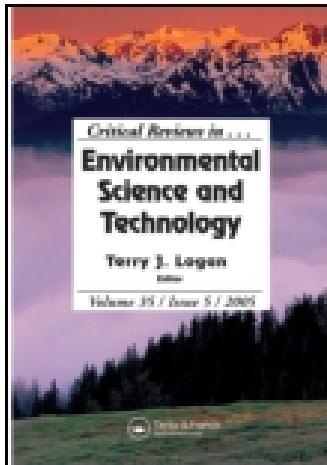


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Cryptosporidium: Its biology and potential for environmental transmission

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CRYPTOSPORIDIUM: ITS BIOLOGY AND POTENTIAL FOR ENVIRONMENTAL TRANSMISSION

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I. INTRODUCTION

Protozoans of the genus *Cryptosporidium* are small (2 to 6 μm , depending on stage in life cycle) coccidian parasites that infect the mucosal epithelium of a variety of animals, including man.¹⁻³ Until recently, infections with *Cryptosporidium* spp. were considered rare in animals, and in man they were thought to be the result of an opportunistic pathogen of immune-deficient persons outside its normal host range.⁴⁻⁸ Our concept of *Cryptosporidium* has changed within the past 4 years to that of a significant and widespread cause of gastrointestinal illness in man and several animal species, especially calves and lambs.¹⁻³

In immunocompetent human beings, at least one species of this protozoan genus may produce mild to severe diarrhea lasting from several days to more than 1 month.^{9,10} In immune-deficient persons, especially those with the acquired immune deficiency syndrome (AIDS), cryptosporidiosis usually presents a prolonged, life-threatening cholera-like illness.^{9,11} Diarrhea in many of these patients becomes irreversible and the fluid loss is excessive with 3 to 6 ℓ /day being common and as much as 17 ℓ of watery feces per day having been reported.¹² Recent reports of respiratory¹³ and biliary¹⁴ cryptosporidiosis demonstrate that this protozoan is not confined to the gastrointestinal tract of immune-deficient persons. To date, no effective therapy has been found;¹² thus, the finding of cryptosporidiosis in the immune-deficient host often carries an ominous prognosis.

The recent recognition of *Cryptosporidium* as a widespread pathogen of man and animals has spawned over 200 articles within the past 4 years. No attempt has been made in this communication to discuss all of the data contained in these publications, however, it does address much of our present understanding of the biology of *Cryptosporidium* spp. and the potential for environmental transmission of cryptosporidiosis. The initial sections of this paper present the taxonomy, life cycle, and epidemiology of the parasite. This is followed by a discussion of the diseases *Cryptosporidium* spp. produce in animals and man, and how they are diagnosed and treated.

II. TAXONOMY

Organisms of the genus *Cryptosporidium* are small protozoans presently assigned to the family Cryptosporidiidae, suborder Eimeriorina, order Eucoccidiorida, subclass Coccidiina, phylum Apicomplexa.¹⁵ Other true coccidia (Eimeriorina) known to infect man include *Toxoplasma gondii*, *Isospora belli*, and *Sarcocystis* spp. Clarke, in 1895, may have been the first to observe a species of *Cryptosporidium*.¹⁶ It is possible that the minute bodies he called "swarm spores" lying free upon the gastric epithelium of mice were actually the

motile merozoites of *Cryptosporidium muris*, the type species named and described 15 years later by Tyzzer.^{17,18} During the ensuing 75 years, approximately 18 additional species of *Cryptosporidium* were named primarily on the assumption that they were host specific (see Reference 15 for a list of named species).

Cross-transmission studies (see Reference 15 for a list of these studies) demonstrating little or no host specificity for isolates of this coccidian obtained from calves, lambs, and man prompted several investigators to consider *Cryptosporidium* as a single-species genus.^{2,19} More recently, Levine¹⁵ consolidated the 19 named organisms into 4 species, one each for those infecting fish (*C. nasorum*), reptiles (*C. crotali*), birds (*C. meleagridis*), and mammals (*C. muris*). The validity of this consolidation should be questioned for several reasons. The original description of *C. crotali*²⁰ was based on oocysts in the feces of a snake. However, from the standpoint of morphology, these resistant stages were probably sporocysts of *Sarcocystis* sp.^{21,22} Also, oocysts indistinguishable from *C. muris* and *C. parvum*, both originally described from mice by Tyzzer,^{18,23} have been recovered from calves. The oocysts (7.4 × 5.6 µm) and sporozoites (11.1 × 1 µm *in situ*) of *C. muris* are easily distinguished from the oocysts (5 × 4.5 µm) and sporozoites (4.9 × 1.2 µm *in situ*) of *C. parvum*.²⁴ *C. muris* infects the gastric glands of the abomasum of calves, cows, and steers^{24a} and has not been associated with diarrheal illness, even in heavily infected animals passing large numbers of oocysts.²⁴ In contrast, *C. parvum* infects the intestinal mucosa of calves and is known to produce severe diarrhea in this and other mammalian hosts. On the basis of oocyst morphology it is the latter species, not *C. muris*, that is associated with all of the previously well-documented cases of cryptosporidiosis in mammals.²⁴ Thus, at the present time, the species producing clinical illness in man and other mammals should be referred to as *C. parvum*, or as *Cryptosporidium* sp. if there is not enough morphologic and/or life cycle data to relate it to Tyzzer's description.²³ This conservative approach has been adopted in the present communication. However, the author is aware that careful studies of the proposed differences in host specificity, sites of infection, and pathogenicity among mammalian isolates¹⁻³ may result in the validation of additional species. Along with this conservative approach, the designation of a particular parasite as an isolate rather than a strain is preferable.

III. LIFE CYCLE AND PARASITE MORPHOLOGY

Just 3 years after the 1907 report¹⁷ of *C. muris* in the gastric glands of laboratory mice, Tyzzer presented much of its life cycle in amazing detail.¹⁸ The most salient features of this classic investigation, as well as a subsequent paper²³ of *C. parvum* infecting the small intestine of mice, included descriptions of a single generation of schizogony, micro- and macrogametogony, fertilization, oocyst wall formation, sporogony, excystation, and experimental transmission by oocysts. Approximately 60 years later, Tyzzer's statement,¹⁸ "It is remarkable that an organism so minute should show so great structural variation and present modes of propagation analogous to those found in organisms very much larger", was vividly confirmed by an ultrastructural study of *C. wrairi* in the guinea pig.²⁵ Vetterling et al.^{22,25} did not find oocysts in the feces of infected guinea pigs and suggested that the oocysts described by Tyzzer were, in reality, second generation meronts containing four merozoites. This discrepancy was partially resolved when Pholenz et al.,²⁶ studying naturally infected calves, and Iseki,²⁷ investigating naturally infected cats, found sporulated oocysts within enterocytes and free in the feces. More recently, Angus et al.²⁸ demonstrated that their isolate of *Cryptosporidium* sp. produced oocysts in experimentally infected guinea pigs. They proposed that the extended, incomplete endogenous development of *C. wrari* reported by Vetterling et al.²² could have been the behavior of an aberrant strain of the organism in a species which is perhaps not the natural host. Although the existence of oocysts was no longer questioned, the just-mentioned authors could not agree as to the number of generations of meronts.

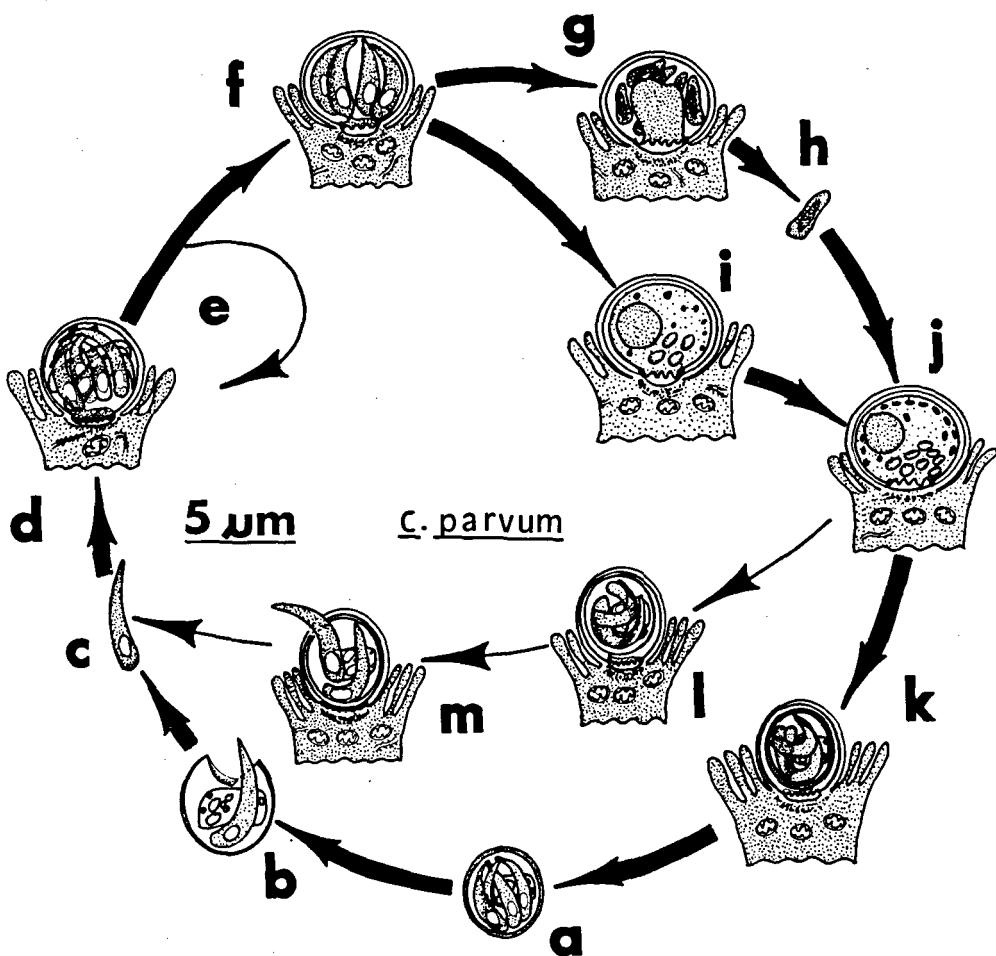


FIGURE 1. The life cycle of human and calf isolates of *Cryptosporidium parvum* in experimentally infected mice. (a) Sporulated oocyst in feces; (b) excystation in intestine; (c) free sporozoite in intestine prior to penetration into the microvillous region of an ileal enterocyte; (d) type I meront (6 or 8 merozoites); (e) recycling of type I merozoite; (f) type II meront (4 merozoites); (g) microgamont, with approximately 16 microgametes; (h) microgamete fertilizes macrogamete (i) to form zygote (j). Approximately 80% of the zygotes form thick-walled oocysts (k) which sporulate within the host cell. Almost all thick-walled oocysts pass unaltered in the feces and are the resistant forms that transmit the infection to another host. About 20% of the zygotes do not form an oocyst wall; their sporozoites are surrounded only by a unit membrane (l). Sporozoites within autoinfective, thin-walled oocysts (l) are released into the intestinal lumen (m) and reinitiate the endogenous cycle (at c).

