

## Review Article

**NEWLY RECOGNIZED PATHOGENS in AIDS  
and NON-AIDS PATIENTS:  
CRYPTOSPORIDIUM, CYCLOSPORA, MICROSPORIDIA**

(Received, 5 May 1997)

**U. Över, Ph.D.\*\* / G. Söyletir, M.D.\***

\* Professor, Department of Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey.

\*\* Specialist, Department of Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey.

**INTRODUCTION**

Although Protozoa are well known cause of worldwide diarrhea in immunocompetent and immunocompromised patients, certain genera have emerged as human pathogens in the last decades especially after AIDS epidemic (1-2). In this review it is our aim to call attention of physicians and clinical microbiologists to the following protozoa that can cause diarrhea with increasing tendency.

*Cryptosporidium parvum* causes acute and selflimited diarrhea in immunocompetent patients but chronic and life-threatening diarrhea in immunocompromised patients (1-3). *Cyclospora* is now well established as a cause of traveler's diarrhea and endemic disease in developing countries (1). *Microsporidia* species are being recognized in many cases of previously undiagnosed diarrhea (1).

**CRYPTOSPORIDIUM**

Cryptosporidiosis is now acknowledged as an important cause of diarrhea in humans (1-4). Currently it is evident that *cryptosporidium* infects gastrointestinal and respiratory epithelium in humans and other animals, causing severe and sometimes fatal diarrhea; especially in the immunocompromised host (4).

*Cryptosporidium* was first described in 1895 by Clark as swarm spores in the gastric epithelium of mice. And then Tyzeer described *Cryptosporidium muris* in the gastric glands of mice, and *Cryptosporidium parvum* in the small intestine of mice in 1907 and 1912 respectively (1,2,4).

Human infection with *cryptosporidium* was not described until 1976 but described in an

immunocompetent child and immunodeficient patient on this date (1). By 1982 several outbreaks of cryptosporidial diarrhea were reported in both AIDS patients and from a cluster of immunocompetent veterinary students who had acquired the infection via handling infected calves (2,4).

**Biology**

*Cryptosporidium* is an apicomplexan protozoan parasite grouped with coccidia and related to such protozoans as *toxoplasma*, *isospora*, *eimeria* and *sarcocystis* (1,4,5). Similar to *toxoplasma*, *cryptosporidium* is not host specific and infects a wide range of mammals (4).

The genus of *cryptosporidium* include probably seven species and two of them (*C.muris*, *C.parvum*) infect mammals and the others (*C.baileyi*, *C.meleagridis*, *C.wrairi*, *C.serpentis*, *C.nasorum*) infect avian and reptiles (1,2). Thus, *C.muris* is found in the stomach of mice and *C.parvum* causes diarrheal disease or asymptomatic infection in humans and many other mammals (1,4).

The organism resides in a parasitophorous vacuole close to the microvillus border of the host cell (2-4). The life cycle of this parasite is similar to that of other true coccidia (e.g. *Eimeria* and *Isospora* spp) infecting mammals in that it can be divided into six major developmental events: (a) excystation, the release of infective sporozoites; (b) merogony, the asexual multiplication within host cells; (c) gametogony, the formation of microgametes and macrogametes; (d) fertilization, the union of microgametes and macrogametes; (e) oocyst wall formation to produce an environmentally resistant stage that transmits infection from one host to another; and (f) sporogony, the formation of infective sporozoites within the oocyst wall (3).

The mature oocysts of *C. parvum* are infectious when passed in the feces and are about 4 to 5  $\mu$ m. Approximately 20% of the oocysts of *C. parvum* within the host enterocysts do not form a thick oocyst wall. The four sporozoites of this autoinfective stage are surrounded only by a single unit membrane. The presence of autoinfective, thin-walled oocysts and type I meronts are believed to be the life cycle features of *C. parvum* responsible for the development of severe infections in hosts exposed to only a small number of thick-walled oocysts and for persistent life-threatening disease in immunodeficient persons who are not exposed repeatedly to these environmentally resistant forms (2,3).

### Epidemiology

*Cryptosporidium* species are transmitted by ingestion of oocysts excreted in the feces of humans and animals (6). Zoonotic transmission of the parasite has been well documented (1, 7, 8). Calves, cats and dogs transmit *C. parvum* to humans, however; person to person transmission by fecal - oral route is also considerable way of transmission (1 - 3). Water contamination is considered the most important mode by which *Cryptosporidium* is transmitted (6,7). Recent studies indicate that *Cryptosporidium* oocysts are present in 65% - 97% of surface waters (rivers, lakes, etc.) tested throughout the USA (6). Contaminated water has been implicated in several community outbreaks of cryptosporidiosis in the United States and the United Kingdom and is a well-recognized cause of traveller's diarrhea and nosocomial infection in elderly patients (7,9-14).

Eradication of *Cryptosporidium* from water supplies is problematic, as the oocysts are resistant to the usual concentrations of the common disinfectants (2-4). The usual purification procedures (chlorination or ozonation) apparently are ineffective in sterilizing *Cryptosporidium* oocysts. Oocysts are 30 times more resistant to chlorine than cysts of *giardia* (2).

