R*eview articles*

Protozoan parasites of the intestinal tract: A review of Coccidia and Microsporida

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Ubiquitous in nature, members of the Coccidia and Microsporida are being reported with increasing frequency in the immunocompromised as well as the immunocompetent population. These protozoans are primarily waterborne, but foodborne disease has also been reported. These organisms are responsible for acute, as well as protracted, cases of watery diarrhea with various other related sequelae. The Coccidia includes three genera—*Cryptosporidium*, *Isospora*, and *Cyclospora*. The latter two are of lesser importance in terms of morbidity and mortality. The Microsporida includes genera (*Enterocytozoon, Encephalitozoon*) only recently recognized as important agents of disease. Unlike the Coccidia, these organisms are more restricted to the immunocompromised population. Increased incidence and numbers of patients with prolonged diarrhea due to these forms indicate the need for increased clinical vigilance with regard to prevention, diagnosis, and treatment.

(Keywords: Coccidia, Microsporida)

The description of "new" agents of human disease and nomenclature changes occur among all groups of pathogenic organisms. These changes are to be expected with advances in technology that enable more precise diagnosis of infectious diseases and greater knowledge of genetic relationships among the etiologic agents. In addition, the human immunodeficiency virus (HIV) pandemic and the related expansion of the immunocompromised population has contributed to an increase in the numbers of individuals infected with opportunistic pathogens. Increased travel opportunities and population migration due to natural and man-made calamities (for example, civil wars in Africa and elsewhere) have also served to expose more humans to these organisms. This is especially evident in gastrointestinal diseases,

with greater numbers of reported cases due to protozoans.

These emerging pathogens are members of two groups, the Coccidia and the Microsporida, commonly referred to as coccidians and microsporidians, respectively. The newly recognized importance of these organisms dictates that healthcare personnel develop a greater knowledge of them. This review presents essential information related to the natural history of these forms and the nature of the disease processes associated with them. In describing the disease presentations, an emphasis is placed on the immunocompetent patient, although the immunocompromised patient will also be included to a lesser extent. For readers with particular interest in disease manifestations in the immunocompromised host population, with an emphasis on the acquired immunodeficiency syndrome (AIDS), an extensive literature is available.[1-10]

Etiologic agents
The intestinal coccidians and microspo-

ridians of humans are listed in *Figure 1*. Members of these groups are generally described as intracellular organisms that are common parasites of various vertebrates, including humans. The role of animals as reservoirs for human infections is not totally clear, but because related (possibly identical) organisms are commonly seen in livestock, feral animals, and pets, a strong possibility exists regarding their etiology for human disease. In addition, the taxonomic designations for the various organisms have been altered as new knowledge of relationships is reported; additional changes in nomenclature will probably occur.

The morphology of these forms is similar. Although the intracellular forms can be detected in biopsied specimens, the excreted stage can furnish the information required for specific diagnosis. The diagnostic forms (oocyts) of the coccidians can be distinguished by size differences and careful scrutiny of their structures using light microscopy.

The microsporidians produce a much smaller and therefore more difficult to detect, form (spore). Nonetheless, they can be detected by trained individuals conducting a vigilant search. The diagnostic forms are shown diagrammatically in *Figure 2*. The coccidian oocyst may not contain sporocysts (such as *Cryptosporidium*) but all species contain the sporozoite form. The "hatched" sporozoite develops into the ensuing life form. The microsporidian spore lacks these life-forms; instead, each spore injects its contents into the susceptible host cell to initiate the infection.

The strong presumption is that all of these organisms are transmitted primarily through the water supply. Essentially cosmopolitan, these organisms are more common in tropical and subtropical than in temperate climates and are more common in developing countries than in industrialized ones. The literature generally supports this perception.[11-13] However, thorough population-based studies of prevalence or incidence are lacking.

