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# Persistent Cryptosporidiosis in Horses with Severe Combined Immunodeficiency

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**Cryptosporidial infections were established in five young foals with severe combined immunodeficiency following oral administration of $10^8$ *Cryptosporidium parvum* oocysts. All foals shed oocysts (average of $8 \times 10^6$ to $2 \times 10^8$/g of feces) until death. Inflammation and *C. parvum* organisms were observed in the common bile duct, duodenum, jejunum, and ileum. Since foals with severe combined immunodeficiency lack functional T and B lymphocytes and are incapable of antigen-specific immune responses, they are well suited for evaluating the pathogenesis and treatment of persistent cryptosporidiosis.**

*Cryptosporidium parvum*, a coccidian that parasitizes mucosal epithelial cells, was first recognized as a human pathogen in 1976 (25, 26). The importance of this organism has escalated since the recognition of its common occurrence in immunocompetent individuals and in patients with AIDS or other forms of immunodeficiency (8–10, 12, 16, 38). Cryptosporidiosis in immunocompetent hosts is characterized by self-limiting diarrhea with transient anorexia and weight loss (8, 10, 12, 16, 38). Conversely, immunodeficient patients typically develop persistent cryptosporidial infections resulting in life-threatening, chronic diarrhea (8–10, 12, 16). *C. parvum* infection is not restricted to the gastrointestinal tract of immunodeficient hosts, as infections of the biliary tree and respiratory tract may also occur (8–10, 12, 16, 20). Since *C. parvum* sporozoites and merozoites cyclically autoinfect mucosal epithelium (9, 11, 12) and since effective chemotherapy is unavailable (6, 7, 9, 19), immunodeficient patients develop persistent disease without reexposure to exogenous oocysts (9, 11, 12).

Currently, supportive care with oral or intravenous hydration is provided to animals and humans with cryptosporidiosis because of the lack of effective anticryptosporidial agents (10, 12, 20, 38). Assessment of therapeutic protocols has been slowed by the absence of a convenient animal model of persistent cryptosporidiosis (8–10). Adult immunocompetent laboratory mice are resistant to *C. parvum*, and suckling mice develop asymptomatic self-limiting infections (11, 13, 35). Adult laboratory mice immunosuppressed by cyclophosphamide also develop minimal cryptosporidial infections (35). Rat models of persistent cryptosporidiosis have been reported, but the rats must be exogenously immunosuppressed by the administration of hydrocortisone acetate, cyclophosphamide, or dexamethasone (4, 31, 32). Persistent *C. parvum* infections can be established in immunodeficient nude mice and in BALB/c mice treated with anti-CD4 monoclonal antibodies or a combination of anti-CD4 plus anti-CD8 monoclonal antibodies (21, 37).

The purpose of this study was to characterize a large-animal clinical model of persistent cryptosporidiosis in an immunodeficient host. Here we report clinical signs of severe persistent diarrhea associated with oocyst shedding in foals with severe combined immunodeficiency (SCID foals) that closely resemble the clinical signs observed in immunodeficient patients with cryptosporidiosis. Since SCID foals lack mature T and B lymphocytes and are incapable of antigen-specific immune responses (5, 24), they appear ideally suited for evaluating the pathogenesis of persistent cryptosporidiosis (18, 23, 36).

The *C. parvum* isolate used in these experiments was originally provided by H. Moon and D. Woodmansee (National Animal Disease Center, Ames, Iowa) and is infectious for humans, newborn mice, and calves (22, 34). The isolate was maintained by passage in neonatal calves. The purification of oocysts from calf feces was done as previously described (34), with minor modifications. Potassium dichromate was not used, and feces were processed within 36 h of collection. The extracted oocyst suspension was stored at 4°C for up to 4 weeks in Hanks' balanced salt solution containing 10,000 U of penicillin, 0.01 g of streptomycin, 0.05 mg of amphotericin B, and 500 U of nystatin per ml to prevent microbial overgrowth. Oocysts were treated as previously described with peracetic acid to kill nonoocyst microbial agents prior to inoculation of foals (22, 34). Treated oocysts were resuspended in Hanks' balanced salt solution and used as an inoculum within 1 h.

SCID foals were obtained from a herd of Arabian horses heterozygous for the SCID trait (29, 30). SCID foals were identified and maintained by guidelines previously established for their care (27). The foals received weekly intravenous administration of equine plasma, beginning at 10 to 14 days of age. They also received daily antibiotic therapy beginning at 7 days of age, consisting of 384 mg of trimethoprim and 1,536 mg of sulfamethoxazole (Bactrim; Roche Products, Inc., Nutley, N.J.). SCID foals were infected at 3 weeks of age by oral administration of $10^8$ purified oocysts via a nasogastric tube. At the onset of oocyst shedding, antibiotic therapy was switched from trimethoprim-sulfamethoxazole to a combination of procaine penicillin G (24,000 U/kg of body weight twice daily) and gentamicin (5 mg/kg twice daily) given intramuscularly to prevent development of microbial resistance.

