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# Diarrhea Due to *Cryptosporidium* Infection in Artificially Reared Lambs

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Severe diarrhea which lasted 7 to 12 days occurred in 40 of 48 artificially reared lambs within 5 to 12 days of birth, and 16 of them died. Of 16 diarrheic fecal samples examined, 10 contained *Cryptosporidium* oocysts and 1 contained rotavirus, but no other known enteropathogen was detected. Upon histological examination, cryptosporidia were found in the ilea of three affected lambs, and in one of them, villous atrophy and fusion, with epithelial cross-bridging between villi, were present in the distal small intestine. Diarrhea was induced in two specific pathogen-free lambs by oral inoculation with fecal homogenate containing *Cryptosporidium* oocysts. Both the small and the large intestines became infected with the organism, and associated lesions included stunting, fusion, and deformities of villi in the distal small intestine, with replacement of columnar enterocytes by immature cuboidal cells. Subclinical infections were induced in newborn specific pathogen-free mice and rats. Judged by these data, the lamb-derived *Cryptosporidium* sp. is similar to those recovered from calves, deer, and humans.

Diarrhea in young domestic animals is most commonly attributed to a few enterotoxigenic *Escherichia coli* serotypes, rotavirus, and coronavirus acting singly or in combination. Recent reports suggest that *Cryptosporidium* sp., a member of the enteric coccidian group, may also be a candidate. *Cryptosporidium* sp. is widespread, and it occurs in many animal species (5). It is an extracellular parasite adhering to the brush borders of enterocytes, has a rapid life cycle in domestic animals, and appears to lack host specificity (13).

Most commonly, *Cryptosporidium* sp. has been associated with diarrhea in calves (7, 9, 11, 15) but there are several reports of its occurrence in enteric disease of other species, such as lambs (2), deer (Tzipori et al., submitted for publication), and humans (6, 8, 14).

Experimental infection of lambs with *Cryptosporidium* sp. isolated from calves induced intermittent diarrhea, culminating in death of lambs infected at birth and growth retardation in older lambs (S. Tzipori, K. W. Angus, E. W. Gray, and I. Campbell, Am. J. Vet. Res., in press).

This is the first report of diarrhea attributed to *Cryptosporidium* infection in artificially reared lambs. The clinical manifestation and pathogenesis of the disease in lambs experimentally inoculated with lamb *Cryptosporidium* sp. is also described.

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## MATERIALS AND METHODS

**Case report.** A total of 48 lambs (40 Texel and 8 North Ronaldsay) born over a period of 12 weeks were removed from their dams immediately after birth and fed cow colostrum. They were subsequently maintained on ewe milk substitute (Nutrilamb; Scottish Agricultural Industries, Edinburgh, Scotland) given twice daily. The first 8 lambs born were Texels and were all reared uneventfully, but each of the following 40 lambs (83%) had diarrhea, and 16 (10 Texels and 6 North Ronaldsay) subsequently died. The first case of diarrhea occurred in a lamb 9 days old; lambs subsequently born began to scour between 5 to 12 days after birth and had intermittent diarrhea lasting 7 to 16 days. Mortalities were mostly associated with diarrhea of 2 to 3 weeks in duration and reduced milk intake.

Two months after the last diarrheic lamb had recovered, more than 100 suckled lambs were born in the same building over a period of 4 weeks, but none of these developed diarrhea. Four orphan lambs belonging to this group had to be reared artificially, however, and three of these developed diarrhea 5 to 7 days after birth. During the same period, a number of cases of diarrhea occurred in suckled calves on the same premises.

The initial investigation of this outbreak was limited to bacteriology and necropsies. The bacteriological results were inconclusive, but necropsy findings suggested that deaths were associated with severe enteritis and digestive upsets. Portions of fixed intestine from three 1- to 2-week-old lambs (two dead, one killed with sodium pentobarbitone) were submitted for histopathological examination.