The investigations discussed above, along with recent studies of the development of calf and human isolates of *C. parvum* in mice,^{3,29} chicken embryos³⁰ and cell culture,³¹ and of an avian isolate of *Cryptosporidium* sp. in chickens³² have revealed that, like the other true coccidia (Eimeriorina), the life cycle of *Cryptosporidium* sp. can be divided into six major developmental events: excystation (release of infective sporozoites from oocysts), merogony (asexual replication), gametogony (gamete formation), fertilization, oocyst wall formation, and sporogony (sporozoite formation). Since the life cycles of *C. parvum* and the chicken isolate of *Cryptosporidium* sp. differ primarily in the number of types of meronts, the remaining discussion will concentrate on the former species. The life cycle of *C. parvum* is illustrated in Figure 1 and photomicrographs and transmission electron micrographs of developmental stages of this parasite in the mucosal epithelium of the ileum of experimentally infected mice are shown in Figure 2 (2 to 26) and Figures 5 (29 to 39) and 6 (40 to 49),

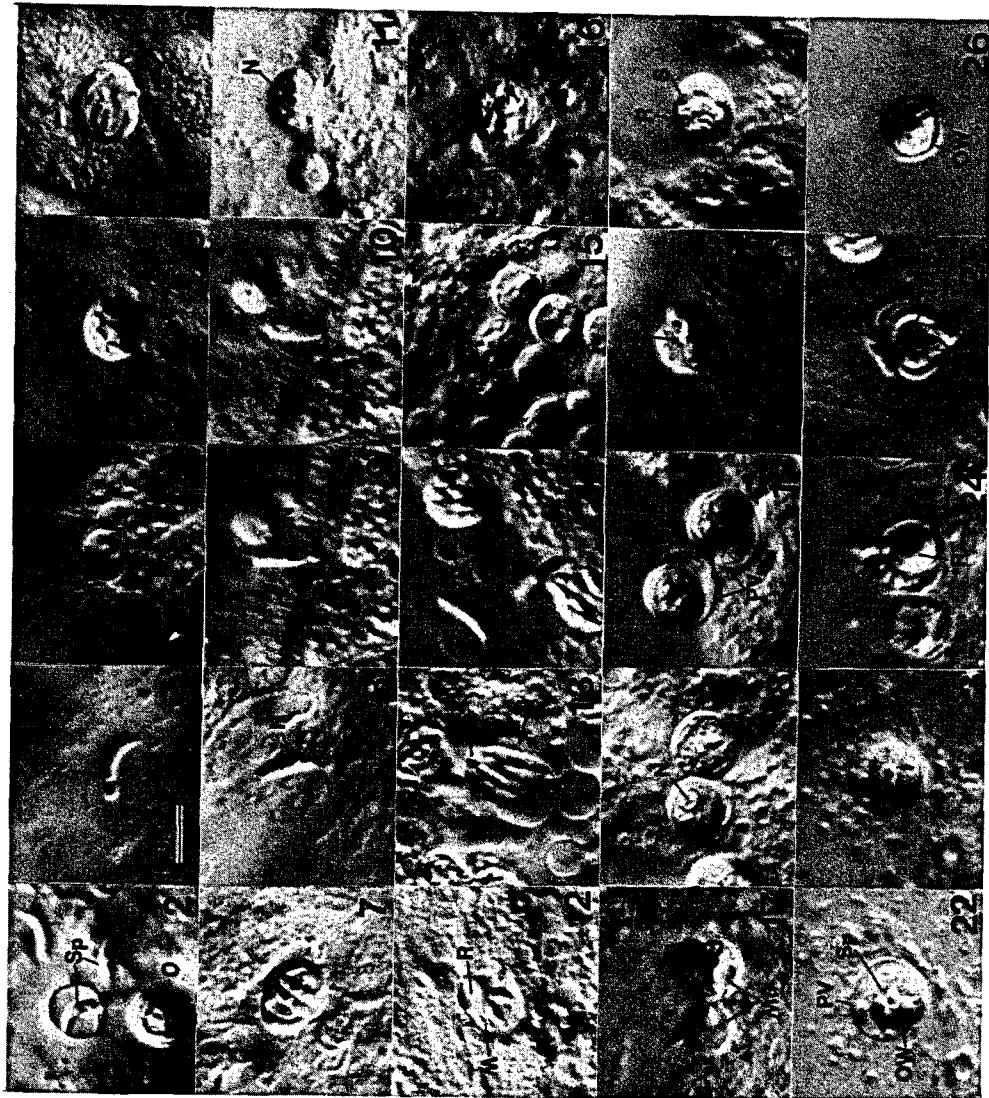


FIGURE 2 (2-26).

FIGURE 2 (2-26). Nomarski interference contrast photomicrographs of developmental stages of *C. parvum* in mucosal scrapings of the small intestines of experimentally infected, suckling mice. (2) Sporozoites (Sp) free and excysting from opening (arrow) in oocyst wall and an intact oocyst (O), 8 hr postinoculation. (3) Free sporozoite showing posterior location of nucleus (N). (4) Uninucleate meront surrounded by hypertrophied microvilli (MV). (5) Immature type I meront with six of the nuclei (N) in focus. (6) Mature type I meront with eight merozoites. (7) Mature type I meront with six merozoites. All merozoites are in focus because this meront has flattened with coverslip pressure. (8) Merozoite attached to host cell. Arrow points to site of host-parasite attachment. Note position of nucleus (N). (9) Merozoite penetrating into microvillous border of an enterocyte. The nucleus (N) of the merozoite is located near the middle of the cell. The anterior of the parasite has formed an invagination of the microvillous border of the host cell (arrow). (10) Merozoite in (8) photographed several minutes later, showing a subsequent phase of penetration into the microvillous border. The parasite nucleus (N) has migrated posteriorly and there is a prominent band of material (arrow) in the host cell cytoplasm just beneath the parasite. (11) Immature type II meront with four nuclei (N) near the periphery. The arrow points to the attachment of the meront to the base of the parasitophorous vacuoles. (12) Type II meront with four merozoites (M) budding from the residuum (R). (13) Mature type II meront with four merozoites compressed flat by coverslip pressure. Note the nuclei (N) of the merozoites and the small residuum (R). (14) Mature type II meronts showing arrangement of merozoites like the segments of an orange. Nuclei (N) of the merozoites are aligned in the center of the meront. (15) Microgamont with microgametes (Mi) beginning to bud from the surface. (16) Microgamont with microgametes (Mi) free in the parasitophorous vacuoles and a large residuum (R) attached to the base of the parasitophorous vacuoles. (17) Two macrogametes (Ma) each with an attached microgamete. Arrow points to microgamete in focus. (18) Macrogamete containing a large eccentric nucleus (N) and a microgamete (arrow). (19) Two unsporulated oocysts with thick-walls (OW). Both oocysts are within prominent parasitophorous vacuoles (PV). (20) Early stage of sporogony showing separation of a granular oocyst residuum (R) from the sporont. (21) Oocyst with oocyst residuum (R) and four sporoblasts (S). (22) Sporulated oocyst within a PV. Note the thick oocyst wall (OW) and four sporozoites, one of which is shown in a longitudinal optical section (Sp). (23) Different optical section of oocyst in (21) showing the other three sporozoites (arrows). (24) One of only a few thick-walled oocysts observed releasing sporozoites (arrows). Note the large oocyst residuum (R) and the posterior of an escaping sporozoite within the oocyst. (25) One of many oocysts with a thin oocyst wall (TOW) or membrane which readily ruptured, releasing sporozoites. Note the characteristic posterior position of the sporozoite nucleus (arrow) and the oocyst residuum (R). (26) Thick-walled oocyst free in intestinal lumen. Note the prominent thick oocyst wall (OW). (Adapted from Reference 29.)

respectively. Additional details of the life cycle of *C. parvum* are presented below, with an emphasis on those events that have been confused or misinterpreted in the literature.

A. Excystation

A key event in the life cycle of any coccidian is excystation, i.e., the release of infective sporozoites from environmentally resistant oocysts. Excystation usually occurs in the lumen of the gastrointestinal tract following ingestion of oocysts by a suitable host. In most coccidian species examined to date, excystation may also be triggered *in vitro* by exposing oocysts to conditions that simulate the gastrointestinal environment of the host (i.e., reducing conditions, CO₂, host body temperature, trypsin, and bile salts). Much of our understanding of this process is based upon *in vitro* excystation studies of coccidian species belonging to the families Eimeriidae³³⁻³⁵ and Sarcocystidae³⁶⁻³⁸ whose oocysts possess sporocysts, each containing infective sporozoites. Such investigations have revealed that there are two major steps in excystation. The first is alteration of oocyst wall permeability, allowing influx of proteolytic enzymes and bile salts. This can be triggered by exposure to host body temperature and CO₂ and is often accomplished by a peeling away or dissolving of the micropyle, a thinning of the oocyst wall at one pole. The first step can also be accomplished mechanically by grinding off the oocyst wall, releasing sporocysts. The second step involves release of sporozoites from sporocysts by the action of pancreatic enzymes and/or bile salts. Sporozoites of eimeriid coccidia such as *Eimeria* spp. and *Isospora* spp. escape through an opening in one pole of the sporocysts that is formed by degradation of a plug, the Stieda body. It is believed that trypsin degrades the Stieda body and that bile salts stimulate sporozoite motility. Species of coccidia assigned to the family Sarcocystidae have sporocysts whose walls are composed of four plates joined by sutures. Trypsin and/or bile salts cause dissolution of the sutures and sporozoites escape between the collapsed plates.

The generic name *Cryptosporidium* was chosen by Tyzzer¹⁸ because the oocysts of *C. muris* contained no sporocysts, i.e., the sporozoites are naked within the oocyst wall. Oocysts of *C. parvum* release their sporozoites within the small intestine of experimentally inoculated mice^{3,29} and *in vitro* when incubated in solutions containing a bile salt and trypsin.³⁹ Table 1 demonstrates that, at mammalian body temperature, sodium taurocholate plus trypsin are the major factors stimulating excystation of *C. parvum* sporozoites *in vitro*. These data also demonstrate that, unlike oocysts of many *Eimeria* and *Isospora* spp., preincubation of *C. parvum* oocysts in a reducing solution and 50% CO₂ to alter oocyst wall permeability is not necessary for excystation to occur. Similar findings have been reported by others.³⁹ In a recent paper, Fayer and Leek⁴⁰ reported that excystation of sporozoites of *C. parvum* readily occurs by incubating oocysts in water at 37°C and that exposure to reducing conditions followed by bile salts and pancreatic enzymes was not necessary. Data presented in Table 2 indicate that these authors may not have been measuring excystation, but rather the structural and functional integrity of the oocyst wall suture (see below) of improperly stored oocysts. Storage of partially cleaned oocysts in tap water results in the growth of bacteria and fungi which release, among other things, a variety of proteolytic enzymes. With time, these microbial enzymes may degrade the oocyst wall suture to a point that it no longer prevents rapid influx of water when these forms are placed in a hypoosmotic environment. This would explain the authors' observations of a higher percentage of excysted (= osmotically ruptured) oocysts incubated in water compared to those incubated in saline. In addition, sporozoites released from oocysts incubated in water do not remain viable (Table 2), they become short and swollen prior to rupturing.

