Infection with *Cryptosporidium* spp. in Humans and Cattle in Manitoba

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ABSTRACT

Between October 1, 1983 and October 31, 1984, fecal specimens from 3656 persons with enteritis and 182 calves, representing 148 herds having a neonatal diarrhea problem, were examined for oocysts of *Cryptosporidium* spp. Oocysts were found in 1% of human and 25% of bovine specimens. All infected persons were immunocompetent. Children under five years of age had an infection rate of 25/100,000 compared to 1.4/100,000 in older people ($p < 0.005$). Rates in northern communities were four to seven times as high as those in southern Manitoba. Human infections occurred most commonly in late summer and fall. In beef calves infection occurred in winter and spring, the calving season in Manitoba. Epidemiological association between the infection in people and in cattle could not be established.

Key words: Cryptosporidiosis, human, calves, Manitoba, epidemiology, diarrhea.

RÉSUMÉ

Cette étude s'étalait sur la période du 1er octobre 1983 au 30 octobre 1984 et elle consistait à rechercher des oocystes de *Cryptosporidium* spp., dans les fèces de 3656 personnes atteintes d'entérite et de 182 veaux issus de 148 troupeaux aux prises avec un problème de diarrhée néonatale. Les auteurs trouvèrent de tels oocystes dans 1% des échantillons des humains et dans 25% de ceux des veaux. Toutes les personnes infectées s'avérèrent immunocompétentes. Les enfants âgés de moins de cinq ans affichèrent un taux d'infection de 25/100 000, comparativement à 1,4/100 000 pour leurs congénères plus âgés ($P < 0,005$). Le taux d'infection se révéla de quatre à sept fois plus élevé dans les localités du nord du Manitoba que dans celles du sud. Les cas humains survinrent surtout vers la fin de l'été et à l'automne, tandis que chez les veaux de boucherie, on les enregistra au cours de la période vêlage qui, au Manitoba, implique l'hiver et le printemps. Il s'avéra impossible d'établir une relation épidémiologique entre les cas humains et bovins.

Mots clés: cryptosporidiose, humains, veaux, Manitoba, épidémiologie, diarrhée.

INTRODUCTION

Recently, interest in *Cryptosporidium* spp. as causes of diarrhea in neonatal animals, particularly calves, and more recently in humans, has grown rapidly (1,2,3,4,5). Cryptosporidiosis in considered important in the neontaal diarrhea syndrome of calves (1,6,7,8).

Cryptosporidial infection has been recognized in calves in Manitoba for several years; organisms were observed histologically in intestines of calves submitted to the Provincial Veterinary Laboratory. This diagnostic procedure is very insensitive (2,6). We used acid fast staining of fecal smears (9), to determine the extent of this infection in Manitoba beef and dairy herds, and to evaluate its contribution to losses due to neonatal diarrhea. Cryptosporidial infection had not been diagnosed in people in Manitoba prior to this study. However, it had been recognized elsewhere (10) in persons having diarrheal disease, some of whom acquired the infection while in contact with infected calves (5,11). We undertook to determine the presence of human cryptosporidiosis in Manitoba, and to examine for epidemiological association between human and bovine cases. We also describe clinical and epidemiological features of the infection in both hosts.

MATERIALS AND METHODS

SPECIMEN SELECTION

A total of 3656 fecal specimens, representing 3593 people having diarrhea, submitted to Cadham Provincial Laboratory were examined. We also examined 182 bovine fecal specimens, representing 148 herds with neonatal diarrhea problems, submitted to the Provincial Veterinary Laboratory. These included 152 submissions from beef calves (124 herds) and 30 from dairy calves (24 herds). Each bovine specimen represented a problem in a herd

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where a variable number of animals had neonatal diarrhea. Ten farms were visited to collect additional fecal specimens from neonates and from older animals. This was done to assess the management on these farms, and to determine whether asymptomatic infections, which could serve as a source for the neonates, were present. Seven of the ten farms had previously reported neonatal diarrhea.

