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Giardiasis and Cryptosporidiosis - Recent Literature with a Focus on Nitazoxanide

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Abstract

Nitazoxanide is a Food and Drug Administration approved Thiazolide to treat parasitic infections such as giardiasis and cryptosporidiosis. Since nitazoxanide is available as a liquid suspension, this medication is uniquely suited for the treatment of children. However, there has been a paucity of published studies since its approval. We completed a systematic literature search using a variety of sources including PubMed to identify studies reporting the impact of nitazoxanide in treating parasitic infections in children ages 1 to 11 years. A similar search was conducted on other available treatment for parasitic infection. In total, 28 publications were found to meet the criteria above for nitazoxanide. A similar number of papers were identified to address other therapy commonly used regardless of product information on approved use. Despite the ease of administration and superiority of nitazoxanide to other Non FDA approved therapies for the treatment of Giardia or Cryptosporidium among children, this medication continues to be underutilized. These data indicated the need for additional clinical studies to raise awareness.

Keywords: Giardia; Giardiasis; Cryptosporidium; Cryptosporidiosis; Epidemiology; Diagnosis; Treatment

Introduction

Giardia lamblia (also called Giardia intestinalis or duodenalis) is a flagellate protozoa that generally causes a self-limited and often persistent illness that is typified by diarrhea and other related gastrointestinal maladies [1]. It is the leading cause of intestinal parasitic infection in humans in the United States [2]. Although less frequently identified than giardiasis, cryptosporidiosis is a similar persistent diarrheal illness caused by protozoa of the genus Cryptosporidium [3]. Cryptosporidiosis is also characterized by diarrhea, that may be intermittent or continuous, and voluminous, and may include vomiting and low-grade fever [4,5]. Giardiasis and cryptosporidiosis are considered 'neglected diseases' by the World Health Organization [6].

Giardiasis is commonly treated with metronidazole or tinidazole; however, the tablet formulations can be difficult to administer in children because of pediatric dose requirements, possess an unpalatable metallic taste, and may cause adverse effects such as nausea [7,8]. In addition, resistance has been documented in G. lamblia [9-11]. Apart from nitazoxanide, there is no other commonly recommended specific treatment for cryptosporidiosis in young children.

Pediatric infections with intestinal parasites such as G. lamblia and Cryptosporidium spp. appear more frequently than commonly perceived and have been understudied [12]. It is believed that general awareness of these persistent parasitic infections may be low. A recent Centers for Disease Control and Prevention survey of obstetrician-gynecologists on their clinical and epidemiologic knowledge of cryptosporidiosis in pregnancy found that only 44% of respondents reported that prolonged, intermittent diarrhea would lead them to consider cryptosporidiosis in a differential diagnosis [13]. In addition, questions about the availability of an FDA-approved treatment were the most frequently missed questions among the survey respondents. A similar survey among pediatricians was recently published that also revealed a general low awareness of parasite induced diarrhea and confirms the CDC findings [14]. Less than 1% of pediatricians in this survey considered parasites in their initial differential diagnosis and only one in 10 pediatricians considered parasites if the diarrhea was considered persistent. Unpublished survey data in caregivers also confirms the CDC findings (Black J. personal correspondence). This lack of awareness and failure to recognize and treat is intensified by the recent reports of long term negative consequences of parasitic infection [15,16]. In this review, we address recent reports on the epidemiology, diagnosis, and treatment of giardiasis and cryptosporidiosis with a focus on nitazoxanide.

Methodology

A search was conducted by Salamandra, LLC for published information pertaining to the use of nitazoxanide in the pediatric population in May of 2015. The searches were performed by a trained research associate using ProQuest Dialog™ and included BIOSIS Previews® (1926 to present), EMBASE® (1947 to present) and MEDLINE® (1946 to present). The search identified a total of 345 unique citations. All of the titles and abstracts were reviewed by a medical professional (M.D.) to identify clinical trials, meta-analyses, or recent reviews (within 10 years) of information pertaining to the use of nitazoxanide in the treatment of diarrhea caused by infections of Giardia lamblia (G. lamblia) or Cryptosporidium parvum (C. parvum)

in children ages >1 and ≤12 years old. The majority of citations described in vitro studies, animal studies, studies evaluating nitazoxanide in off-label use conditions, or were older reviews or case reports (greater than 10 years). In total, 28 publications were found to meet the criteria above.

A secondary review was also performed using only MEDLINE® to capture recent publications (within 10 years) that included metronidazole, tinidazole, and paromomycin. All titles were reviewed by the authors for appropriateness for inclusion in this manuscript. A general review was also performed for Giardia, giardiasis, Cryptosporidium, and cryptosporidiosis with no limit on the date of the citation.

Cryptosporidium

Epidemiology

Cryptosporidium has emerged as an important cause of diarrheal illness especially among children and patients with immune deficiencies. It is an intracellular protozoan first described in 1976. The mean prevalence rate for Cryptosporidium infection in general population is 1%-3% in North America but considerably higher in resource-poor nations (5%-10%). In the US, it is one of the most common causes of water-borne diseases with an estimate of 750,000 cases occurring each year [17]. The recent Global Enteric Multicenter Study (GEMS) of children under 5 years old in developing countries found Cryptosporidium to be among the top four causes of moderate-to-severe diarrhea and that such diarrhea is a "high risk factor for linear growth faltering and death" [18].

Currently, 20 Cryptosporidium species have been identified, but only 6 are considered to be human pathogens: C. hominis, C. parvum, C. emegridis, C. felis, C. canis, and the C. rabbit genotype. The majority of human infections are with C. parvum and C. hominis [19]. Acquisition of infection occurs mainly after ingestion of oocyst from contaminated water or food, or direct contact with human or animal feces [19].

Symptoms range from self-limited gastroenteritis in immune-competent humans to prolonged chronic illness in immune-compromised patients. The incubation period is about 2-10 days and commonly results in watery diarrhea with abdominal pain, nausea, and vomiting. Occasionally, non-specific symptoms such as headaches, myalgia, fatigue, and low-grade fever occur. Extra-intestinal manifestations of the disease are rare, but have been noted in patients with HIV infection, particularly involving the biliary tract, liver, pancreas, joints, and respiratory system [20]. Cryptosporidiosis is often under diagnosed in children with pre-existing irritable bowel disease and can cause significant illness leading to hospitalization [21].

The main site of the infection is the small intestine but can be widespread throughout the gastrointestinal track, with extra-intestinal sites. Cryptosporidium invades the luminal border of enterocytes and causes changes in the villous architecture with mononuclear cell infiltration of lamina propria. Pathogenesis is multifactorial consisting of a direct effect of the parasite on the immune system, causing inflammation, which results in poor absorption and enhanced secretion. Finally, Cryptosporidium also causes apoptosis of intestinal epithelium [22].