**Microbiology.** Full microbiological examinations were undertaken on 16 fecal samples submitted towards the end of the outbreak. Fourteen of these were swabs taken from live lambs, and two were large bowel contents from dead lambs. Fecal swabs were also obtained from the four artificially reared orphan lambs belonging to the second group and from six suckled calves with diarrhea.

All samples were examined for enteric viruses by enzyme-linked immunosorbent assay and electron microscopy, for *Cryptosporidium* oocysts by Giemsa-staining of fecal smears (11), and for enterotoxigenic *E. coli* by demonstration of the adherence antigen K99 (4).

**Animal inoculations.** Two 1-day-old specific pathogen-free (SPF) lambs, housed in plastic gnotobiotic isolators, were each inoculated orally with 2 ml of homogenate containing *Cryptosporidium* oocysts and free of other known enteropathogens prepared from feces of diarrheic lambs (20%, vol/vol).

One litter each of newborn SPF mice and SPF rats were inoculated orally with 0.1 and 0.2 ml, respectively, of the fecal homogenate.

**Necropsy.** The two SPF lambs were killed 3 (no. 1) and 7 (no. 2) days after inoculation. Gut samples were taken for histopathology, immunofluorescence, and transmission electron microscopy, as previously described (Tzipori et al., in press).

Portions were taken from five equally spaced sites along the small intestine, and one portion each was taken from the cecum, colon, and abomasum while the lamb was under deep anaesthesia.

**Enzymology.** Gut sections were taken from the upper, middle, and lower small intestine for measurements of lactase and sucrase activity (3).

## RESULTS

**Microbiology.** A total of 10 of the 14 fecal swabs from live lambs and both samples of large intestinal contents from the dead lambs contained *Cryptosporidium* oocysts, as observed in Giemsa-stained fecal smears. The oocysts, measuring 3 to 4 $\mu$m, were indistinguishable from those observed for other species infected with cryptosporidia.

Oocysts were observed also in samples from two of the three scouring artificially reared lambs from the second group and from three of the six diarrheic suckled calves. Rotavirus was found in the feces of one lamb in which no oocysts were detected and in the feces of two of the six diarrheic calves. No enterotoxigenic *E. coli* was cultured from any of the fecal swabs.

**Histopathology of field material.** Intestinal sections obtained from three lambs (two dead, one alive) revealed numerous cryptosporidia adhering to enterocytes of the terminal ileum. Infection was also apparent in the jejunum and large intestine of the lamb which was killed. Lesions in the distal small intestine of this lamb consisted of partial villous atrophy and fusion (Fig. 1), with numerous epithelial cross-bridges between adjacent villi. Infection of the large intestine (Fig. 2) was not associated with any obvious pathological changes.

**Animal inoculations.** Three days after inoculation, the two SPF lambs became depressed, and diarrhea developed shortly afterwards. Their milk intake was reduced markedly, and *Cryptosporidium* oocysts were seen in their feces from day 3 until they were killed. Lamb 1 was killed 18 h after the onset of clinical signs. Its intestine was congested, the mesenteric lymph nodes were edematous, and the lumen of the small intestine contained large amounts of blood. Numerous cryptosporidia were seen embedded in the brush borders of enterocytes from the upper jejunum to the terminal ileum (Fig. 3) but the large bowel was not infected. Stunting and fusion of villi and replacement of enterocytes by immature cells were observed only in the lower jejunum.

Lamb 2 remained depressed and continued to excrete oocysts and to scour intermittently until

