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Concurrent infections of *Giardia* and *Cryptosporidium* on two Ohio farms with calf diarrhea

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Abstract

Giardia and *Cryptosporidium* infections were diagnosed by immunofluorescence assay on two Ohio dairy farms with calf diarrhea problems. On the first farm, all nine diarrheic calves sampled once in June had *Giardia* cysts in their feces. On the second farm, all five diarrheic calves examined at the beginning of the diarrhea outbreak in March had *Giardia* infection. When resampled, the overall infection rate of normal and diarrheic calves was 82.4% in April, and 40.0% in August after the diarrhea subsided. Positive calves ranged from 11 to 164 days of age, and 22.2% of them were as young as 1 to 3 weeks of age. Eight of nine diarrheic calves (88.8%) on the first farm had *Cryptosporidium* infection. Lower infection rates (<30%) were found on the second farm. Six of 10 positive calves were 11–22 days old, three were 164–177 days old, and one was 71 days old. Five of these 10 positive calves were also positive for *Giardia* infection.

Five diarrheic calves on the northern Ohio farm and one diarrheic calf on the central Ohio farm were treated with metronidazole after failing to respond to antibiotic therapy. Clinical improvement was observed in all calves within 48 h after the start of treatment. The high *Giardia* infection rates and intensities in calves of a wide age range and the clinical response to metronidazole suggest that *Giardia* infection contributed to the outbreaks of diarrhea.

Key words: *Giardia* spp., *Cryptosporidium* spp.; Cattle-Protozoa; Diarrhoea; Epidemiology-Protozoa

Introduction

Diarrhea is the most important cause of calf morbidity and mortality (Heath, 1992b) and is primarily caused by infectious agents (Tzipori, 1981). The role of *Cryptosporidium* infection in calf diarrhea is well established (Angus, 1990). It is one of the most important enteropathogens in calves in many parts of the world, second only to rotavirus (Reynolds et al., 1986; Snodgrass et al., 1986). Concurrent infection with two or more agents occurs frequently

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under field conditions (Tzipori, 1981; Reynolds et al., 1986; Snodgrass et al., 1986).

In humans and small animals, *Giardia* infection is common and is frequently associated with the occurrence of diarrhea (Kulda and Nohynkova, 1978; Kirkpatrick, 1987; Zajac, 1992). Its prevalence and pathogenic role in large domestic animals, however, are not clear, largely because of a lack of studies (Kirkpatrick, 1989). Recently there has been much debate about the zoonotic potential of giardiasis (Bemrick and Erlandsen, 1988; Faubert, 1988). As a result, interest in the prevalence of *Giardia* infection in farm animals is increasing (Buret et al., 1990; Sullivan et al., 1988).

The earliest reports of *Giardia* infection in cattle were in South Africa (Fantham, 1921), Austria (Supperer, 1952) and Italy (Botti, 1956). Studies in Switzerland and Canada during the last decade suggested that *Giardia* infection in calves was probably widespread (Nesvadba et al., 1982; Willson, 1982; Gasser et al., 1987; St. Jean et al., 1987; Taminelli and Eckert, 1989; Buret et al., 1990). *Giardia* infection in cattle was also reported in India (Deshpande and Shastri, 1981), Czechoslovakia (Fischer, 1983; Pavlasek, 1984; Nikitin et al., 1991), Egypt (Sullivan et al., 1988) and the former Soviet Union (Nikitin et al., 1991). On several occasions *Giardia* infection has been implicated as the cause of diarrhea in calves (Willson, 1982; St. Jean et al., 1987).

The occurrence of *Giardia* in cattle in the United States has been documented only once. Davies and Hibler (1979) reported that 10.3% of 58 cattle (age unspecified) in Colorado were infected. Recently, outbreaks of diarrhea associated with *Giardia* and *Cryptosporidium* infections occurred on two cattle farms in Ohio. These outbreaks and results of fecal examinations are described in this report.