Diagnosis

Polymerase Chain Reaction (PCR) is now considered the "gold standard" to detect cryptosporidial DNA and will also identify the organism at a species level, unfortunately, PCR based tests are not yet readily available. The most commonly used diagnostic method to screen for Cryptosporidium is the detection of oocytes or antigens in stool samples. Generally, stool microscopy for ova, cysts and parasites will not detect Cryptosporidium oocytes. The healthcare providers should specifically request testing for Cryptosporidium. Rather than using direct microscopy, the laboratory will identify oocytes in stool specimens using acid-fast or direct fluorescent antibody staining (DFA). Alternatively enzyme immunoassays can be used to detect oocyst antigens and may be superior to acid-fast stain. The sensitivity and specificity of the enzyme immunoassay is comparable to the PCR [21].

Treatment

Although there are promising therapeutic agents and targets for cryptosporidial treatment, robust therapies that clear the infection completely are still lacking. Unique patient populations such as immunocompromised individual including HIV-infected patients, organ transplant recipients, patients on chemotherapy, or those who have primary or secondary immunodeficiency are in need of effective drug therapy. Three antibiotics (paromomycin, azithromycin, and nitazoxanide) have been the subject of clinical trials. There is limited data in the use of nitazoxanide in immunocompromised children.

Current treatment options for cryptosporidiosis are limited and only one drug, nitazoxanide, a thiazolide drug with broad antiparasitic activity, has been approved by the FDA in immunocompetent patients. Nitazoxanide works by inhibiting pyruvate ferredoxin oxidoreductase, an essential enzyme in the anaerobic metabolism of the parasite. Nitazoxanide and its metabolite tizoxanide are more active against the extracellular sporozoite stage, whereas tizoxanide glucuronide acts primarily on intracellular development of the parasite [23-25].

Administration of nitazoxanide after a meal alters the pharmacokinetics of tizoxanide, resulting in a shorter duration to reach maximum concentration and a higher area under the plasma concentration-time curve [24,26]. Because of this, it is recommended that nitazoxanide is given with food. Nitazoxanide does not significantly inhibit P450 enzymes in vitro and, therefore, no significant drug interactions are anticipated during concurrent use with medications that are metabolized by cytochrome P450 [26]. The efficacy of nitazoxanide in treating cryptosporidiosis in immunocompetent patients has been well established by three double-blind placebo-controlled studies [27-29].

In a study from Egypt, 50 adults and 49 children were treated with nitazoxanide or placebo for diarrhea caused by C. parvum [27]. Nitazoxanide was administered orally 500 mg twice a day for 3 days in adults and adolescents, 200 mg twice a day for 3 days in children ages 4-11 years, and 100 mg twice a day for 3 days in children ages 1-3 years. Seven days after initiation of therapy, the diarrhea resolved in 39 of 49 (80%) patients in the nitazoxanide group compared to 20 of 49 (41%) patients in the placebo group (P<0.0001). Diarrhea resolved in 80% of patients receiving nitazoxanide within 3 or 4 days of treatment initiation. In a subsequent trial by the same group in Egypt among 90 outpatients ≥12 years of age with Cryptosporidium-induced diarrhea or enteritis, nitazoxanide 500 mg or placebo as tablets twice daily for 3 days were randomly compared, while another group received

nitazoxanide 500 mg twice daily as an oral suspension for 3 days [29]. Twenty-seven of the 28 patients (96%) receiving nitazoxanide tablets responded clinically compared to 11 of 27 (41%) patients who received placebo (P<0.0001). Twenty-six of the 28 patients (93%) who received nitazoxanide were free of Cryptosporidium oocysts in each of the two post-treatment stool samples compared to 10 of 27 patients (37%) who received placebo (P<0.0001). The clinical response rate with the nitazoxanide suspension was 87% (27 of 31 patients). A third study in Zambia was performed among in children with cryptosporidial diarrhea who were HIV-seropositive (n=50) or HIV-seronegative (n=50). Most of the patients were <3 years and malnourished [28]. A 3-day course of nitazoxanide significantly improved the resolution of diarrhea, parasitological eradication, and mortality in HIV-seronegative children [28].

Treatment outcomes in immune-deficient patients, especially AIDS patients, have been less satisfactory. The second arm of the previously described study in Zambia of HIV-seropositive children did not show a significant benefit from nitazoxanide in post-treatment fecal examinations. Parasitological cure rate was significantly superior with both nitazoxanide 500 mg (63%, P=0.016) and 1000 mg (67%, P=0.013) twice daily compared to placebo (25%). An open study in Mali suggested that nitazoxanide may be effective against the parasite in stage 4 AIDS patients with "light" cryptosporidial infections but failed in "heavily infected" stage 4 AIDS patients [31]. Nitazoxanide was effective in the treatment of a 31-year-old AIDS patient with systemic cryptosporidiosis and sclerosing cholangitis. Cholestasis symptoms resolved after a 12-week course of nitazoxanide 500 mg twice daily [32].

A large compassionate use clinical trial was conducted in the USA to make nitazoxanide available to patients with acquired immune deficiency syndrome-related cryptosporidiosis [33]. A total of 365 patients were enrolled at 165 study centers. The duration of treatment was 1-1528 days (median 62 days). Among the 357 patients included in the intent-to-treat analysis, 209 (59%) achieved a sustained clinical response while on treatment. Clinical responses were closely associated with Cryptosporidium-negative stools (P<0.0001). No safety issues were identified at doses up to 3000 mg/day or for long durations of treatment.

Nitazoxanide was used to treat Cryptosporidium infection in five patients with inflammatory bowel disease who were on immunosuppressive medications. Three of them responded with complete resolution of symptoms within 5 days after the initiation of treatment with nitazoxanide, while the other two patients had significant improvement in stool frequency and output [21].

The aminoglycoside, paromomycin, has been one of the agents used to treat cryptosporidial disease in AIDS patients. In the first double-blind, placebo-controlled, crossover study in 10 AIDS patients with cryptosporidial diarrhea, there was a statistically significant improvement in diarrhea and reduction in oocyst excretion with paromomycin group [34]. Subsequently, the AIDS Clinical Trials Group conducted a larger double-blind, placebo-controlled study in 34 AIDS patients with cryptosporidiosis [35]. Seventeen patients received paromomycin 500 mg four times a day orally and 18 patients received placebo for 3 weeks. There were no significant differences with respect to efficacy outcome measure between the groups. Three patients on nitazoxanide (17.6%) and two patients on placebo (14.3%) had a complete response.

Azithromycin has been used in an open study of a limited number of AIDS patients with cryptosporidiosis [36]. Treatment for 14 days cleared oocyst excretion in stools in five of 13 patients and all showed symptomatic improvement within 7 days of treatment onset.

