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NOTES

Cryptosporidium: Evidence for a Single-Species Genus

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Cryptosporidium, isolated from calves with diarrhea, infected, with or without causing enteritis, seven different species of animals.

The protozoan *Cryptosporidium* (family Cryptosporidiidae [2]) was first described by Tyzzer in 1907 (10), and has since been identified in at least 12 different hosts, including mammals, birds, and reptiles. Like other enteric coccidia, cryptosporidia are considered to be host (12) and site (11) specific. Their life cycle has been outlined in guinea pigs (12) and calves (6) and resembles that of other coccidia (2). Cryptosporidia differ, however, from other enteric coccidia by being extracellular organisms (6), adhering to the microvillous borders of enterocytes of the small intestine. Recent association of cryptosporidia with diarrhea in calves (4, 7, 8), lambs (1), foals (9), and humans (3, 5) has stimulated interest in these parasites. The causative role of cryptosporidia in calf diarrhea, confirmed in two outbreaks (in press), prompted the experimental investigation reported here.

Cryptosporidium oocysts were found in Giemsa-stained smears of diarrheic calf feces (in press). A homogenate of these feces with phosphate-buffered saline given orally to a 10-day-old calf caused diarrhea. Electron microscopy and culture of feces and gut contents from this calf failed to reveal known enteric viruses of enterotoxigenic *Escherichia coli*. *Cryptosporidium* oocysts were demonstrated in stained fecal smears. Homogenates of ileal contents in phosphate-buffered saline (20%, vol/vol) from the experimental calf were stored at 4°C before they were used to inoculate orally seven different species (Table 1).

All seven species became infected with *Cryptosporidium* as demonstrated by (i) presence of oocysts in stained fecal smears and (ii) parasites attached to microvillous borders of host enterocytes, observed by optical and electron microscopy (Fig. 1 and 2). Age-matched control animals which were similarly monitored shed no oocysts in the feces; the intestines were free of histological abnormalities, and the animals showed no signs of diarrhea. Diarrhea, however, occurred only in lambs, piglets, and calves. Lambs were most severely affected, and those less than 5 days old at inoculation died after protracted diarrhea and wasting, possibly because they were specific pathogen free and deprived of colostrum. In lambs, piglets, and calves the most consistent histological changes in the intestinal mucosa were congestion, moderate to severe villous atrophy of the affected area, fusion of villi, replacement of columnar enterocytes by low cuboidal cells, and infiltration of neutrophils. Attached cryptosporidia were most commonly found in the lower small intestine, but other sites were often infected, including upper jejunum (lambs, piglets, chicks, caecum, and spiral colon (lambs, piglets). In lambs examined 5 to 7 days postinoculation parasites were found adhering to both small and large bowels. Rats, mice, guinea pigs, and chicks did not develop diarrhea or any other obvious illness. The infection and shedding of oocysts in the feces lasted much longer in suckling rats than in any of the other species. In rats, mice, guinea pigs, and chicks the histological changes varied from no apparent lesions to moderate villous atrophy and infiltration of mononuclear cells.

To determine whether cryptosporidia obtained after passage in one species could retain pathogenicity for the original host, two 1-day-old, specific pathogen-free lambs were inoculated with gut contents from infected rats. Both lambs became infected and developed intractable diarrhea within 2 days postinoculation. Oocysts from rats also infected mice and vice versa, and the cryptosporidia retained virulence through three serial passages in specific pathogen-free lambs.

Four additional isolates were examined; three were isolated from field cases of diarrhea (two calves, one lamb) obtained from areas geographically remote from each other, and the fourth isolate was obtained from an adult human suffering from acute vomiting and diarrhea. The two calf isolates infected baby rats and mice; the lamb and the human isolates infected mice and

TABLE 1. *The response of seven animal species to experimental oral inoculation with cryptosporidia from calves*[a]

Species	No. infected/no. of controls	Age (days)	Status	Inoculum (ml)	Oocyst shedding		Diarrhea	
					Appearance (days p.i.)	Duration (days)	Appearance (days p.i.)	Duration (days)
Lamb (SPF)	6/6	1	CD	2	2–5	7	2–5	7[b]
Calf (Convent.)	2/2	10	CF	10	9	2	9	3[c]
Pig (Convent.)	2/2	2	CF	2	4	10	3	3
Rat (SPF)	20/8	1	CF	0.02	5	16	±	3
Mouse (SPF)	9/9	1	CF	0.01	5	8	–	–
Guinea pig (SPF)	7/1	1	CF	0.1	7	4	–	–
Chick (Convent.)	4/6	1		0.5	7	1	–	–

[a] The recipient animals were specific pathogen free (SPF) or conventional (Convent.), colostrum fed (CF) or colostrum deprived (CD). p.i., Postinoculation.

[b] All died or were killed when moribund.

[c] One died and one was killed.