Materials and methods

Animal history

Cryptosporidium infection was suspected by practicing veterinarians in outbreaks of diarrhea on two farms after calves failed to respond to antibiotic therapy. They referred the problem to The Ohio State University Veterinary Hospital for laboratory confirmation.The first outbreak occurred on a private dairy farm in northern Ohio in early June 1992. The farm had about 60 male Holstein calves 2–6 weeks old at the time of the outbreak. They were purchased from dairy farms in New York and Florida shortly after their birth. These calves were then raised together in large pens and later sold to children participating in farm animal fairs or 4-H programs. At sampling, about 30% of calves were affected. Major symptoms were diarrhea, lethargy and mild dehydration. Diarrhea lasted 5–7 days.

The second outbreak occurred on The Ohio State University dairy farm in central Ohio. Holstein and Jersey calves on this farm were separated from their mothers shortly after birth and kept in individual calf hutches over gravel until weaning at eight weeks of age. After weaning, they were moved to large outdoor stalls with shelter. Diarrhea began in March 1992, in hutch calves. Four of seven calves in hutches had chronic diarrhea, which recovered spontaneously after about 1 month. Affected animals also showed ill-thrift, lethargy, dehydration and weight loss. Problems continued in animals born later. At the end of April, there were nine calves in hutches, four of them still having diarrhea. Diarrhea gradually disappeared in summer.

Fecal samples

Calves on the northern Ohio farm were sampled only once. Nine rectal fecal samples were taken from nine diarrheic calves from 5 to 16 June 1992. Refrigerated samples were sent to our laboratory by hand delivery or overnight express mail.

Calves on the central Ohio farm were sampled three times on 26 March, 30 April and 26 August 1992. At the beginning of diarrhea outbreak, five samples were taken from five diarrheic hutch calves (12–25 days old). In the middle of the outbreak, nine hutch calves (11–60 days old, four with diarrhea) and eight stall calves (67–177 days old, no diarrhea) were sampled. After the outbreak, samples from 11 hutch calves (2–39 days old) and nine stall calves (62–170 days old) were taken. Samples from both farms were analyzed for *Cryptosporidium* and *Giardia* infections. No bacteriological or virological examinations of fecal samples were performed.

Immunofluorescence assay

Fecal samples were examined for *Giardia* cysts and *Cryptosporidium* oocysts by a direct immunofluorescence staining technique, using a commercial kit from Meridian Diagnostics, Inc., Cincinnati, OH. Approximately 2 g of feces were mixed with about 30 ml distilled water. After straining through a layer of cheese cloth, the suspension was centrifuged at 1000 *g* for 10 min. A thin film of fecal smear was made with the sediment on a slide. It was then stained with the immunofluorescence stain.

Stained fecal smears were examined at $\times 200$ and $\times 400$ magnifications by epifluorescence microscopy. The entire smear (about 80 mm^2) was searched for cysts or oocysts. Infection intensity was classified by infection indexes based on the number of cysts or oocysts detected per $\times 400$ field (0.11 mm^2): 0 for negative; 1 for 1–2 cysts or oocysts; 2 for 3–5 cysts or oocysts; 3 for 6–7 cysts or oocysts; and 4 for ≥ 8 cysts or oocysts (Arrowood and Sterling, 1989; Xiao et al., 1993).

Observations of Giardia cysts

Some positive fecal samples were examined by zinc sulfate flotation to observe the structure of *Giardia* cysts. Twenty cysts were measured at ×400 to estimate the size of cysts.

Results

All nine samples from diarrheic calves on the northern Ohio farm had *Giardia* cysts (Table 1). Infection intensities ranged from light to severe (infection index 1–4). Eight calves also had *Cryptosporidium* oocysts, but a lower infection intensity (infection index 1).

