Emerging Causes of Traveler's Diarrhea: *Cryptosporidium*, *Cyclospora*, *Isospora*, and *Microsporidia*

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Travel is a risk factor for acquiring infection with a spore-forming protozoa: *Cryptosopridium*, *Cyclospora*, *Microsporidia*, and *Isospora*. Certain travel destinations have a high disease burden and intense exposure. Patients present with persistent diarrhea and a history of recent travel to a developing country in the tropics. Very mild infections may be underdiagnosed and may cause typical traveler's diarrhea. In a patient with a history of travel and persistent diarrhea unresponsive to the usual antibiotic and antidiarrhea treatment, stool studies for all four of these protozoa infections should be performed. If immune status is normal and the disease is mild, symptomatic therapy may suffice. Effective treatment is available for *Cyclospora*, *Microsporidia*, and *Isospora*.

Introduction

Travelers develop at least two travel-related gastrointestinal syndromes [1•,2•,3]. The first is acute traveler's diarrhea, often appropriately called "traveler's diarrhea" [1•,2•]. Loose or liquid stools occur in the first few weeks of travel, frequently associated with nausea, vomiting, abdominal pain, fever, malaise, and other constitutional symptoms. The disease usually runs a self-limited course. This very common illness poses a small risk of severe dehydration and toxicity, but a great risk of interfering with travel plans. Traveler's diarrhea is remarkably attenuated by antimotility agents and antibiotics [2•]. The cause is almost always a bacteria or virus. Pretravel counseling on prevention and treatment are warranted. In fact, doctors help patients with travelers diarrhea before rather than after the disease.

The second travel-related gastrointestinal syndrome is less common and is called "persistent diarrhea in returned travelers" [1•,3]. It represents only about 5% of all travel-related diarrhea. The diarrhea begins a little later in travel or after returning home and lasts over 2 weeks. The doctor is consulted after the disease has been present for too long to be routine traveler's diarrhea. It has a different spectrum of causes, with protozoa deserving important diagnostic consideration [1•,3–5,6•]. The diagnostic algorithm can be complex and treatment frequently requires a specific diagnosis.

This review presents evidence that the spore-forming protozoa—*Cryptosopridium*, *Cyclospora*, *Microsporidia*, and *Isospora*—are well documented causes of persistent diarrhea in returned travelers [1•,3,6•]. It explains why travel increases the risk of infection with these organisms, and how the risk is modified by destination, activity, and time of stay. The review speculates on the possibility that some of the 50% of undiagnosed traveler's diarrhea may in fact be due to low-intensity infections with these protozoa. An update on the most effective diagnostic and treatment methods for these organisms is presented. This review uses the term *Cryptosporidium* for both genotypes of *Cryptosporidium parvum* and other *Cryptosporidium* species that may cause human disease. *Microsporidia* is used to refer to *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* genotypes associated with human intestinal infections [7]. *Cyclospora* is *Cyclospora cayetanensis* and *Isospora* is *Isospora belli*. For readers interested in detailed discussions on taxonomy, various species and genotypes, microbiology, epidemiology, and other clinical syndromes, excellent recent reviews with comprehensive references are available for *Cryptosporidium* [8,9], *Cyclospora* [10], *Isospora* [11], and *Microsporidia* [7,12].

These Protozoa Cause Persistent Diarrhea in Returned Travelers

All four of the spore-forming protozoa cause diarrhea in patients with a history of travel. Important milestones in their collective history have been summarized [13]. Human intestinal *Isospora* infection, often associated with travel to the tropics, has been known for many decades. The first publication of human cases of intestinal *Cryptosporidium*, *Cyclospora*, and *Microsporidia* occurred only 26, 23, and 17 years ago, respectively. By 1997 information emerging from case reports and small case series implicated *Cryptosporidium* [14–20] and *Cyclospora* [21–28] as

