cattle practitioner and the clientele. There are two categories of information presented: 1. What I know and 2. What I think I know. Because the latter category undoubtedly exceeds the former, each consumer of this information must continue to think critically on the subject.

We'll labor through some of the details and periodically, get to the questions implied by the title, namely direct impact of cryptosporidiosis on production, and the perhaps even greater impact of regulation on the horizon designed, allegedly, to keep cryptosporidial oocysts (and *E. coli*, etc.) away from any watershed.

Cryptosporidiosis Historically

Cryptosporidium is a protozoan in the same suborder as Eimeria, and has similar life cycle stages. Cryptosporidia were found first in the stomach of a mouse in 1907.[1] The organism was named, *"Cryptosporidium muris"*. Shortly thereafter, a second *Cryptosporidium* was found, this time in the intestine of laboratory mice.[2] This species was named, *"Cryptosporidium parvum,"* and today, it is known to cause intestinal cryptosporidiosis with diarrhea, in calves, lambs, humans, literally all mammals; the over 1000 references have been reviewed.[3]

Recent history finds the incrimination of cryptosporidia in human diarrheal disease at the end of the seventies and into the eighties.[4] Most notable have been critical to fatal cases in immunodeficient patients[5] and massive outbreaks of diarrhea in the general population due to contaminated city water supplies.[6] Cryptosporidial *(C. Parvum)* involvement in calf enteritis[7] was recognized during this same period and the hunt for treatments has been vigorous, though largely fruitless.[8]

In the mid-eighties, *Cryptosporidium muris* was re-discovered, this time as an infection of the abomasum of cattle[9] and the stomach of a few other species in zoos. Based on the gross and microscopic damage in the abomasum, it appeared that in cattle, digestion efficiency of the abomasum would be reduced, which means dollars lost to the cattle business. In fact, the above rediscovery was in a pen of 88 steers which had performed poorly. The obvious questions are being addressed, though progress has been slow. At this time, the most useful designations for the organism from cattle might be "Bovine *C. muris* or *C. muris*-like organism. This is because we don't know that this isolate is, in fact, identical to the original *C. muris* in mice.

Host and Geographic Considerations

Cryptosporidia infect every animals species; assume that fact, for practical purposes. But the intestinal forms, presently designated *C. parvum* in mammals and *C. baileyi* in birds, have dominated the reports. The organism is worldwide and it might safely be said that all infant livestock and infant wild animals are exposed. Anticryptosporidial titers are common in many species[10] and there are many reports of cryptosporidiosis in wild animals.

Humans as well are exposed,[11] probably on a regular basis, but it might be that in places like the urbanized United States, a large part of the adult population is susceptible due to lack of exposure. In former times, many lived a little closer to the "land." We suspect that one is resistant after an initial exposure, but repeat infections have been reported, second and third episodes being relatively mild, clinically. Asymptomatic infections in humans and calves have been reported.[12,13]

In general, intestinal cryptosporidiosis of animals affected only the young (2nd week of life) whereas humans of any age can contract the infection. It is important for veterinarians in their public health role to realize that the elderly humans[14] and those on chemotherapy should avoid exposure to livestock with cryptosporidiosis. These categories of people can suffer severe, life-threatening cryptosporidiosis.

Diagnosis

Put simply, virtually every calf that is 7-14 days old has cryptosporidial oocysts in the feces at some point, and perhaps suffers cryptosporidial diarrhea. Just look over the fence at the diarrheal feces behind these calves and say, "CRYPTO." You probably can assume that rotavirus and perhaps coronavirus are there too.

But if you want a specific diagnosis of crypto, concentrate the oocysts via fecal flotation with saturated sugar solution or use an acid-fast stain of fecal smears. The flotation method is pretty sensitive and you can school yourself using known positive samples; the microscope must be adequate.

I prefer the acid-fast stain of the fecal smear. You can't miss if you consider that your objective is to find the oocysts in just one calf's stool. If one calf has it, then intestinal cryptosporidiosis is pretty much a way of life on that farm.

Often there are several to many calves in the 7-14 day old category. I take one slide, some wood applicator sticks and place many fecal streaks on the one slide. The fecal smear can be as small as a 5 mm dot from the end of the wood stick. That way you can get lots of samples on one slide.

