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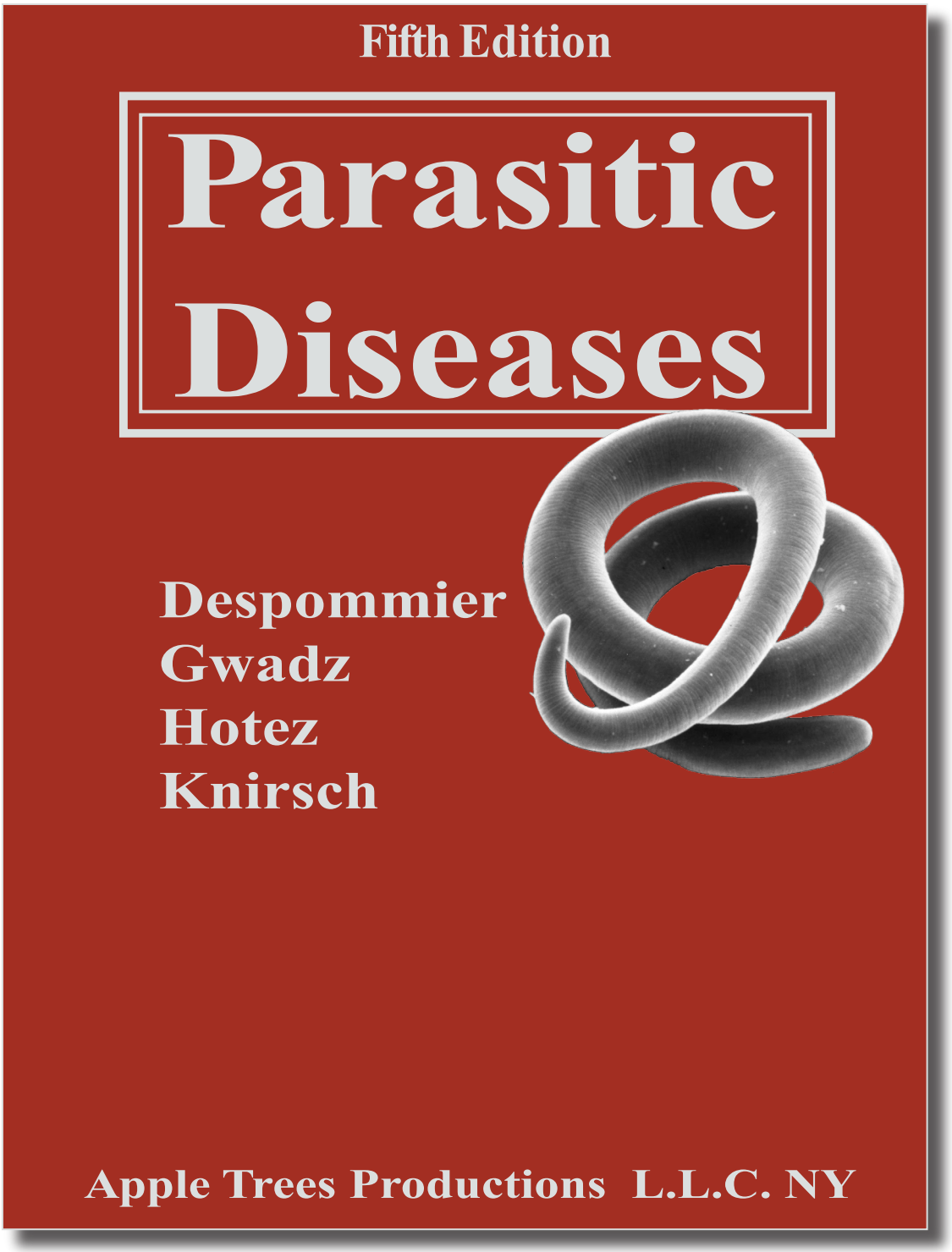


10. *Cryptosporidium parvum* (Tyzzer, 1929)

