Bovine Gastric and Intestinal Cryptosporidiosis: Present Situation

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Introduction

This writer intends to provide information which will be useful or may be useful, next week in the practices of cattle practitioners. Comments on epidemiology, control and zoonotic potential of *Cryptosporidium parvum* (intestinal pathogen) were solicited for an oral presentation at the recent meeting of the American Association of Bovine Practitioners. But, from day-to-day bovine practice standpoint, there isn't much to say about this intestinal pathogen, despite the publication of about 1000 articles on cryptosporidiosis in the past 10 years. In fact, William Current and Lynn Garcia stated recently in a review article[1] (357 references) that, "Despite the large number of recent papers and the large number of laboratories throughout the world devoting significant effort in *Cryptosporidium* research, our present understanding of this protozoan parasite is very limited." Dubey, Speer and Fayer have published a complete reference book (1047 ref.) on cryptosporidiosis.[2] Many of the details of the following may be found among the references in these two sources.

Some preliminary informaton on the newly-discovered gastric pathogen of cattle, *Cryptosporidium muris* was added to the above-mentioned talk and has been added to this paper. Our understanding about this gastric cryptosporidial parasite is also limited because it simply hasn't been studied. Mention of the parasite here and results of preliminary investigations should stimulate some interest in promoting further study of this possibly significant chronic parasitism of the stomach of production animals.

Cryptosporidia are coccidial protozoan parasites related to the well-known eimerial coccidia; in other words, they are in the suborder, Eimeriorina. The original definitions were of the gastric and intestinal forms, *C. muris* and *C. parvum,* respectively, isolated from mice. Today the *C. parvum* designation is applied to isolates which cause enteritis in numerous mammalian species including humans. The *C. muris* organism differs from the enteric *C. parvum* not only in location (stomach) but in size and shape of oocyst. (See section on *C. muris).*

Epidemiology

As with other coccidia, the spread of cryptosporidia is by the fecal-oral route. The excreted oocyst survives well in cool moist environments but does not need a sporulation period outside the host; the oocysts are excreted fully sporulated and are immediately infective for susceptible hosts. Significant is the fact that thin-walled oocysts within the host are auto-infective, thus providing a mechanism for massive magnification of the infection within the host. In calves, the clinical diarrheal episode usually begins in the second week of life and usually is over by the end of week 3 of age. Oocyst shedding at a rate of about a million per gram of feces occurs during the first several days of diarrhea, and may continue at a lesser rate through week 4 after birth. Oocysts have been found in normal feces of recovered calves, but also reportedly in normal feces of calves that had had no diarrheic episode. At this writing, the author is firmly of the opinion that any fecal sample from a bovine animal over 4 weeks old which is reported as positive for cryptosporidia, is positive for *C. muris,* the abomasal form, and not the intestinal *C. parvum.* Some laboratory technicians do not yet recognize the size differences.

Cryptosporidium parvum, though known primarily as an enteric pathogen, has the ability to inhabit many moist membranes, including conjunctiva, upper and lower respiratory tract, stomach (in immunosuppressed humans), biliary and pancreatic ducts. One investigator even reported finding oocysts in the urine of calves with cryptosporidial diarrhea, and post mortem, in the lining of ureters, bladder and renal tubular system[3]. Airborne transmission has been documented in chickens with *Cryptosporidium baileyi* infection of the respiratory tract. Though not yet proven, it is entirely plausible that a few (500?) oocysts placed in the conjunctival sack would result in a progressive infection from that site to nasal cavity to throat to intestine. Inhaled oocysts may first result in tracheo-bronchial infection with coughing up and swallowing of exudate and subsequent intestinal infection. Additional moist sites of replication for *C. parvum,* experimentally, are the chorioallantoic membrane of embryonated chicken eggs and certain cell lines in culture.

