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Experimental Intrauterine Infection of Adult BALB/c Mice with *Cryptosporidium* sp.

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Inoculation of adult, female BALB/c mice with 2×10^5 bleach-treated *Cryptosporidium* sp. oocysts isolated from calf feces resulted in infection of the uterine mucosa in more than 50% of the animals. *Cryptosporidium* sp. completed the entire life cycle in the uterus, and infectious oocysts were passed into the vagina. Two methods of application were used to establish intrauterine infection. The inoculum was either injected into the uterus after abdominal surgery or intracervically instilled. Mice were susceptible at all phases of the sexual cycle, but the highest infection rates were obtained during estrus and ddiestrus. Parasites were demonstrated as early as 5 days postinfection. Phagocytic cells in the uterine lumen and in the vagina contained *Cryptosporidium* sp. Phagocytosis may be an important immune response and a mechanism of parasitic clearance. These results suggest that *Cryptosporidium* sp. is a potential pathogen of the reproductive tract.

Cryptosporidium sp. is a coccidian parasite which infects a wide variety of mucosal surfaces including those of the tonsils (2), pharynx (31), salivary and esophageal glands (31), esophagus (23), stomach (23), large and small intestine (13, 27), gall bladder (25), pancreatic ducts (18), biliary tract (18), bursa of Fabricius (8, 28), nasal cavity (16), infraorbital sinus (10, 15), trachea (6, 12, 15), bronchi (6), conjunctiva (12), and kidney (9). Known hosts include over 20 different species of mammals, in addition to birds, reptiles, and fish (23, 24, 32). The parasite will also complete its life cycle in cell cultures (4, 33) and embryonated chicken eggs (5). Infection by *Cryptosporidium* sp. can cause diarrhea or respiratory disease in animals and humans, particularly immunosuppressed individuals such as patients suffering from the acquired immune deficiency syndrome (3, 19, 22, 23, 32).

Cryptosporidium infections of the reproductive tract have not previously been demonstrated, but the observation of an infection of a 1-day-old gazella suggests that intrauterine infections of fetuses may occur (7). We report an experimental infection of mouse uterine mucosa with a strain of *Cryptosporidium* isolated from calf feces.

MATERIALS AND METHODS

Inoculum. Feces containing oocysts of *Cryptosporidium* sp. were collected from experimentally infected calves, suspended in potassium dichromate, and stored at 4°C for up to 3 months. Oocysts were isolated on sucrose step gradients, treated with cold, full-strength commercial bleach (5.25% sodium hypochlorite) for 5 min in an ice bath, and washed 3 times in Dulbecco phosphate-buffered saline. Bleached oocysts were adjusted to a concentration of 1×10^6 oocysts per ml in Dulbecco phosphate-buffered saline and held at 4°C for up to 7 days before use.

Experimental animals. Regular estrus was induced in adult (at least 8 weeks of age), female BALB/c mice by exposure to a 12-h light-dark cycle in the presence of separately caged males. To monitor the stage of estrus and to eliminate the possibility that the animals were spontaneously infected with cryptosporidia, vaginal flushes were taken daily beginning 8 days before inoculation. The fluids were air-dried on glass slides, stained with Giemsa and Diff-Quik (Diff-Quik stain set, American Scientific Products, McGaw Park, Ill.), and examined under the light microscope.

Experimental design. Mice in group A were inoculated during estrus and necropsied 3, 5, 6, or 7 days postinoculation (p.i.). Seventeen mice in group A were surgically inoculated. After anesthesia with methoxyfluorane, the uterus was exposed through an abdominal midline incision and each uterine horn was injected with 0.1 ml of the inoculum by using a tuberculin syringe. Nineteen mice in group A were inoculated through the cervix. By using a plastic tube as speculum, a capillary pipette connected to a tuberculin syringe was inserted through the cervical orifice, and 0.1 ml of oocyst inoculum was instilled into each uterine horn.

Animals in group B were divided into four subgroups of eight mice each. Subgroups were inoculated through the cervix with 0.1 ml of the oocyst inoculum per uterine horn during proestrus, estrus, metestrus, or diestrus. Vaginal flushings were taken daily from day 4 p.i. and stained with Giemsa and Diff-Quik. All mice were necropsied at day 7 p.i.