Feces were collected to monitor oocyst shedding via a modified Kinyoun acid-fast stain (17) and to determine the number of oocysts shed per gram of feces. Fecal samples

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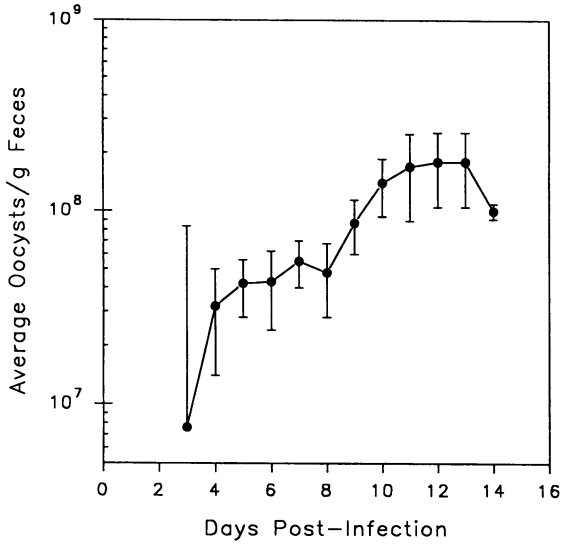


FIG. 1. Daily average shedding of *C. parvum* oocysts (per gram of feces) by SCID foals. Values were calculated from the average daily shedding of each individual foal (up to six samples per foal per day) and expressed as the mean ± standard error. The large error seen on day 3 postinfection is due to the inclusion of zero values for foals that were not shedding oocysts at this time.

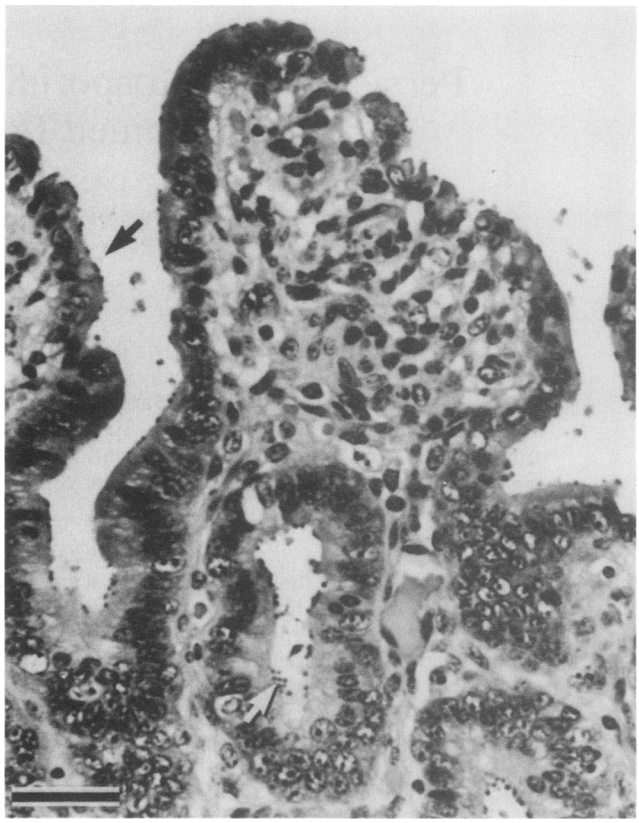

FIG. 2. Photomicrograph of jejunum from a SCID foal orally infected with $10^8$ *C. parvum* oocysts. Note *Cryptosporidium* organisms (arrows) in the superficial mucosa. Bar, 40 μm.

were obtained six times daily (if available) and then stored without fixation at 4°C. Analysis was performed within 3 to 6 weeks. Measured amounts of feces ranging from 0.1 to 1.0 g were suspended in 10 ml of 0.02 M phosphate-buffered saline (PBS) (pH 7.4) containing 0.5 N NaCl. This was further diluted 1/10 in PBS, and oocysts were counted with a hemacytometer by differential interference-contrast microscopy. Fecal samples were also obtained from each foal prior to infection to monitor the presence of other enteropathogens. No etiologic agents that would have accounted for the severe diarrhea, except for *C. parvum*, were identified by electron microscopy, culture, or fecal flotation (17–22).