Nitazoxanide is the only drug approved for use in the treatment of C. parvum infection. The recommended dosage is 7.5 mg/kg twice daily for 3 days in children ≤11 years and 500 mg twice daily for 3 days in patients >12 years of age. It is best that the medication is taken with food. It is available as a liquid suspension for children and as a tablet formulation. In immunocompromised patients, longer duration of treatment or combination therapy can be considered [25].

Giardia

Epidemiology

Giardia lamblia (syn. G. duodenalis, G. intestinalis) is a flagellated protozoan that is the most common cause of intestinal parasitic infection in children living in resource-limited settings [37,38]. G. lamblia is also the most common intestinal parasite of humans identified in the United States and can be one of the most common infections associated with foreign travel among US residents [37-40]. The pathogenicity of G. lamblia has been debated since the parasite was first identified. It generally causes a self-limited clinical illness (i.e., giardiasis) typically characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption; asymptomatic infection also occurs frequently. However, case reports and epidemiologic studies have associated giardiasis with the development of long-term, more chronic immune-mediated disease including chronic enteric disorders, functional gastrointestinal disease, allergies, chronic fatigue, and reactive arthritis.

Importantly, from the public health perspective, the burden and cost of acute giardiasis in the United States as well as in Europe continue to be substantial [38,40-42]. An estimated 1.2 million cases occur annually according to CDC assessments [38,39,43]. Each year, hospitalizations resulting from giardiasis cost about $34 million; additionally, each ambulatory care visit for giardiasis costs $121-$273, depending on the patient's type of health-care insurance coverage [44,45]. Because giardiasis is the most commonly reported intestinal parasitic infection in the United States and no substantial declines in incidence have occurred in recent years, new epidemiologic studies are needed to identify effective public health measures that are able to reduce the overall burden of giardiasis [38,40,46].

In characterizing the epidemiology of Giardia, it is critical to understand proper "case" definition which is defined by the CDC are as follows: a confirmed case of giardiasis (i.e., one that has a positive laboratory finding) is defined as the detection of G. lamblia organisms, antigen, or DNA in stool, intestinal fluid, tissue samples, biopsy specimens, or other biological sample [38,39]. All confirmed or probable cases of Giardia should be reported to the CDC by each of the State Health Departments and healthcare providers are required to report identified or probable cases to their State Health Department [38,39].

The acquisition of giardiasis remains common in geographic areas with poor sanitary conditions and limited water-treatment facilities [38,39,47,48]. The prevalence of giardiasis has been reported to be as high as 20%-40% in resource-limited settings with the highest rates occurring among children <5 years of age.43 In a number of epidemiological studies, many individuals with G. lamblia identified in

stool samples are asymptomatic, an important point for consideration by the clinician in determining sources of infection within families when treating an index case. In population-based studies, Giardia can be more commonly identified in the stool of asymptomatic individuals compared to those with acute diarrhea [39,40,49].

In order to understand the epidemiology of Giardia, it is important to understand its life cycle [50,51]. Giardia species have two morphological forms: cysts and trophozoites. Cysts are the infectious form of the parasite; they are fecally excreted and can survive for prolonged periods in moist environments. After cyst ingestion, usually via the fecal-oral route, excystation occurs in the proximal small bowel with release of trophozoites. Trophozoites are pear-shaped, binucleate, multi-flagellated parasite forms capable of division by binary fission; they are mainly localized in the proximal small bowel. An adhesive disk on the ventral surface of the trophozoite facilitates its attachment to the mucosal surface of the duodenum and jejunum, although it does not invade the mucosal epithelium. Trophozoites that do not adhere to the small bowel move to the large intestine, where they revert to the infectious cyst form; conjugated bile salts appear to promote encystation. Cysts are passed back into the environment in excreted stool; in the setting of diarrhea, trophozoites can also be found in the stool. Following cyst ingestion, infections have an incubation of a week or more before symptoms of acute giardiasis may develop.

An essential component of the control of infection from a public health perspective is an understanding of the sources and routes of transmission in different geographical regions [38,42,52-54]. The ingestion of Giardia cysts typically occurs through the consumption of fecally-contaminated food or water or through person-to-person (or, to a lesser extent, animal-to-person) transmission [38,42,44,52-56]. The cysts are infectious immediately upon being excreted in feces. The infectious dose that is necessary to establish colonization in the new host is low; ingestion of 10 cysts has been reported to cause infection. Infected persons have been reported to shed 108-109 cysts in their stools per day and to excrete cysts for months [38,40,44-49]. Bovines are considered potential sources of infection for humans, because species and genotypes of Giardia infecting humans have also been isolated from cattle in molecular parasitological studies [47,57-61]. However, species and genotypes of Giardia of bovids, and the extent of zoonotic transmission in different geographical regions in the world, are still relatively poorly understood [57,58].

As investigators characterize more features of the human microbiome and its role in health and disease, an emerging clarity is arising among our ability to understand the diametrically opposed associations between Giardia and acute versus persistent diarrhea and a poorly understood potential for long-term sequelae, including impaired child growth and cognitive development [48,62-67]. The mechanisms driving these protozoal-associated long-term clinical outcomes remain to be fully characterized but recent advances suggest that variability in Giardia strains, host nutritional status, the composition of microbiota, co-infecting enteropathogens, host genetically determined mucosal immune responses, and immune modulation by Giardia are all relevant factors influencing disease manifestations after Giardia infection [16,62,68].

A recent report from the CDC provides information depicting the current epidemiology of Giardia infection [38]. Reasons given by these authors for the decrease in rates during 2011-2012 could include changes in transmission patterns, a recent change in surveillance case definition, increased uptake of strategies to reduce waterborne transmission, or a combination of these factors. Geographical differences might suggest actual regional differences in Giardia transmission or more importantly, the variation in surveillance capacity across states. Six states did not report giardiasis cases in 2011-2012, representing the largest number of non-reporting states since giardiasis became nationally notifiable in 2002. Finally, and an important point of clinical relevance for the pediatrician, is that giardiasis was reported more frequently in younger children. This observation might reflect increased contact with contaminated water or ill persons, or as speculated with respect to the changing human microbiome in children under age 3 years, a growing lack of immunity in young children [67,69-71].

Diagnosis

The identification of Giardia in humans, animals, and environmental reservoirs can be difficult in the past. There are some recent data to suggest that molecular approaches might serve as better vehicles for detection [38,40-53,55,56,60,61,69,72,73]. Giardia expresses considerable genetic variability represented by eight genetic clusters termed 'assemblages' (A-H). These assemblages are host restricted and can be zoonotic with A and B assemblages infecting humans and animals around the globe. The knowledge of the molecular epidemiology of human giardiasis in areas around the world is not well characterized and the usefulness of PCR to detect this pathogen in fecal samples remains controversial.