Cryptosporidial infection has been described in both immunocompromised and immunocompetent hosts. Infection is very common in children younger than 2 years, but the true prevalence of cryptosporidial infection in various populations is unknown (2-4). Many prevalence studies have shown that cryptosporidiosis occurs more frequently in underdeveloped countries and is the most frequently reported enteric protozoan parasite. The infection rate for cryptosporidial diarrheal disease is estimated 1-4.3 % in developed countries, whereas it is about 3-20 % in underdeveloped countries (1,2,4,7). Although there are limited prevalence studies in Turkey, the cryptosporidial infection rate in children has been found to be higher than that in adult population (15-

18). In our hospital we found that twelve of 480 diarrheal patients (2.5%), were positive for *Cryptosporidium* oocysts in their stool samples and this rate reached to 9.5% in children under 3 years of age (15). Hospital based studies indicate that cryptosporidiosis is diagnosed for 10 - 20 % of AIDS patients with diarrhea (1,2,4,6).

### Clinical Disease

The spectrum of clinical illness with cryptosporidial infection ranges from asymptomatic carriage of oocysts to life - threatening cholera - like enteritis complicated by biliary tract involvement (4,7). In immunocompetent persons, it is manifested as an acute, self limiting diarrheal illness lasting 7-14 days, and is often accompanied by nausea, abdominal cramps and low - grade fever. However in patients with AIDS cryptosporidiosis is generally chronic and more severe; the voluminous watery diarrhea is often debilitating and may be accompanied by severe abdominal cramps, weight loss, anorexia, malaise and low - grade fever (6,10,19).

A distinctive feature of cryptosporidiosis is the recurrence of diarrhea as observed in the Milwaukee outbreak (10). After a clinical course of diarrhea, immunocompetent persons, shed the oocysts for 7 days, and there may be an intermittent shedding of oocysts asymptotically for up to 2 months. Therefore, one possible explanation for recurrence of symptoms in humans may be related to the presence of a specific stage of the protozoan or the expression of a specific pathogenic factor (10).

After an incubation period of 2 to 14 days cryptosporidial enteritis begins with frequently watery nonbloody stools with volumes up to 25 L/d. Chronic diarrhea often leads to profound weight loss (7).

Malabsorption and dehydration can cause serious problems in immunocompromised patients. The severity of symptoms seems to correlate with quantitative levels of oocyst excretion. The gall bladder and biliary tract can become involved causing sclerosing cholangitis (1 - 3, 20).

Pulmonary and pancreas involvement with *Cryptosporidium* has also been reported, but whether the organism is a pulmonary pathogen or a respiratory tract colonizer is unclear (7, 21).

### Diagnosis

Identification of the oocysts in stool samples is still the best diagnostic method for symptomatic patients (1, 2, 4, 7). Three or more specimens are sometimes needed as oocyst secretion varies from day - to - day



(1, 2, 22). Stool concentration techniques that are useful for the diagnosis of cryptosporidiosis include flotation of oocysts in Sheather's sugar solution, zinc sulfate or saturated sodium chloride. The other stool concentration techniques using sedimentation include formalin - ether and formalin - ethyl acetate (2, 3, 7).

Acid - fast staining is the most effective and convenient method for identification of the oocysts. Many other staining methods have been used including fluorescent stains (Fig 2.) (1 - 4, 7). Immunofluorescent staining with a commercial monoclonal antibody is used with good sensitivity and specificity as are EIA kits for the detection of oocysts in the stool (23-26). Specificity has become an important issue in view of recent data indicating that *Cyclospora*, also acid fast, can be mistaken for *Cryptosporidium* by the inexperienced microscopist (4,7).

Presently, serologic diagnosis is not helpful but may be useful in detecting those who have had exposure to *cryptosporidium* and for seroepidemiologic studies (1,2,4,7,27-29).

## Treatment

Intestinal infection with *Cryptosporidium* in the immunocompetent patients is frequently associated with acute - self-limiting diarrhea and does not require specific treatment (1-4,7). Sometimes, nonspecific antidiarrheal agents such as loperamide (imodium), diphenoxylate (Lomotil), bismuthsubsalicylate (pepto-bismo) are used for symptomatic therapy in these patient population (1-4,7,30). Unfortunately the treatment of cryptosporidiosis in immunocompromised patients can be an extremely vexing problem that responds to few treatment modalities. To date there is no known specific therapy for cryptosporidiosis. Although there are limited numbers of in vitro models for screening drugs, over 100 agents have been assessed for efficacy in cryptosporidiosis and none of them has success for complete eradication of oocysts from the host (1-4,7,31-35).

Initially, attention was focused on the macrolide spiramycin because of anecdotal reports of success with this agent. But further studies demonstrated the high rate of adverse effects of this drug (4,7,36).