Vaginal flushings from three group B mice, shown to be positive for *Cryptosporidium* sp., were pooled and used to orally inoculate six 7-day-old CF-1 mice. Mice were killed 6 days p.i., and fecal smears were examined for *Cryptosporidium* oocysts as previously described (11). Samples of large and small intestines were examined for the presence of developmental stages of the parasite by routine histological methods.

Postmortem procedures. At necropsy, mucosal scrapings were taken from each uterine horn and stained with Giemsa and a modified acid-fast stain (1, 14). Transverse sections of each uterine horn were prepared for morphologic examination. One section was immediately immersed in cold 3% glutaraldehyde in cacodylate buffer, cut into 2-mm-thick slices, and processed routinely for transmission electron microscopy. Adjacent sections were fixed in 10% neutral buffered formalin and processed for histologic examination. Samples of the miduterine horn of selected animals were opened longitudinally, pinned flat to plates of dental wax,

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TABLE 1. Results of intrauterine infection of group A mice inoculated in estrus

Route of inoculation	No. of positive mice/total no. of mice necropsied at day p.i.			
	3	5	6	7
Intrauterine injection after abdominal surgery	0/4	2/4	4/4	5/5
Intracervical instillation	0/4	3/5	3/5	4/5

and immediately immersed in cold glutaraldehyde. After 1 to 2 h of fixation, tissues were washed in cacodylate buffer, treated with tannic acid (30), dehydrated in alcohols of increasing concentration and, in the final step, freon, critical-point dried, sputter-coated with gold, and viewed with a Cambridge 200 Stereoscan microscope.

RESULTS

Developmental stages of *Cryptosporidium* sp. were found in the uterus of 21 (58%) of the mice in group A (Table 1). Infections were demonstrated by all diagnostic methods in 16 of the mice, whereas the remainder were judged positive by mucosal scrapings only. In group B, 20 (63%) mice were shown to be infected, and parasites were seen in mucosal scrapings more often than in histological sections (Table 2). *Cryptosporidium* oocysts were found in vaginal secretions either free or phagocytized in cells (Fig. 1), beginning on day 4 p.i. and continuing to necropsy on day 7 p.i. A total of 7 of the 20 infected mice in group B were shedding *Cryptosporidium* oocysts at day 4 p.i., 8 were shedding the oocysts at day 5 p.i., 11 did so at day 6 p.i., and 16 did so at day 7 p.i.

Giemsa and acid-fast stained mucosal smears were equally efficient in demonstrating infection, but life-cycle stages could be differentiated only with the Giemsa stain (Fig. 2). Sections of resin- or paraffin-embedded tissue revealed multiple parasites measuring 1 to 5 μm in diameter in the brush border of epithelial cells. The microscopic appearance of the parasites in the uterus was compatible with their appearance in the digestive and respiratory tracts. In some animals the uterine epithelium was regular, with cuboidal to columnar cells and no or very mild inflammatory response in the lamina propria. In others, the mucosal surface had multiple foci of epithelial necrosis, epithelial cells were vacuolated, parasitized cells were sloughing into the lumen, and fusions of mucosal folds were common (Fig. 3). In these cases, the endometrium and myometrium were moderately to severely infiltrated by leukocytes, lymphocytes, eosinophils, and macrophages, and a few mast cells. Parasites were present at the luminal aspect of deeper mucosal glands which were dilated, contained cellular debris, were lined with flat to cuboidal epithelial cells, and had frequent mitotic figures. A prominent increase in the number of intraepithelial leukocytes was observed. Some of these cells contained eosinophilic granules, but were larger than eosinophilic granulocytes and were without a lobulated nucleus.