The oocyst walls of *C. parvum* from calves and man,²⁹ *C. muris* from calves²⁴ and mice,¹⁸ and *Cryptosporidium* sp. from chickens³² have a single suture at one pole which spans one third to one half the circumference of the oocysts. During excystation, the suture partially dissolves and the margins of the oocyst wall adjacent to the suture separate, forming a cleft-

Table 1
**EFFECTS OF PREINCUBATION IN REDUCING ATMOSPHERE
 AND OF SODIUM TAUROCHOLATE (BILE SALT) AND
 TRYPSIN ON EXCYSTATION OF OOCYSTS OF A HUMAN
 ISOLATE (REFERENCE 9, CASE 14) OF *C. PARVUM*^a**

Treatment group	Preincubation ^b Cys-HCl + CO ₂	Incubation ^c			Excystation ^d (%)
		Bile salt	Trypsin	PBS only	
A	+	+	+	0	73
B	0	+	+	0	64
C	0	0	+	0	34
D	0	+	0	0	29
E	0	0	0	+	2.5

^a Oocysts had been stored in 2.5% K₂Cr₂O₇ at 4°C for 3 months prior to excystation studies.

^b Preincubation of oocysts was in 0.02 M cysteine-HCl in normal saline in an atmosphere of 50% CO₂ for 16 hr at 37°C.

^c Incubation was in 0.75% (w/v) sodium taurocholate (bile salt) and/or 0.25% (w/v) trypsin in phosphate buffered saline (PBS, pH 7.4) or in PBS only at 37°C for 90 min in an atmosphere of air.

^d Percent of excystation was determined microscopically and is expressed as the number of empty oocysts (excysted) observed in two separate counts of 100 total (excysted plus intact) oocysts.

like opening through which the sporozoites escape (Figures 2 [2], 3 [27], and 4 [28]). Similar observations have been reported in a recent scanning electron microscopic study of oocysts and excysting sporozoites of a calf isolate of *C. parvum*.⁴¹

Based on the chemical/physical stimuli triggering excystation of *Cryptosporidium* oocysts and on the presence of a suture in the oocyst wall, one may conclude that these resistant forms are similar to sporocysts of sarcocystid coccidia. These observations prompted Reduker et al.⁴¹ to hypothesize that *Cryptosporidium*, like *Sarcocystis*, may be passed from the host as sporocysts. Others^{42,43} have proposed that these resistant forms should be called sporocysts because, when passed from the host, they contain naked sporozoites. Our studies on the development of the oocyst wall²⁹ (reviewed below) and our observations of sporulation within host cells^{24,31,32} demonstrate that it is not technically correct to refer to these forms as sporocysts. If these resistant forms are true sporocysts, the bilayered oocyst wall must disappear soon after it is formed and a similar-appearing sporocyst wall must be constructed. Since these events do not occur during oocyst wall formation and sporogony,²⁹ the resistant forms of *Cryptosporidium* passed from the host should be referred to as oocysts.

B. Merogony

Merogony, the asexual replicative phase culminating in the formation and release of invasive merozoites, begins soon after sporozoites (Figures 2 [3] and 3 [27]) of *Cryptosporidium* spp. take up residence in the microvillous border of host epithelial cells. Sporozoite and merozoite invasion are remarkably similar. Invasion of host enterocytes by type I merozoites of *C. parvum* is shown in Figures 2 (8 to 10) and 5 (32). After becoming surrounded by a host cell-derived parasitophorous vacuole membrane, these invasive forms round-up into uninucleate meronts (Figures 2 [4] and 5 [33]). Uninucleate meronts and subsequent developmental stages assume an intracellular, extracytoplasmic location within the host cell; intracellular because each parasite is surrounded by a host cell-derived parasitophorous vacuole membrane, and extracytoplasmic because all developmental stages are

Table 2

**IN VITRO EXCYSTATION OF HUMAN ISOLATE (REFERENCE 9, CASE 14)
OF *C. PARVUM* AT DIFFERENT TIMES AFTER STORAGE AT 4°C IN 2.5%
(W/V) K₂Cr₂O₇, TAP WATER WITH ANTIBIOTICS, OR TAP WATER**

Storage medium	Time of storage (days)	Preincubation (H ₂ O, 4 hr, 37°C)		Incubation (EF, 1 hr, 37°C)*	
		Excystation ^b (%)	Sporozoites/ no. oocysts ^c	Excystation ^b (%)	Sporozoites/ no. oocysts ^d
K ₂ Cr ₂ O ₇ 2.5%	1	1.3	1.5	92.5	136.0
	14	0.8	0.5	90.3	182.5
	28	2.5	0.5	94.8	196.3
	56	6.3	5.5	93.0	185.8
	100	10.8	6.0	87.3	101.8
	140	13.0	5.8	94.0	81.0
H ₂ O + antibiotics ^e	1	2.3	1.0	82.5	122.3
	14	4.0	2.8	92.0	173.3
	28	1.3	0.5	92.5	183.3
	56	7.5	12.8	82.3	133.3
	100	5.0	8.5	85.8	88.0
	140	9.5	4.8	78.0	51.5
H ₂ O	1	2.0	0.5	86.8	117.5
	14	4.5	3.8	91.3	144.8
	28	3.8	2.5	82.3	178.3
	56	5.5	7.0	86.5	179.3
	100	14.8	6.3	79.8	86.3
	140	9.5	12.8	79.5	21.3

* Incubation was in excystation fluid (EF) comprised of 0.75% (w/v) sodium taurocholate plus 0.25% (w/v) trypsin in Hank's balanced salt solution. Incubation for 1 hr at 37°C occurred following the 4 hr preincubation in water at 37°C.

^b Excystation (%) is expressed as the mean percentage of excysted (empty) oocysts observed microscopically in four samples of 100 total (excysted plus intact) oocysts obtained from each sampling period and time.

^c Sporozoites per 100 oocysts is the mean number of sporozoites counted while observing 100 total oocysts in four separate samples. Sporozoites released during preincubation were short, swollen, nonmotile, and appeared dead.

^d Sporozoites per 100 oocysts are mean values determined as described in footnote c. Most sporozoites released during incubation in EF were long, slender, and motile.

^e Antibiotics added to water were penicillin (500 IU/mℓ), streptomycin (500 µg/mℓ), and amphotericin B (125 µg/mℓ).

confined to the microvillous region of the host cell.^{29-31,42,43} A network of microfilaments, similar to those comprising the terminal web of the host cell, forms beneath the parasite and may play a role in preventing its relocation into the perinuclear region, the area occupied by intracellular forms of most coccidian species.

Following nuclear divisions, meront nuclei migrate to the periphery and merozoites begin to form by a budding process termed ectomerogony (Figures 2 [5, 11, and 12] and 5 [35 to 37]). Fully formed merozoites have an apical complex composed of rhoptries, micronemes, a conoid, and preconoidal rings, which are organelles commonly found in invasive forms of other coccidian species (Figure 5 [38 and 39]). Merozoites and sporozoites of *C. parvum* lack subpellicular microtubules and conoidal rings, the proposed microtubule organizing center for these subpellicular supportive elements that are so prominent in invasive forms of other coccidia. Type I merozoites of *C. parvum* may recycle or they may form type II meronts whose merozoites do not recycle but develop directly into gamonts (sexual stages).²⁹⁻³¹

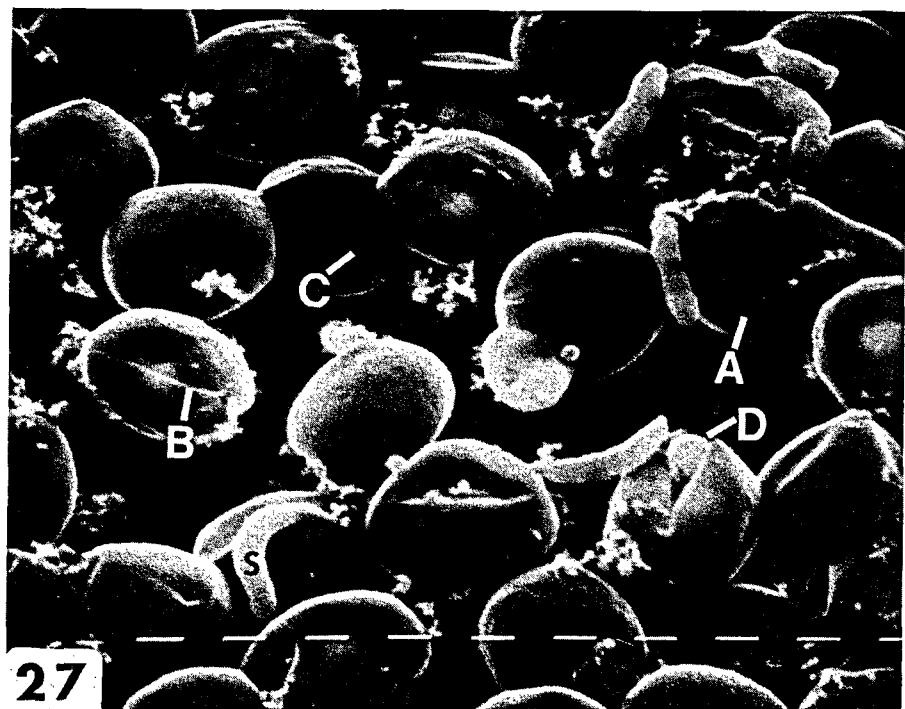


FIGURE 3 (27). Scanning electron micrograph of oocysts of a human isolate of *C. parvum* (Reference 7, case 14). Oocysts were cleaned and concentrated from the feces of an experimentally infected calf, preincubated in H₂O at 37°C for 2 hr, and then incubated in 0.75% sodium taurocholate plus 0.25% trypsin (w/v) in PBS (pH 7.4) for 40 min at 37°C. Parasites were then deposited on nucleopore filters, fixed with 3% w/v glutaraldehyde in phosphate buffer (pH 7.4), and processed for scanning electron microscopy. The oocyst wall suture begins to dissolve, forming an indentation (A). Further dissolution results in the formation of a cleft (B) which widens (C). Sporozoites (S) escape through the widened cleft (D). Lines at the bottom of the micrograph represent 1 μm.

C. Gametogony and Fertilization

The majority of type II merozoites of *C. parvum* that enter host cells develop into macrogametes, whereas the remainder form microgamonts which produce microgametes, the male counterpart of the sexual cycle. Each microgamont produces approximately 16 non-flagellated, bullet-shaped microgametes (Figures 2 [15 and 16] and 6 [41]) which attach to (Figure 2 [17]) and penetrate (Figure 2 [18]) macrogametes. Although observation of fusion of macro- and micronuclei has not been reported for any species of *Cryptosporidium*, it is assumed that fertilization occurs prior to formation of the oocyst wall. Macrogametes are characterized by the presence of polysaccharide storage granules (amylopectin) in the basilar region of the cell (Figure 6 [42]) and two types of wall-forming bodies near the periphery (Figure 6 [43]).

