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## Cryptosporidiosis in a Cotton Rat (*Sigmodon hispidus*)

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**ABSTRACT:** Cryptosporidiosis, previously unreported in cotton rats (*Sigmodon hispidus*), was observed in one of nine cotton rats from Pryor, Oklahoma (USA). Infection was confined to the large intestine. Microscopically, numerous cryptosporidians measuring 2 to 3  $\mu$ m in diameter were intimately associated with the luminal surface of colonic and rectal mucosae. The affected lamina propria of the large intestine was diffusely infiltrated by eosinophils, lymphocytes and macrophages. Ultrastructurally, numerous trophozoites and a single schizont were observed. Microvilli were displaced by the presence of cryptosporidians at the attachment site.

**Key words:** Cryptosporidiosis, ultrastructure, pathology, cotton rats, *Sigmodon hispidus*.

Cryptosporidiosis, a coccidial parasite disease caused by various *Cryptosporidium* spp. has been recognized in several mammalian, reptilian and avian species (Angus, 1983; Davis and Jenkins, 1986; Fayer and Ungar, 1986). Because of clinical disease in humans with acquired immune deficiency syndrome, the disease has gained increased interest. Diarrhea has been associated with infection in lambs, calves, pigs, rhesus monkeys, mice, rats, turkey poults and humans (Fox et al., 1984; Angus et al., 1985; Aboul-Magd et al., 1989; Ungar et al., 1990; Moody et al., 1991). Lethargy, rough hair coat, failure to gain or maintain weight, and weight loss have been reported in natural and experimental infections of mice, rats and rabbits (Harkness and Wagner, 1989). Furthermore, a progressive infection, fatal within 4 mo with weight loss and intermittent diarrhea has been reported in adult athymic mice (Ungar et al., 1990) and immunocompromised rats (Aboul-Magd et al., 1989). A lowered mucosal enzyme activity of the small intestine has been associated with cryptosporidiosis in guinea pig and calves (Manning et al., 1984; Barker and Dreumel, 1985).

*Cryptosporidium* spp. develops in the absorptive surface of intestinal epithelial cells and is associated with villus atrophy of varying severity, blunting and fusion of villi, and hypertrophy of crypts of Lieberkuhn (Gibson and Wagner, 1986; Harkness and Wagner, 1989; Moody et al., 1991). Cryptosporidial infection is associated with loss and displacement of the enterocyte microvilli by the parasite (Van Kruiningen, 1988). Initially, a pentalamellar membrane fusion site develops at the base of the protozoa between the parasite's outer plasma membrane and host membrane. Later, the host membrane disintegrates and directly contacts absorptive cell cytoplasm (Harkness and Wagner, 1989; Marcial and Madara, 1986).

No report of cryptosporidiosis in cotton rats (*Sigmodon hispidus*) has been found in the literature. We describe cryptosporidiosis in a male cotton rat.

Nine cotton rats were collected from typical ungrazed, tall grass prairie in Pryor, Oklahoma (95°17'N, 36°15'W). Animals were returned to the laboratory, fasted overnight, humanely killed by cervical dislocation, and necropsied. Liver, kidneys, lungs, heart, spleen, stomach, duodenum, jejunum, ileum, colon, cecum, rectum, brain and muscle samples were fixed in Carson's modified Milloning's phosphate buffered formalin (Sheehan and Hrapchak, 1980). Six-micron thick sections of paraffin embedded tissue were stained with hematoxylin and eosin (H&E) and permanently mounted on glass slides for microscopic examination. Formalin-fixed tissues (1 mm cube) of colon and rectum were post-fixed for 2 hr in Karnovsky's fixative (Karnovsky, 1965) and prepared for transmission electron microscopy. Fif-

ty-five nm sections of each sample were cut with glass knives on a Sorvall MT 6000 Ultracut ultramicrotome (Research Manufacturing Company, Phoenix, Arizona, USA), mounted on glass microscope slides, and stained with Mallory's stain (Richardson et al., 1960). Areas containing *Cryptosporidium* spp. were selected for thin-sectioning at 0.55 nm, stained with uranyl acetate and lead citrate (Pillsbury, 1980), and examined with a Jeol 100C transmission electron microscope (Jeol Ltd., Tokyo, Japan).

One of nine cotton rats had *Cryptosporidium* spp. in the large intestine with no recognized clinical signs. The liver was fragile, pale and enlarged with accentuated lobular pattern. All other organs (lung, kidneys, heart, stomach, small intestine, brain, muscle samples and genital organs) appeared normal. There was a moderate enlargement of the centrilobular and mid-zonal hepatocytes in the liver, including enlarged nuclei and increased cytoplasmic vacuolations; there also were multifocal aggregates of lymphocytes and macrophages. No *Cryptosporidium* spp. was observed in the liver.

Macroscopically, the intestinal mucosa and contents of this *Cryptosporidium* spp.-infected cotton rat were normal. Microscopically, the colonic and rectal mucosa were diffusely infiltrated by mixed inflammatory cells (eosinophils, lymphocytes, macrophages). No such lesions were observed in the esophagus, stomach, duodenum, jejunum and ileum. Rarely, focal necrosis of superficial mucosa and ectatic crypts containing cellular debris and neutrophils were present in the large intestine. Numerous cryptosporidian trophozoites were intimately associated with the luminal surface of the epithelium of the large intestine; most were approximately 2 to 3  $\mu\text{m}$  in diameter and were easily seen in sections stained with hematoxylin and eosin.