TABLE 2. Results of intracervical infection of group B mice inoculated at different stages of sexual cycle

State of sexual cycle when inoculated	No. of positive mice/total no. of mice necropsied at day 7 p.i. as judged by:	
	Mucosal scrapings	Histologic examination
Proestrus	4/8	3/8
Estrus	7/8	4/8
Metestrus	3/8	2/8
Diestrus	6/8	4/8

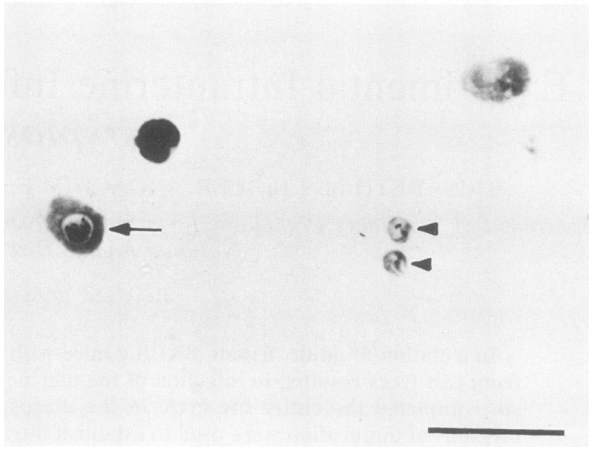

FIG. 1. Phagocytized (arrow) and free (arrowhead) cryptosporidia in Giemsa-stained vaginal flushing, day 7 p.i. Bar = 20 μm.

Trophozoites, schizonts, micro- and macrogametes, and oocysts of *Cryptosporidium* sp. were identified by transmission electron microscopy (Fig. 4 and 5). Microvilli were displaced at the sites of attachment, and the typical attachment zone (26) was seen (Fig. 5). Phagocytic cells in the uterine lumen contained degenerate remnants of parasites (Fig. 6).

Scanning electron microscopy revealed parasites on the mucosal surface. The parasites could be distinguished from mucus droplets by characteristic folds in the parasitophorous envelope. Merozoites within schizonts were seen when the parasitophorous envelope ruptured during processing (Fig. 7).

Cryptosporidium oocysts were found in the feces of all six mice inoculated with vaginal flushings. Large numbers of developing parasites were observed in the cecum and colon

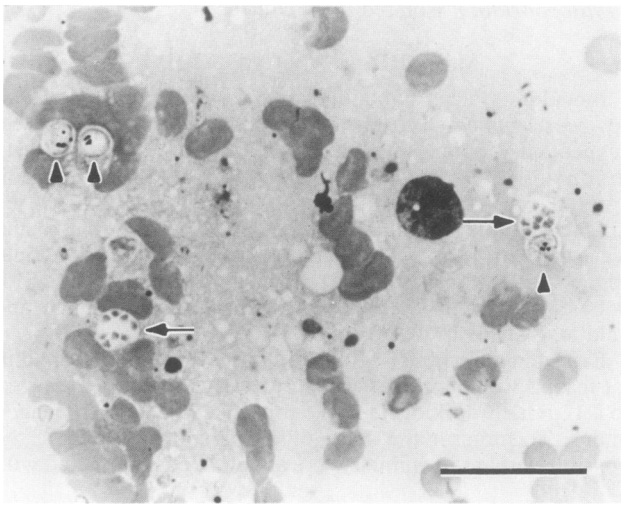

FIG. 2. Different stages of life cycle in Giemsa-stained scrapings of uterine mucosa, day 7 p.i. Arrow, Schizonts; arrowhead, macrogametes. Bar = 20 μm.

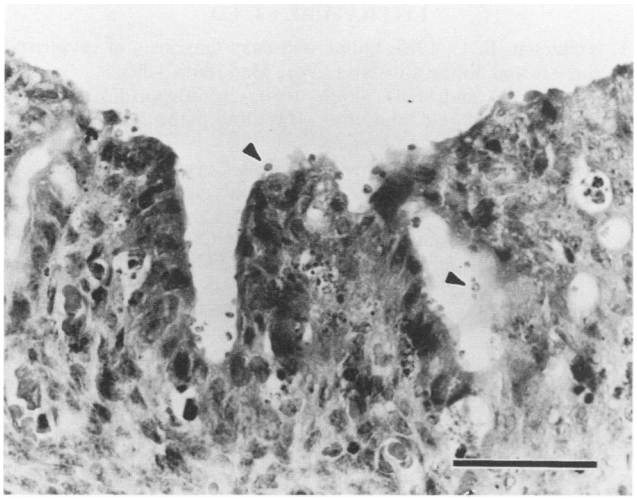

FIG. 3. *Cryptosporidium* sp. (arrowhead) detectable on the surface of uterine epithelium, day 6 p.i. Note focal necrosis and mild inflammatory response. Bar = 50 μm.

of these animals, but few were seen in the small intestine. None of the mice developed diarrhea.