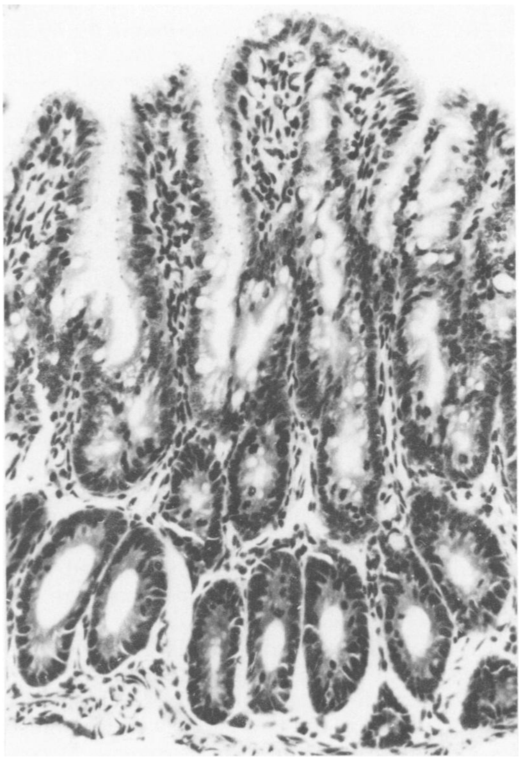

FIG. 1. *Jejunum of North Ronaldsay lamb naturally infected with cryptosporidia. Note the stunting and fusion of villi. Hematoxylin and eosin stain;* ×*150.*

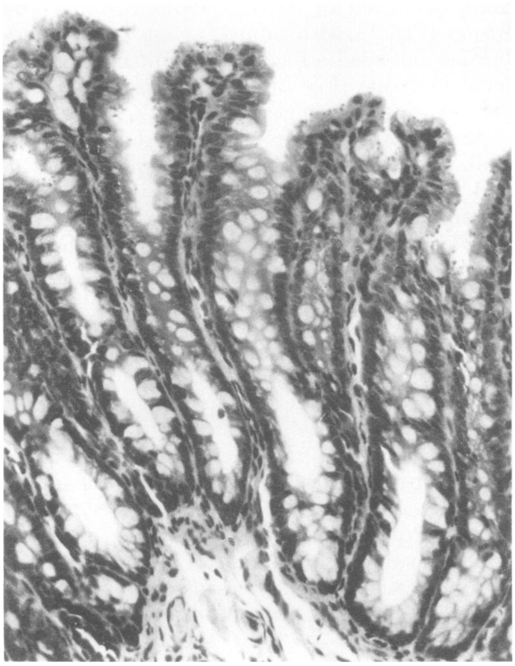

FIG. 2. *Cecum of the lamb described in the legend to Fig. 1, showing cryptosporidia attached to the mucosal surface. Hematoxylin and eosin stain; ×150.*

it was killed, 4 days after the onset of diarrhea. During this period, its milk intake dropped from 1,600 ml to 400 to 800 ml per day. This lamb was emaciated but not dehydrated; its intestine was congested and lacked tone, but contained no blood. The entire small and large intestines were infected with typical *Cryptosporidium* organisms, and mucosal lesions were present in the lower jejunum and ileum. The latter site was most severely affected; marked stunting and fusion of villi and epithelial cross-bridging between adjacent villi occurred (Fig. 4 and 5). The deformed villi were covered by immature cuboidal epithelial cells. The only pathological change in the large intestine was the presence of aggregates of mononuclear cells in the lamina propria. The abomasal mucosa was very heavily infiltrated with neutrophils, and pus exuded from many glands, but no cryptosporidia were observed.

Cryostat sections stained by indirect FAT did not show any reaction with rotavirus and K99-specific hyperimmune rabbit sera. Fecal samples taken daily from the two lambs were examined for enteric viruses and enterotoxigenic *E. coli*, with negative results. Inoculation of litters of mice and rats resulted in *Cryptosporidium* infection without diarrhea. Shedding of *Cryptosporidium* oocysts in the feces of laboratory animals lasted for 7 to 13 days after inoculation. Histological sections taken from the upper, middle, and lower small intestine contained numerous typical organisms adhering to the brush borders of enterocytes.

**Enzymology.** Lactase activity was reduced in both infected lambs, as compared with the control (Table 1). Sucrase activity was reduced only in lamb 2.