Cryptosporidium parvum
The species designation usually accepted for the etiologic agent of human cryp-

Figure 1. *Intestinal coccidia and microsporida of humans.*

tosporidiosis is *Cryptosporidium parvum*. The parasite was first described in 1907 from a laboratory mouse[14]; it has additionally been reported in chickens, turkeys, guinea pigs, horses, calves, pigs, sheep, monkeys, dogs, and cats. Whether the identities of these forms as human-associated *C parvum* will stand up to the closer scrutiny of molecular methods remains to be determined. This well-traveled organism can be described as a cause of zoonotic disease, based on various reports.[15-18] However, it is probably more commonly transmitted person-to-person or by waterborne spread, although an outbreak associated with food-borne transmission has also been reported.[19] Abundant literature on cryptosporidiosis has appeared during the past decade and some rather extensive reviews are available.[4,20-23] Among the plethora of references are a number pertaining to the well-publicized outbreak that occurred in Milwaukee, Wis.[24-26]

This outbreak, as well as earlier ones in Texas[27] and Georgia,[28] underscore the danger of waterborne transmission of *C parvum*. Person-to-person spread is especially noted within the gay community, but it is also a particular hazard in the day care setting.[29-34]

These organisms, like other coccidians, are characterized by the production of an oocyst form that eventually contains the infective sporozoites. When ingested, these forms can initiate an infection of the small intestine, the severity of which is pronounced in persons infected with the HIV.[20] In a study of healthy volunteers, the infective dose was similar to that for *Giardia (lamblia) intestinalis*, a mean dose of 132 oocysts.[35] *C parvum* differs from its coccidian relatives in that its developmental stages occur in an intracellular, extracytoplasmic location, rather than deep within the host's cells. The reinfection of host cells by merozoites occurs, and in addition there are thin-
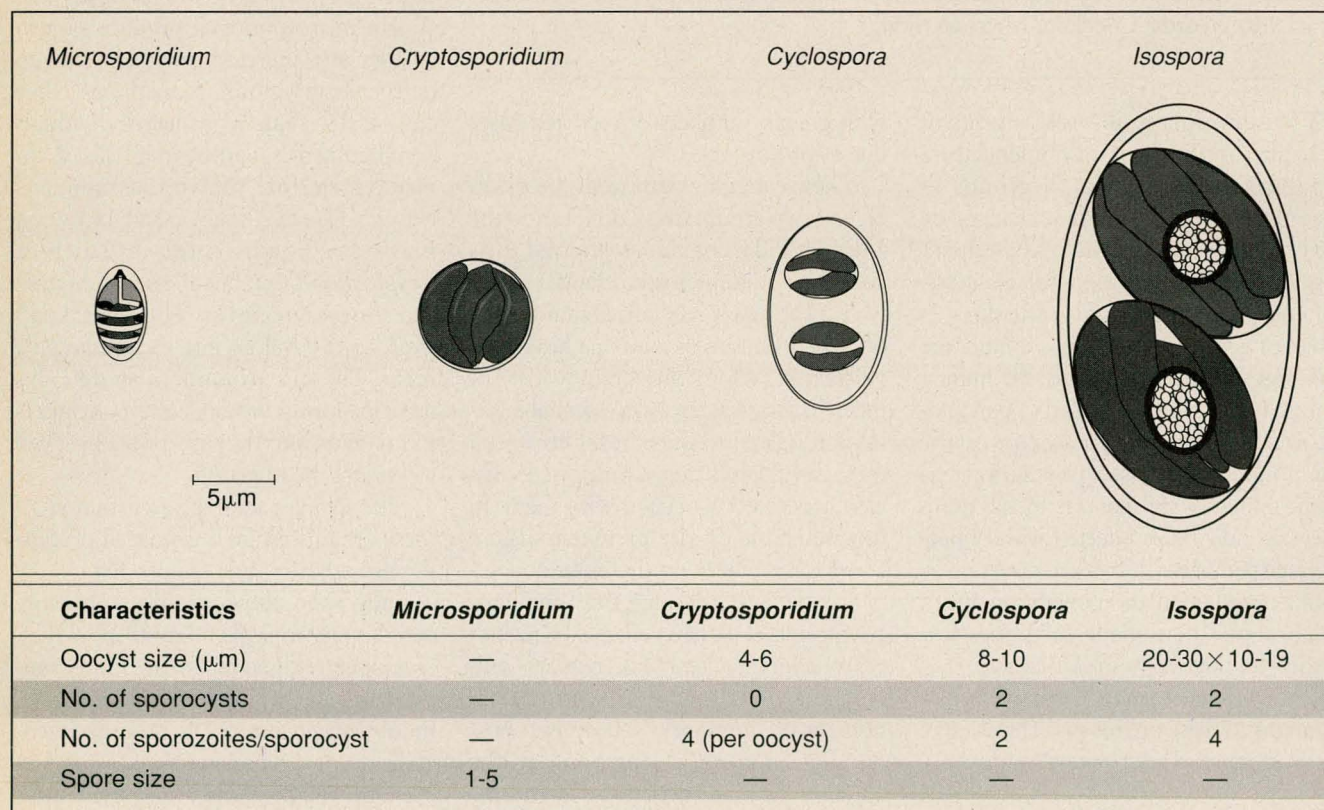