D. Oocyst Wall Formation and Sporogony

By definition, macrogametes become oocysts when one or more layers of the oocyst wall can be recognized. Figure 6 (44 to 46) shows the bilayered oocyst wall of *C. parvum* being formed. The oocyst wall is composed of two layers and three unit membranes. Apparently, type I wall-forming bodies fuse with membrane two and empty their contents between membranes one and two to form the outer layer of the oocyst wall. Similarly, it is believed that type II wall-forming bodies fuse with membrane three and release their contents between

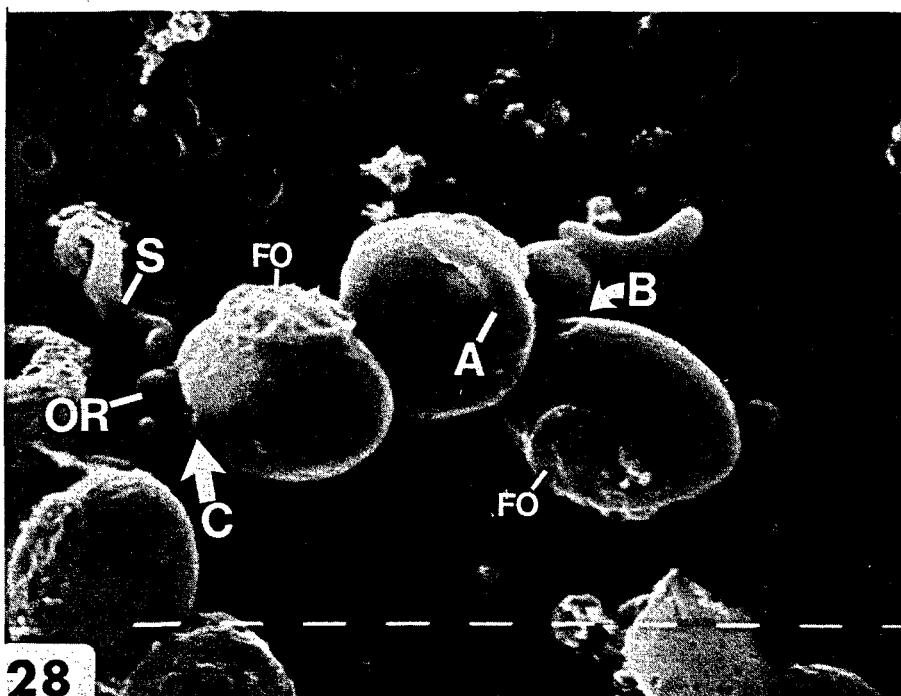


FIGURE 4 (28). Scanning electron micrograph (SEM) of oocysts of a *Cryptosporidium* sp. of chickens obtained from experimentally infected chicken embryos and subjected to in vitro excystation and SEM as described in the legend of Figure 3 (27). Dissolution of the suture forms a cleft (A) which widens (B) allowing the release (C) of sporozoites (S) and often the oocyst residuum (OR). Note the feeder organelle (FO) is still attached to the oocyst wall. Lines represent 1 μ m.

membranes two and three to form the inner layer of the oocyst wall. A section through the suture in the inner oocyst wall is shown in Figure 6 (46). During excystation, this suture dissolves and the oocyst wall collapses inwardly to form an opening through which the sporozoites exit (Figures 3 [27] and 4 [28]).

The process of oocyst wall-formation occurs in approximately 80% of the oocysts of *C. parvum* and is responsible for the production of thick-walled oocysts.²⁹ The thick-walled oocysts sporulate within the host cell (Figure 6 [47]) and virtually all of them pass unaltered through the gut of the host (Figure 6 [48]). These thick-walled forms, i.e., the oocysts that are detected in the feces of infected animals, are highly resistant and maintain their infectivity for mice and cell cultures for at least 16 months if stored at 4°C in a 2.5% aqueous solution of potassium dichromate.^{3,29} Thick-walled oocysts are the environmentally resistant forms that transmit the infection from one susceptible host to another.

In addition to the thick-walled form, transmission electron microscopy has revealed the presence of a thin-walled oocyst (Figure 6 [49]) in enterocytes of mice experimentally infected with *C. parvum*.^{3,29} In contrast to the resistant forms which have two distinct layers in the oocyst wall, the sporozoites and residuum of the thin-walled oocysts are surrounded by only a single unit membrane. This unit membrane ruptures soon after the thin-walled oocysts are released from the host cell, and the free sporozoites penetrate into adjacent epithelial cells and reinitiate the endogenous cycle.^{3,29} Autoinfective, thin-walled oocysts are not passed in the feces. Similar autoinfective, thin-walled oocysts of *C. parvum* are also produced in experimentally infected chicken embryos.³⁰

C. parvum and a *Cryptosporidium* sp. of chickens can also complete their developmental

cycles in extraintestinal sites. Respiratory and biliary infections of *C. parvum* have been reported in AIDS patients.^{13,14} Introduction of oocysts of a calf isolate of *C. parvum* into the trachea and eyes of suckling pigs resulted in heavy infections of the tracheal and conjunctival epithelium.⁴⁴ Introduction of sporozoites excysted from oocysts of *Cryptosporidium* sp. isolated from the gastrointestinal tract of chickens into the eyes and nares of 1-day-old and 1-month-old chickens results in upper respiratory and conjunctival epithelium infections.^{44a} Thick-walled oocysts develop in each of these extraintestinal sites, suggesting a developmental cycle similar to that observed in the gastrointestinal tract.

The presence of autoinfective oocysts (~20% of the oocysts found in host cells) along with type I meronts that recycle are life cycle features of *C. parvum* that make it unique among the coccidia infecting warm-blooded vertebrates. It is believed that these two features explain why a small number of oocysts can produce severe infections in susceptible hosts and why immune-deficient persons may develop persistent, life-threatening infections in the absence of repeated oral exposure to thick-walled oocysts. The lack of host and even organ specificity and the zoonotic potential of *C. parvum* are features which also make the epidemiology of this protozoan unique.

IV. EPIDEMIOLOGY

Since *Cryptosporidium* was recognized only recently as a human pathogen, our knowledge of its epidemiology is somewhat limited and is confined to information relative to its mode of transmission, potential sources of infection, and prevalence in man and his domesticated animals.

Studies of experimental infections in farm and laboratory animals have clearly established that cryptosporidiosis in mammals may be transmitted by oocysts (thick-walled) of *C. parvum*²⁴ which are fully sporulated and infective at the time they are passed in the feces.^{3,9,29} Oocysts of *C. parvum*, like those of other coccidian species, are resistant to most disinfectants used in hospitals and laboratories.² Infectivity of *Cryptosporidium* sp. oocysts was reported to be destroyed by ammonia, formal saline,⁴⁵ freeze-drying, and exposure (30 min) to temperatures below freezing (-20°C) and above 65°C .² In the author's laboratory, full-strength commercial bleach is used as a disinfectant. Exposure of oocysts to full-strength bleach (5.25% sodium hypochlorite) for 10 min or longer at room temperature destroys their infectivity for suckling mice.^{45a} If one uses bleach as a disinfectant, however, the final concentration should be above 70%. Incubation of oocysts in solutions of 20% commercial bleach (1.05% sodium hypochlorite) for 12 min in an ice bath did not prevent subsequent release of viable sporozoites under conditions of in vitro excystation.³⁹ These data demonstrate that routine chlorination of drinking water should have no effect on the infectivity of *Cryptosporidium* sp. oocysts. Although heat (hot water $>80^{\circ}\text{C}$ or autoclaving) and full-strength bleach may destroy infectivity of *Cryptosporidium* sp. oocysts, there is a need for the identification of a disinfectant for medical instruments that cannot tolerate such harsh treatments.

Within the past 4 years, studies have clearly shown that calves are a source of human infection,^{9,46,47} and it has been suggested that companion animals such as rodents, puppies, and kittens are also reservoir hosts.^{9,10} This, coupled with a large list (Table 3) of animals reported to be infected with what is believed to be a parasite that readily crosses species barriers, has led to the view that zoonotic transmission accounts for most human infections. This view is probably correct for persons living and working in environments where they are exposed to fecal contamination from potential reservoir hosts. However, zoonotic transmission cannot explain the large number of infections among urban dwellers whose exposure to animal feces is minimal.

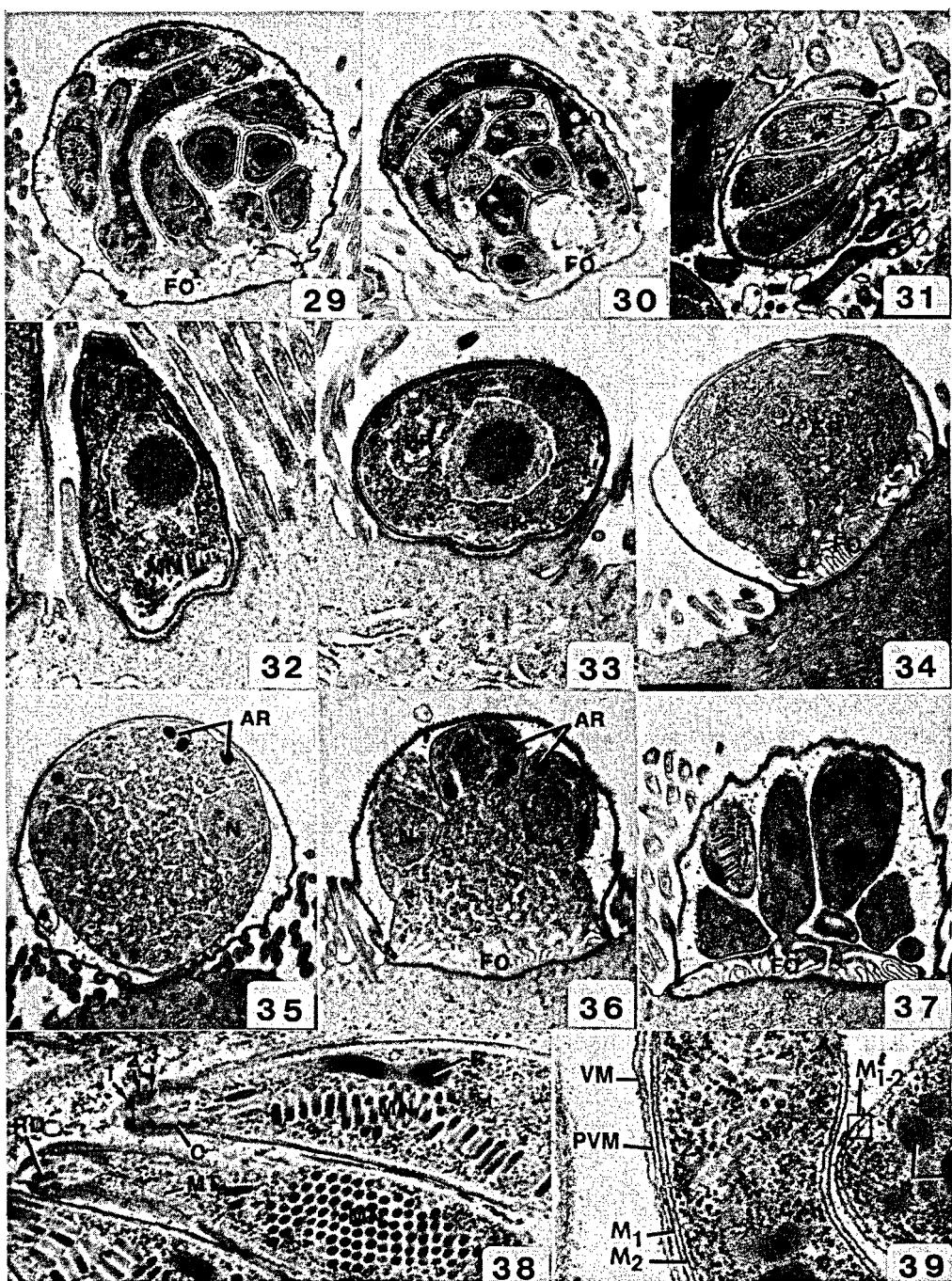


FIGURE 5 (29-39).

