Diarrhea in Young Red Deer Associated with Infection with *Cryptosporidium*

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In an outbreak of diarrhea among 82 artificially reared red deer calves, 56 developed the disease and 20 subsequently died. During the outbreak 80% of diarrheal and 50% of apparently healthy calves excreted cryptosporidial oocysts in feces. The coincidence of infection with *Cryptosporidium* and clinical diarrhea suggested a causal relationship. Histologic examination of intestinal sections from a necropsied deer calf showed lesions consistent with field and experimental cryptosporidiosis in other species. The deer *Cryptosporidium* subclinically infected newborn mice; in indirect immunofluorescence tests, it could not be distinguished from a calf *Cryptosporidium*.

Diarrhea has been noted among captive deer, which appear to be susceptible to enteropathogens, such as *Escherichia coli* [1] and rotavirus [2], that commonly infect other domestic species. Infection with *Cryptosporidium* has been reported among several species of mammals, birds, and reptiles [3] but has not previously been observed in deer. Although *Cryptosporidium* is classified with other enteric coccidia, it has some distinctive characteristics. It is an extracellular parasite that infects the brush borders of enterocytes [4], has a rapid life cycle and lacks host specificity [5], and is pathogenic for much younger animals than the age group generally affected by other coccidia. Features of cryptosporidial infections that make recognition difficult are that the oocysts are small (diameter, 4 μ m) and they are excreted in small numbers. *Cryptosporidium* has been associated with diarrhea in several mammalian species. Outbreaks have been most commonly observed among calves [4-8], but there have been reports of similar occurrences among lambs [9, 9A], and humans [10-12]. Experimental cryptosporidiosis in newborn lambs in-

duces fatal disease [13] with severe histopathologic lesions [13A]. In contrast, experimental exposure of laboratory animals to *Cryptosporidium* induces only subclinical infection [13].

In the present communication, an association between severe diarrhea in artificially reared red deer calves and infection with *Cryptosporidium* is described. In addition, the serologic relationship between the *Cryptosporidium* isolated from the deer to a bovine *Cryptosporidium* is reported.

Materials and Methods

Case history. During December 1979 an outbreak of diarrhea in suckled beef calves occurred on a research station in Scotland, which, after investigation, was attributed to cryptosporidiosis [14]. During June-July 1980, 82 red deer calves, up to one week in age, were caught and brought to the research station to be reared artificially. They were divided into small groups, housed in pens indoors, and fed ewes' milk substitute (Nutrilamb®; Scottish Agricultural Industries, Edinburgh, Scotland) three to four times a day with access to dried grass and concentrate pellets. Within two weeks, a few of the calves developed diarrhea, and over the next four weeks 56 (68%) had diarrhea that lasted two to 14 days. Twenty animals (25%) died during this period.

Concurrently two suckled deer calves aged eight to 10 days and reared naturally outdoors were found dead. Ileal and cecal contents from both calves were obtained for microbiologic examination.

One three-week-old live deer calf which had been scouring intermittently for two weeks was

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necropsied. The animal appeared to have recovered after an initial seven days of illness but relapsed two days later for two days, appeared to recover for two days, and then became bloated and constipated. On the 14th day it began to scour again and refused milk. On arrival at the laboratory a day later, it appeared alert and was not scouring. During the two-week period of illness, three fecal samples had been collected, and the calf had been treated with ampicillin, neomycin, chloramphenicol, and sulfamethazine. Samples for histopathologic and electron microscopic examination and for cryostat sectioning were taken from seven intestinal sites from the calf while it was under anesthesia [13].

Microbiologic examination. Fifty-six fecal swabs obtained during the outbreak (34 from scouring and 22 from nonscouring calves), 58 fecal swabs collected at the end of the outbreak, and ileal and cecal contents of two dead, naturally reared calves were examined for microbes. All samples were examined for enteric viruses by direct electron microscopy with use of negative staining [15], for rotaviruses by enzyme-linked immunosorbent assay, for cryptosporidial oocysts in direct smears treated with Giemsa stain by light microscopy using an oil-immersion lens [8], and for the adherence antigen (K99) of enterotoxigenic *E. coli* by a slide agglutination method [16].