EXAMINATION PROCEDURES

Fecal specimens were smeared on glass slides, air dried, fixed in 100% methanol and stained by Kinyoun's acid-fast procedure (9). All slides were scanned for typical acid-fast spherical organisms using 400x magnification, with confirmation at 1000x magnification. All bovine specimens were examined for bacterial pathogens by routine cultural procedures, and for viral pathogens by electron microscopy. Examinations for other pathogens in human specimens were performed, by routine procedures, as requested by the submitting physician.

COLLECTION OF CLINICAL AND EPIDEMIOLOGICAL INFORMATION

Questionnaires were sent to the attending physicians of human cases and to the owners of infected cattle. For human cases we requested information on age, sex, onset and duration of diarrhea, frequency of bowel movements, association with fever, vomiting and colic, other illnesses including enteric infections, and history of travel or contact with animals. For bovine cases we requested information on herd morbidity and mortality, association with the purchase of calves, onset and duration of diarrhea, response to treatment, history of neonatal diarrhea in previous years, and indication of associated diarrheal illness in humans.

DATA ANALYSIS

In humans, an estimate of the incidence of cryptosporidiosis by age and sex was made by comparing the number of positive specimens with the number examined for the corresponding period of time. Since all bovine specimens were also examined for viruses, association of cryptosporidial infection and viruses was also assessed. The Chi square test for significance was used to establish differences between groups. Relative risk was calculated with the formula R.R. = number positive/number examined (group A): number positive/number examined (group B).

RESULTS

HUMAN CRYPTOSORIDIOSIS

During the period October 1, 1983 to October 31, 1984, 39 persons (~1%) were shedding cryptosporidial oocysts. Table I illustrates the age and sex distribution, of cryptosporidial infection and rates per 100,000 population. The apparent predilection for males in patients ≤ 5 years of age and for females in patients > 5 years of age were not significant. The overall predilection for patients ≤ 5 years of age as compared to those > 5 years of age was significant ($P < 0.025$). The relative risk for patients ≤ 5 years old as compared with those > 5 years old was 2.29. The age range was from two months to 71 years; there were only four cases in patients over 30 years of age. Rates per 100,000 population among patients ≤ 5 years of age, were nearly 18 times as great as in those > 5 years of age ($p < 0.0005$).

Human cryptosporidiosis occurred province-wide, however 24 of the 39 patients (61.5%) lived in rural areas. Sixteen persons (13 < 2 years of age) were from northern native reserves and eight were from farms. Fourteen were identified as urban residents, of which 11 lived in Winnipeg; four were children aged one to three years, all of whom attended the same day care center. One was a pediatrician and one was a nurse attending sick children. Table II shows rates of infection per 100,000 population on the basis of the region of Manitoba where patients lived. These data indicated a major difference in rates of infection between northern and southern communities. The former had an infection rate of 13.6/100,000 whereas the latter had a rate of only 3.4/100,000 in rural areas and 2.0/100,000 in Winnipeg. Differences between the northern and both southern populations were highly significant ($p < 0.0005$), but differences between rural and urban southern populations were not significant.

TABLE I. Age and Sex Distribution of Human Cryptosporidial Infection, in Manitoba, for the Year Ending October 31, 1984

Age[a]	Males[b]	Females[b]	Total[b]
≤ 5	17/918 (1.85)	8/787 (1.16)	25/1605 (1.5)[c]
> 5	5/900 (0.56)	9/1088 (0.82)	14/1988 (0.7)[c]
Total	21/1818 (1.15)	17/1775 (0.96)	39/3593 (1.06)

[a]In years
[b]Number positive/number examined (% positive)
[c]$p < 0.025$ and relative risk = 2.29

TABLE II. Human Cryptosporidial Infections, by Manitoba Region, for the Year Ending October 31, 1984