Cryptosporidium parvum is not very host-specific, with isolates from calves and humans, for example, easily

infecting numerous other species of inoculated mammals including rats and mice. The *C. muris* organism from rats in Japan was transmitted to mice, dogs and cats without difficulty. Cryptosporidia, source unknown, have been found in public water supplies of many towns in the USA and Britian, in association with epidemics of cryptosporidial diarrhea in thousands of residents. Some of the involved water treatment facilities involved have met federal and state standards for treatment methods.

On dairies, cows apparently have been incriminated as carriers of *C. parvum,* though the information has not been published. Wild mice on dairies have been incriminated as sources of cryptosporidia which caused diarrhea in inoculated calves.[4] In the author's unpublished experience, about 30 bull calves taken from the maternity pens of a dairy were raised in isolation and did not contract cryptosporidial diarrhea, whereas, all heifer calves born in those pens and taken to the calf barn contracted the disease. The heifers (about 2000 over a 12 year period) on the test dairy have consistently contracted cryptosporidiosis, but have never been ill enough to require treatment; deaths do not occur. Concurrent infection with rotavirus and/or coronavirus is the rule on this dairy.

In the cow/calf situation, the cows undoubtedly carry the cryptosporidia since herds which calve on frozen ground where cattle have been absent for a significant time still suffer calf cryptosporidiosis. Reports of beef calf cryptosporidiosis with acute dehydration and death in the absence of other detected enteric pathogens, suggest that cryptosporidia can be killers. Either these calves are more susceptible genetically, or there are some relatively virulent cryptosporidia (strains have not yet been defined), or there are in fact other significant undetected pathogens, or significant complicating stressors are being discounted. Such stressors might be wind or wet or cold.

Many reports suggest that there may be some seasonal pattern to the occurrence of dairy calf cryptosporidiosis, but they vary in their assessment. Other reports suggest no seasonal pattern.

Control

Practically speaking, there are no published, well-controlled studies which show that any drug will significantly affect the course of enteric cryptosporidiosis. About 100 various drugs have been tried, including those that are effective against other coccidians. Hyperimmune bovine colostrum has been helpful on a trial basis in clearing a couple of immunodeficient humans of intestinal cryptosporidia.[5] The colostrum was administered directly into the duodenum.

Anecdotes from enterprising veterinarians suggest that Deccox (decoquinate, Rhone-Poulenc) reduces the time course of cryptosporidial diarrhea in calves and reduces the oocyst numbers shed in feces. A similar account suggested that the same drug, when fed to beef cows in several herds prior to calving and through the calving season, was associated with a marked reduction in calf scours, compared with the previous season; cryptosporidia were the only enteric pathogens found previously in association with devastating scours outbreaks; the possible role of non-infectious factors must be considered.

Cryptosporidial oocysts are resistant to a myriad of basic disinfectant chemicals and commercial products. Considering the task of trying to chemically treat the environment of the calf house or hutch, the 4 products which showed some effect are probably not practical. The 4 products are hydrogen peroxide, a chlorine dioxide-based cold sterilant (Exspor), a two phase product producing amonia (Oo-cide) and ozone.[2]

The author has determined that drying of oocyst-laden calf feces for 4 days eliminated infectivity of the oocysts for infant mice,[6] so that thorough cleaning of calf hutch surfaces followed by drying for several days might eliminate that source of infection for subsequent calves. As mentioned above, the transmission of cryptosporidiosis in the dairy situation is perhaps not dependent on what happens in the maternity pen but the nursery or calf rearing area. It is well-known that where the young congregate, as in human day-care centers, cryptosporidial diarrheal outbreaks are common.

Zoonotic Potential

Circumstantial evidence indicates that humans can acquire cryptosporidiosis from animals; veterinary students are common examples. Human to human transmission is common as well. Immunocompetent humans, while they may contract fairly severe cryptosporidial diarrhea associated with a "flu-like" illness, usually recover quickly and completely from the disease. In contrast, humans with immune deficiencies, congenital, acquired or chemotherapy-induced, may suffer from protracted and life-threatening infections; cryptosporidiosis of the intestine and possibly the respiratory system, is an example. There is some evidence that the elderly may be especially susceptible to severe cryptosporidiosis.