Characteristics	Microsporidium	Cryptosporidium	Cyclospora	Isospora
Oocyst size (μm)	—	4-6	8-10	20-30 × 10-19
No. of sporocysts	—	0	2	2
No. of sporozoites/sporocyst	—	4 (per oocyst)	2	4
Spore size	1-5	—	—	—

Figure 2. *Comparative morphology of the diagnostic forms of microsporidian and coccidian agents of intestinal disease (Adapted from Gardiner CH, Fayer R, Dubey JP:* An Atlas of Protozoan Parasites in Animal Tissues. Handbook No. 651, US Department of Agriculture, Washington DC, 1988; *and Garcia LS, Bruckner DA:* Diagnostic Medical Parasitology. *Washington, DC, ASM Press, 1993.)*
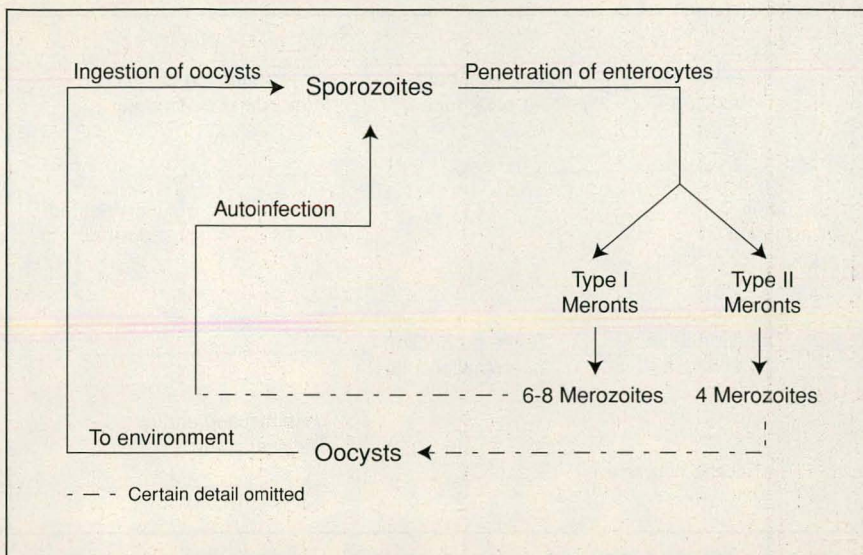
Figure 3. *The life cycle of* Cryptosporidium parvum *in humans.*

Diagram labels: Ingestion of oocysts → Sporozoites; Penetration of enterocytes; Autoinfection; Type I Meronts; Type II Meronts; 6-8 Merozoites; 4 Merozoites; To environment; Oocysts; – – – Certain detail omitted

Checklist

☐ *Encephalitozoon* spp
Intestine, cornea,kidney, nasal sinus, central nervous system; disseminated disease

☐ *Enterocytozoon* spp
Intestine

☐ *Nosema* spp
Cornea

☐ *Pleistophora* spp
Muscle

☐ "*Microsporidium*" *spp
Cornea

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*Designation used for insufficiently characterized isolates.

Figure 4. *Reported sites of human infection with microsporida.*

walled oocysts that can excyst within the patient's intestine, with the potential for overwhelming autoinfection (*Figure 3*).

The generally used permanent stains (trichrome, iron hematoxylin) for intestinal protozoan parasites do not adequately stain the cryptosporidia. The oocysts and their internal sporozoites are detectable in wet mounts, but this requires a skill level that may be lacking in laboratories unaccustomed to diagnosis of the cryptosporidia. The modified acid-fast stain is usually adequate for detecting the relatively small oocyst, but it will not reveal the internal morphology and is, therefore, a presumptive test. Definitive diagnosis is possible with various immunolabeling and fluorescent antibody methods that are commercially available. These techniques are highly specific, but sensitivity varies depending on the kit used.[36] In addition, the added expense may not be justified unless one is dealing with a population at higher risk for presence of the infection.