Paromomycin, a poorly absorbed oral aminoglycoside antibiotic appears to be well tolerated and has provided symptomatic relief in a subset of HIV-infected individuals, at a total dose of 2 g/d (37-43). Azithromycin in high doses also appears to be effective both in animal models and early human trials. Azithromycin should be obtained through

Pfizer's compassionate program because the preparation that is used for cryptosporidiosis does not contain lactose and is different from commercially available azithromycin (zithromax) (44).

In addition it has been shown that antibodies to cryptosporidial antigens in the form of hyperimmune bovine colostrum and dialyzable leukocyte extract (immune DLE) prepared from the lymph node cells of calves immunized with *C.parvum* have provided significant relief of diarrhea in some AIDS patients as well as in animal studies (2,3,45). Additional studies are needed to confirm the observation noted in these patients and to determine whether such treatment results in augmentation of cellular immunity to *C.parvum* (46).

Despite significant progress over the past decade, much still needs to be learned about this enigmatic parasite. Clinically, the most important priority is to identify efficacious therapy. For this, we need to develop useful in vitro and in vivo models that will help in understanding mechanisms of immunopathogenesis and thus provide a rational basis for designing useful therapeutic agents.

## CYCLOSPORA

As with *cryptosporidium*, *cyclospora* is now well established as a cause of diarrhea in humans affecting children and adults, including HIV-infected patients. Since 1986, there have been a number of reports linking diarrheal illness in both immunocompetent and immunocompromised hosts to an acid-fast organism resembling a "large *cryptosporidium*" (7, 47). This organism has been called a cyanobacterium (blue- green algae) and a coccidian - like body (CLB), but recent ultrastructural data suggest that it belongs to the genus *Cyclospora* (7, 48-52).

## Biology

Ortega and colleagues have assigned the organism to the coccidian genus *Cyclospora* on the basis of its sporulation characteristics (1, 7, 51,52). Members of the genus *Cyclospora* have two sporocysts per oocyst and two sporozoites per sporocyst (51,52). It is sometimes referred to as *Cyclospora cayetanensis* derived from that of the institution where much of the original epidemiological and taxonomic research was done.

Organisms are excreted in the stool in the form of oocysts. Fecal leukocytes are not seen in stool preparations. Oocysts are wrinkled spheres measuring 8-10 microns in diameter and resemble

large cryptosporidia (51,52). Complete sporulation with the appearance of sporozoites within sporocysts, occurs between days 7 and 12 in culture (51,53).

### **Epidemiology**

In prospective cohort studies in Lima, Peru, 6-18 % of children were found to be infected with *Cyclospora*, but only 22 % of these were symptomatic (51). However, populations in endemic areas may be less susceptible to the organism, with high ratios of asymptomatic carriers to symptomatic patients, and non endemic populations have a 100 % attack rate (53). *Cyclospora* has been discovered in immunocompetent adults, and children and in individuals with human immunodeficiency virus infection (48,50-52,54)

*Cyclospora* is now a well accepted cause of traveler's diarrhea (7,52,55,56). Although a study of 6525 stool specimens in Chicago hospital microbiology laboratory revealed the organism in 34 (0,2 %) specimens, including 20 specimens from the hospital dormitory outbreak, the prevalence of *Cyclospora* infection is not well known (7,50,52).

Contaminated water is a common source of infection and sewage has been reported as the source in an immunocompetent patient in the USA (50, 52, 55,57). The peak seasonality of infection is between April and September (7, 50, 52,55 )

### **Clinical Disease**

Recent studies have revealed *Cyclospora* is associated with a self-limited diarrheal illness of 2 to 6 weeks duration in immunocompetent hosts and chronic intermittent diarrhea in immunocompromised patients, specifically patients with HIV infection (7, 50-52).

The incubation period of illness is between days 1 and 11 (52,55). Except for prolonged intermittent diarrhea, the clinical presentation of patients infected with this organism was quite similar to the presentation of those infected with *C. parvum* (51). Clinical illness is characterized by watery diarrhea, often accompanied by extreme fatigue, anorexia, abdominal pain, bloating, flatulence and weight loss. (7,50,52,53,58). Most patients usually do not have fever although the latter has been reported (52). Hoge et al. reported a median of six stools/day per patient (51). The mean duration of shedding of the organism by children in Peru was

23 days (51). In the Chicago outbreak, the acute episodes of diarrhea, nausea and vomiting lasted a mean of 6.6 days, but patients had relapsing diarrhea

for several weeks following the appearance of the initial symptoms (52).

### **Diagnosis**

Currently, microscopic examination of a stool sample is the only way to diagnose *Cyclospora* infection (7,51,52). *Cyclospora* oocysts are round, acid fast and may be distinguished from *Cryptosporidium* by their larger diameter (8 to 10 mm) (1,7,47,51,52).

In unstained wet mounts, they appear as nonrefractile spheres containing globular inclusions (51). *Cyclospora* may also be identified by its ability to autofluorescence when exposed to UV illumination (49).