In a recent study of 145 Turkish children who were asymptomatic or symptomatic, PCR analysis revealed 22 G. lamblia isolates with assemblage A, B, and AB in 50.0%, 31.8%, and 18.2% of children, respectively [73]. There was no significant ($P>0.05$) association between specific assemblages and symptoms, although there was between the age of the child and assemblage AB ($P=0.001$). In another recent study among asymptomatic children from rural Colombia, 181 fecal samples revealed 13% positivity for Giardia by microscopy but 76%-80% by PCR depending on the molecular marker with no concordance between microscopy and PCR [60].

Molecular assays for rapid screening and the identification of causative agents have also been studied. One recent study, among others, demonstrates the accuracy of a “rapid” molecular assay to standard methods for parasite detection [74]. This study compared matched asymptomatic controls and cases in patient with persistent diarrhea by molecular multiplex testing with the Luminex® Gastrointestinal Pathogen Panel (GPP) to microscopy and rapid antigen detection tests. The results suggest that multiplex PCR assays can be a useful screening tool, but that positive results likely need to be confirmed by independent methods to discriminate active infection from asymptomatic fecal shedding of nucleic acid.

From a practical standpoint, it is important for the clinician to recognize that Giardia cysts can be excreted intermittently, thus, multiple stool collections (i.e., three stool specimens collected on separate days) can and do increase test sensitivity. Use of concentration methods and trichrome staining might not be sufficient to identify Giardia because variability in the concentration of organisms in stool can make this infection difficult to diagnose [37,51-54,67-73,75-78].

The traditional method is the microscopic identification of trophozoites and cysts on direct smears or concentrated specimens of stools. Trophozoites are fragile and more likely to be present in unformed stools as a result of rapid intestinal transit time. Stools should be examined fresh, or placed in preservative fluid. Laboratories can reduce reagent and personnel costs by pooling specimens

submitted for detection of Giardia from different patients before evaluation by EIA. Because variability in concentration of Giardia organisms in stool can make infection difficult to diagnose, fecal immunoassays that do not require microscopy (EIA) and rapid immunochromatogenic cartridge assays, which are rapid and more sensitive and specific, are preferred by many laboratories. Direct fluorescent antibody (DFA) testing is an extremely sensitive and specific detection method, and is considered the benchmark for accuracy by many laboratories. Other immunodiagnostic kits that do not require microscopy (e.g., enzyme immunoassay [EIA] testing and rapid immunochromatographic cartridge assays) also are available; they do not take the place of routine ova and parasite examination and DFA. Antigens shared by trophozoites and cysts excreted in stool specimens include the 65-kd antigen used in commercial diagnostic assays. Only molecular testing (e.g., PCR) can be used to subtype Giardia [47,74-77].

When giardiasis is suspected and stool specimens are negative, the string test, duodenal aspiration, or biopsy at the time of upper endoscopy can be performed [78-80]. In a fresh specimen, trophozoites usually can be visualized on direct wet mount. Duodenal biopsy is an optimal method for diagnosis. The biopsy sample can be used to make touch preparation for identifying Giardia in tissue sections and for histologic examination, and to identify abnormalities not associated with Giardia. Small intestinal biopsy should be considered in patients with characteristic clinical symptoms, negative stool and duodenal fluid specimens, and, one of the following: abnormal radiographic findings (such as edema and segmentation of the small intestine), abnormal lactose tolerance test, absent sIgA, hypogammaglobulinemia, or achlorhydria [79,81-84].

Identification of Giardia can be difficulty because of intermittent excretion of cysts. Additionally, medications, including antimicrobial agents, antacids, and ant-diarrheal compounds, and certain enema and laxative preparations, can interfere with identification of the organisms by altering morphology or by causing a temporary disappearance of the parasites from stool specimens. Such compounds should be withheld for 48-72 hours before collection of the stool for identification of Giardia. Because contrast material, such as barium used for imaging studies, also masks the presence of parasites, stools should be examined before performing tests with these materials [50,60,74,77].

Treatment

Treatment of G. lamblia infection currently relies on a small number of drug classes. The nitroheterocyclic class of pharmacologic agents, in particular metronidazole, represented the front-line treatment for Giardia infection over the last 40 years [85]. The management of Giardia infection in children and adults requires an accurate diagnosis to exclude several conditions that can mimic chronic giardiasis. Because Giardia is an easily transmissible infectious disease, optimal treatment should entail a public health-based approach which includes the recognition of the known modifiable causes of this health condition, assessment of symptoms and potential complications, their treatment utilizing, if necessary, a multidisciplinary team, and an ongoing monitoring for the effect of therapy - weighing the efficacy of individual drugs - all of these together may lead to a successful treatment of chronic giardiasis. Children with acute or chronic diarrhea with failure to thrive [36,79,87], malabsorption [81,87-89] or other gastrointestinal tract symptoms should be treated with an anti-protozoal agent if Giardia is identified in a stool or duodenal specimen [36,90,91].

Several drugs can be used to treat Giardia infection [85]. Effective treatments include metronidazole, tinidazole, and nitazoxanide. Alternatives to these medications include paromomycin, [92-94] quinacrine, [94,95] and furazolidone [96]. Some of these drugs may not be routinely available in the United States. Different factors may shape how effective a drug regimen will be, including medical history, nutritional status, and condition of the immune system. Therefore, it is important to discuss treatment options with the family of a child with identified Giardia infection.

In addition, there are a number of other approaches that have been used which both eradicate the organism and provide symptomatic relief to the infected patient. In particular, gastric acid-suppression agents, proton pump inhibitors, like omeprazole have been successfully employed in Giardia treatment [97,98]. Specifically, omeprazole enters the cytoplasmic compartment of the trophozoites and inhibits cellular triosephosphate isomerase activity in a dose-dependent manner. Such inhibition takes place concomitantly with the cytotoxic effect caused by omeprazole on trophozoites. A relatively recent study demonstrated that omeprazole was effective in vitro against drug-resistant and drug-susceptible strains of G. lamblia.

The nitroimidazoles, metronidazole and tinidazole have been used as specific antigiardial agent. Tinidazole, which is used as a single-dose therapy, was approved by the FDA in 2004 for children ≥3 years of age and adults for treatment of giardiasis. Nitazoxanide was approved by the FDA in 2003 for use in children with giardiasis and cryptosporidiosis, and more recently for giardiasis treatment in adults. Quinacrine and furazolidone are not available from any US manufacturer, although the drugs can be obtained from several US compounding pharmacies. Furazolidone, a nitrofuran derivative and a monoamine oxidase inhibitor, is approved by the FDA for treatment of giardiasis. Paromomycin [92-94,99], a luminally active aminoglycoside that is not absorbed, is less effective than the other agents but can be used for treatment in pregnant women. Albendazole [89,100-102] is useful for treatment when multiple intestinal parasites are identified or suspected.