Manitoba Region	Cases	Population[a]	Rate[b]
A. Winnipeg	7	610,391	1.15[d]
B. Norman	17	124,800[a]	13.62[d,e]
C. Nonurban southern regions	9	291,147	3.09[e]
1) Eastman	3	78,569	3.81
2) Central	2	92,395	2.16
3) Interlake	3	69,716	4.30
4) Parkland	1	50,467	1.80

[a]Manitoba Health Services Commission, Statistics 1984
[b]Per hundred thousand population
[c]Population of Norman Region plus "Status Indian" population of Manitoba
[d]Significantly different ($p < 0.0005$)
[e]Significantly different ($p < 0.0005$)

TABLE III. The Monthly Distribution of Cases of Cryptosporidial Infection in Humans and Calves During a Period from October 1, 1983 to October 31, 1984

Month	Human[a]	Beef Calves[a]	Dairy Calves[a]
October	4/35 (11.4)	N.S.	N.S.
November	3/79 (3.8)	0/2	3/4 (75.0)
December	2/82 (2.4)	N.S.	N.S.
January	0/61	0/5	8/16 (50.0)
February	1/150 (0.67)	4/16 (25.0)	3/4 (75.0)
March	0/328	8/51 (15.7)	2/2 (100)
April	0/372	8/39 (20.5)	1/2 (50.0)
May	0/372	8/39 (20.5)	N.S.
June	3/370 (0.81)	N.S.	N.S.
July	0/578	N.S.	N.S.
August	9/475 (1.9)	N.S.	N.S.
September	11/525 (2.1)	N.S.	2/2 (100)
October	6/511 (1.2)	N.S.	N.S.
Total	39/3593 (1.06)	28/152 (18.4)	19/30 (63.3)

[a]Number positive/number examined (% positive)
N.S. = no submissions

TABLE IV. Mortality Rate in Neonatal Diarrhea Associated with Cryptosporidial Infection in Cattle During the Period from October 1, 1983 to October 31, 1984

Class[a]	Mortality (%)
A	62/414 (15.0)[b,c]
B	23/41 (56.0)[b]
C	4/11 (36.4)
D	22/37 (59.5)[c]

[a]A. producing only beef (11 farms), B. producing primarily dairy products (5 farms), C. producing beef and dairy products in segregated herds (2 farms) and D. producing beef and dairy products in unsegregated herds (7 farms)
[b]Significantly different ($p < 0.0005$)
[c]Significantly different ($p < 0.0005$)

Most human cases occurred in the late summer and fall (Table III).

Twenty of 38 questionnaires (52%) sent to physicians, were returned. Only two people gave a history of travel outside of Manitoba. Two people believed their illness was water related, and three children from farms had consumed unpasteurized milk. Fourteen of 20 patients had animal exposure; three were in contact with cattle, five with cats, eight with dogs, and one with a budgie. Two infected children had contact with a puppy which was shedding cryptosporidial oocysts. Diarrhea was reported among family contacts of some human cases. On five occasions when cryptosporidiosis was diagnosed in cattle herds, owners admitted that there had been coincident human diarrheal disease, in either animal attendants or family members. We were not able to determine the etiology of these illnesses; no fecal specimens were received.

Some patients had other associated illnesses, including one 14 year old male with Ewing sarcoma and a 71 year old female with chronic lymphocytic leukemia, but neither was immunodeficient when examined. One child had been hospitalized for an ocular condition; another had an upper respiratory infection. Responding physicians reported associated enteric pathogens in only four patients; one had *Aeromonas hydrophila*, one had *Shigella sonnei*, and one had *Taenia saginata*; one had recently been successfully treated for *Campylobacter jejuni*.

All 20 patients had an acute watery diarrhea which was self limiting. The duration of illness was four days to 12 weeks and less than two weeks in 12 of 20 cases. The number of bowel movements per day varied from four to more than 20, but was less than 13 in three quarters of the cases. Two patients had blood in their stool, two had recurrent diarrhea, and four children were hospitalized due to dehydration. Diarrhea was associated with fever in 12/20, vomiting in 12/20 and colic in 9/20.