risks to travelers. Almost all of these case reports were about patients with persistent diarrhea in a returned traveler. To find out the relative frequency with which protozoa were found in returned travelers, Jelinek *et al*. [29] recruited 795 German nationals who had a recent history of travel. 2.8% Had stools positive for *Cryptosporidium* and 1.1% were positive for *Cyclospora*. All subjects with positive stools had the clinical syndrome of persistent diarrhea in a returned traveler. The length of diarrhea in the *Cryptosporidium* cases averaged 36 days and the length of diarrhea in the five *Cyclospora* cases averaged 20 days. In addition to the 15 patients with *Cryptosporidium* and the five patients with *Cyclospora*, 74 had *Giardia* and 74 had amoeba. *Isospora* was present but was very rare [29]. Although prolonged diarrhea in travelers is a recognized presentation for *Isospora* [30,31], the number of cases is much smaller than for *Cryptosporidium* and *Cyclospora*. The investigators concluded that the spore-forming protozoa were rare infections in travelers but should be tested for in patients with prolonged diarrhea [29]. Since their report, more cases of *Cryptosporidium* [32,33•] and *Cyclosopra* [34,35] in travelers have been described.

In Jelinek *et al*. [29], *Microsporidia* were not found. However, *Microsporidia* has been found in travelers [36••,37–42] more frequently than *Isospora* in recent years. The best estimate of frequency comes from the study by Müller *et al*. [36••]. They examined stools from 148 returned travelers in Germany with both fluorescent microscopy and polymerase chain reaction (PCR). Nine were positive by PCR , of which five were positive by light microscopy. Therefore, using the most sensitive technique to evaluate persistent diarrhea in a returned traveler, the frequency of *Microsporidia* infection was found to be 5%. A prior study using only light microscopy showed one *Microsporidia* infection out of 750 returning Swiss travelers [42]. Three of 40 immunocompetent patients with persistent travel related diarrhea were positive by light microscopy (confirmed by PCR) in a small case series from Europe [37].

Risk of Infection Is Related to Destination, Activity, and Length of Stay

Travelers to certain destinations enter an environment of potential intense exposure to the spore-forming protozoa. Exposure is a combination of infected people, infected environment, and contact facilitated by poor sanitation and water supplies.

Cryptosporidium

Every country in the world has a "*Cryptosporidium* problem" to some degree. *Cryptosporidium* may cause 2% of all diarrhea in healthy persons in developed countries (7% in children) and 6% of diarrhea in developing countries (12% in children) [9]. Infection rates in AIDS patients are much higher. The number of oocysts needed to cause infection is very small. The main risk for sporadic infection in developed countries with good water supplies are exposure to infected people (for example, child care institutions and swimming pools) or animals (especially cattle and lambs) [43•]. Unlike the other spore-forming protozoa, *Cryptosporidium* oocysts are highly infectious when they are excreted in stool. Formation of an infectious particle does not require time for sporulation in the environment. Thus, the amount of cryptosporidiosis in a community is a measure of the amount of fecal-oral contamination in that community.

Another source of cryptosporidiosis, presumably in all countries, is contaminated water supplies. Many large outbreaks attributed to municipal water supplies in the United States have been reported. The combination of its ubiquitous occurrence in the environment and its relative resistance to filtration and chlorination means that this organism will emerge in epidemic form if there is a breakdown in water supply and sanitation systems [8]. Oocysts can survive in aquatic environments for a long time. Oocysts of *Cryptosporidium* are found in 87% of untreated water samples in the United States, so they must be high in developing countries.

Whether a matter of epidemics through water supplies or endemic through fecal-oral contact with infected persons and animals, there do seem to be regions of highly endemic disease [9] for cryptosporidiosis. In Kampala, Uganda, 29% of children with diarrhea had stools positive for *Cryptosporidium* compared with 8.5% of controls [8]. In children with persistent diarrhea it was 31%. Studies of diarrhea in infants and children in developing countries indicate it is especially common in Guinea Bissau and northeastern Brazil. The studies of Ortega *et al*. [44•,45] have demonstrated the high infection rate among poor infants and children in Peru. Most recently they followed a total of 368 infants and children of all different ages for a mean of 2.4 years [44•]. The cohorts were well described in terms of housing, sanitation, water, and contact with animals. They collected weekly stool samples and carried out more intensive stool collection if diarrhea developed. Thirty-eight percent had at least one episode of *Cryptosporidium* infection and 33% of all children had at least one episode of *Cyclospora* infection. Their studies indicated that *Cryptosporidium* infection occurred early in infancy, which is consistent with direct person to person transmission as the main method of infection. (*Cyclospora* infection, while just as frequent, peaked later in childhood, which is consistent with environmental exposure.) The high frequency of infection in this community represents an enormous reservoir of infection for travelers entering a situation of well known fecal-oral contact.