Introduction

The genus Cryptosporidium comprises a very large group of closely related obligate intracellular parasites that cause transient diarrheal disease in most mammal species throughout the world, including humans. All are transmitted through fecally contaminated food and water.[1,2,3] Most species have broad host ranges. Eight species have been shown to infect humans on a regular basis: *C. parvum*, *C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *Cryptosporidium* pig and cervine species. [4-10] The majority of human infections are caused by *C. parvum* (sometimes referred to as *C. hominis*), which also infects sheep, cattle, birds, rodents, and non-human primates. This chapter will concentrate on *C. parvum,* with the assumption that disease in humans caused by other related species gives a similar clinical picture. In 1993, the city of Milwaukee, Wisconsin experienced the largest waterborne outbreak of diarrheal disease ever documented in the United States. Over 400,000 people suffered from infection with *C. parvum*.[11] In immunocompetent infected individuals, the most serious manifestation of infection is diarrhea of short duration, although sometimes severe. In contrast, infants, non-AIDS immunocompromised adults, and people suffering from HIV/AIDS often experience severe, protracted diarrhea, sometimes resulting in death.[12] *C. parvum* can be grown axenically in vitro, using monolayers of epithelial cells.[13, 14] The genome of *Cryptosporidium hominis (parvum*) has been determined.[15, 16]

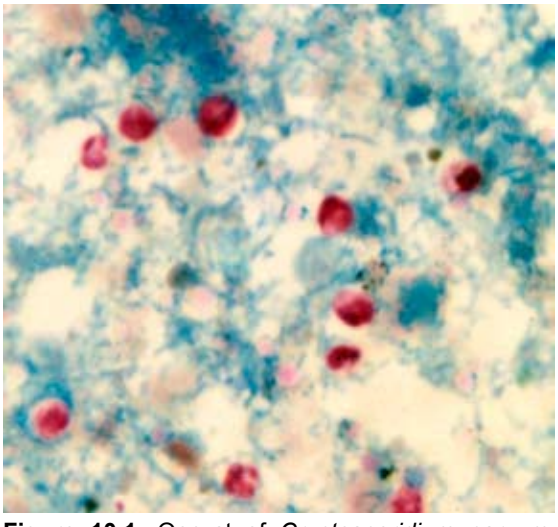


Figure 10.1. Oocyst of *Cryptosporidium parvum*. Cold acid fast stain. 5 µm.

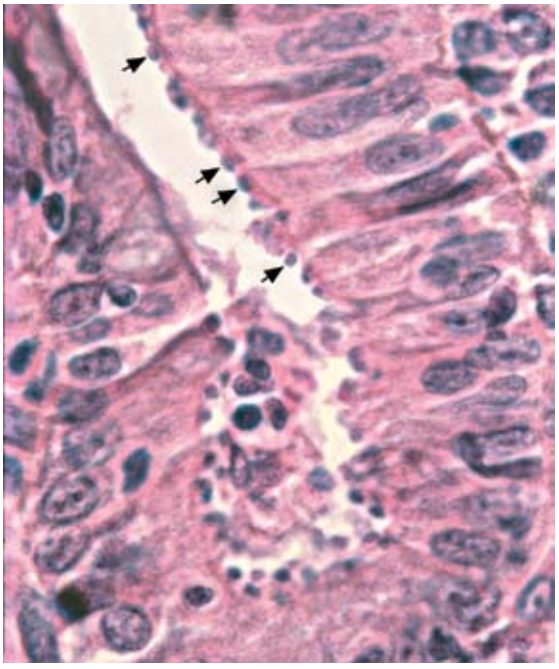


Figure 10.2. Histologic section of small intestine of patient suffering from HIV/AIDS, infected with *C. parvum* (arrows). Courtesy J. Lefkowitch.

Historical Information

Tyzzer, in 1907,[17] provided a description of Cryptosporidium based on histologic sections of mouse intestine, in which the parasites were observed attached to the epithelial cells. The pathogenic characteristics of Cryptosporidium were not recognized until much later, when Slavin, in 1955,[18] established that this protozoan caused diarrhea in turkeys. Nime and coworkers, in 1976,[19] described human diarrheal disease due to Cryptosporidium, and Meisel and colleagues, in 1976,[20] were the first to report it in immunocompromised human hosts.

Life Cycle

A comprehensive review of the biology of *C. parvum* is available.[21] Infection begins when the host ingests thick-walled sporulated oocysts (Fig. 10.1), each of which contains four sporozoites. A minimum of 30 oocysts are necessary to initiate infection,[22] while the calculated ID50 for healthy volunteers was 132 oocysts.[23]

The sporozoites excyst when the oocyst enters the small intestine. Little is known regarding excystment in vivo. A protein-plugged suture in the cyst wall blocks the escape route for sporozoites.[24] in vitro, excystment occurs after exposure to 37° C or by pretreatment of

purified oocysts with either sodium taurocholate and trypsin,[25] or with sodium hypochlorite (bleach) alone, followed by introduction into culture medium. Oocysts treated with bleach can be inhibited from excysting by exposure to human α-1-anti-trypsin inhibitor[26] or inhibitors of arginine aminopeptidase.[27] Like other enteric parasites with resistant outer structures (e.g., eggs of helminths and cysts of Giardia and Entamoeba), alteration of the outer surface may be a prerequisite for the organism to receive environmental cues, triggering the synthesis of enzymes of parasite origin required for emergence.