*Cyclospora* organisms can be concentrated by centrifugation in formalin - ethyl acetate or by sucrose flotation in Sheather's solution. Formalin preserved organisms are acid fast, staining with both Kinyoun and Ziehl-Neelsen methods but staining best with the modified carbol - fuchsin technique that is used to stain *Cryptosporidia*. Organisms stain variably; the stain color ranges from unstained to mottled pink to deep red (1,7,47,51,52).

### **Treatment**

Preliminary studies suggest that trimethoprim - sulfamethoxazole (TMP - SMX) is efficacious in the treatment of *Cyclospora* infections (1,50,59). Wurtz et al. reported one HIV-positive patient whose symptoms resolved following a course of oral TMP - SMX (50). Madico and colleagues reported treating five patients with oral TMP - SMX at a dosage of 5/25 mg/(kg.d) (59).

### **MICROSPORIDIA**

Microsporidia are very primitive eukaryotic, obligate intracellular protozoans, infecting every major animal group, especially insects, fish and mammals (7,60). The majority infect the digestive tract but reproductive, respiratory, excretory, and nervous system infections are well documented as are infections of connective tissue and muscle (4,7,60). Between 1959 and 1989, six cases of microsporidiosis were reported in humans. Since 1985 over 100 cases of microsporidiosis have been documented in patients with AIDS (7,60,61). There is now an increasing recognition of microsporidia as important opportunistic pathogens in AIDS patients (1,4,7,60). As with cryptosporidiosis, it is likely that microsporidiosis is a common infection in humans but is self - limited or asymptomatic in normal hosts.



## Biology

In the Protozoa subkingdom, the phylum Microspora consisting of approximately 90 genera and close to 1000 species are commonly referred to as microsporidia, and the disease they produce is known as microsporidiosis (4,7). Microsporidia belonging to five genera have been identified as infecting variety of organ systems in humans (Table I) (1,4,7,60,61).

**Table I.** Organ involvement with various species of microsporidia

Species	Organ involvement
Enterocytozoon bienewisi	GI, SP
Septata intestinalis	GI, UT, disseminated
Nosema spp.	E, disseminated
Pleistophora	M
Encephalitozoon cuniculi	GI, UT, E, CNS, disseminated
Encephalitozoon hellem	E, UT, SP, disseminated

GI; gastrointestinal tract including liver and gall bladder, SP: sinopulmonary involvement; UT: urinary tract, E: eye, CNS: central nervous system, M: muscle

In addition, the generic term microsporidium is often used for organisms in which sufficient material is unavailable to allow the establishment of the correct taxonomic name (4).

Microsporidian spores range from 1-20  $\mu\text{m}$  in diameter and are usually ovoid or piriform. The genera infecting mammals tend to be 1 to 2  $\mu\text{m}$  (4, 7, 62). Molecular biologic studies of microsporidian organisms have focused on rRNA structure and pointed out that nucleated spores possess prokaryote-like ribosomal RNA but lack mitochondria and golgi (4, 7, 63). All microsporidian spores contain a characteristic coiled polar tube. The number of coils varies among the different microsporidia, but all use this structure to invade host cells. The sporoplasm (nucleus) is then injected from the spore through the polar tube into the enterocyte, thus initiating infection. The organism replicates inside the enterocytes, within a parasitophorous vacuole and eventually causes cell lysis with dissemination of new spores either throughout the host or into the environment. Spores that are released into the environment can remain viable for up to 4 months (7). Classification of the various microsporidian species is based on spore size, arrangement of the nucleus, and mode of replication within the host cell.

## Epidemiology

The true prevalence of infection has yet to be defined. Prior to AIDS epidemic, it occurred mainly in patients who travel or reside in tropical areas (4,7). There have several reports on human infections with

microsporidia in non- AIDS patients since 1973 (4). In these infections various species of this protozoon were identified from different body sites such as lungs, stomach, small and large bowel, kidneys, adrenal glands, liver, cornea, urine (4). Recently, numerous cases of microsporidian keratitis have been identified from immunologically normal patients (61).

Various genera mostly *Enterocytozoon bienewisi* have been reported in HIV-infected patients throughout the United States, Europe, Africa, and South America with increasing frequency, with prevalence rates as high as 15 % to 33 % among patients with chronic diarrhea (64-68). Molina et al. found that nine of the 18 patients with AIDS had infections of the small intestine with *E. bienewisi*. Although the majority of microsporidian cases were intestinal infection in AIDS patients, cases of hepatitis, peritonitis, keratopathy have been described (4).

Little is known about transmission. Ingestion of contaminated feces or urine is believed to be important, and direct inoculation has been implicated in corneal infection (7).

## Clinical Disease

Non-AIDS patients most often have ophthalmologic or central nervous system involvement (4, 7). The majority of HIV infected individuals develop microsporidian enteritis clinically indistinguishable from cryptosporidiosis (7). The majority of these microsporidian enteritis have been identified as *E. bienewisi* (4). A diagnosis of small intestinal microsporidiosis should be suspected in any HIV-infected patient with AIDS and chronic, watery, nonbloody diarrhea without fever (65). In addition to intermittent or persistent diarrhea exacerbated by oral intake, abdominal cramping, bloating and anorexia are common (4, 7).