The veterinarian's role is to know when cryptosporidiosis is present and to inform human contacts, especially high-risk groups mentioned above, of the zoonotic potential. This may in fact be a legally binding obligation. A judicial ruling reportedly stated that a veterinarian, involved in a situation where a clinically-manifested infectious disease was present, was responsible to detect the infectious agent involved in order to warn of the potential danger (to neighboring herds should the fence be breached). The fence apparently failed, the neighboring purebred herd was affected and damages were collected from the veterinarian for negligence in completely assessing the situation.

Abomasal Cryptosporidiosis Due To *Cryptosporidium muris*

Cryptosporidium muris, Apicomplexa, Cryptosporidiidae, was described in 1907, and shown to inhabit only the stomach of mice.[7,8] The similarity of a cattle feces-source oocyst to that originally-described gastric parasite was verified[9] and the site of the generation of this oocyst in cattle was shown to be the abomasum or true stomach[10] and the parasitism tentatively was linked with reduced weight gain in a pen of 88 feedlot steers and selected steers in feedlot nutrition trials. In order to begin to assess the importance of *C. muris* infection in cattle, the following national survey (partly completed) of cattle populations was sponsored by the American Veterinary Medical Association Foundation. The objective, simply, was to find out if the parasite existed in cattle populations throughout the USA.

Materials and Methods

Food animal veterinary faculty and private veterinary practitioners were contacted throughout the United States in order to gain access to dairies and feedlots for the purpose of collecting fecal samples from pens. Trips to various regions of the country were for about 2 weeks each.

Samples were collected from individual, fresh fecal deposits by thrusting the end of a wood applicator stick (1 stick per 2 samples) into the feces and then touching the end of the stick to a glass slide. Up to 144 dots of feces (8 rows of 18 dots each) fit on one side. The slides, labelled with assigned farm and pen numbers, were air dried and transported to the University of Idaho's Caine Veterinary Teaching and Research Center for staining and light microscopic examination.

Slides were heat-fixed by three passes through a full flame and acid-fast stained using the AFB Kit (Logos Scientific, P.O. Box 12449, Las Vegas, NV 89112). After drying, the 3-5mm dots were examined at 100X. Each dot was 2-3 fields wide and was perused in 3-5 seconds for the distinct red oocysts of *C. muris.* Key features of the oocysts in this preparation are: 1. The oocyst is oval and about twice the size of *C. parvum.* 2. There is usually a fine unstained halo around the sharply-outlined oocyst. 3. There is a distinct acid-fast granularity to the internal structure; the granular redness is most intense at the periphery, but is faded centrally manifested as a rarefied central zone within the oocyst. Not all oocysts are stained in this typical way, perhaps due to orientation of the oocysts in the dried smear and other factors.

In order to label a specimen as positive, at least two typical oocysts, arbitrarily, were necessary. Practice with known positive specimens is recommended. The acid-fast technique was chosen over concentration/flotation because it is almost as reliable as flotation (87% as reliable in this investigator's laboratory), and relatively simple and inexpensive, considering the number of samples examined and the objective of this survey.

Results and Discussion

Dairies and feedlots were not selected by any particular criteria. Individually identifiable manure deposits, and therefore, collected samples, in general numbered about 1-1.5 per animal in the pen. Various factors impacted this availability, e.g. concentration and activity level of the animals and recent scraping of the pens or barns. Summary data was tabulated in tables 1 & 2. Total samples were 95,875 with 1,317 (1.4%) positive; dairy samples, overall, had about twice the prevalence of feedlot samples. At least 50% of the feedlot or dairy premises in any given state yielded positive samples. The percent positive samples from dairies were relatively high in the eastern states, compared with percent positive samples from states in the western USA. Many selected dairies in the East were relatively small (50-100 milking cows), utilized pasture more than western dairies.