BOVINE CRYPTOSPORIDIOSIS

Forty-seven (25.8%) of the bovine specimens representing 38 (25.7%) of the herds examined were found positive for cryptosporidial oocysts. Twenty-eight of 152 (18.4%) submissions from beef calves, and 19 of 30 (63.3%) from dairy calves were positive. Table III compares the seasonal occurrence of cryptosporidiosis in beef and dairy calves to that in humans. There was some overlap between season in humans and dairy calves.

Cryptosporidiosis in calves was commonly associated with enteric viruses. Ten of 47 cases (21.0%) were positive for rotavirus, 11 (23.4%) for coronavirus and four (8.5%) were positive for toga-like viruses. Approximately 27% of the 47 calves were also infected by one or more pathogenic viruses.

Twenty-five of 38 questionnaires (65.8%) sent to owners of positive herds were returned. Three owners suggested a link between diarrhea in their calves, and the purchase of calves from other farms or cattle markets. This practice occurred on 12 additional farms, most commonly in herds which also had other indicators of poor management, such as comaintainance of beef and dairy animals, and animals of various ages and species (i.e. cattle, swine and poultry). There was an ~15% case fatality rate with *Cryptosporidium*-associated diarrhea in beef herds. In dairy and mixed herds, case fatality rates ranged from 35-60%. Table IV shows the mortality rate in four classes of cattle herds. Eight owners reported that neonatal diarrhea had been worse in 1984 than in previous years. An additional four indicated no neonatal diarrhea problem in the previous two years. Onset of diarrhea occurred during the first week of life, and had a duration of less than two weeks in most herds, but varied considerably even within herds.

Clinical signs in calves included yellow to green watery diarrhea commonly progressing to dehydration and death within one to two days. When recovery occurred, the diarrhea tended to become brown and there was a long convalescence, with relapses. Various antimicrobial agents, fluids and electrolytes were administered orally, parenterally or often simultaneously, in the treatment of neonatal diarrhea. Treatment results were variable.

Examination of fecal specimens identified additional cases of cryptosporidial infection on nine of ten farms visited; ten neonatal calves with diarrhea, 15 nondiarreic calves and three older calves that previously had diarrhea. In 18 instances a few oocysts were found in the feces of asymptomatic adult cattle. A chronically ill (possibly "bovine virus diarrhea") two year old bull in a beef herd, and two neonatal calves in a dairy, were excreting numerous cryptosporidial oocysts. No neonatal diarrhea was previously reported from either farm, but the calves had loose stools when the specimens were collected. A few oocysts were also detected, in asymptomatic yearling and adult animals, on two farms where cryptosporidial infection had not already been associated with neonatal diarrhea. On two farms with infected cattle, fecal specimens from barnyard hens contained cryptosporidial oocysts.

DISCUSSION

During the same time period in which 1% of human specimens examined were positive for cryptosporidial oocysts, approximately 1.5% and 4% respectively of diarrheal specimens examined for *Campylobacter* and *Salmonella* at the same laboratory, were positive (Cadham Provincial Laboratory Records, 1983-84). Therefore *Cryptosporidium* spp. have a frequency comparable with these well documented enteric pathogens.

Several studies (12,13,14,15,16) reported cryptosporidial oocysts in diarrheal specimens in various geographic locations. Many reports suggested close association between cryptosporidiosis and immunodeficiency (10,17,18,19,20). Our data are similar to those of others who described this infection in patients with no recognized immunodeficiency (5,11,12,13).

The source of infection in humans is usually unknown. Our data suggested possible person-to-person and zoonotic transmission. As transmission of cryptosporidial oocysts from infected calves to humans occurs (11,21), it was remarkable that only one human case was confirmed during the spring, when ~25% of submissions from diarrheic calves were positive. Two children were possibly infected by contact with the infected dog. The dog was acquired only four or five days prior to the occurrence of diarrhea in the first child. The basement of the home was widely contaminated with dog feces when the specimens were collected.