DISCUSSION

Inoculation of bleach-treated *Cryptosporidium* oocysts resulted in infection of the uterine mucosa in more than 50% of the experimental mice. The parasites completed their entire life cycle in the uterus, and infectious oocysts were passed into the vagina. To our knowledge, this is the first report of infections of the reproductive tract by this parasite. We assume that our failure to demonstrate infections 3 days p.i. was because the numbers of parasites developing at that time were below the sensitivity of our diagnostic methods. Numbers of parasites clearly increased on days 5 through 7.

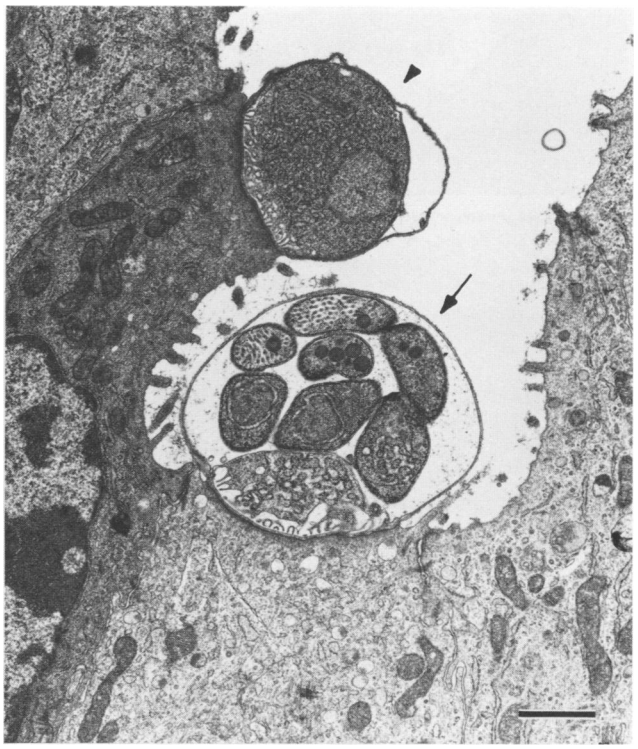

FIG. 5. Uterine epithelial cells with trophozoite (arrowhead) and schizont (arrow). Bar = 1 μm.

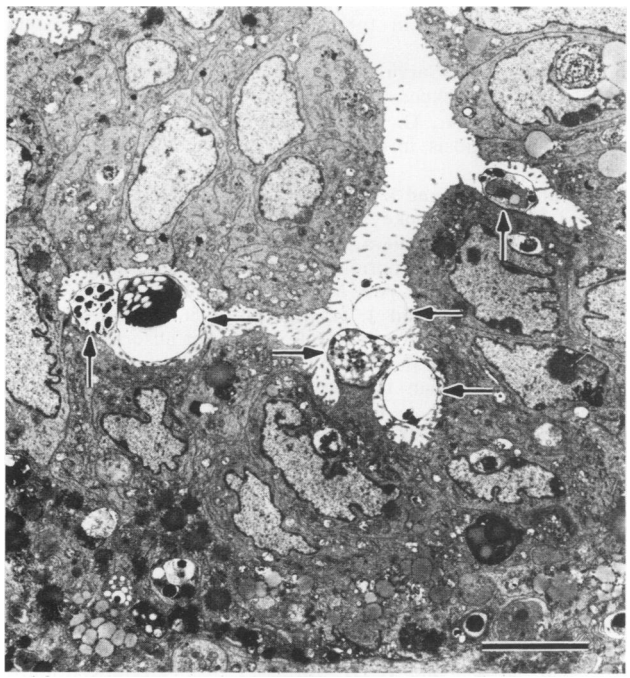

FIG. 4. Different stages of life cycle of *Crytosporidium* sp. within uterine epithelial cells: microgametocyte (↑), macrogametocyte (→), and oocysts (←). Bar = 5 μm.