Isospora belli

Although universal agreement does not prevail regarding the number of species of *Isospora* that can cause human disease, most reports designate *I belli* for the intestinal cases. A review of this genus has recently been published.[37] Cosmopolitan in its geographic distribution,

this microorganism is considered to be only a human parasite; no nonhuman reservoir hosts have been identified. This coccidian inhabits the epithelial cells of the small intestine and has a life cycle that is very similar to that of *Cryptosporidium*. However, unlike *C parvum*, it exists within a parasitophorous vacuole in the infected cell.[38]

The organism is rarely reported in the United States; for example, it has not been reported in Iowa since 1993.[39] This absence may reflect a true lack of regular occurrence; its oocysts are relatively large (20-30 μm × 10-19 μm) and are less likely to be overlooked in a properly conducted stool examination if fresh smears with high contrast or phase contrast microscopy are used. However, unless the laboratory technologist is forewarned, these microscopic techniques may not be used and thus the microorganism might be overlooked. As with *Cryptosporidium*, this organism will stain with the modified acidfast stain but not with the common stains for intestinal protozoans.

Cyclospora cayetanensis

The most recent candidate for media coverage is *Cyclospora cayetanensis*.[40] This coccidian also affects the small intestine; it was first reported in 1979 in Papua, New Guinea.[41] It has been reported with increasing frequency since

then. Although it has been described in a variety of ways (coccidian-like bodies, cyanobacterium-like bodies, or blue-green algae), it now has the more certain designation of *C cayetanensis*. Although its life cycle has yet to be determined definitively, it is probably typical of that seen with other intestinal coccidians. Unlike the others, however, *Cyclospora* outbreaks have been associated with foodborne spread.[42] Food items associated with clinical disease have included seasonal produce, particularly fresh fruits.[43-45] However, waterborne spread of this organism is also likely.[46-49] Person-to-person spread is unlikely because of the time (days to weeks) required for oocyst maturation in the environment.

The modified acid-fast stain is not as effective for this species as for the other intestinal coccidians. This organism exhibits a highly variable staining reaction, and some oocysts will not be visualized.

Microsporida

Obligate intracellular parasites, the microsporidians occur predominantly

in immunocompromised patients. The taxonomy of these species is even less settled than for their coccidian relatives. Their extensive host range means that species have been reported in every class of vertebrate as well as many invertebrates.[6] Before the AIDS pandemic, the microsporidians were generally unreported as causes of human disease.[50] In terms of gastrointestinal disease, the numbers of reported cases for immunocompromised hosts is more than 500, compared with less than 20 for immunocompetent hosts. The numbers are likely higher for both groups, but currently available methods for diagnosis limit the ability to effectively screen for these organisms' presence. The diagnostic forms ("spores") are quite small (1 to 4 μm), but they are visible in histologic sections or stool specimens with the use of various stains and light microscopy.[51,52] Identification to the actual species level depends on electron microscopy, fluorescent antibody, or polymerase chain reaction (PCR) methods.[53-56]

Currently, four genera (*Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, and *Nosema*) have documented associations with human disease; a fifth designation (*Microsporidium*) is not a valid genus, but it is used for those isolates with insufficient characteristics (*Figure 4*). A fifth genus, *Septata*, was recently described as a human pathogen.[57,58] However, the use of PCR and other techniques has demonstrated its identity with *Encephalitozoon*.[59,60]

The unique biology of the microsporidian features a highly resistant spore form, which when ingested or possibly inhaled, will evert a polar tubule. The extruded tubule, upon contact with the host cell membrane, permits the infectious form to enter the cell. Although the process is not necessarily a true injection of the parasite's protoplasm, it certainly serves that function. Replication and reinfection of other cells then occur with the eventual production of the spore form and its release to the environment. Some species are associated with disseminated disease and the mechanism(s) for this process is not fully understood. A typical life cycle is shown in *Figure 5*.
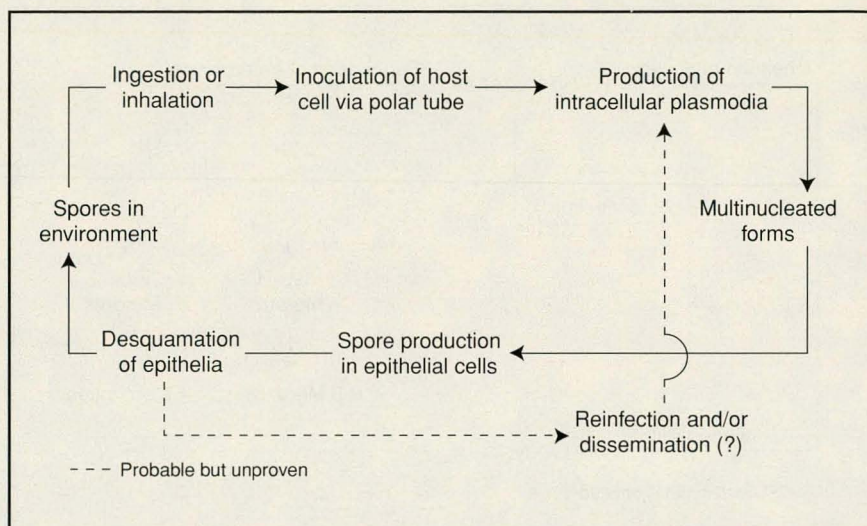