Because the intestinal contents of the dead suckled deer contained large amounts of blood, they were also checked for salmonellae and clostridia.

Inoculation of mice. Two litters of newborn, specific pathogen-free C57 mice were inoculated orally with 0.1 ml (20% [vol/vol] in 0.5% phosphate-buffered saline) of feces containing cryptosporidial oocysts from a diarrheal red deer calf. Fecal smears from the mice were examined daily for cryptosporidial oocysts. Between four and 14 days after inoculation, randomly selected mice from each litter were killed, smears prepared from the large bowel were treated with Giemsa stain and examined, and sections from the upper, middle, and lower intestine taken for histologic and electron microscopic examination. To confirm that the mouse colony was free from naturally acquired cryptosporidial infection, age-matched, conventionally reared control mice were killed before and after the experimental period.

Serology. The indirect immunofluorescence test

was used for serologic examinations. Cryostat-cut sections obtained from a specific pathogen-free lamb that was heavily infected with *Cryptosporidium* derived originally from calves [14] were reacted with 12 convalescent-phase sera from red deer that were recovering from diarrhea. Fluorescein-conjugated pig antiserum to sheep γ -globulin, known to cross-react with deer γ -globulin (H. W. Reid, personal communication), was used as an indicator. Cryostat-cut sections of intestine from uninfected, specific pathogen-free lambs were used as negative controls. The 12 red deer sera were diluted 1:10 and the conjugate 1:40 before the test. This system was tested previously with a large number of positive sera obtained from specific pathogen-free lambs experimentally infected with calf *Cryptosporidium* and with negative sera from uninfected, specific pathogen-free lambs. It had also been tested against K99-specific hyperimmune rabbit serum and did not cross-react (S.T., unpublished observation).

Results

Fecal examinations. Cryptosporidial oocysts measuring about 4 μ m in diameter were observed in fecal smears from 27 (80%) of the 34 deer calves with diarrhea and from 11 (50%) of the remaining 22 apparently healthy animals during the outbreak. However, by the end of the outbreak only one of 58 fecal swabs was positive for oocysts.

Five of the 56 fecal smears from diarrheal deer calves during the outbreak contained astrovirus-like particles [17]. These particles were observed in the feces of two scouring calves, one of which was also excreting cryptosporidial oocysts, and three normal calves, one of which was shedding oocysts. No other viral or bacterial enteropathogen was observed.

The ileal and cecal contents of the two suckled deer calves which contained large amounts of blood were negative for salmonellae and clostridia, but all samples contained large numbers of cryptosporidial oocysts.

Pathology. In the necropsied deer calf, the small intestine was slightly congested and the mesenteric lymph nodes were enlarged. Both the small and large intestines were infected with cryptosporidia; typically, organisms were attached to the microvillar borders of enterocytes, as demonstrated by light and electron microscopy (figure 1).

Figure 1. Jejunal enterocytes from an artificially reared red deer calf that had been intermittently diarrheal for two weeks. *Left*, cryptosporidia (arrows) in the microvillar borders (hematoxylin and eosin, $\times 125$); *right*, an attached cryptosporidial trophozoite in the upper ileum (formalin fixation and lead citrate; bar = 2 μm).

Cryptosporidia were most numerous in the cecum and colon and were also present, although in smaller numbers, in the jejunum and upper ileum. Very few organisms were seen in the terminal ileum, with none in the two most proximal intestinal sites.

Atrophy was almost total in the villi of the ileum (figure 2, top), which showed fusion of villi and striking elongation of crypts. Changes were much less severe in the jejunum, and some infected areas had a normal morphology. Focal areas of villous atrophy were present in the villi of the upper jejunum; however, these areas were associated with adherence of bacteria but not with the presence of cryptosporidia. The lamina propria of the small intestine contained moderate numbers of mononuclear cells.