Some studies indicate that the frequency of *Cryptosporidium* infection (and other parasites) is not only influenced by place but also by time of exposure. Herwaldt *et al*. [46•] followed 36 Peace Corp workers in Guatemala with monthly stool examinations to determine the rate at which they were infected with enteric parasites. Eighty-nine per-

cent of all subjects had at least one parasite in the stool during the 2-year period. Most of the infections were with nonpathogenic organisms. This high infection rate is a measure of fecal-oral exposure. Fifty-three percent became infected with at least one pathogen; 30% developed a stool positive for *Cryptosporidium*. In a parallel study, 47% showed seroconversion for *Cryptosporidium* antibodies [46•]. Stool positivity for *Cryptosporidium* was higher than for *Giardia lamblia* (16.7%) and *Entamoeba histolytica/dispar* (5.6%). Interestingly documented stool infection with *Cryptosporidium* did not occur until after an average of 322 days. If they had used only the modified acid fast stain and not the immunofluorescent stain, then only 20% of the *Cryptosporodium* infections would have been recognized. It is likely that very low-intensity infections were missed.

Certainly there are not only risky places and times but also activities. The high-risk activities associated with routine travelers diarrhea (eating in indigenous restaurants, using unprotected and untreated water, close contact with people with high disease prevalence) must also influence the frequency of *Cryptosporidium* infection. However, even within countries where *Cryptosporidium* is known to be highly endemic, there may to be pockets of increased infection. A potential example (not yet shown to be significant to travelers) is illustrated by some recent small studies in Uganda. Ugandans (not travelers) who live and work in contact with the gorillas of Bwindi forest have a high prevalence of *Cryptosporidium* in their stools (21%) [47]. Stool positivity rates for *Cryptosporidium* in a nearby unexposed villlage (3%) and in the general Ugandan adult population (6%) are much lower. It appears that cattle that graze in the forest may be a reservoir of *Cryptosporidium*, which infects the gorillas in the nearby game reserve [48].

Cyclospora

The geographic epidemiology of this organism is best illustrated by three narratives related to risk places: travelers and expatriates in Nepal, raspberries from Guatemala, and children in Peru. A series of articles from Kathmandu, Nepal, reported a high frequency of prolonged diarrhea due to *Cyclospora* among travelers and expatriates [49,50]. The attack rate of infections was high among travelers and foreign residents, with stool positivity rates up to 11% depending on the season of the year. The spectrum of illness was broad, including both asymptomatic infections and prolonged severe diarrhea. Consumption of untreated water was a risk factor for infection among travelers and residents who were identified at two outpatient clinics in 1992. But this means of transmission only accounted for about a quarter of the infections. Frank contamination of a water supply in Pokhara, Nepal caused an epidemic of cyclosporiasis among British military expatriates in 1994 [51].

The risk of infection varied according to risk times. The onset of the warm rainy season marked the time of most numerous infections. In addition, the length of residence directly increased risk of illness. *Cyclospora* oocysts require time in the environment to sporulate into an infectious particle. Person-to-person transmission does not occur. However, the exact reservoir of infection in Nepal (and other travel destinations) is not clear. *C. cayetanensis*-like oocysts have been found in sewage water and from vegetable washings [52]. Although the role of animals is unknown, *C. cayetanensis*-like oocysts have also been recovered from mice, rats, chickens, and dogs [52]. These are only very preliminary data.

Guatemala has been identified as a risk place not because of frequent infections in those who travel there, but because of what has happened when raspberries from Guatemala travel to the United States. There were important and well-publicized epidemics of acute cyclosporiasis most every year from 1995 to 2000 linked in some way to raspberries imported from Guatemala [53•]. The organism has not only been identified in the stools of infected patients but also on the fruit in some cases [53•]. Washing does not reliably remove the organisms from fruit. Several Guatemalan farms were identified as the sources of the contaminated raspberries. In spite of Food and Drug Administration (FDA) inspection of some farms, the exact mode of the contamination for any of the epidemics still has not been discovered. One survey among raspberry farm workers and families in Guatemala found a prevalence of 2.3%. But another study failed to find any infections [54]. The larger prevalence rate has been attributed to screening children rather than adults, larger sample size, and seasonal fluctuation of *Cyclospora* infections [55]. A small case-control study from Guatemala shows that *Cyclospora* may cause up to 6% of acute diarrhea episodes of Peace Corp workers in Guatemala [56]. There are huge gaps in our understanding of the biology and epidemiology of this organism.