All five samples from diarrheic calves on the central Ohio farm had *Giardia* cysts in March, with infection indexes of 1–4 (Table 1). No *Cryptosporidium* infection was detected at that time. One month later, the *Giardia* infection rate was still high, with eight out of nine hutch calves (four with diarrhea) and six out of eight stall calves positive. The infection intensity remained constant. Two hutch calves and three stall calves also had *Cryptosporidium* infection of low infection intensity. Four of these five *Cryptosporidium*-positive calves also had *Giardia* infection. When the farm was re-examined 4 months later, the infection rate and intensity of *Giardia* had decreased, while the infection rate of *Cryptosporidium* remained the same, but with increased

Table 1
Prevalence of *Gardia* and *Cryptosporidium* infections in calves on two Ohio farms with diarrhea problems

Farm/calf group	No. examined	Date	Giardia[1]					Cryptosporidium[1]				
			0	1	2	3	4	0	1	2	3	4
North Ohio farm												
Diarrheic calves	9	5/6/92 to 16/6/92	0	4	2	2	1	1	8	0	0	0
Central Ohio Farm												
During diarrhea												
Diarrheic hutch calves	5	26/3/92	0	3	0	1	1	5	0	0	0	0
Hutch calves	9	30/4/92	1	4	2	1	1	7	1	1	0	0
Stall calves	8	30/4/92	2	5	0	0	1	5	3	0	0	0
After diarrhea												
Hutch calves	11	26/8/92	9	2	0	0	0	7	1	0	0	3
Stall calves	9	26/8/92	3	6	0	0	0	8	1	0	0	0

[1]Number of calves infected with *Giardia* or *Cryptosporidium* by infection index: 0, negative; 1, 1–2 cysts or oocysts per ×400 field; 2, 3–5 cysts or oocysts per ×400 field; 3, 6–7 cysts or oocysts per ×400 field; 4, ≥8 cysts or oocysts per ×400 field.

intensity. Overall, positive calves ranged from 11 to 164 days in age for *Giardia* infection and 11 to 177 days for *Cryptosporidium* infection.

Giardia cysts in calves during the outbreaks were 11.65 ($\pm$ 1.04) $\times$ 7.23 ($\pm$ 0.73) microns in size. Judged by the size, shape and median bodies, they appeared to belong to the *Giardia duodenalis* group (Filice, 1952).

Five diarrheic calves on the northern Ohio farm were treated with metronidazole at 25 mg/kg three times daily for 3 days. One diarrheic calf on the central Ohio farm was also treated with 25 mg/kg metronidazole three times daily for 5 days. Symptomatic improvement was obtained on both occasions 48 h after the start of treatments.

Discussion

Diarrheic calves examined on two Ohio farms had a 100% *Giardia* infection rate in the early stages of the outbreaks. The infection rate of normal and diarrheic calves on the second farm was still 82.4% in the middle of the outbreak, and 40.0% after the diarrhea had subsided. These infection rates are much higher than those previously reported (Davies and Hibler, 1979; Deshpande and Shastri, 1981; Nesvadba et al., 1982; Pavlasek, 1984; Sullivan et al., 1988; Taminelli and Eckert, 1989; Buret et al., 1990) and was probably due to the use of a more sensitive detection method. In most prior studies, zinc sulfate or other flotation methods were used for the detection of *Giardia* cysts in feces. Experience in our laboratory has shown that the zinc sulfate flotation method is less sensitive than immunofluorescence assay for the detection of *Giardia* infection.

Infection with *Cryptosporidium* was also found on both Ohio farms. On the northern farm, eight of nine animals (88.8%) examined were positive. Lower infection rates ($<$ 30%) were found on the central farm. The infection intensity of *Cryptosporidium* was much lower than that of *Giardia* at the time of diarrhea, although it increased on the second farm after the diarrhea had subsided. Among the 10 calves positive for *Cryptosporidium* infection on the central Ohio farm, six were 11–22 days old, three were 166–177 days old, and one was 71 days old. This differs from previous reports, where oocyst excretion in calves older than 1 month was considered rare (Henriksen, 1989; Angus, 1990).