A review of comparative trials on treatment of giardiasis reported that the cure rates for patients treated with metronidazole (n=219) was higher than for those treated with furazolidone (n=150) (92% vs. 84%, $P<0.001$) . Approximately 7% of metronidazole-treated patients and 10% of the furazolidone-treated patients had side effects that were adverse enough for the study subjects to report. A meta-analysis of 31 publications on treatment of giardiasis also showed that metronidazole administered >3 days appears to achieve a better parasitologic cure than do other long-term treatment regimens. Single-dose tinidazole was as effective as longer term treatments. The clinical response rate of tinidazole was 85% compared with 80% for metronidazole (tinidazole package insert). In one study, furazolidone, which is available in a pediatric liquid formulation, had a cure rate of 92% after a 10-day course. Metronidazole can be formulated into liquid preparations by special request at compounding pharmacies but taste can be a significant factor in limiting adherence to the prescribed treatment regimen. Asymptomatic excreters of Giardia cysts are generally not treated except in specific instances, such as repeated antibiotic treatment failures (possibly related to decreased antibiotic absorption), outbreak control, and prevention of household transmission by toddlers to pregnant women, or people with hypogammaglobulinemia or cystic fibrosis [89,103,104].

More recently, oral administration of the probiotic L. casei in conjunction with albendazole further reduced the Giardia infection as was evident by the restored normal gut morphology. This suggests that probiotics and antiprotozoal drugs in combination may be the better alternative therapy for treatment of gastrointestinal diseases and enhanced recovery.

First-line treatment typically should be a nitazoxanide. In a relatively recent placebo-controlled study [105], 100 children mean age 3.3 years with infectious diarrhea were treated with an oral nitazoxanide suspension twice daily for 3 days. Resolution of symptoms was faster, and there was an overall shorter duration of illness in the nitazoxanide treated cohort. Moreover, disease resolution was more effectively achieved in the nitaxoxanide-treated group whether G. lamblia was identified or in cases where no pathogen determined; underscoring the potential usefulness of this agent for empiric therapy of persistent diarrheal illness in children.

Discussion

G. lamblia and Cryptosporidium spp. remain important human pathogens. These parasites continue to cause disease and long-term sequelae worldwide. When Giardia or Cryptosporidium infection are suspected there are a number of newer diagnostic tests available, such as DNA based tests or immunofluorescence (DFA), that can assist the clinician in reach a rapid and accurate diagnosis. The initial clinical evaluation should include a thorough history including details of exposures (including recreational water exposure), travel, precedent antibiotic, and medication use. Supportive measures for the treatment of children with symptomatic giardiasis or cryptosporidiosis include correction of fluid and electrolyte abnormalities. If Giardia or Cryptosporidium is confirmed or highly suspected; antimicrobial eradication therapy for symptomatic patients is recommended. There is still controversy, as with a number of other enteric pathogens, regarding treatment for asymptomatic patients with either parasites. However, to prevent the spread of infection, treatment of asymptomatic carriers who are food handlers, household contacts of pregnant women or immunocompromised individuals, or children in a daycare or other settings who might transmit infection to others is clearly indicated. For eradication of Giardia we suggest nitazoxanide, metronidazole, or tinidazole as the drugs of choice for initial therapy. As discussed in this review, alternatives include albendazole, paromomycin, and furazolidone.

At present, it is not recommended to repeat the stool examination for parasite clearance in patients whose symptoms resolve. Patients with recurrent diarrhea should undergo re-evaluation of the stool for parasites before empiric retreatment since the diarrhea may be related to lactose intolerance rather than recurrent Giardia. The optimal approach to relapse after treatment remains to be determined; however, it is suggested that the clinician employ treatment with a drug from a different class. Alternative approaches include treatment with a second course of the original agent, use of a proton pump inhibitor (e.g., omeprazole) or to utilize a probiotic during the course of therapy, as well as treatment with a longer course or higher dose of the original agent, or treatment with a combination of drugs. Finally, person-to-person spread of giardiasis can be prevented through strict hand washing, care with diaper disposal, and treatment of symptomatic patients. Water-borne Giardia infection can be prevented through effective treatment of drinking water. Individuals with giardiasis should refrain from using recreational water venues until they have been asymptomatic for 2 weeks. Most importantly, since giardiasis is a reportable infection with public health impact, the clinician's local health department should always be contacted when an outbreak of giardiasis is suspected.

Conclusion

Nitazoxanide is the only drug FDA approved for use in children for the treatment of both C. parvum and G. lamblia infection. The recommended dosage is 7.5 mg/kg twice daily for 3 days in children ≤11 years and 500 mg twice daily for 3 days in patients >12 years of age. It is available either as a liquid suspension or as a tablet formulation. In immunocompromised patients, longer duration of treatment or combination therapy can be considered. There is a need for additional clinical studies to raise awareness among practitioners regarding the availability of an FDA approved liquid medication to treat these infections.

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References

1. Thompson RC (2000) Giardiasis as a re-emerging infectious disease and its zoonotic potential. Int J Parasitol 30: 1259-1267.
2. Kappus KD, Lundgren RG Jr, Juranek DD, Roberts JM, Spencer HC (1994) Intestinal parasitism in the United States: update on a continuing problem. Am J Trop Med Hyg 50: 705-713.
3. Chen XM, Keithly JS, Paya CV, LaRusso NF (2002) Cryptosporidiosis. N Engl J Med 346: 1723-1731.
4. Pitlik SD, Fainstein V, Garza D, Guarda L, Bolivar R, et al. (1983) Human cryptosporidiosis: spectrum of disease. Report of six cases and review of the literature. Arch Intern Med 143: 2269-2275.
5. Fayer R, Ungar BL (1986) Cryptosporidium spp. and cryptosporidiosis. Microbiol Rev 50: 458-483.
6. Savioli L, Smith H, Thompson A (2006) Giardia and Cryptosporidium join the 'Neglected Diseases Initiative'. Trends Parasitol 22: 203-208.
7. Gardner TB, Hill DR (2001) Treatment of giardiasis. Clin Microbiol Rev 14: 114-128.
8. Lalle M (2010) Giardiasis in the post genomic era: treatment, drug resistance and novel therapeutic perspectives. Infect Disord Drug Targets 10: 283-294.
9. Lemée V, Zaharia I, Nevez G, Rabodonirina M, Brasseur P, et al. (2000) Metronidazole and albendazole susceptibility of 11 clinical isolates of Giardia duodenalis from France. J Antimicrob Chemother 46: 819-821.
10. Upcroft JA, Dunn LA, Wright JM, Benakli K, Upcroft P, et al. (2006) 5-Nitroimidazole drugs effective against metronidazole-resistant Trichomonas vaginalis and Giardia duodenalis. Antimicrob Agents Chemother 50: 344-347.
11. Tejman-Yarden N, Millman M, Lauwaet T, Davids BJ, Gillin FD, et al. (2011) Impaired parasite attachment as fitness cost of metronidazole resistance in Giardia lamblia. Antimicrob Agents Chemother 55: 4643-4651.
12. Barry MA, Weatherhead JE, Hotez PJ, Woc-Colburn L (2013) Childhood parasitic infections endemic to the United States. Pediatr Clin North Am 60: 471-485.
13. Domjahn BT, Hlavsa MC, Anderson B, Schulkin J, Leon J, et al. (2014) A survey of U.S. obstetrician-gynecologists' clinical and epidemiological knowledge of cryptosporidiosis in pregnancy. Zoonoses Public Health 61: 356-363.
14. Attias E, Czinn SJ Harro C, Munoz FM, Sockolow RE, et al. (2015) Emerging Issues in Managing Pediatric Parasitic Infections: An

Assessment of Clinical and Epidemiological Knowledge of Giardiasis and Cryptosporidiosis. Pediat Therapeut 5: 254.