## DISCUSSION

Two earlier reports of *Cryptosporidium* infection in lambs described intestinal lesions associated with attachment of *Cryptosporidium* organisms to the intestinal mucosa of three animals (1), and the organisms were detected in one of two scouring lambs submitted for necropsy (2). However, this is the first report to incriminate *Cryptosporidium* sp. as a possible cause of diarrhea in a sizable group of lambs. There was a close association between the outbreak of diarrhea and excretion of oocysts in the feces. The *Cryptosporidium* organisms in lamb feces induced clinical illness and pathological changes in SPF lambs resembling those which occurred in the field outbreak and those found in lambs experimentally infected with a *Cryptosporidium* sp. isolated from calves (Tzipori et al., in press; K. W. Angus, S. Tzipori, and E. W. Gray, unpublished data).

*Cryptosporidium* sp. has been shown to lack host specificity (13), and bovine and ovine cryptosporidia in sections of intestine were serologically indistinguishable, as assessed by indirect immunofluorescence tests on infected gut, using convalescent sera (S. Tzipori, I. Campbell, and D. Sherwood, unpublished data). Fecal homogenates containing oocysts from both species also infected laboratory animals in an identical manner. Thus, calves and lambs may be capable of infecting each other under field conditions, as is the case with enterotoxigenic *E. coli*. It follows that results obtained with the calf *Cryptosporidium* sp. in lambs (Tzipori et al., in press) are probably valid for interpretation of the lamb *Cryptosporidium* infection in lambs. *Cryptosporidium* sp. from calves induced acute diarrhea in newborn lambs, whereas in older lambs, diarrhea was less acute but tended to relapse, and weight gain was affected (Tzipori et al., in press). Additionally, *Cryptosporidium*-induced enteritis appeared to be far more severe in experimentally infected lambs than enterotoxigenic *E. coli*, rotavirus (10; Tzipori et al., submitted for publication), or astrovirus (12) infections.

It is doubtful that rotavirus had a role in the pathogenesis of diarrhea in this outbreak in