FIGURE 5 (29-39). Transmission electron micrographs (TEM) of meronts in the microvillous region of enterocytes (for 29 to 31, magnification $\times 10,800$) and TEM showing major features of merogony and merozoite fine structure (32 to 39). (29) Type I meront with eight merozoites; two of which are in the final stages of budding from the residuum. The basilar portion of the residuum is modified into a feeder organelle (FO). (30) Type I meront with six merozoites and a feeder organelle (FO) within the parasitophorous vacuole. (31) Section through anterior of the four merozoites (arrows) of a type II meront. (32) Merozoite in final stages of penetrating into microvillus region. Note the nucleus with a large nucleolus (Nu) and the vacuolated cytoplasm previously occupied by micronemes (MN) (magnification $\times 21,600$). (33) Early uninucleate meront with dilated endoplasmic reticulum (ER) and a nucleus with a large nucleolus (Nu) (magnification $\times 16,850$). (34) Late-stage uninucleate meront with extensive ER and an eccentric nucleus with a nucleolus (Nu) that is less prominent than that of the previous stage. The plasma membrane of the parasite has folded extensively to form the feeder organelle (FO) at the base of the parasitophorous vacuole (magnification $\times 14,760$). (35) Meront with two nuclei (N) without nucleoli and with anlagen of the rhoptries or micronemes (AR) near the periphery (magnification $\times 11,660$). (36) Type II meront showing three or four merozoites budding from the surface opposite the feeder organelle (FO). Within each budding merozoite is a nucleus (N) and anlagen of rhoptries or micronemes (AR) (magnification $\times 12,100$). (37) Nearly mature type II meront with three merozoites free in the parasitophorus vacuoles and one in the final stages of budding from the feeder organelle (FO). Merozoite nuclei have prominent nucleoli (Nu) (magnification $\times 14,760$). (38) Higher resolution of anterior portion of type II merozoites shown in (28). The apical complex is composed of three preconoidal rings (arrows 1, 2, 3), a conoid (C), two rhoptries (R), and numerous micronemes (MN). Note the microtubules (MT) accompanying what appears to be a duct from a rhoptry (RD) leading to a nipple-like projection at the anteriormost end (magnification $\times 33,260$). (39) Section through the posterior of several merozoites showing the arrangement of membranes. VM, outer villous membrane; PVM, parasitophorous vacuole membrane; M₁, outer plasma membrane of merozoite; M₂, inner plasma membrane present only in the posterior half of the merozoite (note its absence in [38]). Rhoptries (R) and micronemes can also be seen (magnification $\times 43,920$). (Adapted from Reference 29.)

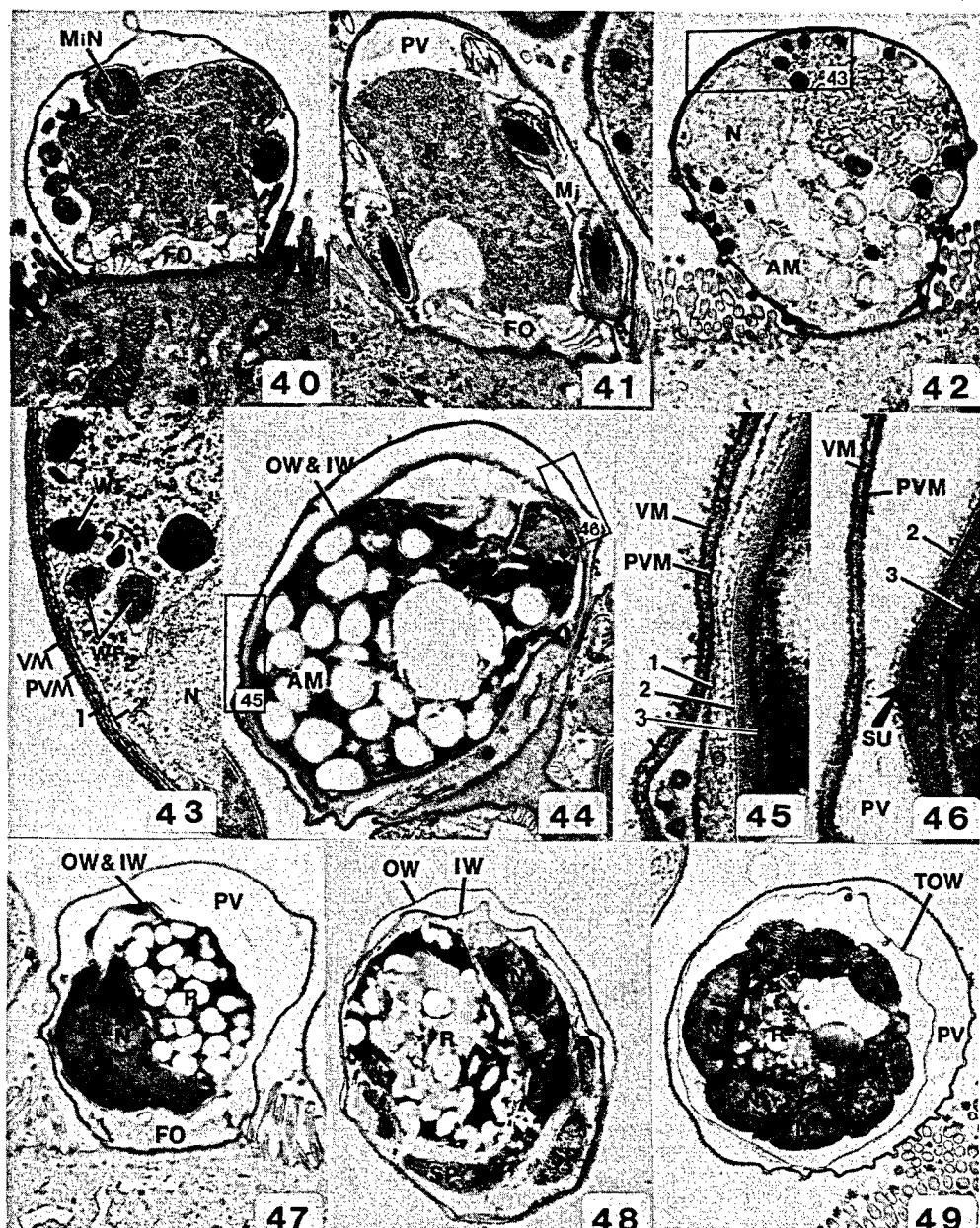


FIGURE 6 (40-49).

FIGURE 6 (40-49). Transmission electron micrographs of sexual stages of *C. parvum* in enterocytes. (40) Immature microgamont with compact nuclei (MiN) near the periphery of what will be the large residuum (R) whose basilar region has formed the feeder organelle (FO) (magnification $\times 11,520$). (41) Nearly mature microgamont with microgametes (Mi) free in the parasitophorous vacuole (PV) and budding from the residuum (R). The parasite membrane nearest the host cell has folded extensively to form the feeder organelle (FO) (magnification $\times 13,540$). (42) Macrogamete with an eccentric nucleus (N) and an accumulation of amylopectin-like bodies (AM) near the feeder organelle (magnification $\times 12,020$). (43) Enlargement of the boxed portion of the macrogamete in (39) showing two types of wall-forming bodies (WF₁, WF₂). Membranes surrounding the macrogamete include, from outside to inside, the villous membrane (VM), the parasitophorous vacuole membrane (PVM), the outer plasmalemma of the parasite (1) and a short segment membrane 2 forming just beneath membrane 1 (magnification $\times 30,960$). (44) Unsporulated, thick-walled oocyst within a parasitophorous vacuole. Amylopectin-like granules (AM) are numerous and some smaller lipid-like bodies (L) are present. The arrangement of membranes associated with the outer (OW) and inner (IW) oocyst wall in the boxed areas are shown in (42) and (43) (magnification $\times 12,600$). (45) The oocyst is contained in two host cell membranes, the villous membrane (VM) and the parasitophorous vacuole membrane (PVM). The outer wall (O) of the oocyst is limited by parasite membranes 1 and 2, whereas the inner wall (I) is limited by membranes 2 and 3 (magnification $\times 61,200$). (46) Portion of oocyst with membrane 1 and a portion of the outer oocyst wall missing. The suture (SU) in the inner oocyst wall represents the site of oocyst wall collapse during excystation (see Figure 1). VM, villus membrane; PVM, parasitophorous vacuole membrane; (2, 3) membranes 2 and 3 of oocyst wall; PV, parasitophorous vacuole (magnification $\times 61,200$). (47) Sporulated, thick-walled oocyst within a parasitophorous vacuole (PV). Two of the four sporozoites with posteriorly located nuclei (N) and a residuum (R) with amylopectin-like granules are enclosed within the outer and inner oocyst wall (OW and IW). The feeder organelle (FO) is still firmly attached to the base of the PV (magnification $\times 8,640$). (48) Thick-walled oocyst shed in feces. The outer (OW) and inner (IW) oocyst wall surrounds the sporozoites (SP) and residuum (R) both of which contain amylopectin-like granules. Note the posterior position of the sporozoite nucleus (N) (magnification $\times 10,800$). (49) Thin-walled, autoinfective oocyst within a parasitophorous vacuole (PV). The thin oocyst wall (TOW) consists of a single unit membrane surrounding the four sporozoites (three of their nuclei [N] are labeled) and the residuum (R) containing many amylopectin-like granules (magnification $\times 10,800$). (Adapted from Reference 29.)