Based on transmission electron microscopy, numerous cryptosporidians occurred

at the luminal border of epithelial cells in the large intestine. The microvilli of epithelial cells were displaced by the presence of parasites at the attachment site. Numerous trophozoites and a single schizont were the only parasite stages observed in the section (Figs. 1, 2). Trophozoites were variable in shape and measured 1.99 to 2.39  $\mu\text{m}$  by 2.39 to 3.31  $\mu\text{m}$ ; they were recognized by the presence of a nucleus with a nucleolus, endoplasmic reticulum, and Golgi Anlagen (Vetterling et al., 1971). Trophozoites were attached to the apical surface of enterocytes. There were broad, electron-dense attachment zones at the interface between parasite and host cells. The schizont measured 2.70 by 3.39  $\mu\text{m}$ ; it was identified by the presence of membrane-bound merozoites free within the parasitophorous vacuole. These ultrastructural features are characteristic of *Cryptosporidium* spp., as described by Vetterling et al. (1971).

In laboratory animals (guinea pigs, rats, mice, rabbits, ferrets), developmental stages of *Cryptosporidium* spp. may be seen from the pylorus or duodenum to the cecum with the highest concentration in the ileum (Rehg et al., 1988; Harkness and Wagner, 1989). The small intestine was not affected in this cotton rat. It is not known whether the mild eosinophilic inflammation of colon and rectum in the absence of helminth parasites, that we observed in this cotton rat was associated with *Cryptosporidium* spp. infection. However, similar observations have been reported in ferrets (Rehg et al., 1988).

Six of the nine cotton rats, including the one with cryptosporidiosis, were collected from a site contaminated with Aroclor 1254, a polychlorinated biphenyls (PCB) mixture; the other rats were collected from an uncontaminated site with ecologically similar habitat, approximately 0.8 km from the contaminated site. The soil concentration of Aroclor 1254 was more than 800 ppm in some areas (United States Corps of Engineers, 1987). Tissue PCB concen-

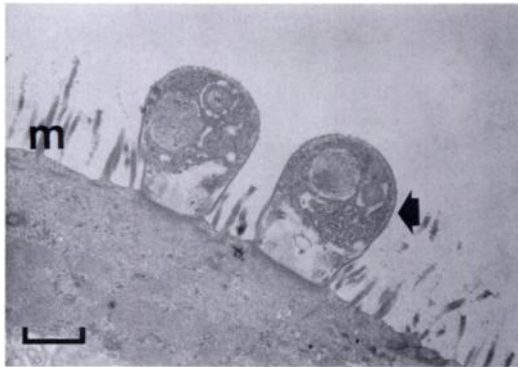


FIGURE 1. Electron micrograph of cotton rat intestine with two trophozoites (arrow) attached to the epithelial cell and displacing microvilli (m). Uranyl acetate and lead citrate. Scale bar = 1  $\mu$ m.

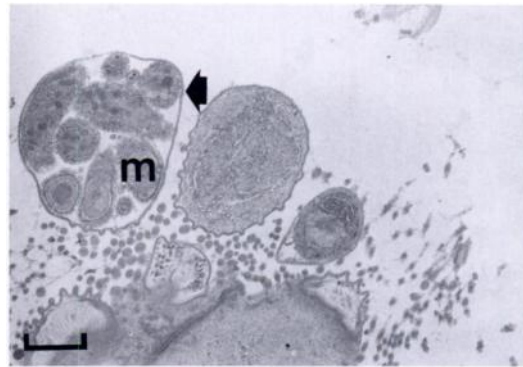


FIGURE 2. Electron micrograph of a schizont (arrow) containing merozoites (m) separated by artifact from an epithelium. Uranyl acetate and lead citrate. Scale bar = 1  $\mu$ m.

trations of contaminated cotton rats (1.42 to 12.36 ppm) greatly exceeded the concentrations in uncontaminated rats (<0.05 ppm). Hepatic changes in this infected cotton rat were similar to those seen in the remaining contaminated rats and probably were related to the Aroclor 1254 exposure. The hypertrophy of hepatocytes with increased organelle content has been reported in PCB-fed rats and rabbits (Sleight and Sanger, 1976; Raber and Carter, 1986). Others (Cooper et al., 1984; Fukushima and Helman, 1984) suggest that *Cryptosporidium* spp. acts as an opportunist in immunocompromised hosts. In another study, cryptosporidial infection was induced in cyclophosphamide-treated immunosuppressed rats which remained infected as long as they received cyclophosphamide (Rehg et al., 1987). It is possible that exposure to Aroclor 1254, a known immunotoxin, predisposed this cotton rat to cryptosporidiosis. However, we did not evaluate the immunological status of the host and, therefore, cannot confirm immunodeficiency nor speculate further on its role in the development of cryptosporidiosis.

The source of infection in this cotton rat is not known. Interspecies transmission of *Cryptosporidium* spp. is well documented (Tzipori et al., 1980; Iseki et al., 1989), but

no attempt was made in this instance to determine the source of infection.

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