The studies of Peruvian children documented the frequency and severity of this infection in shanty towns near Lima [44•,45]. Community-based prospective surveys have shown infection rates in young children from 6% to 18%; about one quarter had diarrhea [45]. Much lower rates were fund in alternative sites and seasons. Peak infection frequency appears to coincide with the warm, dry season. A 2-year prospective study showed that 33% of all children had at least one episode of *Cyclospora* infection [44•]. One fourth of the *Cyclospora* infections were symptomatic with diarrhea. *Cyclospora* infection incidence was highest after age 2 years. Environmental exposure, rather than person-to-person transmission, is most important for infection transmission. The search for the environmental reservoir continues [57]. The role of animals is controversial [44•]. The situation in Peru, Nepal, and Guatemala for *Cyclospora* transmission is probably common throughout similar places in the tropics, and this represents a substantial risk to travelers.

Microsporidia

There are no risk places or activities directly linked to *Microsporidia* infection. Although animal reservoirs for human disease do not seem to be important, swine and

monkey infections with *E. bieneusi* have recently been identified [7]. Infections have been found in indigenous people in Niger (0.8%), Argentina (7%) [58], and Mexico (8%) [59]. When *Microsporidia* is looked for with sensitive tests, like PCR, it is found [60]. Its small size and relative low virulence probably makes it unrecognized in most human infections. The main reason for believing that *Microsporidia* is undiagnosed in acute and persistent disease in healthy people is its high prevalence in a well-identified risk group: patients with AIDS. Worldwide *E. bienuesi* (and less commonly *E. intestinalis*) infection is found in 7% to 50% of patients with HIV infection, depending on the severity of immune dysfunction, presence of diarrhea, and the method of identification [7]. The prevalence of *Microsporidia* in HIV-positive patients tested in a variety of ways has recently been reported as 13.1% to 32.4% in Mali (immunofluorescent stain) [58], 51% in Zimbabwe (PCR) [61], and 11% in Thailand (trichrome) [62]. Recent reviews have pointed out the major gaps in our knowledge of *Microsporidia* disease [7,12]. Little is known about natural reservoirs, mode of transmission, epidemiology, host response, and spectrum of illness. The real risk to travelers is hard to estimate.

Isospora

Detailed knowledge of the epidemiology of *Isospora* infection is lacking, including route of infection, reservoirs, risk places, and risk times. The large oocysts sporulate outside of the body. Person-to-person transmission is probably rare. *I. belli* only infects humans. Infection in normal individuals is documented [63]. As in the case of *Microsporidia*, prevalence among a high-risk population can serve as a marker of the degree of exposure in the environment. The fact that it is a prominent organism in HIV infection in the tropics means that there is some level of infection in those countries. *Isospora* has commonly been found in AIDS patients with diarrhea in Brazil (9.9%), Zaire (12%), Zambia (16%), and Haiti (12%). These rates are much higher than in developed countries, suggesting that travel to the tropics may increase risk, even for healthy travelers.

These Protozoa May Cause Typical Acute Traveler's Diarrhea

Although *Escherichia coli* is most frequently found as the etiology of traveler's diarrhea, there are a large number of patients (about 50%) in which no pathogen can be identified. Surely a significant number are due to calicivirus, rotavirus, or adenoviruses, the agents that cause so much epidemic and sporadic acute gastroenteritis. Until just a decade ago the cause of most epidemics of acute viral gastroenteritis was unknown [64]. The use of molecular diagnostic techniques such as broadly reactive PCR has demonstrated that over 90% of epidemics of viral gastroenteritis are due to calicivirus [64,65]. Is it possible that the use of very sensitive, broadly reactive diagnostic techniques would demonstrate that many of the episodes of acute self-limited diarrhea of travelers are due to mild and low-intensity infection with *Cryptosopridium*, *Cyclospora*, *Microsporidia*, or *Isospora*?