Figure 5. *The life cycle of a "typical" microsporidian.*

Clinical considerations
Coccidial infections
The forms of intestinal coccidiosis are similar clinically, regardless of the etiologic agent. The difference in presentation generally relates to the patient's immune status. The infection is self-limited in the immunocompetent host, but it is protracted in the immunocompromised host, particularly when the individual is HIV-positive.[20,38,61] The clinical manifestations of isosporiasis and cryptosporidiosis are indistinguishable.[38,62] The immunocompromised patient presents with chronic, profuse watery diarrhea; anorexia and weight loss commonly occur. Peripheral eosinophilia has been associated with isosporiasis but not with cryptosporidiosis.[38] This association is possibly spurious since concomitant helminthic infection may be present. Both infections will usually result in acute, self-limited intestinal diseases in immunocompetent hosts.[4,63] However, more protracted forms of these diseases have been described in apparently immunocompetent patients.[4,64] In eight outbreaks of cryptosporidiosis (consisting of a total of 221 patients), the duration of diarrhea ranged from 1 to 60 days with a median of 2 weeks.[4] Others have reported similar numbers,[65,66] but with possibly longer duration of symptoms in adults than in children.[66] In isosporiasis, duration of the infection is 6 weeks to 6 months.[12] In one exceptional case of isosporiasis, intermittent diarrhea of 20 years' duration and persistent eosinophilia were described.[67]

Sudden onset, associated malaise, and infrequent fever are useful clues for cyclosporiasis.[68] In a series of 20 immunocompetent patients with cyclosporiasis, the mean duration of illness was 35 days, with a range of 10 to 120 days[69]; in two Peruvian patients, the self-limited diarrhea lasted 3 to 4 weeks.[70]

Microsporidial infections
Unlike human intestinal coccidiosis, microsporidial infections of the gastrointestinal tract have been reported only in severely immunocompromised patients.[71-81] Certain genera are limited to very few tissues, while others can infect multiple organs and are associated with disseminated disease (*Table 2*). It appears that these organisms are rarely capable of causing intestinal disease in immunocompetent hosts, but it is also too early in our accumulation of knowledge to determine whether this situation will remain unchanged. Only one documented case due to *Enterocytozoon bieneusi* in an immunocompetent patient has been reported.[82] In that patient, the diarrhea (4 to 5 soft stools per day) was self-limited, resolving after 2 weeks. Nevertheless, the concept of emerging diseases related to increased travel opportunities and an increasing immigrant population warrants the need for clinical

awareness pertaining to both immuno-competent and immunodeficient hosts.

Documentation of risk groups is more clearly shown for cryptosporidiosis than for the other infections, with foreign travelers, animal handlers, children in day care centers, and immunocompromised patients (particularly AIDS patients) at greater risk. Based on our limited knowledge regarding the biology of the other related protozoans, it is reasonable to conclude that these same population groups are also at higher risk of infection with these other coccidian and microsporidian parasites. Certainly, the expanding list of etiologic agents for traveler's diarrhea must now include the agents described herein. The most reliable indicator for their omission from the differential diagnosis is absence of leukocytes or erythrocytes in the fecal specimen. Even though these protozoans are intracellular, they are not invasive in the immunocompetent host. The intracellular habitat does, however, stimulate a circulating antibody response, at least in the case of *C parvum* infections.[35,83-86] Although more sensitive procedures for diagnosis such as antibody detection and PCR are available, most laboratories will continue to rely on the classic light microscopic techniques for the forseeable future. Therefore, the clinician must be knowledgeable regarding these pathogens in order to alert the laboratory technologist to search for the possible presence of these microorganisms.

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