15. Wensaas KA, Langeland N, Hanevik K, Mørch K, Eide GE, et al. (2012) Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. Gut 61: 214-219.
16. Hanevik K, Wensaas KA, Rortveit G, Eide GE, Mørch K, et al. (2014) Irritable bowel syndrome and chronic fatigue 6 years after giardia infection: a controlled prospective cohort study. Clin Infect Dis 59: 1394-400.
17. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, et al. (2011) Foodborne illness acquired in the United States-major pathogens. Emerg Infect Dis 17: 7-15.
18. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, et al. (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382: 209-222.
19. Chalmers RM, Davies AP (2010) Minireview: clinical cryptosporidiosis. Exp Parasitol 124: 138-146.
20. López-Vélez R, Tarazona R, Garcia Camacho A, Gomez-Mampaso E, Guerrero A, et al. (1995) Intestinal and extraintestinal cryptosporidiosis in AIDS patients. Eur J Clin Microbiol Infect Dis 14: 677-681.
21. Vadlamudi N, Maclin J, Dimmitt RA, Thame KA (2013) Cryptosporidial infection in children with inflammatory bowel disease. J Crohns Colitis 7: e337-343.
22. Farthing MJ (2000) Clinical aspects of human cryptosporidiosis. Contrib Microbiol 6: 50-74.
23. Gargala G, Delaunay A, Li X, Brasseur P, Favennec L, et al. (2000) Efficacy of nitazoxanide, tizoxanide and tizoxanide glucuronide against Cryptosporidium parvum development in sporozoite-infected HCT-8 enterocytic cells. J Antimicrob Chemother 46: 57-60.
24. Stockis A, De Bruyn S, Gengler C, Rosillon D (2002) Nitazoxanide pharmacokinetics and tolerability in man during 7 days dosing with 0.5 g and 1 g b.i.d. Int J Clin Pharmacol Ther 40: 221-227.
25. Navarrete-Vázquez G, Chávez-Silva F, Colín-Lozano B, Estrada-Soto S, Hidalgo-Figueroa S, et al. (2015) Synthesis of nitro (benzo) thiazole acetamides and in vitro antiprotozoal effect against amitochondriate parasites Giardia intestinalis and Trichomonas vaginalis. Bioorg Med Chem 23: 2204-2210.
26. Anderson VR, Curran MP (2007) Nitazoxanide: a review of its use in the treatment of gastrointestinal infections. Drugs 67: 1947-1967.
27. Rossignol JF, Ayoub A, Ayers MS (2001) Treatment of diarrhea caused by Cryptosporidium parvum: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. J Infect Dis 184: 103-106.
28. Amadi B, Mwiya M, Musuku J, Watuka A, Sianongo S, et al. (2002) Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. Lancet 360: 1375-1380.
29. Rossignol JF, Kabil SM, el-Gohary Y, Younis AM (2006) Effect of nitazoxanide in diarrhea and enteritis caused by Cryptosporidium species. Clin Gastroenterol Hepatol 4: 320-324.
30. Rossignol JF, Hidalgo H, Feregrino M, Higuera F, Gomez WH, et al. (1998) A double-'blind' placebo-controlled study of nitazoxanide in the treatment of cryptosporidial diarrhoea in AIDS patients in Mexico. Trans R Soc Trop Med Hyg 92: 663-666.
31. Doumbo O, Rossignol JF, Pichard E, Traore HA, Dembele TM, et al. (1997) Nitazoxanide in the treatment of cryptosporidial diarrhea and other intestinal parasitic infections associated with acquired immunodeficiency syndrome in tropical Africa. Am J Trop Med Hyg 56: 637-639.
32. de la Tribonnière X, Valette M, Alfandari S (1999) Oral nitazoxanide and paromomycin inhalation for systemic cryptosporidiosis in a patient with AIDS. Infection 27: 232.
33. Rossignol JF (2006) Nitazoxanide in the treatment of acquired immune deficiency syndrome-related cryptosporidiosis: results of the United States compassionate use program in 365 patients. Aliment Pharmacol Ther 24: 887-894.
34. White AC Jr, Chappell CL, Hayat CS, Kimball KT, Flanigan TP, et al. (1994) Paromomycin for cryptosporidiosis in AIDS: a prospective, double-blind trial. J Infect Dis 170: 419-424.
35. Hewitt RG, Yiannoutsos CT, Higgs ES, Carey JT, Geiseler PJ, et al. (2000) Paromomycin: no more effective than placebo for treatment of cryptosporidiosis in patients with advanced human immunodeficiency virus infection. AIDS Clinical Trial Group. Clin Infect Dis 31: 1084-1092.
36. Kadappu KK, Nagaraja MV, Rao PV, Shastry BA (2002) Azithromycin as treatment for cryptosporidiosis in human immunodeficiency virus disease. J Postgrad Med 48: 179-181.
37. Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev 24: 110-140.
38. Painter JE, Gargano JW, Collier SA, Yoder JS; Centers for Disease Control and Prevention (2015) Giardiasis surveillance -- United States, 2011-2012. MMWR Surveill Summ 64 Suppl 3: 15-25.
39. Harvey K, Esposito DH, Han P, Kozarsky P, Freedman DO, et al. (2013) Surveillance for travel-related disease--GeoSentinel Surveillance System, United States, 1997-2011. MMWR Surveillance summaries 62: 1-15.
40. Schlagenhauf P, Weld L, Goorhuis A, Gautret P, Weber R, et al. (2015) Travel-associated infection presenting in Europe (2008-12): an analysis of EuroTravNet longitudinal, surveillance data, and evaluation of the effect of the pre-travel consultation. The Lancet Infect Dis 15: 55-64.
41. Pensabene L, Talarico V, Concolino D, Ciliberto D, Campanozzi A, et al. (2015) Postinfectious functional gastrointestinal disorders in children: a multicenter prospective study. J Pediatr 166: 903-907.
42. Iqbal A, Goldfarb DM, Slinger R, Dixon BR (2015) Prevalence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in diarrhoeic patients in the Qikiqta ni Region, Nunavut, Canada. Int J Circumpolar Health 74: 27713.
43. Yoder JS, Gargano JW, Wallace RM, Beach MJ; Centers for Disease Control and Prevention (CDC) (2012) Giardiasis surveillance--United States, 2009-2010. MMWR Surveill Summ 61: 13-23.
44. Berrilli F, Di Cave D, N'Guessan R, Kaboré Y, Giangaspero A, et al. (2014) Social determinants associated with Giardia duodenalis infection in southern Cote d'Ivoire. Eur J Clin Microbiol Infect Dis 33: 1799-1802.
45. Barry MA, Weatherhead JE, Hotez PJ, Woc-Colburn L (2013) Childhood parasitic infections endemic to the United States. Pediatr Clin North Am 60: 471-485.
46. Lv Z, Wu Z, Zhang L, Ji P, Cai Y, et al. (2015) Genome mining offers a new starting point for parasitology research. Parasitol Res 114: 399-409.
47. Bouzid M, Halai K, Jeffreys D, Hunter PR (2015) The prevalence of Giardia infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Vet Parasitol 207: 181-202.
48. Dorevitch S, DeFlorio-Barker S, Jones RM, Liu L (2015) Water quality as a predictor of gastrointestinal illness following incidental contact water recreation. Water Res 83: 94-103.
49. Boggild AK, Geduld J, Libman M, Ward BJ, McCarthy AE, et al. (2014) Travel-acquired infections and illnesses in Canadians: surveillance report from Can Trav Net surveillance data, 2009-2011. Open Med 8: e20-32.
50. Showler AJ, Wilson ME, Kain KC, Boggild AK (2014) Parasitic diseases in travelers: a focus on therapy. Expert Rev Anti Infect Ther 12: 497-521.
51. Wright SG (2012) Protozoan infections of the gastrointestinal tract. Infect Dis Clin North Am 26: 323-339.
52. Macchioni F, Segundo H, Gabrielli S, Totino V, Gonzales PR, et al. (2015) Dramatic decrease in prevalence of soil-transmitted helminths and new insights into intestinal protozoa in children living in the Chaco region, Bolivia. Am J Trop Med Hyg 92: 794-796.
53. David ÉB, Guimarães S, de Oliveira AP, Goulart de Oliveira-Sequeira TC, Nogueira Bittencourt G, et al. (2015) Molecular characterization of intestinal protozoa in two poor communities in the State of São Paulo, Brazil. Parasit Vectors 8: 103.
54. Daniels ME, Shrivastava A, Smith WA, Sahu P, Odagiri M, et al. (2015) Cryptosporidium and Giardia in Humans, Domestic Animals, and Village Water Sources in Rural India. Am J Trop Med Hyg 93: 596-600.