Table 1. Bovine *Cryptosporidium muris* in fecal samples at Dairies, by State.

State	# of Dairies	# Positive[b]	Total Samples	Total Positive	% Positive
Arizona	2	2	3,271	23	0.7
California	14	12	8,539	149	1.7
Idaho	17	9	6,997	68	1.0
Montana	8	5	1,696	12	0.7
New Mexico	4	4	4,368	46	1.1
Washington	17	11	7,867	48	0.6
Connecticut	14	12	1,943	74	3.8
New York	18	9	2,446	76	3.1
N. Carolina	10	5	2,343	32	1.4
Ohio	12	6	3,135	39	1.2
Pennsylvania	21	15	3,705	83	2.2
Virginia	13	12	2,500	117	4.7
TOTALS	150	102	48,810	767	1.6[a]

[a]Average percent.
[b]A dairy was positive for *C. muris* if 1 fecal sample was positive.

Fewer feedlots than dairies were visited. Cattle at feedlots generally numbered in the many thousands and were from a wide geographic range. An attempt was made to sample pens representative of a variety of sources. Cattle from all beef producing states and from Mexico were postive; seemingly Holstein calf pens yielded relatively more positive samples, perhaps related to the dairy background.

While prevalence of *C. muris* within cattle populations in the USA appears to be low, certain pens of cattle

Table 2. Bovine *Cryptosporidium muris* in fecal samples from Feedlots.

State	Feedlots Visited	Feedlots Positive	Total # Samples	#/% Positive	# Pens Sampled	#/% + Pens
Arizona	2	1	1,670	9/0.5	20	2/10
California	5	4	5,522	65/1.2	45	19/42
New Mexico Texas	5	4	8,152	58/0.7	60	12/20
Ohio	4	3	772	8/1.0	6	3/50
N. Carolina	2	1	474	7/1.5	5	2/40
Washington	2	2	1,258	22/1.7	6	5/83
Montana	6	5	3,570	54/1.5	30	16/53
Idaho	3	3	21,728	299/1.4	130	82/63
Colorado	1	1	3,918	28/0.7	27	[a]12/44
TOTALS	30	24	47,064	550/1.2	329	153/47[b]

[a]Eleven of the 15 negative pens were undersampled at a rate of < 1 sample per 2 animals in the pen.
[b]Average percent.

had a relatively high prevalence. Thirty-one percent of the milking cows on one dairy in Connecticut were positive. Several other dairies in the East had positive percentages of 10-20% among milking cows. Unusual was a pen of about 50 weaned calves in Virginia that had 29 positive of 105 samples. On dairies where positive samples were found in calf pens, adult pens were always positive. The finding of positive samples from adult pens was not necessarily accompanied by the finding of positives in pens of younger cattle on dairies; in many cases heifers were raised elsewhere and not available for testing. One pen of 100 milking cows in California had 16.6% positives; other pens of 100 cows at that dairy had 12.2%, 10%, 8.8%, 6.6%, 5.5%, respectively.

Almost half of the total feedlot pens yielded positive samples. However, prevalence within pens of feedlot cattle was relatively high (10-15%) only in a few instances. For example, a pen of cross-bred beef calves in California had 11.8% positive samples. Both dairy and beef backgrounds were represented among the sampled pens of feedlots.

Given the data presented here, those who wish to procure *C. muris* oocysts should be able to identify chronically-infected dairy cattle by use of the acid-fast fecal smear technique. Such affected cattle, though not ill may be fading in production, and it may be important to know why. The dairy environment has the most promise for finding *C. muris*-infected cattle since dairy cows are relatively more likely to be infected, dairy cows are individually indentified, usually by numbered ear tag, and they are regularly restrained.

The parasitism appears to be chronic in cattle; the author as of this writing has possession of 3 cattle which have shed the oocyst of *C. muris* for over 18 months at a rate of about 1 million per gram of feces.

References

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