Sporozoites attach to the surface of epithelial cells (Fig. 10.2), most likely aided by numerous proteins secreted from their rhoptries and micronemes. A monoclonal antibody, designated 3E2, binds solely to the apical complex of the organism (the region where microneme- and rhoptre-specific proteins exit from the parasite), and inhibits invasion in vitro.[28] On Western Blot analysis, this antibody recognizes numerous epitopes, ranging from 46 kDa to 1300 kDa. Furthermore, a purified microneme-specific mucin-like 900 kDa glycoprotein can prevent invading parasites from attaching to their target cells when employed in competitive inhibition studies.[29]

After the sporozoite attaches to the cell surface, microvilli in the area immediately adjacent to the parasite fuse and elongate, enveloping the parasite to create a unique intracellular environment (Fig. 10.3). This event may also be triggered by apical end-associated secreted proteins. A specialized membrane structure develops at the interface between the parasite and the host cell. Nutrients are thought to pass through this region, since parasite-specific ABC transporters have been identified there by means of immunofluorescent monoclonal antibodies.[30]The sporozoite differentiates into the type I meront (Fig. 10.4) and division ensues, producing four haploid merozoites. The merozoites are released and attach to new epithelial cells, now differentiating into Type II meronts. Macrogamonts and microgamonts (pre-sex cells analogous to the gametocytes of plasmodia) are produced inside these new meronts. Following their release, microgamonts fuse with macrogamonts, forming thick-walled zygotes termed oocysts. This stage sporulates within the large intestine, and four haploid sporozoites are produced. Oocysts can also be thin-walled. In this case, they sporulate and excyst within the same host, producing an autoinfection that may endure for months to years. Even in these cases, however, thick-walled oocysts are produced as well.

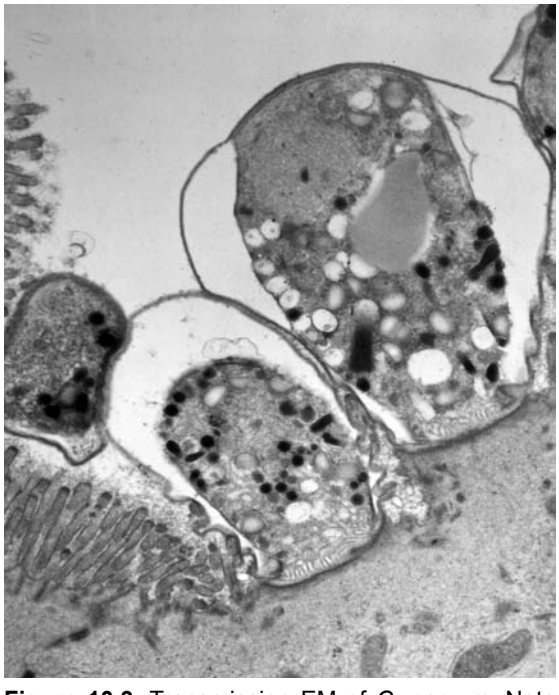


Figure 10.3. Transmission EM of *C. parvum.* Note microvillus-derived membranes encasing parasites (arrows). Courtesy J. Lefkowitch.