The current status of diarrhea in travelers is illustrated by a recent report by Hill [66•]. He studied 784 Americans who consulted with his travel medicine clinic before traveling to a variety of international destinations. They all received education for prevention and treatment of travelers diarrhea according to standard recommendations. The travelers were offered prescriptions for antidiarrheal agents and antibiotics to take if symptoms occurred with a certain severity and frequency. Most of the trips were for a few weeks; 80% were for less than 30 days. All were for less than 3 months. Patients traveled to 123 countries but the following countries made up about 58% of the travel destinations: Kenya, India, Nepal, Thailand, Tanzania, Brazil, Ghana, Ecuador, Egypt, Guatemala, Hong Kong, Singapore, Peru, Indonesia, and South Africa. Diarrhea was the most common health complaint and was reported by 46%. Most diarrhea began 7 to 10 days after arrival and lasted 2 to 4 days. About 80% of all affected travelers treated themselves, either with antidiarrhea medication or antibiotics or both. The travelers had the impression that treatment was effective. Nine percent of diarrhea or loose motions lasted more than 7 days; 4.6% lasted more than 2 weeks. Persistent diarrhea in a returned traveler (greater than 2 weeks with liquid stools) occurred in 4%. The major predictors of diarrhea were duration of the journey and the destination. Each additional day of travel was associated with an increased risk of diarrhea of about 1.7%.

Several observations on Hill's study can be made. First, recent advances in prevention and treatment have improved the traveler's prospects of a more successful and healthy journey, but travel still poses a risk of both acute and persistent diarrhea. Second, the advice and treatment are directed toward traveler's diarrhea: early onset, short-lived, and antibiotic responsive [2•]. The list of bacterial etiologies are well known, and these agents represent 90% of the identifiable causes of traveler's diarrhea. But to reiterate, in many large series of diarrhea associated with travel, a bacterial cause cannot be determined [1•,2•]. A group such as Hill's has never been followed prospectively using the most sensitive stool tests for the detection of spore-forming protozoa. Mild, self-limited diarrhea could be due to low-intensity infections with these organisms. Third, persistent diarrhea occurred in 4% of Hill's returned travelers. It is likely that many of these are protozoa infections. The studies of spore-forming protozoa in developing countries quoted above demonstrate that asymptomatic and mild infections predominate. If these lasting infections represent only the more severe cases of infections, then many of the self-limited, typical traveler's diarrhea may also be due to protozoa.

There is a great risk of underestimating the frequency of these infections. The concept of low-intensity infection is best described for cryptosporidiosis. Normal human vol-

unteer studies using serologic response [67] and studies in communities using serologic data [68] show that stool examination is a very insensitive method of identifying infections, especially in patients who have had prior infection. All patients at some time in their illness excrete organisms at a concentration below the level of detection by stool examination. In longitudinal studies in Guatemala, seroconversion rates exceeded stool positivity rates by about 50% [46•]. If the investigators had used only the modified acid fast stain (insensitive) and not the immunofluorescent stain (more sensitive), then only 20% of the *Cryptosporidium* infections would have been recognized [46•]. It is likely that low-intensity infections were missed. In AIDS patients the intensity of intestinal infection (number of oocysts per gram of stool) with *Cryptosporidium* varies from patient to patient [69], and the severity of intestinal structural abnormalities and gut function defects are related to the intensity of infection [70,71].

The relationship between stool positivity and clinical spectrum of illness in cryptosporidiosis is illustrated by comparing studies that used different methods to identify cases. In a case-control study of using stools from people who went to the doctor, the average patient experienced a 3-week illness of severe watery diarrhea and belly pain, with peak stools of 10 per day [43•]. In the prospective human volunteer studies the average episode of cryptosporidiosis was a 3-day illness with a peak of six stools per day [67]. In the Milwaukee epidemic of cryptosporidiosis the unconfirmed cases (stool negative, but exposure and timing highly suggestive) had 4-day illness with a peak of eight stools per day. Confirmed cases (stool positive) had a 12-day illness with peak diarrhea severity of 19 stools per day. It is reasonable to suggest that some of the typical traveler's diarrhea with negative stool examinations could easily be mild cases of cryptosporidiosis. A recent study of travelers diarrhea in Mexico showed that 3% was due to *Cryptosporidium* [33•]. This study was not designed to identify very light infections.