55. Boontanom P, Pipatsatitpong D, Tan-Ariya P, Mungthin M, Siripattanapipong S, et al. (2014) Incidence and risk factors of Giardia duodenalis infection in an orphanage, Thailand. Trop Biomed 31: 525-533.
56. Asher AJ, Holt DC, Andrews RM, Power ML (2014) Distribution of Giardia duodenalis assemblages A and B among children living in a remote indigenous community of the Northern Territory, Australia. PLoS One 9: e112058.
57. Abeywardena H, Jex AR, Gasser RB (2015) A perspective on Cryptosporidium and Giardia, with an emphasis on bovines and recent epidemiological findings. Adv Parasitol 88: 243-301.
58. Cardona GA, de Lucio A, Bailo B, Cano L, de Fuentes I, et al. (2015) Unexpected finding of feline-specific Giardia duodenalis assemblage F and Cryptosporidium felis in asymptomatic adult cattle in Northern Spain. Vet Parasitol 209: 258-263.
59. Ye J, Xiao L, Wang Y, Guo Y, Roellig DM, et al. (2015) Dominance of Giardia duodenalis assemblage A and Enterocytozoon bieneusi genotype BEB6 in sheep in Inner Mongolia, China. Vet Parasitol 210: 235-239.
60. Ramirez JD, Heredia Ramos RD, León CM, Hernandez DC, Moncada LI, et al. (2015) Molecular diagnosis and genotype analysis of Giardia duodenalis in asymptomatic children from a rural area in central Colombia. J Mol Epidemiol Evol Genet Infect Dis 32: 208-213.
61. Schär F, Inpankaew T, Traub RJ, Khieu V, Dalsgaard A, et al. (2014) The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. Parasitol Int 63: 597-603.
62. Bartelt LA, Sartor RB (2015) Advances in understanding Giardia: determinants and mechanisms of chronic sequelae. F1000Prime Rep 7: 62.
63. LaBeaud AD, Nayakwadi Singer M, McKibben M, Mungai P, Muchiri EM, et al. (2015) Parasitism in Children Aged Three Years and Under: Relationship between Infection and Growth in Rural Coastal Kenya. PLoS Negl Trop Dis 9: e0003721.
64. Gough EK, Stephens DA, Moodie EE, Prendergast AJ, Stoltzfus RJ, et al. (2015) Linear growth faltering in infants is associated with Acidaminococcus sp. and community-level changes in the gut microbiota. Microbiome 3: 24.
65. Hoen AG, Li J, Moulton LA, O'Toole GA, Housman ML, et al. (2015) Associations between Gut Microbial Colonization in Early Life and Respiratory Outcomes in Cystic Fibrosis. J Pediatr 167: 138-147.
66. Li Y, Tanner A (2015) Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries. Pediatr Dent 37: 226-244.
67. Sherman MP, Zaghouani H, Niklas V (2015) Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. Pediatr Res 77: 127-135.
68. Jex AR, Koehler AV, Ansell BR, Baker L, Karunajeewa H, et al. (2013) Getting to the guts of the matter: the status and potential of 'omics' research of parasitic protists of the human gastrointestinal system. Int J Parasitol 43: 971-982.
69. Jaeggi T, Kortman GA, Moretti D, Chassard C, Holding P, et al. (2015) Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. Gut 64:731-742.
70. Logan AC, Katzman MA, Balanza-Martinez V (2015) Natural environments, ancestral diets, and microbial ecology: is there a modern "paleo-deficit disorder"? Part II. J Physiol Anthropol 34: 9.
71. Nobel YR, Cox LM, Kirigin FF, Bokulich NA, Yamanishi S, et al. (2015) Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat Commun 6: 7486.
72. Crannell ZA, Cabada MM, Castellanos-Gonzalez A, Irani A, White AC, et al. (2015) Recombinase polymerase amplification-based assay to diagnose Giardia in stool samples. Am J Trop Med Hyg 92: 583-587.
73. Tamer GS, Kasap M, Er DK (2015) Genotyping and phylogenetic analysis of Giardia duodenalis isolates from Turkish children. Med Sci Monit 21: 526-532.
74. Becker SL, Chatigre JK, Gohou JP, Coulibaly JT, Leuppi R, et al. (2015) Combined stool-based multiplex PCR and microscopy for enhanced pathogen detection in patients with persistent diarrhoea and asymptomatic controls from Cote d'Ivoire. Clin Microbiol Infect 21: 591.
75. Beal SG, Couturier MR, Gander RM, Doern CD (2014) Diagnostic Algorithm for the Diagnosis of Pediatric Parasitic Gastroenteritis. J Clin Lab Anal .
76. Jahan N, Khatoon R, Ahmad S (2014) A Comparison of Microscopy and Enzyme Linked Immunosorbent Assay for Diagnosis of Giardia lamblia in Human Faecal Specimens. J Clin Diagn Res 8: DC04-DC06.
77. Maas L, Dorigo-Zetsma JW, de Groot CJ, Bouter S, Plötz FB, et al. (2014) Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR. Clin Microbiol Infect 20: 545-550.
78. Martínez-Gordillo MN, González-Maciel A, Reynoso-Robles R, Montijo-Barrios E, Ponce-Macotela M (2014) Intraepithelial giardia intestinalis: a case report and literature review. Medicine (Baltimore) 93: e277.
79. Albuquerque A (2014) Nodular lymphoid hyperplasia in the gastrointestinal tract in adult patients: A review. World J Gastrointest Endosc 6: 534-540.
80. Abulhasan M, Elshazly TA, Eida M, Albadry A (2013) Giardia intestinalis in patients with nonulcer dyspepsia. Arab J Gastroenterol 14: 126-129.
81. Ventura LL, Oliveira DR, Viana JC, Santos JF, Caliari MV, et al. (2013) Impact of protein malnutrition on histological parameters of experimentally infected animals with Giardia lamblia. Exp Parasitol 133: 391-395.
82. Blackwell AD, Martin M, Kaplan H, Gurven M (2013) Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. Proceedings B 280.
83. Kamda JD, Nash TE, Singer SM (2012) Giardia duodenalis: dendritic cell defects in IL-6 deficient mice contribute to susceptibility to intestinal infection. Exp Parasitol 130: 288-291.
84. Lalle M, Camerini S, Cecchetti S, Blasetti Fantauzzi C, Crescenzi M, et al. (2011) Giardia duodenalis 14-3-3 protein is polyglycylated by a tubulin tyrosine ligase-like member and deglycylated by two metallocarboxypeptidases. J Biol Chem 286: 4471-4484.
85. Kimberlin DW, Brady MT, Jackson MA (2015) Giardia intestinalis (formerly Giardia lamblia and Giardia duodenalis) infections. (30 ed) Elk Grove Village, IL American Academy of Pediatrics.
86. Granados CE, Reveiz L, Uribe LG, Criollo CP (2012) Drugs for treating giardiasis. Cochrane Database Syst Rev 12: CD007787.
87. Speich B, Marti H, Ame SM, Ali SM, Bogoch II, et al. (2013) Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide. Parasit Vectors 6: 3.
88. Astiazarán-García H, Iñigo-Figueroa G, Quihui-Cota L, Anduro-Corona I (2015) Crosstalk between Zinc Status and Giardia Infection: A New Approach. Nutrients 7: 4438-4452.
89. Nabarro LE, Lever RA, Armstrong M, Chiodini PL (2015) Increased incidence of nitroimidazole-refractory giardiasis at the Hospital for Tropical Diseases, London: 2008-2013. Clin Microbiol Infect 21: 791-796.
90. van Lieshout L, Roestenberg M (2015) Clinical consequences of new diagnostic tools for intestinal parasites. Clin Microbiol Infect 21: 520-528.
91. Krumkamp R, Sarpong N, Schwarz NG, Adlkofer J, Loag W, et al. (2015) Gastrointestinal infections and diarrheal disease in Ghanaian infants and children: an outpatient case-control study. PLoS Negl Trop Dis 9: e0003568.
92. Nash TE (2013) Unraveling how Giardia infections cause disease. J Clin Invest 123: 2346-2347.
93. Rossignol JF (2010) Cryptosporidium and Giardia: treatment options and prospects for new drugs. Exp Parasitol 124: 45-53.
94. Lalle M (2010) Giardiasis in the post genomic era: treatment, drug resistance and novel therapeutic perspectives. Infect Disord Drug Targets 10: 283-294.