AIDS patients with microsporidiosis have usually < 100 CD4 cells / mm<sup>3</sup> in their peripheral blood. Recent studies pointed out that microsporidiosis occurred in patients with severe immune deficiency. Thus small intestine microsporidiosis seems to be a late-occurring opportunistic infection in patients with AIDS (1,64, 68,69).The mean duration of symptoms change 6.7 to 12.3 months in several studies. In these studies the mean stool frequency have been found to be 5.7 to 7 per day (68,70,71). Intestinal microsporidiosis in AIDS patients is associated with malabsorption, as evidenced by abnormal D-xylose tests and low vitamin B12 levels (4, 7,66, 68).

Microsporidian invasion of the gallbladder and liver have been reported, and *E. bienewisi* has been found in the biliary tract of HIV-infected patients with

cholangitis (1, 7, 68,72). Clinical manifestations of microsporidial cholangitis are similar to those described for cryptosporidial cholangitis, and, in fact, many patients have co-infection (7, 72,76).

Pulmoner infection with *E. bienewisi* has been diagnosed by bronchoalveolar lavage and transbronchial lung biopsy in one patient with AIDS and intestinal microsporidiosis (7). *Encephalitozoon cuniculi* and *Encephalitozoon hellem* have been found both AIDS and non-AIDS disseminated infection (intestine, bladder, eye, liver, and peritoneum) (4, 7, 73).

*Septata intestinalis* commonly infects the gastrointestinal tract but may also disseminate to distant sites, including the gallbladder, kidneys, urinary bladder, liver and lungs (4, 7).

## Diagnosis

Although the precise diagnosis of microsporidiosis requires biopsy and transmission electron microscopic study of tissue stages and spore ultrastructure, general diagnosis can be made by light microscopy of both Giemsa stained smears and standard hematoxylin/eosin stained paraffin sections prepared from distal duodenal biopsy specimens (1,4,7,63,64). But light microscopic detection of Microsporidia in paraffin sections of mucosal biopsy tissue is difficult because the organisms are small and poorly stained with routinely used histologic stains (4).

Diagnosis of *E. bienewisi* infection from stool examination is also difficult due to the small size of the spores. Recently a new modified trichrome technique for identifying microsporidia in stool samples has been reported (4,7,62,67,74). Zhu et al. have cloned and sequenced the rRNA of *E. bienewisi* and defined polymerase chain reaction primers based on this rRNA. Such probes may also be useful for diagnosis from stool specimens of infected patients (63).

Diagnosis of corneal infections in patients with AIDS has been technically less difficult. Microscopic examination of corneal tissue has revealed multiple gram positive, oval organisms within epithelial cells (4). Immunofluorescent staining of conjunctival scrapings with an *Encephalitozoon hellem* antisera has been used for diagnosis (75).

## Treatment

There is no known effective therapy for microsporidiosis (4,7). Anecdotal reports suggest that metranidazole induces a transient clinical response in

some patients (4,64,76). In one of these reports, 13 patients with microsporidiosis were treated with metranidazole (500 mg three times a day), in 10 of whom treatment led to a substantial improvement or disappearance of diarrhea within days of starting therapy, but did not result in eradication of the parasite in 5 patients who underwent repeat biopsy (64).

More consistent results have been achieved with the broad-spectrum antiparasitic agent, albendazole (68,76). Although treatment with albendazole led to a significant clinical improvement in some patients, *E. bienewisi* was still present in biopsy specimens of the small intestine obtained after treatment (7,76).

Few treatment options exist for corneal infections. *Encephalitozoon hellem* has been reported to respond to topical Fumagillin. There have also been anecdotal reports of successful therapy of ocular microsporidial infection with itraconazole, and fluconazole (4,7,77).

## REFERENCES

1. Adal KA. From Wisconsin to Nepal: *Cryptosporidium*, *Cyclospora*, and *Microsporidia*. *Curr Opin Infect Dis* 1994; 7: 609-615.
2. Current WL, Garcia LS. *Cryptosporidiosis*. *Clin Microbiol Rev* 1991; 4: 325-358.
3. Current WL, Garcia LS. *Cryptosporidiosis*. In: Gutierrez Y, Little MD, eds. *Diagnosis of Important Parasitic Diseases*. *Clin Lab Med* 1991; 11: 873-897.
4. Wittner M, Tanowitz HB, Weiss LM. *Parasitic infections in AIDS patients: Cryptosporidiosis, Isosporiasis, Microsporidiosis, Cyclosporiasis*. *Infect Dis Clin North Am* 1993; 7: 569-586.
5. Garcia LS. *Classification of human parasites, vectors, and similar organisms*. *Clin Infect Dis* 1995; 21: 281-282.
6. Juranek DD. *Cryptosporidiosis: Sources of infection and guidelines for prevention*. *Clin Infect Dis* 1995; 21 (Suppl 1): 57-61.
7. Mannheimer SB, Soave R. *Protozoal infections in patients with AIDS. Cryptosporidiosis, Isosporiasis, Cyclosporiasis, and Microsporidiosis*. *Infect Dis Clin North Am* 1993; 7: 569-586.
8. Lengerich EJ, Addiss DG, Marx JJ, Ungar BLP, Juranek DD. *Increased exposure to Cryptosporidia among dairy farmers in Wisconsin*. *J Infect Dis* 1993; 167: 1252-1255.
9. Hayes EB, Matte TD, O'Brien TR, et al. *Large community outbreak of Cryptosporidiosis due to contamination of a filtered public water supply*. *N Engl J Med* 1989; 320: 1372-1376.