Next, stain the slide using the "AFB Kit" ordered from, VOLU-SOL, 700 West Sunset Road, Henderson, Nevada 89015 (702) 565-1383. Investment is about $22 plus shipping and handling. Heat fix the fecal smears by 3, 1 second passes over a flame, smear side up, flood slide with the carbol fuchsin for 2 minutes, rinse in tap water. Flood with decolorizer for 2-4 seconds, wash in tap water and apply counterstain for a minute or so.

Blot and air-dry the slide and mount a coverglass, for easy viewing of a sharp image at any magnification. I read these dry, without coverglass at about 125X. At higher magnifications, the image is fuzzy if a coverglass is not used. Again, expertise can be gained by schooling beforehand with a known, oocyst-positive fecal sample, and by using as a positive control sample with each test sample. Work with your diagnostic laboratory personnel to identify and store an oocyst control; perhaps they will send your some smears or some formalin-preserved positive feces.

A fluorescein-labelled, monoclonal antibody (Meriflor, Meridian Diagnostics, Cincinnati, Ohio) for diagnosing *C. parvum* is available and in use by various research and diagnostic laboratorians. Also the very sensitive polymerase chain reaction will be applied to explicit diagnosis of as yet undefined strains of cryptosporidia. We want to know that all those oocysts in the local river or lake are from beavers or opossums or raccoons, if that is the case. Right now, the knee-jerk reaction to finding oocysts in surface waters, is to blame cattle.

Transmission, Epizootiology, Epidemiology

Simply put, cryptosporidia follow the fecal-oral transmission route. At times, it appears that veterinary students just passing through the calf facility, no contact directly with the calves, acquire the infection. The oocyst, which is infective when passed in the feces, can be aerosolized. When the sporulated (infective) oocysts are inhaled, they excyst and the life cycle can be completed on respiratory membranes. Autoinfective oocysts generated there are moved to the pharynx, swallowed, and give rise to colonies of cryptosporidia in the intestines, which in turn, generate more autoinfective oocysts. Through such a mechanism, the population of cryptosporidia within an individual can become large though the infecting dose may have been small.

One researcher documented the infection in the urinary tract of calves[17] so splattering urine might be a medium of transfer. Also, we know that cryptosporidia can replicate on a variety of moist membranes, including upper respiratory membranes. Another researcher had a needle blow off a syringe full of cryptosporidial oocysts, felt moist droplets on his face, and developed cryptosporidial diarrhea 5 days later, as predicted.[11]

It is easy to imagine, therefore, the ease with which

cryptosporidial oocysts are transferred. It is not uncommon to see the farm dog feasting on calf feces and licking the milk off the muzzles of the calves. And then it might go play with the kids. We donated an orphan lamb to a youngster and 5 days later, the child had cryptosporidial diarrhea; the lamb had been a little loose at 6 days of age. Transmission from wild mice on dairies to calves has been hypothesized, based on the finding of the cryptosporidia-indected mice in the calf rearing area, and successful creation of cryptosporidiosis in calves exposed to oocysts from the mouse feces.[18]

Unpublished results from pilot studies of calves from a well-managed dairy revealed that calves procured directly from maternity pens did not contract cryptosporidiosis whereas all calves which were raised in the calf barn contracted the disease. This would imply that cows are not frequent carriers of the *C. parvum* organism but that cryptosporidiosis on dairies is mainly a disease perpetuated in the calf-rearing area, as opposed to the maternity area. We have only a single verbal communication that oocysts of *C. parvum* were found via immunofluorescence in an occasional adult dairy cow fecal sample. See section below about *C muris* oocysts in cows with abomasal cryptosporidiosis.

Transmission among the members of human families has been documented,[19] as has spread of cryptosporidiosis (and rotavirus diarrhea) among children and workers at day-care centers.[20] Children are careless, predictably, in their hygienic practices.

To re-emphasize the public health role of veterinarians, the elderly and people undergoing immunosuppressive therapy of some kind are extremely susceptible to cryptosporidial (and other) infections. The clinical syndrome in these people can be life-threatening, so inform your clients with calves, about the risks. Also, a significant percentage of humans with AIDS suffer incredible diarrhea and discomfort due to cryptosporidial infection.[21] The organism infects bile ducts, pancreatic ducts and respiratory system, inflicting additional misery in immunosuppressed persons.