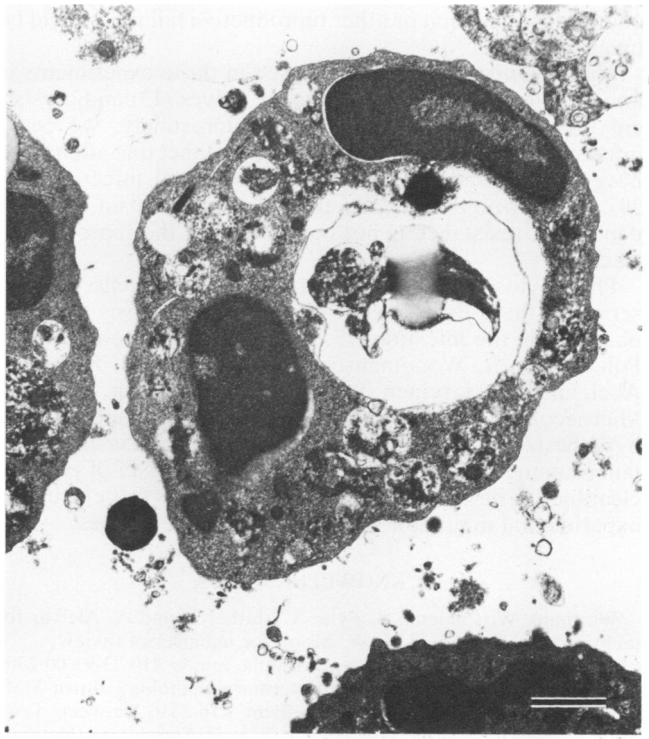

FIG. 6. Phagocytized oocyst in uterine lumen. Bar = 1 μm.

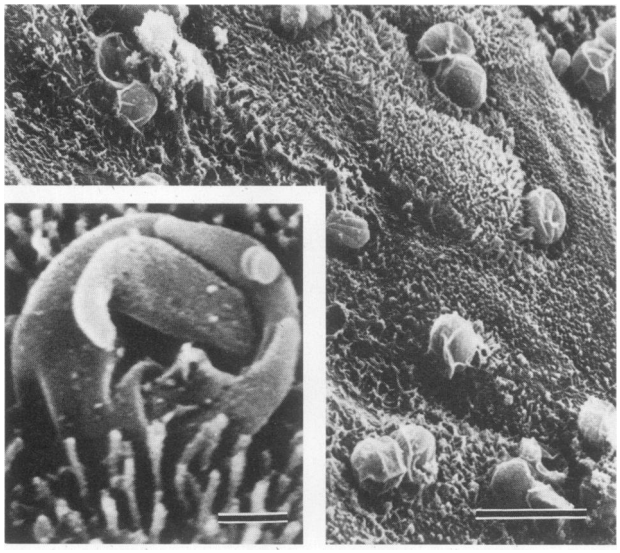

FIG. 7. Numerous parasites embedded in microvilli of uterine epithelial cells. Bar = 10 μm. Inset: ruptured parasite with merozoite. Bar = 1 μm.

Although infections were established at all phases of the sexual cycle, mice appeared to be most susceptible to infection during estrus and diestrus.

Since the uterus is capable of supporting growth of the parasites and endometritis was demonstrated in some cases, cryptosporidia should be considered as potential pathogens of the reproductive tract. Persistence of cryptosporidia in placental membranes or fluids could result in animals being infected at or before birth, which would explain the observation of an infection at the first day of life (7). The possibility that cryptosporidiosis of the reproductive tract could cause abortion or other reproductive failures should be investigated.

The *Cryptosporidium* isolate used in these experiments is known to cause intestinal disease in calves (13) and persistent infections in nude mice (11). Interestingly, we could infect the uterus of adult mice despite the fact that adult mice have a decreased susceptibility to intestinal infection (11, 29). This would indicate that the mechanism of age-dependent resistance is not operational in the reproductive tract.

Phagocytosis of parasites by inflammatory cells was observed in these experiments. Similar phagocytosis has been observed in the intestine (17, 20, 21; H. W. Moon, J. F. L. Pohlenz, D. B. Woodmansee, G. N. Woode, J. Heine, F. Abel, and J. A. Jarvinen, Proc. 10th Intern. Symp. Intestinal Microecology 1985, in press) and respiratory tract (19). We hypothesize that the phagocytosis of parasites is an important immune response and may be a mechanism of parasite clearance. The uterine infection may prove to be a useful experimental model for the study of such responses.

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