95. Wright JM, Dunn LA, Upcroft P, Upcroft JA (2003) Efficacy of antigiardial drugs. Expert Opin Drug Saf 2: 529-541.

96. Tejman-Yarden N, Millman M, Lauwaet T, Davids BJ, Gillin FD, et al. (2011) Impaired parasite attachment as fitness cost of metronidazole resistance in Giardia lamblia. Antimicrob Agents Chemother 55: 4643-4651.

97. Pérez-Villanueva J, Romo-Mancillas A, Hernández-Campos A, Yépez-Mulia L, Hernández-Luis F, et al. (2011) Antiprotozoal activity of proton-pump inhibitors. Bioorg Med Chem Lett 21: 7351-7354.

98. Reyes-Vivas H, de la Mora-de la Mora I, Castillo-Villanueva A, Yépez-Mulia L, Hernández-Alcántara G, et al. (2014) Giardial triosephosphate isomerase as possible target of the cytotoxic effect of omeprazole in Giardia lamblia. Antimicrob Agents Chemother 58: 7072-7082.

99. Meltzer E, Lachish T, Schwartz E (2014) Treatment of giardiasis after nonresponse to nitroimidazole. Emerg Infect Dis 20: 1742-1744.

100. Shukla G, Kaur H, Sharma L (2013) Comparative therapeutic effect of probiotic Lactobacillus casei alone and in conjunction with antiprotozoal drugs in murine giardiasis. Parasitol Res 112: 2143-2149.

101. Swanson SJ, Phares CR, Mamo B, Smith KE, Cetron MS, et al. (2012) Albendazole therapy and enteric parasites in United States-bound refugees. N Engl J Med 366: 1498-1507.

102. Bernal-Redondo R, Martínez-Méndez LG, Mendoza-Chavez A, Velasco-Perales D, Chavez-Munguia B (2004) Evaluation of the in vitro effect of albendazole, metronidazole and nitazoxanide on viability and structure of Giardia lamblia cysts. J Submicrosc Cytol Pathol 36: 241-245.

103. Escobedo AA, Alvarez G, González ME, Almirall P, Cañete R, et al. (2008) The treatment of giardiasis in children: single-dose tinidazole compared with 3 days of nitazoxanide. Ann Trop Med Parasitol 102: 199-207.

104. Yadav P, Tak V, Mirdha BR, Makharia GK (2014) Refractory giardiasis: a molecular appraisal from a tertiary care centre in India. Indian J Med Microbiol 32: 378-382.

105. Rossignol JF, Lopez-Chegne N, Julcamoro LM, Carrion ME, Bardin MC (2012) Nitazoxanide for the empiric treatment of pediatric infectious diarrhea. Trans R Soc Trop Med Hyg 106: 167-173.