Pathophysiology

We have a little experimental evidence as to how cryptosporidia might affect intestinal function and cause some of the clinical signs. Vitamin A absorption is decreased, mucosal lactase and alkaline phosphatase are reduced, and trans-enterocyte fluid path permeability is increased.

Specific virulence factors have not been identified, nor has work been done which would allow us to define "strains" of cryptosporidia. But we wonder when some veterinarians report that in their experience, cryptosporidia alone kill calves. Other experience reveals that cryptosporidia alone cause only a transient, mild syndrome in calves. Perhaps there are, in fact, relatively virulent strains of cryptosporidia.

The life cycle stages, involving schizogony, merogony and sexual reproduction., take place just beneath enterocyte cell membranes, but not within the cytoplasm proper. The parasite occupies space, thus displacing or eliminating significant numbers of the microvilli on the enterocytes. So, surface area is reduced.

Outright necrosis of the mucosal enterocyte does not occur in such a way as to cause denudation of the small intestine villi, cecal or colonic crypts. Perhaps there is shortening of the infected cells's life, with increased rate of cell population turnover. Pathologists report lesions associated with cryptosporidia as inflammatory cell infiltration of mucosa and shortening/blunting of villi.

Hypersecretion of mucus and excessive expulsion of water can occur due to cryptosporidiosis, although asymptomatic infections of calves have been reported.[13] The colon can absorb about 200% of the normal water content and maintain the normal fecal consistency, so that fluid flow rates above that result in loose feces. The diarrhea has been labelled as "secretory." Hemorrhage is not a feature, although flecks of blood will be present in feces as a nonspecific feature of diarrhea in general. These flecks appear to derive from ruptured mucosal capillaries in a rectum irritated by the abnormal content passing through.

Humans with cryptosporidial diarrhea can have a severe syndrome characterized by vomiting, nausea, weakness and severe headache.[22] Pain of various sorts in the abdomen is associated with intestinal spasm and infection of biliary system. There must be some parasite-derived or endogenous toxins at work.

It seems that many calves with cryptosporidiosis can have similar discomforts and depression, although many infected calves remain frisky with good appetite in my experience. We've raised about 3000 Holstein calves, all with cryptosporidiosis; they rarely missed a meal; death loss from all causes is less than 2% at that particular dairy.

Perhaps most common are mixed infections in diarrheic calves. These have been well-documented and include various bacteria, viruses and cryptosporidia. Among these, the cryptosporidia are relatively easy to find and often get the blame for illness and death. Other organisms like rotavirus and coronavirus can be found. We speculate that short clinical courses of diarrhea might be associated with simultaneous infection by these several agents whereas longer clinical courses might result from infections by these agents in succession.

The 1993 calving season was disastrous for many beef producers. The 'dead wagon' was full, electrolyte packets were backordered by many suppliers, and cryptosporidial oocysts were ubiquitous in feces of ill

and dead calves. Cryptosporidia got the blame in many cases and anti-cryptosporidial groups and task forces have sprung up.

This past calving season, 1994, calf losses were minimal, at least in Idaho. Nobody checked, but I'll wager that cryptosporidia were just as available as in 1993. The real difference between the two years was the weather. In 1993, calves were born into snow and mud and bedded down in ice water. In 1994, the calving season weather for most of our producers was ideal.

Anticryptosporidial Measures???

Numerous medications have been tried on cryptosporidia.[8] It seemed logical that anticoccidial agents would work but they have not. Recently, halofuginone reportedly has reduced or eliminated oocyst shedding in infected calves.[25] The drug is not available in the United States yet, but word is that clearance will be sought.

Hyperimmune hen yolk-derived monoclonal antibodies[24] effective against cryptosproidia in mice, are being tested in AIDS patients with cryptosporidiosis. A similar product might become available for calves, depending upon a number of factors, including efficacy and economics.

Cryptosporidial oocysts resist disinfectants under most practical circumstances. Half strength household bleach is one of the few effective disinfectants. Formalin can be effective but its carcinogenic hazard prevents its widespread use.

At present preventive efforts to minimize effects of cryptosporidial and other infections must be based on common, best management practices. These are common sense measures aimed at creating a stress-minimal calf environment, pathogen dose reduction and balanced nutrition for pregnant cows. But if cryptosporidia were eliminated, would serious calf diarrhea outbreaks be history???? Some anecdotal information suggests so, but controlled studies have not been done.