10. Mac Kenzie WR, Schell WL, Blair KA, et al. Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: Recurrence of illness and risk of secondary transmission. *Clin Infect Dis* 1995; 21: 57-62.
11. Soave R. Editorial Response: Waterborne *Cryptosporidiosis*-Setting the stage for control of an emerging pathogen. *Clin Infect Dis* 1995; 21: 63-64.
12. Gatti S, Cevini C, Bruno A, Bernuzzi AM, Scaglia M. *Cryptosporidiosis* in tourists returning from Egypt and the Island of Mauritius. *Clin Infect Dis* 1993; 16: 344-345.
13. Sterling CR, Seegar K, Sinclair NA. *Cryptosporidium* as a causative agent of traveler's diarrhea. *J Infect Dis* 1986; 153: 380-381.
14. Neill MA, Rice KS, Ahmad NV, Flanigan TP. *Cryptosporidiosis*: An unrecognized cause of diarrhea in elderly hospitalized patients. *Clin Infect Dis* 1996; 22: 168-170.
15. Över U, Söyletir G. İshalle seyreden hastalıklarda Kriptosporidyumun rolü. *Flora İnf Hast Kli Mikrobiyo Derg* 1997; 2:98-104
16. Akşehirli ŞG. Kayseri' de 0-5 yaş grubu ishallerde çocuklarda *Cryptosporidium*' un araştırılması. Erciyes Üni Sağlık Bil Enst Mik. Anabilim Dalı-Doktora Tezi. 1995
17. Mülazımoğlu L, Vahaboğlu H, Görgün Ö, Yıldırım İ, Semerci E, Taşer B. Beş yaş altı çocuklarda *Cryptosporidium* Sıklığı. *Türk Mikrobiyol Cem Derg* 1988; 157:551-556
18. Fındık D, Karabayraktar A. Gaita örneklerinde *Cryptosporidium* oocistlerinin araştırılması. *T Parazitoloj Derg* 1994; 18:418-419
19. Newman RD, Zu SX, Wuhib T, Lima AAM, Guerrant RL, Sears CL. Household epidemiology of *Cryptosporidium parvum* infection in urban community in Northeast Brazil. *Ann Intern Med* 1994; 120: 500-505.
20. Smith PD, Quinn TC, Strober W, Janoff EN, Masur H. Gastrointestinal infections in AIDS. *Ann Intern Med* 1992; 116: 63-77.
21. Hawkins SP, Thomas RP, Teasdale C. Acute Pancreatitis: A new finding in *Cryptosporidium* enteritis. *Brit Med J* 1987; 294: 483-484.
22. Clavel A, Arnal AC, Sanchez EC, et al. Evaluation of the optimal number of faecal specimens in diagnosis of *Cryptosporidiosis* in AIDS and immunocompetent patients. *Eur J Clin Microbiol Infect Dis* 1995; 14: 46-48.
23. Arrowood MJ, Sterling CR. Comparison of conventional staining methods and monoclonal antibody-based methods for *Cryptosporidium* oocyst detection. *J Clin Microbiol* 1989; 27: 1490-1495.
24. Baron EJ, Schenone C, Tanenbaum B. Comparison of three methods for detection of *cryptosporidium* oocysts in a low-prevalence population. *J Clin Microbiol* 1989; 27: 223-224.
25. El-Shewy K, Kilani RT, Hegazi MM, Makhlof LM, Wenman WM. Identification of low-molecular-mass coproantigens of *Cryptosporidium parvum*. *J Infect Dis* 1994; 169: 460-463.
26. Goodgame RW, Genta RM, White AC, Chappell CL. Intensity of infection in AIDS-associated *Cryptosporidiosis*. *J Infect Dis* 1993; 167: 704-709.
27. Ungar BLP, Soave R, Fayer R, Nash TE. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *Cryptosporidium* in immunocompetent and immunocompromised persons. *J Infect Dis* 1986; 153: 570-578.
28. Ungar BLP, Gilman RH, Lanata CF, Perez-Schael I. Seroepidemiology of *Cryptosporidium* infection in two Latin American populations. *J Infect Dis* 1988; 157: 551-556.
29. Kuhls TL, Moiser DA, Crawford DL, Griffiths J. Seroprevalence of *Cryptosporidial* antibodies during infancy, childhood, and adolescence. *Clin Infect Dis* 1994; 18: 731-735.
30. Soave R, Weikel C. *Cryptosporidium* and other protozoa including *Isospora*, *Sarcocystis*, *Balantidium coli*, and *Blastocystis*. *Infectious Disease and Their Etiologic Agents*
31. McDonald V, Stables R, Warhurts DC, et al. In Vitro cultivation of *Cryptosporidium parvum* and screening for anticryptosporidial drugs. *Antimicrob Agents Chemother* 1990; 34: 1498-1500.
32. Rohlman VC, Juhls TL, Mosier DA, et al. Therapy with atovaquone for *Cryptosporidium parvum* infection in neonatal severe combined immunodeficiency mice. *J Infect Dis* 1993; 168: 258-260.
33. Laughon BE, Allaudeen HS, Becker JM, et al. Summary of workshop on future directions in discovery and development of therapeutic agents for opportunistic infections associated with AIDS. *J Infect Dis* 1991; 164: 244-251.
34. Lemeteil D, Roussel F, Favennec L, Ballet JJ, Brasseur P. Assessment of candidate anticryptosporidial Agents: an Immunosuppressed rat model. *J Infect Dis* 1993; 167: 766-768.
35. Rehg JE. Anticryptosporidial activity of lasalocid and other ionophorus antibiotics in immunosuppressed rats. *J Infect Dis* 1993; 168: 1566-1569.
36. Wittenberg DF, Miller NM, Van Den Ende J. Spiramycin is not effective in treating *Cryptosporidium* diarrhea in infants: Results of a double-blind randomized trial. *J Infect Dis* 1989; 159: 131-132.
37. Fichtenbaum CJ, Ritchie DJ, Powderly WG. Use of Paromomycin for treatment of *Cryptosporidiosis* in patients with AIDS. *Clin Infect Dis* 1993; 16: 298-300.