Table 3
SOME ANIMALS REPORTED TO BE INFECTED WITH
***CRYPTOSPORIDIUM* spp.**

	Animals species	Ref.
Fishes		
Naso tang (<i>Naso lituratus</i>)	151	
Carp (<i>Cyprinus carpio</i>)	152	
Reptiles		
Red-bellied black snake (<i>Pseudechis porphyriacus</i>)	153	
Corn snake (<i>Elaphe guttata</i>)	154	
Trans-pacos ratsnake (<i>E. subocularis</i>)	154	
Madagascar boa (<i>Sansinia madagascarensis</i>)	154	
Timber rattlesnake (<i>Crotalus horridus</i>)	154	
Birds		
Chicken (<i>Gallus gallus</i>)	107—110	
Turkey (<i>Meleagris gallopavo</i>)	111, 112, 155, 156	
Bobwhite quail (<i>Colinus virginianus</i>)	114, 115	
Peafowl (<i>Pavo cristatus</i>)	113	
Black-throated finch (<i>Poephila cincta</i>)	157	
Domestic goose (<i>Anser anser</i>)	158	
Red-lored parrot (<i>Amazona autumnalis</i>)	159	
Mammals		
Man (<i>Homo sapiens</i>)	1—14, 46—72	
Macaques (<i>Macaca mulatta</i>)	160	
(<i>M. radiata</i>)	160	
(<i>M. fascicularis</i>)	160	
Calf (<i>Bos taurus</i>)	73—91	
Lamb (<i>Ovis aries</i>)	96—100	
Goat (<i>Capra hircus</i>)	9	
Horse (<i>Equis caballus</i>)	105, 106	
Swine (<i>Sus scrofa</i>)	102—104	
Roe deer (<i>Capreolus capreolus</i>)	161	
Red deer (<i>Cervus elaphus</i>)	162	
White tail deer (<i>Odocoileus virginianus</i>)	163	
Fox squirrel (<i>Sciurus niger</i>)	163	
Gray squirrel (<i>S. carolinensis</i>)	164	
Pocket gopher (<i>Geomys bursarius</i>)	163	
Chipmunk (<i>Tamias striatus</i>)	163	
Flying squirrel (<i>Glaucomys volans</i>)	163	
13-Lined ground squirrel (<i>Spermophilus tridecemlineatus</i>)	163	
Beaver (<i>Castor canadensis</i>)	163	
Muskrat (<i>Ondatra zibethicus</i>)	163	
Woodchuck (<i>Marmota monax</i>)	163	
Nutria (<i>Myocastor coypus</i>)	163	
Domestic rabbit (<i>Oryctolagus cuniculus</i>)	165	
Cottontail rabbit (<i>Sylvilagus floridanus</i>)	163	
Exotic undulates (~14 species)	116	
Domestic dog (<i>Canis familiaris</i>)	9, 166	
Coyote (<i>C. latrans</i>)	163	
Red fox (<i>Vulpes vulpes</i>)	163	
Grey fox (<i>Otocyon cinereoargenteus</i>)	163	
Domestic cat (<i>Felis catus</i>)	9, 27, 167	
Striped skunk (<i>Mephitis mephitis</i>)	163	
Raccoon (<i>Procyon lotor</i>)	168	
Black bear (<i>Ursus americanus</i>)	163	

Evidence has been rapidly accumulating that person-to-person transmission of cryptosporidiosis is also common. An accidental infection of a researcher demonstrated that a human isolate of *C. parvum* could be transmitted from person-to-person.⁴⁸ Outbreaks of cryptosporidiosis are being reported among children in day-care centers,^{49,50} several hospital-acquired infections have been investigated,^{10,51} at least one large waterborne outbreak has been documented,⁵² and this protozoan appears to be a cause of travelers diarrhea.^{53,54}

Soon after a waterborne outbreak in San Antonio, Tex.⁵² was investigated, a preliminary study, in conjunction with F. W. Schaefer of the Environmental Protection Agency, demonstrated that oocysts of *C. parvum* can be recovered from water samples by high volume orlon filters⁵⁵ used to trap *Giardia lamblia* cysts.^{55a} Similar filtration methods, coupled with an immunofluorescent detection technique, resulted in the recovery and identification of *Cryptosporidium* sp. oocysts from the secondary effluent of a sewage treatment plant in Tucson, Ariz.⁵⁶ *Cryptosporidium* sp. oocysts have also been identified by an immunofluorescence detection procedure in materials filtered from several rivers in western Washington State.⁵⁷

The epidemiologic features of cryptosporidiosis emphasized above, i.e., transmission by an environmentally resistant cyst (oocyst), the identification of numerous potential reservoir hosts for zoonotic transmission, the demonstration of person-to-person transmission, and documentation of waterborne transmission, are remarkably similar to those of human giardiasis that have come to light during the past decade. The importance of *G. lamblia* as a widespread cause of diarrheal illness in man has been recognized for a number of years, and *Cryptosporidium* is now rapidly ascending a similar recognition curve. Studies conducted recently in selected areas of Australia,⁵⁸ Costa Rica,^{59,60} the U.K.,⁶¹ the U.S.,^{62,63} Brazil,⁶⁴ Peru,⁶⁵ South Africa,⁶⁶ Bangladesh,⁶⁷ Spain,⁶⁸ Denmark,⁶⁹ central Africa,⁷⁰ Liberia,⁷¹ and Haiti⁷² have provided some data on the importance of *Cryptosporidium* sp. as a cause of diarrheal illness in man. In most of these studies, *Cryptosporidium* sp. was the most common parasite found and in several, this protozoan was considered to be the most significant of all known enteropathogens causing diarrheal illness. Other common findings were that children had a significantly higher prevalence of cryptosporidiosis than did adults and that infections were often seasonal, with a higher prevalence during the warmer, wettest months. From the standpoint of infection control, another interesting finding was that small numbers of oocysts may be shed in the feces for up to 2 weeks following resolution of diarrhea. These data alone suggest that *Cryptosporidium* is a major cause of diarrheal illness in humans worldwide.

In light of the information just reviewed, physicians, veterinarians, and others involved in educating the public of risk factors associated with the transmission of infectious diseases should broaden their public health role to include cryptosporidiosis. This role should be approached aggressively because of the apparently high prevalence of the disease, because of the large number of potential reservoir hosts, and because persons with compromised immune systems may develop life-threatening cryptosporidiosis (see below). Physicians should train themselves to recognize when the disease is present in their patients and veterinarians should train themselves to recognize when the infectious agent is present in farm and companion animals so that persons at high risk of developing serious illness can be warned. The typical symptoms in humans and clinical signs in animals are reviewed below (see Section V), along with techniques to diagnose cryptosporidiosis (see Section VI).

V. CRYPTOSPORIDIOSIS

Gastrointestinal and/or respiratory illness has been attributed to *Cryptosporidium* spp. infections in a variety of animals, particularly calves and humans. Clinical features of cryptosporidiosis occurring in these two mammalian species as well as a variety of other animals are discussed below.

A. Calves

Although some may have recognized it sooner, the role of *Cryptosporidium* sp. (*C. parvum*) as a cause of gastrointestinal illness in calves was first presented in the literature by Panciera et al.⁷³ in 1971. From 1976 to 1979, papers published by researchers in Canada,⁷⁴ Iowa,²⁶ and South Dakota⁷⁵ presented data supporting the view that *C. parvum* is a significant component of the neonatal bovine diarrhea complex. All of these studies relied on the recognition of endogenous stages of the parasite in the microvillus border of tissues obtained during necropsy. With the development of techniques to demonstrate oocysts in the feces (see Section VI), numerous papers have appeared within the past 10 years demonstrating that *Cryptosporidium* infections are prevalent and widespread in young calves (for representative papers, see References 75 to 91).

Most reported cases of cryptosporidiosis in calves present the disease as a diarrheal illness resulting in low to moderate mortality and moderate to high morbidity in animals 5 to 15 days of age. *Cryptosporidium* frequently occurs in conjunction with other enteropathogens of the neonatal calf diarrhea complex, such as rotavirus, coronavirus, and enterotoxigenic *Escherichia coli* and *Salmonella* spp. In such multiple infections, the enteropathogens may act synergistically to increase morbidity and mortality. High mortality may also occur when calves with cryptosporidiosis are exposed to extremely cold temperatures.^{75,92} Post-mortem examination of these animals reveals that death is usually due to starvation.^{75,92} Apparently, malabsorption due to cryptosporidiosis prevents calves fed diets that are formulated for warm weather from meeting the additional energy demand when environmental temperatures are unusually low. Thus, when *Cryptosporidium* is associated with high calf mortality, concurrent infections with other enteropathogens, cold weather, and perhaps poor management practices should be considered.

Difficulty in separating or identifying the roles of the different agents of the neonatal calf diarrhea complex, along with the finding of asymptomatic *Cryptosporidium* sp. infections, has led some to question the role of this protozoan as a primary pathogen.⁹³ The controversy as to whether *Cryptosporidium* sp. is a true enteropathogen in the absence of other agents of enteric disease in calves has, to this author's satisfaction, been resolved. The typical clinical signs of diarrhea and malabsorption and the characteristic lesions of villous blunting, fusion, and atrophy noted in naturally and experimentally infected animals also occur in gnotobiotic calves monoinfected with *Cryptosporidium* sp. (*C. parvum*).⁹⁴ Heine et al.⁹⁴ suggested that malabsorption caused by villous atrophy resulting from accelerated loss of epithelium was the basis for cryptosporidial-induced diarrhea in these calves. Similar findings also have been published for gnotobiotic pigs⁹⁵ and gnotobiotic lambs⁹⁶ that were reported to be monoinfected with *Cryptosporidium* sp. Thus, it appears that most of the *Cryptosporidium* isolates studied to date are primary pathogens that cause diarrhea in calves. As with any enteropathogen, one should expect to find some calves infected with *Cryptosporidium* sp. that have no clinical signs of illness. Large numbers of infected calves without diarrhea in a herd would suggest that different isolates of *Cryptosporidium* sp. may vary markedly in their virulence. Such differences in virulence have been suggested by others.^{2,93}

B. Lambs, Kids, Piglets, and Foals

Several other mammals commonly raised on farms have been reported to be susceptible to *Cryptosporidium* sp. (*C. parvum*) infection. Diarrhea associated with high morbidity and mortality has been attributed to natural^{97,98} and experimentally induced^{96,99-101} *Cryptosporidium* sp. infections in young lambs. As with calves, *Cryptosporidium* sp. appears to be an important cause of neonatal diarrhea in lambs. Young goats may also develop severe diarrhea following exposure to *Cryptosporidium* sp. oocysts.⁹

Natural^{102,103} and experimentally induced¹⁰⁴ cryptosporidiosis infections in young pigs have also been associated with diarrheal illness. However, cryptosporidiosis appears to occur

less frequently and to be a much less severe disease in young pigs than it does in calves, lambs, and goats.

Cryptosporidiosis has been associated with diarrheal illness and death in immune-deficient foals¹⁰⁵ and nonfatal diarrhea in immunocompetent foals.¹⁰⁶ The role of *Cryptosporidium* sp. as a cause of neonatal equine diarrhea requires further investigation.