The intensity of infection with *Microsporidia* has also been quantified [72]. Heavy and light infections exist. Müller *et al*. [36••] makes a good case for *Microsporidia* being underdiagnosed. All of the infections in travelers that he studied showed very low spore excretion. The most sensitive stool assay techniques are not usually applied, but when they are, more infections are identified. Further studies of acute traveler's diarrhea using very sensitive tests for all of the spore-forming protozoa would be required to test this hypothesis. However, given the current state of prevention and treatment, the clinical importance of this type of study is debatable.

Diagnosis of Infection in Persistent Diarrhea in a Returned Traveler

The diagnosis of infections with spore-forming protozoa is based on stool studies. Small bowel biopsy may have a role in unusual or difficult cases when there is continued diagnostic uncertainty. Light microscopic tests, autofluorescent stains, monoclonal antibody fluorescent stains, antigen capture enzyme-linked immunosorbent assay (ELISA), and PCR have been used in research studies. The ELISA test is readily available only for *Cryptosporidium*. PCR is not commercially available for any of the organisms, but a combination kit for detection of *Cryptosporidium*, *Cyclospora*, and *E. bieneusi* is possible [5]. Most laboratories will rely on the light microscopic stains: modified acid fast stain for *Cryptosporidium*, *Cyclospora*, and *Isospora*, and modified trichrome for *Microsporidia*. We prefer the nonspecific fluorescent tests for screening. For returned travelers with persistent diarrhea, three negative stools with these tests by an experienced observer probably excludes significant infection. For a demonstration of the modified acid fast stain, modified trichrome stain, and the nonspecific fluorescent stain see http://www.bcm.tmc.edu/gastro/VGICC/protozoa.html.

For the diagnosis of *Cryptosporidium* the assays commonly used in diagnostic laboratories include modified acid fast stain, immunofluorescent stain (Meridian MERIFLUOR assay; Meridian Diagnostics, Inc., Cinncinati, OH) , and antigen capture ELISAs (Alexon ProSpecT assay; Alexon, Inc., Sunnyvale, CA) [4,9]. The easiest to use is the acid fast stain, but it is insensitive. The immunofluorescent stain enhances sensitivity but requires fluorescent microscopy. Antigen capture panel enzyme immunoassay can be performed for *Giardia*, *E. histolytic*, and *Cryptosporidium* (BIOSITE Diagnostics, San Diego, CA) [5].

The simple inexpensive staining technique, modified acid fast stain, is commonly used to identify *Cyclospora* infection [10]. Confusion with *Cryptosporidium* is possible. As stated above, we prefer fluorescent tests for screening. Soave *et al*. [10] have stressed the insensitivity and rudimentary nature of currently available stool tests. *Isospora* infection can be approached in the same way as *Cyclospora*.

Detailed review of the staining procedures for the two intestinal *Microsporidia* infections have been published [7]. The chromotrope stains have been modified to increase the contrast between spores and background material, and to accelerate the process. The major disadvantage is that this is still a time-consuming procedure. The fluorescent staining methods are much quicker. They are just as sensitive as the chromotrope stain but specificity depends on the experience of the observer. Some authors advocate using both the chromotrope and fluorescent stains in a suspicious setting to maximize sensitivity and specificity [7].

If fluorescent microscopy is available then the nonspecific fluorescent tests can be used as described below. This may be a good screening examination, since it is a single sensitive test for all four organisms. Our preferred stain for these four organisms is performed as follows. An aliquot of stool is fixed in 10% formalin. Ten µL of formalin fixed stool is mixed with 10 µL of Fungi-Fluor Solution A (Polysciences, Warington, PA) on a Genetic Systems Fluorescent Microscope Slide (Genetic Systems Corp., Redwood, WA) and