38. White AC, Chappell CL, Hayat CS, Kimball KT, Flanigan TP, Goodgame RW. Paromomycin for Cryptosporidiosis in AIDS: A prospective, double-blind trial. *J Infect Dis* 1994; 170: 419-424.
39. Bissuel F, Cotte L, Montclos M, Rabodonirina M, Trepo C. Absence of systemic absorption of oral paromomycin during long-term, high-dose treatment for Cryptosporidiosis in AIDS. *J Infect Dis* 1994; 170: 749-750.
40. Verdon R, Polianski J, Gaudebout C, Pocardalo JJ. Paromomycin for Cryptosporidiosis in AIDS. *J Infect Dis* 1995; 171:1070
41. Tzipori S, Griffiths J, Theodos C. Paromomycin Treatment against Cryptosporidiosis in patients with AIDS. *J Infect Dis* 1995; 171: 1069-1070.
42. Regh JE. A Comparison of Anticryptosporidial Activity of Paromomycin with that of other aminoglycosides and azithromycin in immunosuppressed rats. *J Infect Dis* 1994; 170: 934-938.
43. Anand A. Cryptosporidiosis in patients with AIDS. *Clin Infect Dis* 1993; 17: 297-298.
44. Regh JE. Activity of azithromycin against Cryptosporidia in immunosuppressed rats. *J Infect Dis* 1991; 163: 1293-1296.
45. Ungar BLP, Ward DJ, Fayer R, Quinn CA. Cessation of Cryptosporidium-associated diarrhea in an acquired immunodeficiency syndrome patient after treatment with hyperimmune bovine colostrum. *Gastroenterology* 1990; 98: 486-489.
46. Das SS, Simpson AJH. Therapy of protozoan infections in patients with impaired immunity. *Cur Opin Infect Dis* 1993; 6: 784-793.
47. Baxby D, Blundell N. Recognition and laboratory characteristic of an atypical oocyst of Cryptosporidium. *J Infect Dis* 1988; 158: 1038-1045.
48. Garcia LS, Brewer TC, Bruckner DA. Fluorescence detection of Cryptosporidium oocysts in human fecal specimens by using monoclonal antibodies. *J Clin Microbiol.* 1987; 25: 119-121.
49. Long EG, White EH, Carmichael WW, et al. Morphologic and staining characteristics of a Cyanobacterium-like organism associated with diarrhea. *J Infect Dis* 1991; 164: 199-202.
50. Wurtz RM, Kocka FE, Peters CS, Weldon-Linne CM, Kuritza A, Yungbluth P. Clinical characteristics of seven cases of diarrhea associated with a novel acid - fast organism in the stool. *Clin Infect Dis* 1993; 16: 136-138.
51. Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. Cyclospora species - A new protozoan pathogen of humans. *N Engl J Med* 1993; 328: 1308-1312.
52. Würtz R. Cyclospora: A newly identified intestinal pathogen of humans. *Clin Infect Dis* 1994; 18: 620-623.
53. Bendall RP, Lucas S, Moody A, Tovey G, Chiodini PL. Diarrhoea associated with Cyanobacterium - like bodies: A new coccidian enteritis of man. *Lancet* 1993; 341: 590-592.
54. Hart AS, Ridinger MT, Soundarajan R, et al. Novel organism associated with chronic diarrhoea in AIDS (Letter). *Lancet* 1990; 335: 169-170.
55. Hoge CW, Shlim DR, Rajah R, et al. Epidemiology of diarrhoeal illness associated with coccidian - like organism among travellers and foreign residents in Nepal. *Lancet* 1993; 341: 1175-1179.
56. Berlin OGW, Novak SM, Porschen RK, Long EG, Stelma GN, Schaeffer FW. Recovery of Cyclospora organisms from patients with prolonged diarrhea. *Clin Infect Dis* 1994; 18: 606-609.
57. Hale D, Aldeen W, Carroll K. Diarrhea associated with Cyanobacterium-like bodies in an immunocompetent host. *JAMA* 1994; 271: 144-145.
58. Pollok RCG, Bendall RP, Moody A, Chiodini PL, Churchill DR. Traveller's diarrhoea associated with Cyanobacterium-like bodies. *Lancet* 1992; 340: 556-557.
59. Madico G, Gilman RH, Miranda E, Cabrera L, Sterling CR. Treatment of Cyclospora Infections with Co-trimoxazole. *Lancet* 1993; 342: 122-123.
60. Shadduck JA, Greeley E. Microsporidia and human infections. *Clin Microbiol Rev* 1989; 2: 158-165.
61. Shadduck JA. Human microsporidiosis and AIDS. *Rev Infect Dis* 1989; 11: 203.
62. Weber R, Bryan RT, Owen RL. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med* 1992; 326: 161-166.
63. Zhu X, Wittner M, Tanowitz HB, Kotler D, Cali A, Weiss LM. Small subunit rRNA sequence of enterocytozoon bienersi and its potential diagnostic role with use of the polymerase chain reaction. *J Infect Dis* 1993; 168: 1570-1575.
64. Eeftinck Schattenkerk JKM, Van Gool T, Van Ketel RJ, et al. Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individuals. *Lancet* 1991; 337: 895-898.
65. Greenson JK, Belitsos PC, Yardley JH, Bartlett JG. AIDS Enteropathy: Occult enteric infections and duodenal mucosal alterations in chronic diarrhea. *Ann Intern Med* 1991; 114: 366-372.
66. Kotler DP, Francisco A, Clayton F, Scholes JV, Orenstein JM. Small intestinal injury and parasitic diseases in AIDS. *Ann Intern Med* 1990; 113: 444-449.
67. Rabeneck L, Gyorkey F, Genta RM, Gyorkey P, Foote LW, Risser JMH. The role of microsporidia in the pathogenesis of HIV-related chronic diarrhea. *Ann Intern Med* 1993; 119: 895-899.