C. Poultry

Cryptosporidium spp. have been reported from the gastrointestinal and respiratory tracts of poultry. Intestinal (bursa of Fabricius and cloaca) cryptosporidiosis appears to be quite common among broiler chickens and has not been associated with overt clinical diseases.¹⁰⁷⁻¹⁰⁹ However, when the organism colonizes the epithelium of the upper respiratory tract of broilers, it may cause significant respiratory disease.¹¹⁰ Respiratory cryptosporidiosis, resulting in high morbidity and mortality, has also been reported for turkeys^{111,112} and peacock chicks.¹¹³

During the past several years, this author has been made aware of a number of outbreaks of respiratory cryptosporidiosis in broiler houses, particularly in the southeastern U.S., and most have resulted in increased morbidity and mortality. Several producers have also suggested that respiratory cryptosporidiosis may also contribute to airsacculitis condemnation of broilers during processing. Although it is not known why sudden outbreaks of respiratory cryptosporidiosis occur, recent studies^{113a} have demonstrated that the same species of parasite commonly found in the bursa of Fabricius of broilers can produce respiratory disease in chickens and turkeys. Approximately 1 week after intranasal inoculation of *Cryptosporidium* sp. oocysts isolated from the bursa of Fabricius of commercial broilers, young chickens and turkeys may develop respiratory disease associated with extensive colonization of the mucosal epithelium by the parasite.^{113a}

In addition to turkeys and chickens, the quail is another commercially reared avian species that is susceptible to clinical cryptosporidiosis. High mortality of young, commercially raised quail has been attributed to *Cryptosporidium* sp. infections of the small intestine¹¹⁴ and the upper respiratory tract.¹¹⁵

D. Companion and Wild Animals

Numerous other species of animals have been reported to be infected with *Cryptosporidium* spp. Of particular interest to physicians, veterinarians, and others interested in the epidemiology of cryptosporidiosis are clinical (usually diarrhea) and nonclinical infections reported in companion animals such as rodents, puppies, and kittens and in the wild mammals such as raccoons, foxes, coyotes, beavers, muskrats, and squirrels (Table 3). Cryptosporidiosis has also been reported as an important cause of neonatal diarrhea in exotic undulates raised in captivity.¹¹⁶

E. Man

Much of the available literature presents cryptosporidiosis as a short-term, often cholera-like, diarrheal illness in immunocompetent persons or as a prolonged, life-threatening, cholera-like, illness in immune-deficient persons.¹⁻⁹ However, the clinical presentation of gastrointestinal cryptosporidiosis in immunocompetent and immune-deficient persons does not always fall into these two divergent categories. Persons with the clinical and laboratory features of AIDS have been known to clear *Cryptosporidium* sp. infections after several months of diarrhea, and persons reported to be immunocompetent have had infections associated with diarrhea lasting for more than 1 month.¹⁰ Mild and asymptomatic infections may occur in immunocompetent⁹ as well as immune-deficient persons.¹¹⁷

1. Immune-Deficient Persons

In most immune-deficient persons, diarrheal illness due to *Cryptosporidium* infection of

the gastrointestinal tract becomes progressively worse and may be a major factor leading to the death of these patients. Such life-threatening infections have been documented in persons with congenital immunodeficiencies such as hypogammaglobulinemia,^{5,8,9} individuals on immunosuppressive chemotherapy,^{4,7,118,119} and patients with AIDS.^{9,11,13,14,120-122} Of the gastrointestinal pathogens identified in AIDS, *Cryptosporidium* is the most ominous in its effects on morbidity and its contributions to mortality.¹²³ Fluid loss by AIDS patients with cryptosporidiosis is often extensive; as noted earlier, 3 to 6 ℥ of diarrheic stool per day is common, and as much as 17 ℥ of watery feces per day has been reported.¹²

In immune-deficient persons with gastrointestinal cryptosporidiosis, developmental stages of the parasite have been found in the pharynx, esophagus, stomach, duodenum, jejunum, ileum, appendix, colon, and rectum.^{4-7,11,12,124} Post-mortem examination of three patients demonstrated that the jejunum was the most heavily infected region of the gut in all cases.^{7,8,124} Histologic lesions observed in heavily infected regions of the small intestine included villous atrophy, an increase in crypt length, and mild-to-moderate mononuclear cell infiltration of the lamina propria.⁴⁻⁸ Similar microscopic lesions have been reported in the small⁹⁴ and large¹²⁵ intestines of gnotobiotic calves monoinfected with *Cryptosporidium* sp. Heine et al.⁹⁴ proposed that malabsorption due to villous atrophy of the small intestine was the basis for cryptosporidial-induced diarrhea in these germ-free calves. In a subsequent study, Pholenz et al.¹²⁵ reported that gnotobiotic calves monoinfected with *Cryptosporidium* sp. also had extensive colonization of the spiral colon by the parasite and that infected regions had marked erosion of the epithelium. Thus, it appears that reduced malabsorption and impaired digestion in the small intestine coupled with malabsorption in the large intestine are the major factors responsible for diarrhea in calves with cryptosporidiosis. Malabsorption has also been documented in immunosuppressed humans with cryptosporidiosis.^{8,11} However, the secretory (often described as cholera-like) diarrhea common to many immune-deficient patients with cryptosporidiosis would suggest hypersecretion into the gut. Slow marker intestinal profusion tests performed on a patient with AIDS and a *Cryptosporidium* infection of the entire small bowel suggested profuse fluid secretion into the duodenum and proximal jejunum and normal reabsorption of water and sodium in the ileum and colon.¹²⁶ To date, the pathophysiologic mechanism(s) of *Cryptosporidium*-induced diarrhea have not been defined. Definitive, systematic investigations of the mechanisms by which *Cryptosporidium* sp. and its metabolites or toxins may alter normal intestinal function of a susceptible animal model are needed.

In the immune-deficient patient, *Cryptosporidium* infection is not confined to the gastrointestinal tract. Acute and gangrenous cholecystitis in AIDS patients has been attributed to *Cryptosporidium* infections of the biliary tree and gall bladder epithelium.^{14,127,128} Respiratory tract infections with *Cryptosporidium* sp. have been associated with chronic coughing, dyspnea, bronchiolitis, and pneumonitis in immune-deficient persons.^{13,129,130} The importance of *Cryptosporidium* sp. as a cause of hepatic and pulmonary disease in immune-deficient and perhaps in immunocompetent persons needs further investigation.

2. Immunocompetent Persons

Most of the 18 cases of cryptosporidiosis in immunocompetent humans reported prior to 1983,^{46,47,131-135} and the numerous cases reported since then,^{48-54,58-72} describe a short-term, flu-like, gastrointestinal illness or a self-limited cholera-like illness. The most commonly reported symptoms in immunocompetent individuals with cryptosporidiosis are profuse, watery diarrhea (cholera-like) and abdominal cramping, nausea, vomiting, anorexia, low-grade fever, and headache (flu-like).

In most well-nourished persons, the diarrheal illness due to *Cryptosporidium* sp. infection lasts from 3 to 12 days. Occasionally, these patients may require parenteral fluid therapy because of extensive fluid loss, and occasionally the diarrheal illness may last for more than 2 weeks. In poorly nourished persons, especially young (less than 2 years old) children,

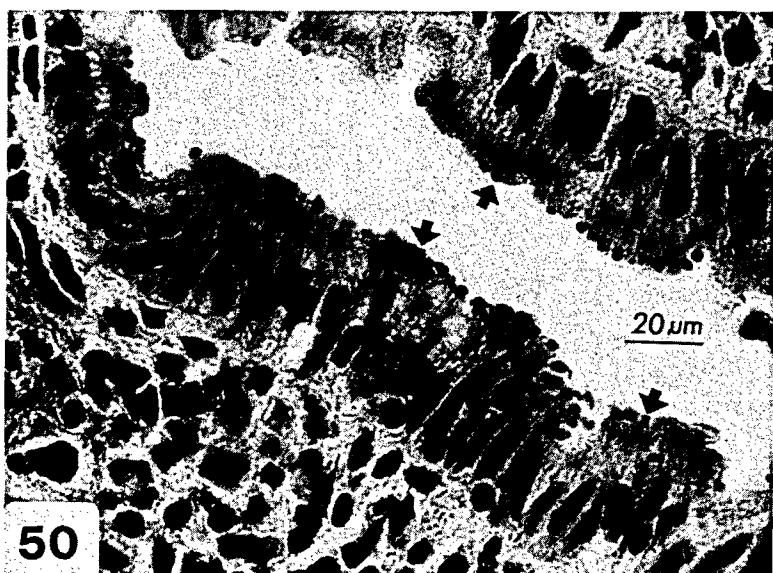


FIGURE 7 (50). Histologic section (stained with H.E.) of a small bowel biopsy from a patient with hypogammaglobulinemia and intestinal cryptosporidiosis.⁹ Arrows point to some of the many endogenous stages of *Cryptosporidium* sp. in the microvillous region of the enterocytes.

oral and parenteral rehydration therapy are often required because of extensive fluid loss. It is also not uncommon for young malnourished children with cryptosporidiosis to have diarrhea lasting for more than 3 weeks. Diarrheal illness is a major cause of morbidity and mortality, especially in young children residing in developing countries. Estimates of Walsh and Warren¹³⁶ suggest that in Africa, Asia, and Latin America there are as many as five billion episodes of diarrhea and five to ten million diarrheal-associated deaths annually.¹³⁶ Although recent surveys demonstrate that cryptosporidiosis is a major cause of diarrheal illness worldwide,⁵⁸⁻⁷² it is not clear to what extent this protozoal disease contributes to morbidity and mortality. Additional, detailed studies employing the proper diagnostic tools should define more clearly the impact of cryptosporidiosis on human health in developing and developed countries.

VI. DIAGNOSIS

Prior to 1980, diagnosis of human cryptosporidiosis was dependent upon identification of the small spherical endogenous stages of *Cryptosporidium* sp. in the microvillous region of enterocytes of intestinal tissues obtained by biopsy and subsequently processed for examination by light- or electron-microscopy (Figures 7 [50] and 8 [51]). Such invasive, time-consuming procedures are no longer necessary since a variety of techniques have been developed for identifying *Cryptosporidium* sp. oocysts in fecal specimens.^{9,77,137-142} Intestinal biopsy is still useful to determine the region of the gut infected with *Cryptosporidium* sp. and to ascertain histopathologic changes that may occur in the affected region.

Stool samples obtained from most hosts (animal and man) with cryptosporidiosis will contain large numbers of oocysts, and use of any of the published concentration^{9,77} or staining¹³⁷⁻¹⁴² techniques should result in a positive diagnosis. However, some stool samples contain only a few oocysts, making it difficult for the medical microbiologist or the veterinary

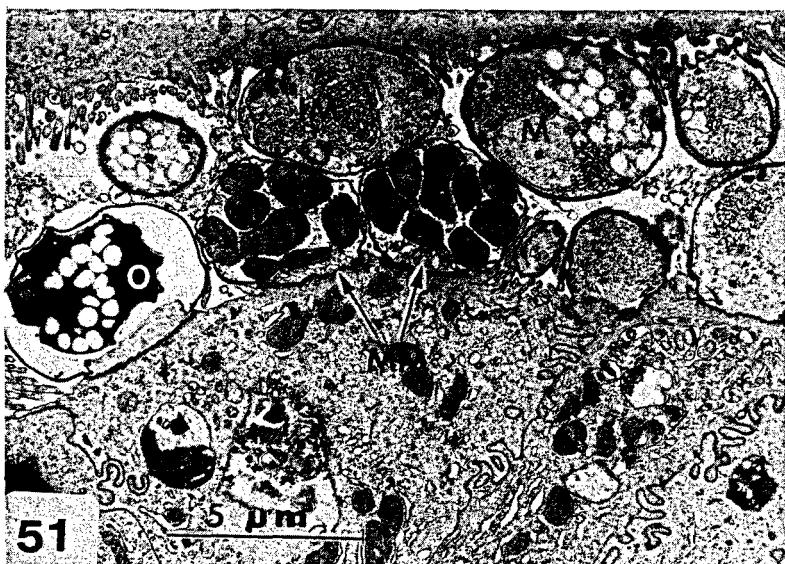


FIGURE 8 (51). Transmission electron micrograph of endogenous stages of *Cryptosporidium* sp. in enterocytes of a mouse 6 days after oral inoculation of oocysts obtained from an immune-deficient patient (Reference 9, case 13). Developmental stages of the parasite shown are an oocyst (O) a macrogamete (M), immature meronts (IM), and mature meronts (MM).

diagnostician to decide if one or two *Cryptosporidium*-like bodies seen in a stained fecal smear warrant a positive diagnosis. This is especially true for many of the modified acid-fast staining procedures because there may be small spherical objects other than *Cryptosporidium* oocysts in some stool samples that appear acid fast. Increased sensitivity can be achieved by using an oocyst concentration technique such as Sheather's sugar flotation.^{9,77} Material concentrated from stool samples can be examined prior to⁹ or after staining.¹³⁷

The most widely used techniques for demonstrating *Cryptosporidium* sp. oocysts in fecal specimens from animals and man are modified acid-fast staining,¹³⁷⁻¹³⁹ negative staining,^{140,141} and Sheather's sugar flotation.^{9,77} The last two procedures are used routinely in this author's laboratory (Figure 9 [52]) because they are relatively simple, rapid, inexpensive, and allow one to distinguish *Cryptosporidium* oocysts from the yeasts commonly found in stool specimens.