spread to a diameter of 9 mm. The sample is placed on a heating block (65°C) and allowed to dry. The sample is briefly rinsed with tap water and returned to the heating block. The smear is covered with TB Auramine M (Becton Dickinson, Sparks, MD) and then TB Decolorizer TM covers the slide for 30 seconds. The slide is rinsed with tap water and the smear is covered with Fungi-Fluor Solution A for 30 seconds at room temperature, then drained. Four drops (approximately 200 µL) of Eosin Yellowish Solution (Fisher Scientific, Fair Lawn, NJ) are added, incubated for 30 seconds, and drained. Two drops of Fungi-Fluor Solution B are added, rocked briefly, and drained. Two more drops of Fungi-Fluor Solution B are added, incubated for 30 seconds, and drained. The smears are repeatedly dipped into tap water until the specimens no longer release a blue color. The smears are air-dried and a drop of low-fluorescence immersion oil is placed on the specimen and cover-slipped. Smears are examined using a fluorescence microscope (BH-2, Olympus, Lake Success, NY) equipped with filter module HP490 (excitation 410–490 nm) and filter module VGI (excitation 330–380 nm) and a mercury vapor lamp for illumination. Stained specimens are examined at X500 and X1000 magnification. *Microsporidia* are examined with filter module VGI. The spores are 1 to 3 mm long and have a blue-white fluorescence. *Cryptosporidium*, *Cyclospora*, and *Isospora* are examined with filter module HP490. All three stain apple-green color. *Cryptosporidium* oocysts measure 6 to 9 µm in diameter, *Cyclospora* 8 to 10 µm, and *Isospora* 20 to 30 µm in length by 10 to 19 µm in width.

Prevention and Treatment

This review points out the many unknown features of the epidemiology of infections with the spore-forming protozoa. However, infection risk seems variably related to person-to-person contact (especially children), contaminated water and food, animals, and swimming pools. Therefore, general recommendations regarding hygiene and food preparation and consumption, similar to those guidelines for prevention of travelers diarrhea, can be made [1•,2•]. The extreme case would be instructions to high-risk persons, such as AIDS patients: meticulously wash hands; consume only boiled water and recently well-cooked food; avoid local restaurants, street vendors, and school cafeterias; do not touch frequently or recently handled food or produce; do not have close contact with local children; do not swim in surface waters or swimming pools. The healthy traveler would have to weigh the risks and benefits of such a rigorous program, especially in light of the absence of conclusive evidence of efficacy.

Empiric self-therapy with antibiotics, based on severity of symptoms, is helpful and appropriate for traveler's diarrhea [1•,2•,66•], but recommended antibiotics are unikely to affect acute disease due to spore-forming protozoa. There is a strong rationale for the use of antimotility agents, but this has not been systematically studied for these infections, especially in healthy travelers. Until more is known about the frequency and severity of spore-forming protozoa infections in travelers, presumptive therapy directed at protozoa is not indicated.

Treatment is likely to be an issue in persistent diarrhea in a returned traveler in whom a positive stool result has been obtained [1•,3,6•]. There is no recommended specific therapy for immunocompetent persons with cryptosporidiosis [9] or microsporidiosis [6]. Antimotility agents for mild cases and fluid support for severe cases have an obvious rationale. For persistent *Cryptosporidium* infection in immunocompetent patients, there is a rationale for combination therpy with paromomycin and azithromycin [9]. Persistent severe *Microsporidia* infection in immunocompetent travelers is quite rare. If encountered, there would be a rationale for therapy with albendazole or fumigillin [73]. *Cyclospora* infection in travelers responds well to a 10-day course of trimethoprim-sulfamethoxazole [10]. Identical treatment is effective for *Isospora* infection. Treatment of these infections in AIDS patients is summarized in recent reviews [9–11,73].

Conclusions

The spore-forming protozoa—*Cryptosporidium*, *Cyclospora*, *Microsporidia*, and *Isospora*—are well documented but infrequently recognized causes of travel-related diarrhea. For *Cryptosporidium* and *Cyclospora*, studies have identified travel destinations associated with increased risk. There are risks associated with food, water, persons, and seasons. These organisms have been identified as a the cause of persistent diarrhea in returned travelers, but they may be a more common but unrecognized cause of typical, mild, and short-lived traveler's diarrhea. Prospective studies using very sensitive stool diagnostic techniques will have to be done to sort out the true frequency and epidemiology of these infections.

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- •• Of major importance

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What happens to travelers to the tropics who get the best advice and prescriptions from a travel medicine clinic before they go? Here is the summary of 784 such subjects. Diarrhea was the most common problem (46%). Some was persistent.

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