By contrast, the large intestine was heavily infiltrated by macrophages and other mononuclear cells; the crypts were dilated and contained few mucus-secreting cells. Cryptosporidia were numerous, both at the mucous surface and in the

crypts. Vasculitis involving submucosal venous branches was also present in the cecum (figure 2, bottom); the walls of affected vessels were heavily infiltrated with neutrophils.

Cryostat-cut sections of intestine failed to react in indirect immunofluorescence tests with serum containing antibody to lamb rotavirus and with K99-specific hyperimmune rabbit serum.

The only enteric pathogens identified in the three fecal samples from this calf during the two-week period of illness were cryptosporidial oocysts.

Inoculation of mice. Mice orally inoculated with feces containing cryptosporidia from the deer excreted oocysts between seven and 10 days later, but they remained clinically healthy. Histologic and electron microscopic examination of mouse intestinal sections revealed widespread infection of the lower small intestine (figure 3).

Serology. The convalescent-phase sera collected from the deer surviving two weeks after the outbreak of diarrhea contained antibody to the bovine *Cryptosporidium*, as demonstrated by the

Figure 2. Intestinal tissue from an artificially reared red deer calf that had been intermittently diarrheal for two weeks and was infected with cryptosporidia (hematoxylin and eosin). *Top*, total atrophy of the villi and elongation of crypts in the ileum ($\times 46$); *bottom*, dilatation of the crypts, profuse infiltration of the lamina propria with mononuclear cells, and phlebitis in the submucosa of the cecum ($\times 115$).

indirect immunofluorescence test. Fluorescence was observed on the surface of villi in sections from infected but not from control deer.

Discussion

The results of the present communication demonstrate, on the basis of the excretion of typical oocysts in the feces and the attachment of typical stages of the organism to the brush borders of enterocytes, that these artificially reared red deer were infected with *Cryptosporidium*.

There is thus strong circumstantial evidence to incriminate *Cryptosporidium* as the cause of diarrhea and mortality in the outbreak. The diarrhea coincided with the period in which 80% of the deer tested were shedding oocysts. If sampling had not been confined to a single collection, the infection

rate may have been higher. It should also be emphasized that the method of detection of infection is not very sensitive—there are generally few oocysts in fecal smears treated with Giemsa stain, and often a positive diagnosis is based on detection of very small numbers. The clinical disease was similar to that observed in field outbreaks of diarrhea associated with *Cryptosporidium* in calves [14], lambs [9A], and experimentally infected, specific pathogen-free lambs [13]. Intestinal lesions in an affected deer were also similar to those observed in experimentally infected lambs [13A].

Not all of the infected deer developed diarrhea, a result which suggests that in cryptosporidiosis—as in infections with enterotoxigenic *E. coli*, rotavirus, and coronavirus—contributing factors (for example, inadequate passive immunity) may play a significant role in precipitating diarrhea.

Figure 3. A microgamete (M) and schizont (S) of *Cryptosporidium* attached to a colonic enterocyte from a mouse infected with a fecal homogenate of cryptosporidia from diarrheal, artificially reared red deer calves. Bar = 2 μ m.

Initially the disease was thought to be confined to artificially reared red deer, as is the case in lambs [9A], but the involvement of a few suckled deer suggests that in the latter group colostral protection was insufficient to prevent infection. A possible explanation for this lack of protection is that the organisms were introduced recently to the deer population. In past years and under the same system of management, diarrhea in young red deer had occurred only sporadically and less severely (W. Corrigan and G. A. M. Sharman, personal communication). Furthermore, the occurrence of an outbreak of diarrhea in suckled beef calves on the same premises six months earlier may have had important epidemiologic significance. Cryptosporidia isolated from those calves and from the deer both infected laboratory animals and were serologically indistinguishable by the indirect immunofluorescence method used.

The role of astrovirus-like particles in this outbreak of diarrhea is uncertain. On the basis of the evidence presented here and its doubtful role as an enteropathogen in other species [18, 19], it was probably only of minor importance.

In conclusion, the present communication describes another possible enteropathogen, *Cryptosporidium*, to be considered in future investigations of outbreaks of diarrhea in deer.

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