Concurrent infections of *Giardia* and *Cryptosporidium* on the two farms were very high among animals sampled. All eight *Cryptosporidium*-positive samples from the northern Ohio farm and five of the 10 *Cryptosporidium*-positive samples from the central Ohio farm occurred in calves with *Giardia* infection. In most previous studies of *Cryptosporidium* infection in animals or humans the investigators have not searched for *Giardia*, although interactions of *Cryptosporidium* with other pathogens such as rotavirus, coronavirus, enterotoxigenic *Escherichia coli* and human immunodeficiency virus are well known (Tzipori, 1981; Reynolds et al., 1986; Snodgrass et al., 1986; Ungar, 1990). Fischer (1983), however, found concurrent infections of *Cryptosporidium* and *Giardia* in healthy calves in Czechoslovakia and Jokipii et al. (1985a,b) reported diarrhea outbreaks in humans caused by concurrent infections of *Cryptosporidium* and *Giardia*. Concurrent infections of *Giardia* and *Cryptosporidium* are probably more common than reported.

Calves on both farms apparently obtained *Giardia* and *Cryptosporidium* infections by a calf-to-calf fecal-oral route. All calves had been separated from their mothers shortly after birth, therefore, cows were an unlikely source of infection. On the northern farm, calves were raised in group pens previously occupied by other calves. It is possible that some of these calves became infected from the surroundings and amplified the environmental contamination by shedding cysts or oocysts in their feces, causing infection of other calves.

On the central Ohio farm, calves kept in individual calf hutches were able to make nose-to-nose contact with calves in neighboring hutches. The hutches had been in the same place for several years for year-round calf raising. Thus the surroundings may have been heavily contaminated with *Giardia* cysts and *Cryptosporidium* oocysts. It is also possible that calves infected in hutches later carried infection to stalls, explaining why calves in both hutches and stalls were infected with *Giardia* and/or *Cryptosporidium* during the diarrhea outbreak. Heath (1992a) suggested that to prevent transmission of infectious agents among calves, single-calf hutches should be placed to avoid nose-to-nose contact.

The reduced infection rate (18.2%) and intensity of *Giardia* in the hutch calves in late summer was probably the result of high temperatures and strong sunshine, since *Giardia* cysts are susceptible to desiccation and sunlight exposure (Zajac, 1992). The stalls, however, were sheltered, muddy, and favorable for the survival of *Giardia* cysts. As a result, infection rates of stall calves remained the same during spring and summer. *Cryptosporidium* oocysts are more resistant to adverse conditions than *Giardia* cysts (Sterling, 1990), and this probably accounted for the infection rates remaining constant.

As bacteriological and virological examinations were not undertaken, it is difficult to make a definite diagnosis of the cause of diarrhea in these two outbreaks. However, the high *Giardia* infection rates and intensities, the wide range in age of affected animals, and the clinical response to metronidazole treatments on both farms suggest that *Giardia* infection contributed to the clinical outbreaks. It should, however, be noted that metronidazole has both anti-bacterial and anti-protozoal activity (Adam, 1991), although animals with diarrhea had previously failed to respond to antibiotic therapy. The role of *Cryptosporidium* in the two outbreaks appeared to be less important because of its lower infection rates and intensities. It is, however, possible that the more acute nature of diarrhea on the northern farm was the result of higher concurrent infection rates of *Giardia* and *Cryptosporidium*.

Although *Giardia* infection has been implicated as the cause of calf diarrhea in some case studies (Willson, 1982; St. Jean et al., 1987), it has not been aggressively investigated in large scale diarrhea outbreaks (Tzipori, 1981; Reynolds et al., 1986; Snodgrass et al., 1986; Heath, 1992b). This may be due to the fact that most investigators held the belief that *Giardia* infection does not occur in young calves. In this report, six of the 27 *Giardia* positive calves (22.2%) on the central Ohio farm were 11-17 days old. *Giardia* infection thus can occur in very young animals and should be considered as a potential pathogen in investigations of neonatal calf diarrhea.

The zoonotic potential of *Giardia* infection in this study is worth noticing. Epidemiological evidence and animal cross-transmission studies suggest that humans can be infected by some strains of *Giardia* from animals (Thompson et al., 1990). Most calves on the northern Ohio farm in the study were to be sold to children participating in animal fairs or 4-H programs. The present results raise important questions regarding human health implications and emphasize the need for more studies on the epidemiology and control of giardiasis at the farm level.

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