The occurrence of a cluster of cases in a day care center in this study was similar to reports from the United States (22), and suggested person-to-person transmission. The case in the child hospitalized for an eye condition might have been a nosocomial infection. The incubation period of cryptosporidiosis is three to seven days (11); this child had been in hospital for more than a month when the specimen was taken. The possibility of transmission of the infection among people in institutions has been shown by Baxby (23), and cases identified in health care workers in our study are similar.

Specimens from children ≤ 5 years old were more than twice as likely to contain cryptosporidial oocysts than specimens from older people, and a conclusion that cryptosporidiosis is primarily an infection of young children may be justified. The rate per 100,000 population in northern areas was almost four times as high as in the nonurban southern areas and seven times as high as in Winnipeg city. The same age distribution was observed in northern cases as for total cases, indicating that northern children have the greatest risk of contracting the infection. These differences may be partly due to poorer sanitary facilities in northern communities. Person-to-person transmission of other enteric pathogens is well documented in these communities and probably also occurs with this parasite. There is a possibility of transmission from dogs or wild animals.

The incubation period was not determined in any cases; no sources of infection were conclusively identified. The child who developed diarrhea three days after drinking unpasteurized milk, and two cases in children in contact with the positive puppy suggest an incubation period of three to five days.

The absence of other enteric pathogens, in most cases, supports previous reports that *Cryptosporidium* is a primary pathogen in immunocompetent humans (12,13,15,16). The severity of the syndrome has also been directly related to the number of oocysts being excreted (14), and we also found oocysts absent or in decreased numbers in recovering patients. In calves 27% of cases occurred coincident with other enteric pathogens. However, the pathogenicity of *Cryptosporidium* for gnotobiotic calves has been established (25). Other workers reported clinical diarrhea in calves coinciding with the shedding of oocysts and its absence in the postpatent period (26). Finding oocysts in one herd not reporting neonatal diarrhea, indicated that cryptosporidial infection is not always associated with clinical disease in cattle.

The seasonality of cryptosporidial infections in humans is difficult to explain. Previous reports indicate a higher incidence of the infection in warm wet months (13,16); this does not closely fit the pattern in Manitoba. The overlap between the seasons, in dairy calves and humans, suggests the involvement of climatic factors, or an associaton between these two populations. In beef calves seasonality was related to the presence of a large susceptible population of neonates; most beef calves in Manitoba are born in winter and spring. The stressful weather conditions which prevail at this time of the year may also be significant.

The mortality data reflect some of the economic effects of neonatal diarrhea in calves, although losses due to subclinical infection, possibly more significant, were not examined. Some types of management appeared to result in more serious losses; the purchase of young calves from undefined sources, was most common in the group of farms having the highest mortality. The purchased calves may have introduced *Cryptosporidium* spp. to some herds.

The source of infection in herd outbreaks is also unknown. The presence of oocysts in adults and yearlings suggests that this organism is highly prevalent in the bovine population. The finding of cryptosporidial oocysts, in specimens from barnyard hens on two farms, suggests that these birds might have been a source for the calves. Levine (27) reported *Cryptosporidium* of birds as a separate species from that of mammals (27). However, Tzipori (28) was able to transmit the infection to six mammalian species and chickens, using oocysts recovered from calf feces, and suggested that *Cryptosporidium* may be a single species genus. This study has suggested some links between human and animal cryptosporidial infection, but did not demonstrate transmission.

More studies are required to determine whether *Cryptosporidium* is a single species genus, and further surveys are needed to determine the sources of the organism for animals and humans. If it is a single species genus, a much wider range of possible sources exist. Other research priorities include studies on the pathogenesis and treatment of cryptosporidiosis.

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