All five SCID foals developed severe watery diarrhea within 2 to 5 days postexposure and began shedding oocysts within 24 h after the onset of diarrhea. Diarrhea and oocyst shedding persisted until termination of the experiment (Fig. 1). Foals were euthanized either at 14 days postinfection (one foal) or earlier if severely debilitated (four foals). At necropsy, tissue sections from the common bile duct, stomach, duodenum, jejunum and ileum (at 100-cm intervals throughout), base and body of the cecum, right ventral colon, sternal flexure, left ventral colon, pelvic flexure, left dorsal colon, diaphragmatic flexure, right dorsal colon, transverse colon, and descending colon were histologically examined for endogenous stages of *C. parvum*. Infection scores of 0, 1, 2, or 3, representing the relative density of organisms per unit length of intestinal mucosa, were assigned to seven regions of the gastrointestinal tract (stomach, common bile duct, duodenum, jejunum, ileum, cecum, and colon) to determine the infectibility of the individual regions of intestine (34). The score was determined by estimating the percentage of the mucosa infected by *C. parvum* (0, absence of infection; 1, 1 to 33% of the mucosa parasitized; 2, 34 to 66% of the mucosa parasitized; 3, >66% of the mucosa parasitized) (34). The average of the individual infection scores for the common bile duct or each individual region of intestine was the mean infection score for that region. The mean infection scores could range from 0 (indicating the absence of infection) to 3 (indicating maximal infection). The mean infection scores for the duodenum, jejunum, ileum, and biliary system were 1.8 ± 1.1, 1.6 ± 0.8, 2.4 ± 0.5, and 1.4 ± 0.5, respectively. *C. parvum* organisms were not observed in the stomach, cecum, or colon.

Lesions in the jejunum and ileum consisted of mild to moderate villous atrophy and multifocal accumulations of neutrophils and cellular debris within intestinal crypts. Numerous *Cryptosporidium* organisms were present in the superficial intestinal mucosa (Fig. 2). Histologic sections of common bile duct revealed ductal hyperplasia with a surrounding neutrophilic and monocytic inflammatory response and numerous cryptosporidial organisms in lining epithelial cells (Fig. 3).

The data demonstrate that SCID foals develop severe diarrhea and persistent cryptosporidial disease following experimental challenge with isolated *C. parvum* oocysts. Infection of the common bile duct (horses do not have gallbladders) in all SCID foals was observed. This is an important observation, since the biliary system is postulated to serve as a reservoir for *C. parvum* in persistently infected immunodeficient patients (8, 36). The clinical signs and lesions in SCID foals infected with *C. parvum* establish this use of SCID foals as a relevant large-animal clinical model of disease in an immunodeficient host. SCID foals may prove valuable for evaluation of the effects of treatment with colostrum, serum, and monoclonal antibodies with demonstrated efficacy in mouse models (1–3, 14, 15, 28, 33) and for

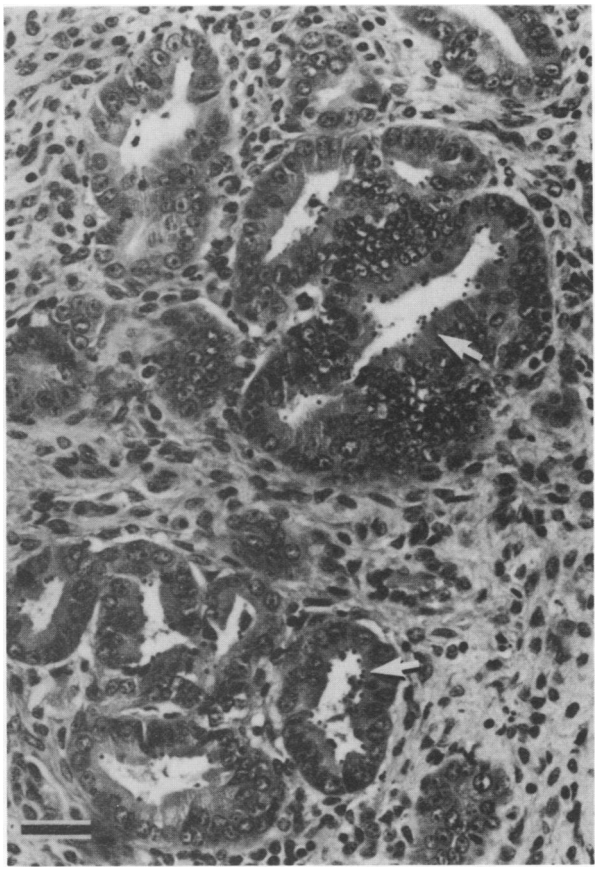

FIG. 3. Photomicrograph of common bile duct from the same SCID foal as for Fig. 2. Note *Cryptosporidium* organisms (arrows) in the superficial mucosa. Bar, 40 μm.

continued study of the pathogenesis of persistent cryptosporidiosis.

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