Any laboratory faced with the task of diagnosing cryptosporidiosis should have one or more persons who become familiar with two techniques, one technique to be used as the primary diagnostic procedure and the second as a back-up procedure for specimens that give confusing results with the first method. Good-quality, *Cryptosporidium*-positive fecal specimens should be available while laboratory personnel are becoming familiar with the techniques of choice and as a reference source as long as the diagnostic procedures are performed. Such samples are available from at least one commercial source for a modest price.¹⁴³ Many laboratories obtain good reference specimens through routine submissions. If stool samples containing *Cryptosporidium* sp. oocysts are stored cold (4°C) in either 10% formalin or in 2.5% K₂Cr₂O₇, they should remain useful for at least 12 months.³

Now that several inexpensive, rapid, and reliable techniques for identifying *Cryptosporidium* oocysts in fecal samples are available, cryptosporidiosis should be considered in the routine diagnosis of diarrheal illness in animals and in man. Veterinarians and veterinary diagnosticians may consider incorporating these diagnostic techniques into health-monitoring

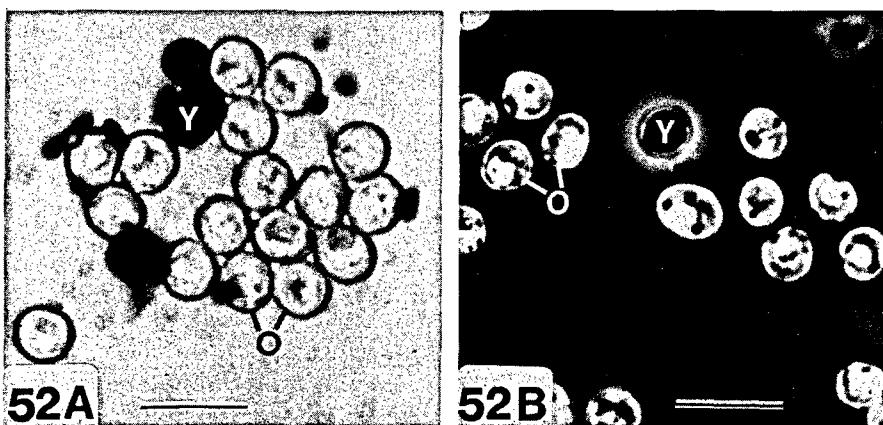


FIGURE 9 (52). Oocysts of *Cryptosporidium* sp. from stool specimens. (A) Brightfield photomicrograph of modified kinyoun's carbol fuchsin negative stain^{140,141} of a fecal smear. Oocysts (O) do not stain and are somewhat refractive. Yeast (Y) and most other debris stain darkly. (B) Phase-contrast photomicrograph of Sheather's sugar flotation of fecal specimen.⁹ Oocysts (O) are bright and refractive and contain one to six small dark bodies. Yeasts (Y) are not refractive. Lines represent 10 μm .

programs for pets, especially if their owners are immunocompromised. Physicians may consider the use of these diagnostic procedures prior to placing patients on immunosuppressive chemotherapy. Delaying immunosuppressive chemotherapy until after a mild or subclinical *Cryptosporidium* sp. infection is cleared may avoid a life-threatening diarrheal illness.

VII. TREATMENT

Treatment of cryptosporidiosis, especially in immune-deficient persons, has been unsuccessful and frustrating in most cases. To date, there have been no controlled studies and all therapeutic information is based on isolated reports. The list of unsuccessful attempts to treat cryptosporidiosis in immune-deficient persons is growing rapidly. Many of these attempts, reviewed in recent papers,^{11,144,145} are listed in Table 4. Some therapies reported to be of value in the treatment of cryptosporidiosis in immune-deficient persons are discussed below.

A. Antimicrobial/Antiparasitic Agents

Of the numerous antimicrobial and antiparasitic drugs administered to immune-deficient persons with intestinal cryptosporidiosis, spiramycin is the only one reported to have some efficacy.

Spiramycin, a macrolide antibiotic administered orally at 3 g/day, was reported to control diarrhea in nine patients with AIDS and cryptosporidiosis.¹⁴⁶ However, most of these patients continued to shed *Cryptosporidium* sp. oocysts in their feces. Many other physicians have reported to this author that spiramycin therapy is of little value in the treatment of cryptosporidiosis in persons with AIDS. These conflicting reports may be explained, in part, by observations of Soave,¹⁴⁷ who found that spiramycin therapy may control diarrhea in some patients treated for cryptosporidiosis early in the progression of AIDS. However, spiramycin therapy does not appear to have any effect on the course of clinical cryptosporidiosis in patients who have had AIDS for several months. Carefully controlled studies are needed to determine if spiramycin therapy is effective in the treatment of cryptosporidiosis in immune-deficient persons.

Table 4
SOME UNSUCCESSFUL ATTEMPTS TO TREAT
CRYPTOSPORIDIOSIS IN IMMUNE-DEFICIENT
HUMANS^{11,144,145}

Antimicrobial/antiparasitic agents	Antidiarrheals
Trimethoprim/sulfamethoxazole	Kaopectate®
Pyrimethamine/sulfadiazine	Opiates
Sulfathalidine	Paragoric
Quinacrine	Cholestyramine
Metronidazole	Immunomodulators
Chloroquine/primaquine	Levamisole
Furazolidone	Transfer factor ^b
Diloxamide furoate	Cimetidine
Diiiodohydroxyquin	Interferon
Thiabendazole	Globulin
Idoquinal	Other
Paromomycin	Gluten-free diet
Salinomycin	Lactose-free diet
Vancomycin	Glucose + amino acids
Cotrimoxazole	
Robenidine	
Tetracycline/doxycycline	
Clindamycin/Pentamidine	
Pentamidine	
Sulfathalidine	
Spiramycin ^a	
Puromomycin	
Piperazine	
Ampicillin	
Erythromycin	
Ketoconazole	
Amprolium	
Nystatin	
Clopidol	
Piperazine	
Thiobendazole	

^a Some have reported that spiramycin may control diarrhea in some AIDS patients (see Section VII and References 146 and 147).

^b Bovine transfer factor therapy was recently reported to result in resolution of diarrhea in six of seven patients with cryptosporidiosis and AIDS (see Section VII and Reference 150).

B. Immunomodulation

In the absence of effective chemotherapy, the major factor determining the severity and duration of a *Cryptosporidium* sp. infection is the immune status of the host. Discontinuing immunosuppressive chemotherapy and restoration of immune function has resulted in clearance of cryptosporidiosis from the intestinal tract of several patients.^{4,118,119} These findings suggest that immunomodulation or passive transfer of antibodies or lymphocytes may be of value in the treatment of cryptosporidiosis in immune-deficient patients.

This author is aware of at least one patient that has been given serum from three persons with high antibody titers to *C. parvum*. In addition, lymphocytes were also obtained from the immune donors, irradiated, and administered to the patient with hypogammaglobulinemia and cryptosporidiosis. Both the immune serum and lymphocyte therapy had no effect on the course of cryptosporidiosis in this immune-deficient patient.

Mata et al.⁶⁰ reported that there was a lower incidence of cryptosporidiosis in breast-fed

infants than in babies fed artificial diets, suggesting that lactogenic immunity may be of some value in preventing cryptosporidiosis in neonates. Lactogenic immunity does not appear to play a role in altering the course of cryptosporidiosis in suckling calves or in suckling mice. In the author's research facility, calves are routinely infected by mixing oocysts of *C. parvum* with the first liter of colostrum fed. These calves then receive an additional 1 to 2 ℥ of colostrum during the next 36 hr. High antibody titers (primarily IgG) were found by indirect immunofluorescence^{122,148} in three of four of the colostrum samples examined. The presence or absence of colostoral antibodies against *Cryptosporidium* sp. had no apparent effect on the course of cryptosporidiosis in these calves.^{148a} Similarly, female mice that recovered from neonatal cryptosporidiosis and that were challenged orally with *C. parvum* oocysts during gestation did not confer protective lactogenic immunity to their suckling pups that were fed *C. parvum* oocysts.¹⁴⁹ Bovine colostrum containing antibodies (IgG) to *Cryptosporidium* sp. administered orally for several days was of no value in the treatment of cryptosporidiosis in one patient with AIDS.^{149a} Additional studies are needed to determine the role of lactogenic immunity in preventing cryptosporidiosis in neonates from man and from monogastric and ruminant animals.

A transfer factor prepared from lymphoid cells obtained from calves immune to *Cryptosporidium* sp. was recently reported to be effective in the treatment of cryptosporidiosis in several AIDS patients.¹⁵⁰ Oral administration of the bovine transfer factor resulted in resolution of diarrhea in six of seven patients with cryptosporidiosis and AIDS. Four of these patients also stopped shedding *Cryptosporidium* sp. oocysts in their feces. One patient did not show any symptomatic or parasitologic improvement during or following bovine transfer factor therapy. More extensive studies are needed to confirm the results obtained in this small number of AIDS patients and to determine if transfer factor therapy is of any value in the treatment of cryptosporidiosis in persons with AIDS and other types of immune deficiencies.

C. Supportive Therapy

As shown in Table 4, antidiarrheal compounds may provide little symptomatic relief in immune-deficient persons with cryptosporidiosis. However, antidiarrheal drugs may lessen the severity of diarrhea in most immunocompetent persons with cryptosporidiosis.

Oral or parenteral rehydration therapy is often required by both immunocompetent and immune-deficient persons with severe cryptosporidial diarrhea, especially young children. Parenteral nutrition may help sustain the nutritional status of some immune-deficient patients with persistent cryptosporidiosis; however, this procedure has not been successful in sustaining many persons with AIDS and cryptosporidiosis. Until an effective drug is found to treat cryptosporidiosis, supportive therapy is the only avenue available to most clinicians.

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