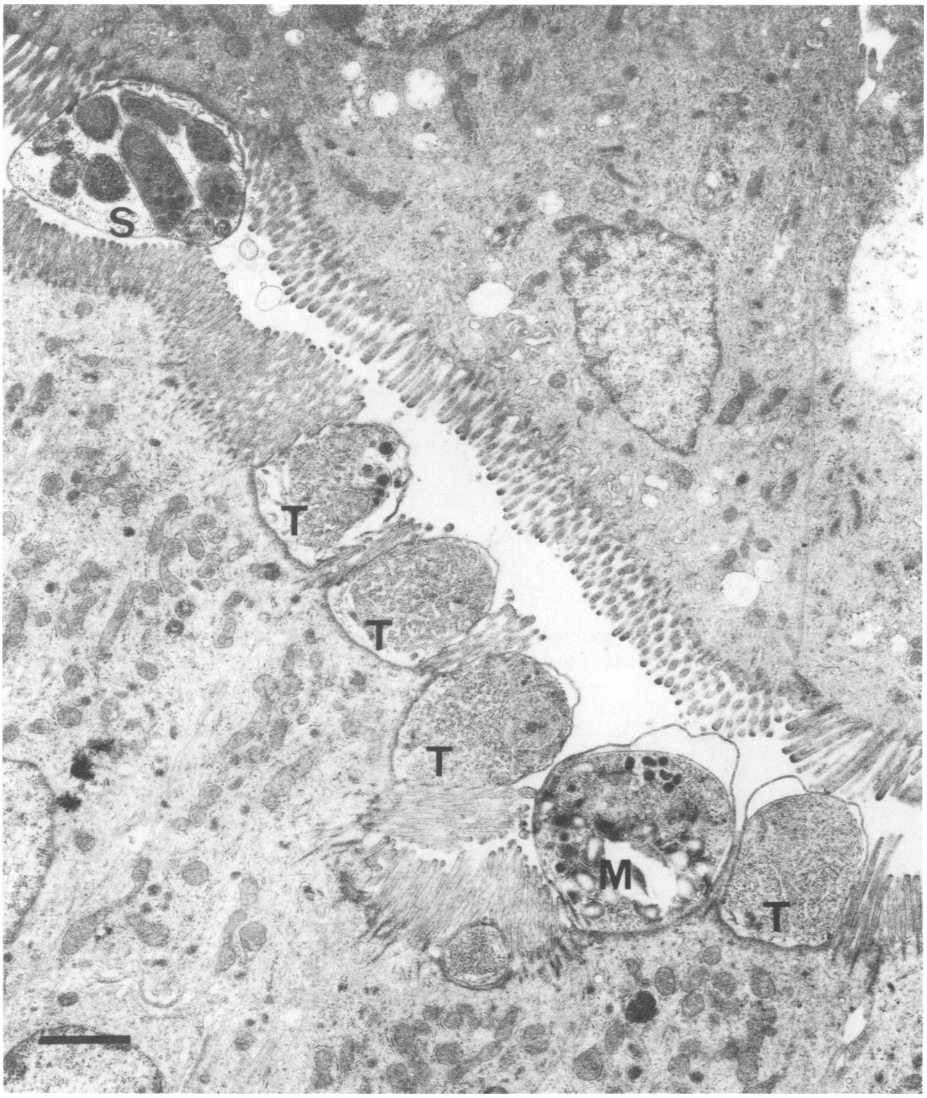


FIG. 3. *Cryptosporidia attached to ileal enterocytes of an experimentally infected SPF lamb. Trophozoite (T), schizont (S), and macrogametocyte (M) stages are all present. Lead citrate stain; ×6,000. Bar = 2 μm.*

lambs, for two reasons. First, virus was observed in only one sample, despite the greater sensitivity of the systems used (enzyme-linked immunosorbent assay and electron microscopy) for detection of rotavirus as compared with the sensitivity of examination of fecal smears for *Cryptosporidium* sp. (Although demonstration of *Cryptosporidium* oocysts is closely correlated with infection, they may be overlooked because they are small in size and few in number.) Second, rotavirus has a short incubation period, and towards the end of an outbreak, such as the one described, diarrhea due to rotavirus infection would have been expected to occur within the first 5 days of life. Affected lambs in the outbreak were at least 5 days old, the majority being 1 to 2 weeks old. Furthermore, in experimental infections of gnotobiotic and SPF lambs, rotavirus and enterotoxigenic *E. coli* induced clinical diarrhea only in lambs under 4 days old, whereas older lambs became subclinically infected. In the same experiments, it was found that in coinfection of older lambs with rotavirus and *Cryptosporidium* sp., the virus played no part in the pathogenesis of diarrhea (Tzipori et al., submitted for publication).

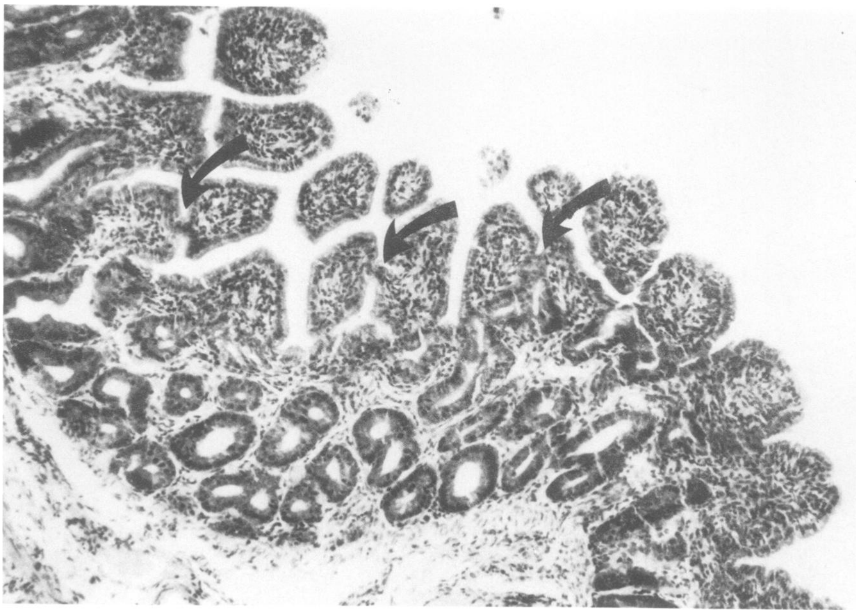

FIG. 4. *Stunted and fused villi in the ileum of the lamb described in the legend to Fig. 3. Note the epithelial cross-bridges (arrows) between adjacent villi. Hematoxylin and eosin stain; ×60.*