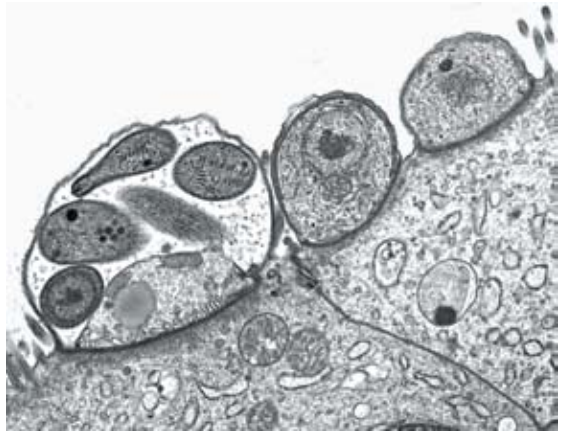


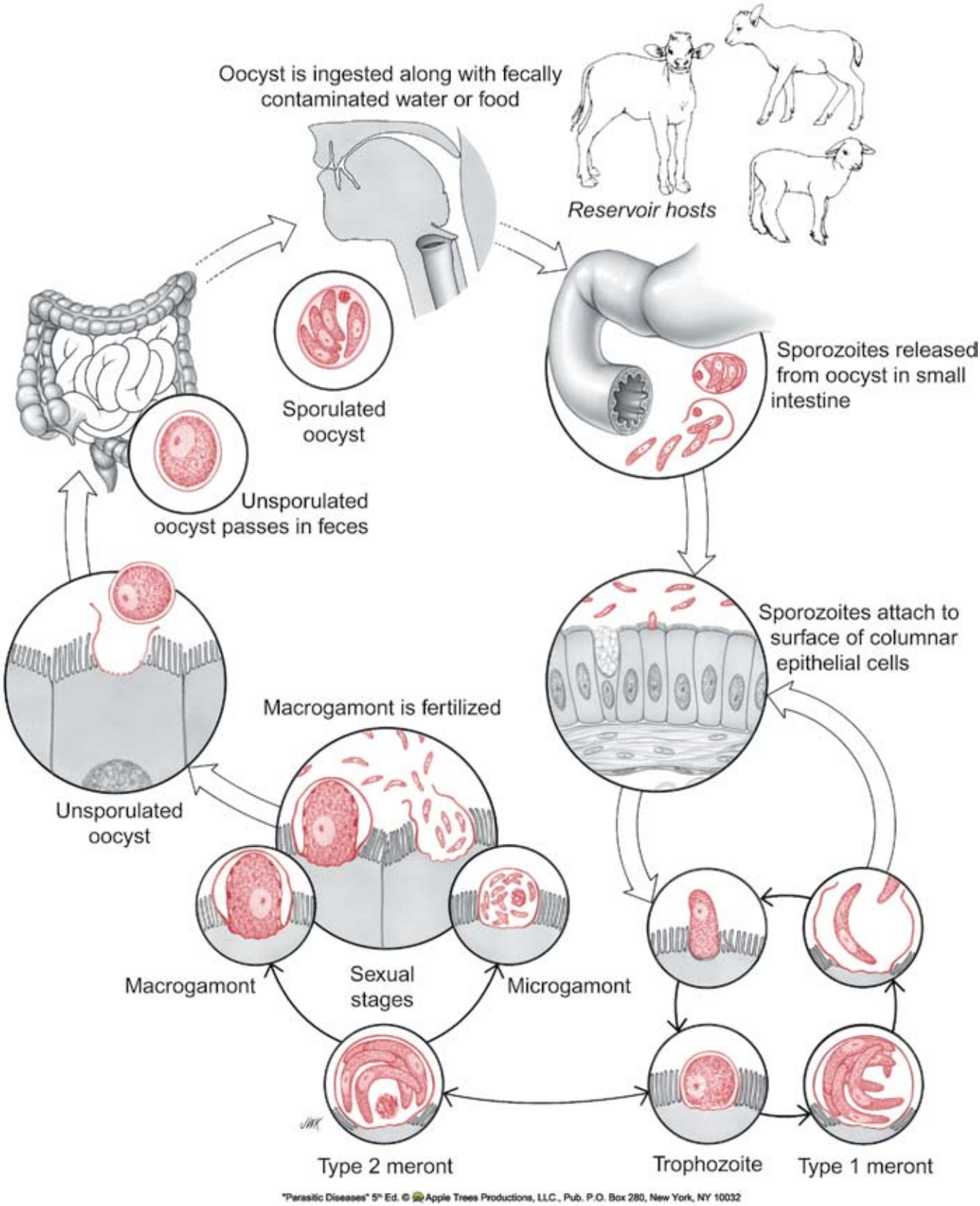
Figure 10.4. Transmission EM of *C. parvum* meronts. Courtesy M. Belosevic.