Treatments of dehydrated, depressed, acidotic calves must be early, continuous, according to the ongoing pathologic process, and appropriate. These include adequate volumes, often gallons, of fluid with electrolyte composition specifically designed to combat acidosis rapidly. Isotonic sodium bicarbonate given intravenously, is the strongest alkalinizer. Commonly, hypokalemia in these cases accounts for a weak response and must be corrected safely. Gram-negative septicemia will compromise response in calves with simple secretory diarrheal illness; such septisemias with endotoxemia must be suspected, detected early and treated vigorously.

Cattle Production and Zoonotic Concerns.

The real risk presented by cryptosporidiosis to the cattle industry may not be what the parasitism is perceived to do to calves, but the perception by society that cattle are the main conveyors of cryptosporidiosis to humans. Activists with hidden agendas certainly will play upon the whipped up feeling that livestock are solely to blame for contaminating surface waters with cryptosporidial oocysts. Nobody has determined the viability and infection potential of oocysts isolated from rivers and lakes. Nor have the source animals been determined; the possibilities are endless.

A recent example of the hysteria was played out in Orange County, California by those who suggested that horseback riding in the mountains resulted in cryptosporidial oocysts in the water supply. We know that immunodeficient foals contract cryptosporidiosis, but the activists maintained, without foundation, that saddle horses are sources, and wanted them banned from the entire watershed.

In the *"Conservation Impact,"* volume 12, No. 1, January, 1994, page 2, the following points were made. "When it comes to water quality and the environment, the word is 'watershed'." It involves, "the entire landscape within a drainage basin." "Residents need to know they have authority to make their watershed a better place for everyone to live and they can exercise that authority by taking the initiative and forming neighborhood alliances." The idea expressed in the article is for people to get together and assess their own impact on their watershed, subscribe to some sort of self-imposed scorecard system and act voluntarily **today**, to protect and enhance the environment.

This plea recognizes the real potential for regulation "tomorrow." If government can declare a "wetlands" of the puddle left after washing your pickup, what is a watershed that needs regulating???? Everywhere a drop of rain falls, I guess. Everywhere a cow pie rests.

Articles in agricultural publications have warned of the need to be proactive, concerning agricultural pollution, the non-point source type. Manure runoff, erosion, sedimentation, and leaching of nitrogen, phosphorus and pathogens, are mentioned (*"Hoard's Dairyman,"* February 10, 1994, page 87).

A lawyer called me a while back and talked of the 400,000 Milwaukeeans who got cryptosporidial diarrhea just after the rain-bloated river washed the local sewer plant into Lake Michigan in the Spring of 1993. He automatically blamed the dairy cattle. And it is possible that dairy cattle, calves at least, which can shed millions of cryptosporidial oocysts, are sources of surface water contamination.

Based on my examination of Wisconsin dairies, and 250 other dairies around the county, **fresh** stool from 1-

2 week old, diarrheic calves shedding billions of crypto oocysts, is not too likely to find its way to the local creek. Some calf raisers do wash their calf manure away to some lower topographic location. Manure of pastured steers, heifers and milking cows is likely to wash to the gullies and creeks, if not deposited there directly.

But the manure of cattle aged 4 weeks or more is unlikely, in my opinion, to contain billions of *Cryptosporidium parvum* oocysts, the zoonotic, intestinal crypto. Therefore, if young calves are the bulk of the treat, the relatively small amount of crypto-rich dairy calf manure ought to be easy to manage in such a way as to almost eliminate dairies as sources of *C. parvum* oocysts.

We have to use the qualifier, "almost," because a recent report by the National Animal Health Monitoring System investigators (January, 1994) documented that some *C. parvum* oocysts can be found by monoclonal antibody technique, in feces of non-diarrheic calves up to about 10 weeks old. Giardia also were present. More work needs to be done to quantify the degree of contamination of cattle pastures by oocysts.

Cattle older than 4 weeks **are** likely sources of large numbers of *Cryptosporidium muris*-like oocysts. This gastric cryptosporidial organism parasitizes abomasal glands for many months and perhaps the lifetime of certain cattle. This was the case for three animals owned by this investigator. A yearling Holstein heifer, a 5-year-old Angus bull and a 10-year-old cull Holstein cow, all had the parasitism for the 2 years I owned them. These animals excreted 1-1.5 million oocysts per gram of feces daily. But remember, this gastric *Cryptosporidium* may be harmless to humans. Time will tell.