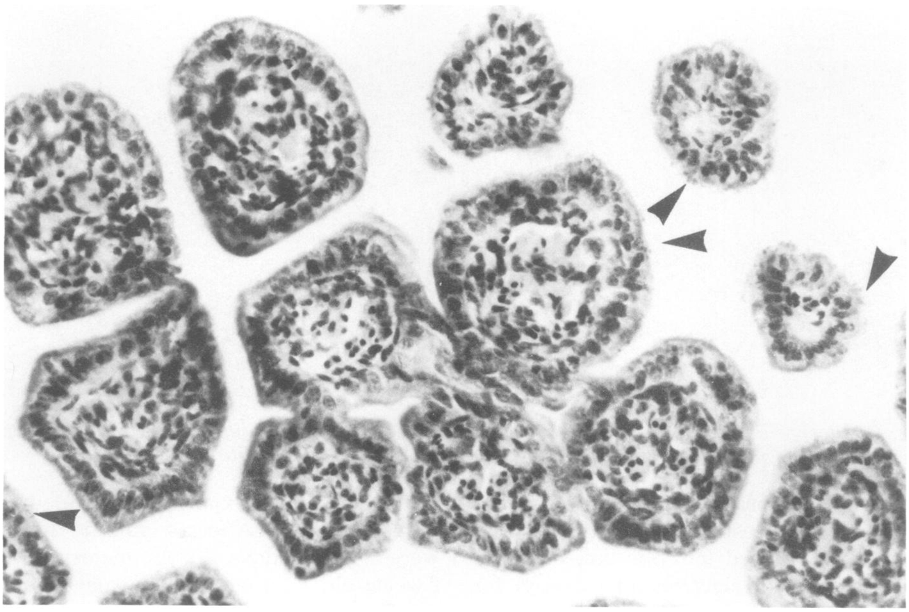

FIG. 5. *Extensive fusion between adjacent villi. Arrows indicate clusters of organisms in the brush borders. Hematoxylin and eosin stain; ×150.*

The age susceptibility under field conditions is unknown, although 30-day-old lambs, experimentally inoculated with *Cryptosporidium* sp. isolated from calves, were only mildly affected (Tzipori et al., in press).

To date, the oldest lambs found to be infected with *Cryptosporidium* sp. were 7 months old (K. W. Angus, unpublished data).

In the field, diarrhea appears to be more prevalent in calves than in lambs, possibly because

TABLE 1. *Level of disaccharidase activity in one control and two experimentally infected SPF lambs infected with Cryptosporidium sp.*

| Lamb | Age (days) | Activity[a] at small intestine site | | | | | |
|---|---|---|---|---|---|---|---|
| | | Lactase | | | Sucrase | | |
| | | Upper | Middle | Lower | Upper | Middle | Lower |
| Control | 7 | 5.09 | 12.90 | 1.40 | 0.55 | 0.50 | 0.31 |
| 1 | 3 | 2.86 | 3.30 | 0.40 | 0.73 | 1.53 | 0.78 |
| 2 | 7 | 2.80 | 1.28 | 0.08 | 0.07 | 0.08 | ND[b] |

[a] Micromoles per minute per gram of wet weight of intestine.
[b] ND, Not detected.

lambs seem to be less susceptible to the same range of enteropathogens. Diarrhea associated with *Cryptosporidium* sp. may be a problem in artificially reared lambs, as this case report suggests that suckled lambs are protected against the adverse effects of infection. Although they were born and reared in premises which had earlier accommodated the affected lambs, none of over 100 suckled lambs contracted diarrhea. In contrast, three of four hand-reared orphan lambs in the group became diarrheic, and *Cryptosporidium* infection was confirmed in two instances.

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