Thick-walled oocysts pass out in feces, and can infect another host. This type of oocyst is environmentally resistant, and can remain viable for months to years in soil, given optimum moisture conditions.[31]

Cellular and Molecular Pathogenesis

Until recently,[32] one of the most perplexing and frustrating aspects of the biology of *C. parvum* was its ability to avoid being affected by a wide variety of drugs.[33, 34] The altered microvillus-derived membrane complex that surrounds the parasites while they are attached to epithelial cells has proven highly impermeable to all chemotherapeutic agents, with the one possible ex-

Cryptosporidium parvum



ception, nitazoxanide. That is why speculation favors the entry of nutrients through the attachment zone between the parasite and the surface of the host cell. The fact that ABC transporters have been identified in this region[30] is further indirect evidence in support of this hypothesis. Cellular or molecular events that result in the alteration of microvilli at the site of attachment have attracted the attention of some research groups.[35] Apparently, Cdc42 (a GTPase) and actin are recruited to the site of attachment early on in the process.[36] Actin then aggregates, forming a kind of platform on top of which the organism then elaborates its complex of membranes. Much more needs to be learned about the mechanism(s) of nutrient acquisition by *C. parvum* before rational drug design aimed at interference with this process can evolve.

Protection against the primary infection develops in individuals whose immune systems are not compromised. At least two classes of antibodies, IgA[37] and IgG,[23] and several cellular-based immune mechanisms are thought to play important roles in the elimination of the parasite from the gut tract, although the precise mechanisms responsible for this have yet to be determined.[38] Healthy human volunteers whose anti-*C. parvum* IgG levels were already present (exposed, immune), required a higher dose of oocysts to become infected, and developed fewer symptoms than their non-exposed (non-immune) counterparts.[23] Studies carried out in experimental infections employing various strains of inbred mice have shown that IL-12,[39] gamma interferon,[38] and perhaps β-defensins,[40, 41] peptides chemically related to magainins,[42, 43] act in conjunction to protect against a challenge infection. Calves fed irradiated oocysts of *C. parvum* were protected from a challenge infection,[44] implying that protection-inducing antigens are present in this stage of the infection. Patients suffering from AIDS do not develop protective immunity. In underserved regions of the tropics, many children born with the HIV virus and who went on to develop AIDS are dying from this opportunistic infection.[45]

Clinical Disease

Two excellent reviews on the clinical aspects of cryptosporidiosis have been published.[46, 47] In immunocompetent individuals disease can vary from a mild to profuse watery diarrhea. Upper abdominal cramps, anorexia, nausea, weight loss, and vomiting are common features of the acute stage of the infection. In those who have already experienced clinical disease and recovered, a second infecting dose of oocysts may be asymptomatic, or they may have only a mild, transient diarrhea. Cryptosporidiosis is self-limited, lasting from several days to one month.

Children are the most severely affected group,[48] as the diarrhea lasts longer, and there is usually some weight loss. Those undergoing cancer chemotherapies suffer worse yet, with protracted, life-threatening diarrhea accompanied by significant weight loss.[49]

Cryptosporidiosis in patients suffering from AIDS is chronic, lasting months and even years, during which patients can lose more than three liters of fluid each day, and are in significant danger of dying; the case fatality rate is 50%. However, death is usually a result of associated conditions, such as malnutrition or superinfection with other pathogens. Extraintestinal infection in the bile duct can cause acalculous biliary disease.

Diagnosis

Definitive diagnosis depends upon two approaches: identification of acid fast-stained oocysts (Fig. 10.1) by microscopy of stool samples[50] and PCR.[51] The latter test can identify cryptosporidium down to the species level. Oocysts are easily isolated from stool by flotation in sugar solution,[52] then stained by acid-fast methods, or used in the IFA test.

Treatment

Nitazoxanide is the drug of choice.[32, 53] Although use of this drug has been limited, so far it appears to be not effective when used to treat infections in HIV/AIDS patients.

Prevention and Control

Without knowledge as to the source of a given outbreak, control and prevention of infection due to *C. parvum* is not possible. In the case of waterborne epidemics,[11] management of watersheds[54] is the long-term solution in situations where the water supply is not filtered. Filtering drinking water is usually effective, but deterioration of filtration equipment and/or lack of proper maintenance can erode any progress made in controlling waterborne infections.[55] Chlorination of water supplies is ineffective against the oocyst, but ozonation kills this stage.[56, 57] In agricultural settings, creation of vegetative barriers to curtail the spread of oocycts is effective.[58] Surveillance is key to keeping public water supplies free of pathogens with environmentally resistant stages (e.g., *Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum*). In this regard, PCR-based testing now allows for the possibility of continuous monitoring of water supplies for *C. parvum*.[59] Urban and suburban pet stores and petting zoos for children are other sources of infection that until very recently have received little attention.

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