It appears that abomasal cryptosporidiosis does result in subpar performance of cattle. The subject is under scrutiny at a dairy where about 100 (10%) of the milking cows have the *Cryptosporidium muris*-like oocysts in the feces. Preliminary studies of these cows showed about a 15% reduction in milk flow, compared with unparasitized, age- and lactation-matched cows (manuscript in preparation).

Previous work revealed that 24 of 30 feedlots sampled (total samples = 47,064) had the *C. muris*-like occyst in some pen samples.[25] The test used was acid-fast staining of fecal smears made from manure deposits in the pens. Overall, 153 (47%) of 329 lots sampled had positive fecal smears, the highest within-lot prevalence being 13%.

We've not looked at performance of significant numbers of parasitized feeder cattle, but as mentioned earlier, the original re-discovery of this gastric cryptosporidial organism was in 88 feeder cattle which gained on average a pound less per day than expected. Also, in a few University of Idaho feedlot steers with gastric cryptosporidiosis, performance has been off, 10 to 50%. Other parasitized steers performed well.

Hypothetically, this variation may be a function of the duration of the infection. It appears that this massive colonization of gastric glands results in a slowly progressive hypertrophy of mucosa with hyperplasia and metaplasia of the functional epithelial cell populations. We know that reduced acidity of the abomasal secretions results, inhibiting the pepsinogen to pepsin conversion; plasma pepsinogen concentration is thus elevated, on average. Inflammation of mucosa and overt clinical signs do not appear to be common features of abomasal cryptosporidiosis due to *Cryptosporidium muris*-like infection.

We collected 48,810 fecal samples from dairy cattle pens across the USA and found that 102 of 150 dairies had some samples positive for oocysts of *Cryptosporidium muris*, based on acid-fast staining of fecal smears. If there were positive samples to be found, they could always be found in the milking cow pens. But it appears that on some dairies, relatively high prevalences of parasitism with *C. muris* can be found among replacement heifers. This is under further study. Hypothetically, infected heifers might be delayed in reaching target weights for breeding.

Opinion/Summary Statements for Emphasis, and Other Facts

1. *Cryptosporidium parvum* is ubiquitous, causes diarrhea in some mammals and can be found in association with piles of dead calves as well as populations of calves which have little illness. Give a newborn calf a dose of oocysts and 4 days later there will be an impressive efflux of watery yellow stool full of oocysts, though the calf may not be very ill.
2. Humans can get cryptosporidiosis from any infected mammalian animal, including each other. Veterinarians have a role in protecting humans, especially the elderly and immunosuppressed persons from exposure to animals likely to be suffering transient intestinal cryptosporidiosis. This pretty much means calves in the second week of life, though they can shed oocysts in normal feces for several weeks.
3. Surface waters contain cryptosporidia from mammalian, avian and reptilian species of animals and possibility from fish. Based on size and some fluorescence microscopy, we think we can separate *C. pravum* from *C. muris*; we can't determine the source mammal of the *C. pravum* isolated from water. Also, we have developed a monoclonal antibody specific for the Bovine *C. muris* as opposed to the *C. muris*-like oocysts from a camel and a Rock Hyrax.

4. Antiquated water treatment facilities pervade the USA and allow cryptosporidial oocysts to pass to consumers. Expect very expensive implementation of newer technologies in water treatment facilities across the USA.
5. All associates in animal agriculture should join in proactive approaches to emerging concerns about animal waste and agricultural pollutions. No use getting regulated out of business if we can manage in an environment-neutral or even beneficial manner.
6. Promising treatment and preventive measures for cryptosporidiosis are on the horizon, at best. Halofuginone, if truly effective will be publicized widely. The hyperimmune hen yolk product might eventually be made cost-effective for calves.
7. You might hear that Deccox, given at 2X dose to mother cows, one month prior to calving, and right through the calving season, will dramatically reduce calf scours problems. You might hear that. The notion is that the drug thwarts cryptosporidia. So does calving in moderate weather with the cows spread over the hills. Controlled trials have not been done.

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