68. Molina JM, Sarfati C, Beauvais B, et al. Intestinal microsporidiosis in human immunodeficiency virus-infected patients with chronic unexplained diarrhea: Prevalence and clinical and biologic features. *J Infect Dis* 1993; 167: 217-221.
69. Drobniwski F, Kelly P, Carew A, et al. Human microsporidiosis in African AIDS patients with chronic diarrhea. *J Infect Dis* 1995; 171: 515-516.
70. Asmuth DM, De Girolami PC, Federman M, et al. Clinical features of microsporidiosis in patients with AIDS. *Clin Infect Dis* 1994; 18: 819-825.
71. Dieterich DT, Lew EA, Kotler DP, Pales MA, Orenstein JM. Treatment with Albendazole for intestinal disease due to enterocytozoon *bieneusi* in patients with AIDS. *J Infect Dis* 1994; 169: 178-183.
72. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993; 328: 95-99.
73. Terada S, Reddy R, Jeffers LJ, et al. Microsporidian hepatitis in the acquired immunodeficiency syndrome. *Ann Intern Med* 1987; 107: 61.
74. Wuhib T, Silva TMJ, Newman RD et al. Cryptosporidial and microsporidial infections in human immunodeficiency virus-infected patients in north-eastern Brazil. *J Infect Dis* 1994; 170: 494-497.
75. Schwartz DA, Visvervara GS, Diesenhouse MC, et al. Pathologic features and immunofluorescent antibody demonstration of ocular microsporidiosis (*Encephalitozoon hellem*) in seven patients with acquired immunodeficiency syndrome. *Am J Ophthalmol* 1993; 115: 285-292.
76. Weber R, Saver B, Lüthy R, Nadal D. Intestinal coinfection with enterocytozoon *bieneusi* and *Cryptosporidium* in a human immunodeficiency virus-infected child with chronic diarrhea. *Clin Infect Dis* 1993; 17: 480-483.
77. Diesenhouse MC, Wilson LA, Corrent GF, et al. Treatment of microsporidial keratoconjunctivitis with topical fumagillin. *Am J Ophthalmol* 1993; 115: 293.