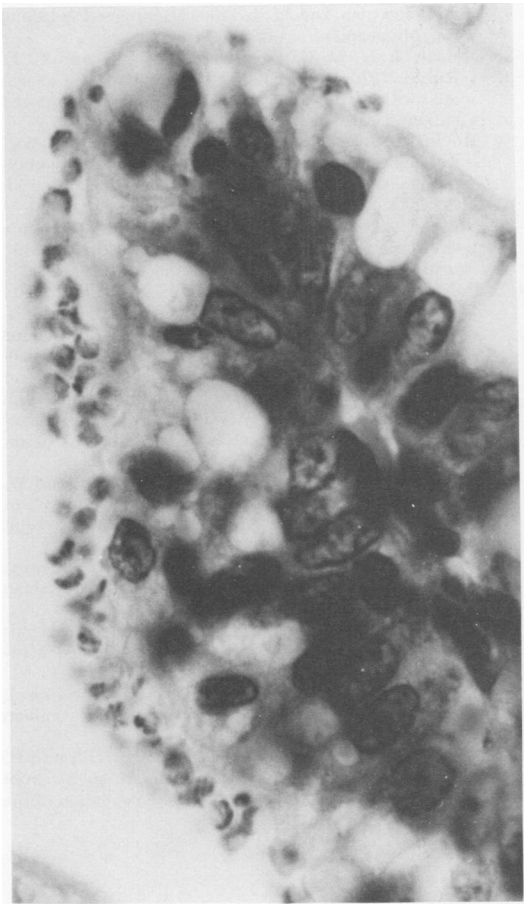

FIG. 1. *Cryptosporidia attached to rat intestinal villus. Oil immersion (×1,000).*

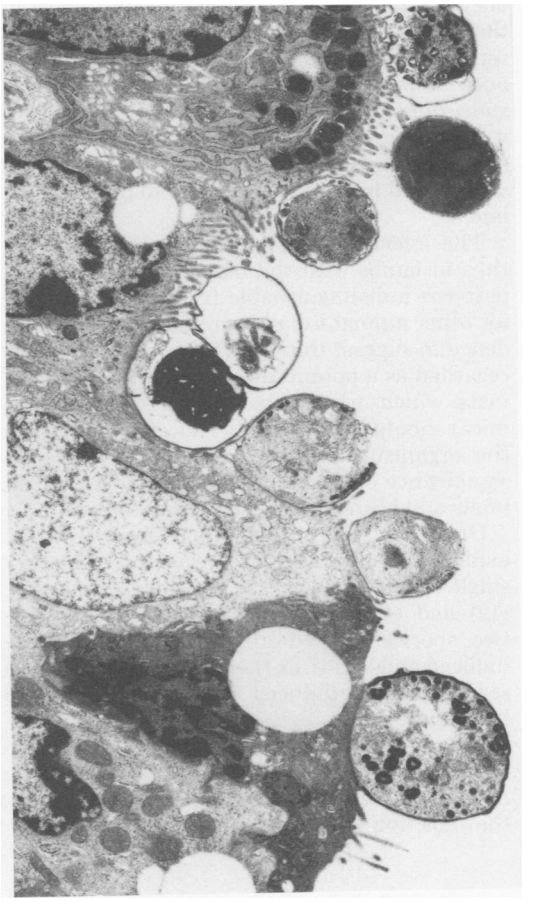

FIG. 2. *Electron micrograph of cryptosporidia adhering to ileal enterocytes of a 5-day-old lamb. From top to bottom: early developing macrogamete, unidentified stage, developing first generation schizont, two oocysts, two trophozoites, and a macrogamete with typical electron-dense polysaccharide granules (×6,000).*

induced diarrhea in lambs. There was little difference in the pathogenesis of infections between animals infected with these four isolates and

those infected with the original calf cryptosporidia. The lack of host specificity is thus a property shared by more than one isolate. It is conceivable that these isolates will propagate in many more species and may have as wide a range of hosts as *Toxoplasma gondii*, a member of the tissue cyst-forming coccidia. The apparent lack of host specificity contradicts the findings of Vetterling and co-workers (12), who were unable to induce infections with cryptosporidia isolated from ileal scrapings from guinea pigs in 3-week-old chicks, turkeys, rabbits, or weanling mice. This discrepancy could be because in our experiments the laboratory animals used were less than 1 day old and were derived from specific-pathogen-free stock.

A major obstacle to pathogenesis studies with cryptosporidia is the difficulty of separating oocysts from other enteropathogens infecting the gut. Other known coccidian oocysts can be separated by flotation or sporulation techniques, both of which were unsuccessful with *Cryptosporidium* (unpublished data). The susceptibility of rats and chicks may overcome this obstacle, as passage in these species removes enteric viruses and bacteria pathogenic to domestic animals.

The infection of mice and induction of diarrhea in lambs with the human isolate followed patterns indistinguishable from those observed for other animal isolates, providing indirect evidence to suggest that crytosporidiosis should be regarded as a potential zoonosis. Species of animals which were subclinically infected (e.g., mice) should be regarded as potential carriers of the organism. The prevalence and hence the significance of cryptosporidiosis in domestic animals and humans are yet to be assessed.

On the basis of our findings there is strong evidence to suggest that *Cryptosporidium* is a single-species genus. *Cryptosporidium wrairi* (12) and *Cryptosporidium bovis* (7), the only two species studied in experimental animals, induced infections in their respective hosts resembling that produced with our calf isolate in both species.

Our results indicate that cryptosporidia are non-host-specific parasites capable of inducing diarrhea in several species of animals, and that the infection is transmitted by the ingestion of oocysts. Infection can be diagnosed by demonstration of oocysts in feces and can be confirmed by inoculation of laboratory animals.

To add another potential pathogen to an increasing list of enteric agents may appear to complicate an already complex syndrome. On the positive side, however, the emergence of cryptosporidia as enteropathogens provides an added line of investigation in otherwise unresolved cases of diarrhea in animals and humans.

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