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Review

Key aspects of coccidia associated with diarrhea in HIV patients

Lucía Quesada-Lobo

Abstract

Intestinal parasites affect mainly developing countries and constitute a public health problem, often related to the lack of efficient health systems, or drinking water sources. These are also enhanced by underlying diseases such as AIDS, which are also present in developed countries. The literature describes that *Cryptosporidium* spp, *Isospora belli* and *Cyclosporacayetanensis* are the parasites most frequently associated with persistent diarrhea in patients with advanced cases of HIV/AIDS.

This group of protozoa requires specific tests for diagnosis; the Ziehl-Neelsen stain is one of the non routine tests that allows their identification. In most cases, it is not performed in the laboratory if not explicitly requested by the doctor.

Keywords: diarrhea, coccidia, *Cryptosporidium*, *Isospora*, *Cyclospora*, AIDS

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Worldwide, 33,4 million people are infected with HIV; most of them live in countries of low and middle income. It has been estimated that in 2010, 2,7 million people were infected.[1] In late stages of the disease, there is a decrease in the number of CD4+ cells, below 200 cells/mm^3 and development of opportunistic infections, tumors, and neurologic complications.[2]

Tropical intestinal parasitoses principally affect developing countries and constitute a public health problem, often related with the lack of an efficient health system, sources of drinking water; but also accentuated by underlying diseases, like HIV, that also affect developed countries. The literature describes *Cryptosporidium spp, Isospora belli* and *Cyclosporacayetanensis* as the parasites most frequently associated with persistent diarrhea and weight loss, in patients with advanced cases of HIV/AIDS.[3-8]

Likewise, it has been described as one of the parasites associated with diarrhea cases in children younger than 5 years of age, with a prevalence that varies from 7,7% up to 85,1%.[3,4] The objective of this review is to highlight the coccidia that play a role as the main agents in episodes of diarrhea in patients with HIV/AIDS, the pathology in these patients and the diagnostic tools.

Authors' Affiliation: University of Costa Rica and Clinical Laboratory, Mexico Hospital[1]

Abbreviations: HIV: human immunodeficiency virus, AIDS: acquired immunodeficiency syndrome

Correspondence:
luquesadalobo@hotmail.com

Isospora belli

The species of the genre *Cystoisospora (Isospora)* are parasitic protozoa that belong in the *Apicomplexa* phylum, which are also known as *coccidia.* The term *coccidia* also involves the species *Cryptosporidium, Toxoplasma gondii* and other members of the *Eimeriorina* suborder. In general terms, *coccidia* are characterized for having complex life cycles. However, members of the genre *Cystoisospora* are capable of completing their life cycle in a single host. The oocysts of the species in the *Cystoisospora* genre contain two sporocysts, each one with four sporozoites. The oocysts of the genre *Toxoplasma* and *Sarcocystis*, among others, are constituted in a similar way, but these parasites are heteroxenous and have an intermediate vertebrate host, unlike *Isospora*, which is the reason they belong in another family of *coccidia.*[5]

It is not possible to determine if the authors in early descriptions referred to *C. belli* or species of *Sarcocystis.* An example of this confusion can be found in a pioneer work about *coccidiosis* of E.R. Becker, published in 1934. Becker includes sketches that exemplify the sporulation of *C. belli*, but when referring to the parasite, he does as *I. hominis.*[5]

Infections by *C. belli* are cosmopolite, but they appear with a higher frequency in tropical and subtropical regions, especially in Haiti, Mexico, Brasil, El Salvador, Africa, Middle East and Southeast Asia.[6,7,8] During a longitudinal study conducted in Los Angeles with patients with HIV/AIDS, it was determined that the prevalence of *C. belli* found among 16.351 patients through 8 years, was of 1%. This prevalence was higher when patients were born in El Salvador (prevalence of 7,4%), Mexico (prevalence of 5,4%) or any other Hispanic ethnicity (prevalence of 2,9%). Patients with previous history of pneumonia by *Pneumocystis jirovecci* resulted less prone to infection with *C. belli*, probably because they received treatment with TMP-SMX. Investigators concluded that this behavior of cystoisosporiasis, of higher prevalence in patients with HIV/AIDS of Latin origin, is due to their travels to their countries, where the disease is more abundant, or because of a recent immigration.[8]

In another research conducted in Port-au-Prince, Haiti, 20 out of 131 (15%) patients with HIV/AIDS with opportunistic infections were infected with *C. belli*.[9] In Sao Paulo, Brasil, of 81 feces samples from immunocompetent patients, all of them resulted negative for *C. belli*; while, from other 81 samples of feces from patients with HIV/AIDS, 13 resulted positive (9,9%).[10] Among developed countries, in Spain, 3 out of 60 samples (5%) of patients with HIV/AIDS with diarrhea were positive for oocysts of *C. belli*.[11]

Domestic animals like the pig, the dog, the cat and the rabbit, are not appropriate definite hosts to harbor the infection.[12,13] Nonetheless, it is unknown if other animals could serve as hosts during transmission of *C. belli*, but the existence of paratenic hosts could explain why infections occur where sanitary measures are correct.[5]

C. belli produces, in immunocompetent patients, an episode characterized by steatorrhea, cephalea, fever, malaise, abdominal pain, vomiting, dehydration and weight loss. Blood in stools is not a common finding, as the peripheral eosinophilia observed in some patients. The disease tends to chronicity, with parasites on feces or in biopsy samples, even months or years later, and recurrence is frequent.[14,15,16]

The disease is more sever in children as compared to adults. There is a record of a child 6 months old that remained with an infection by *C. belli* for 30 weeks, which had a fatal outcome, despite the continuous parenteral nourishment.[17] In this case, the diarrhea was characterized by stools of 1 to 3 liters daily, due to a hypersecretion of cholera-type intraluminal fluid. However, not all infections in children develop that way, and there are multiples cases in which the patient recovers without major problems.[18]

In individuals with other immunocompromises, cystoisosporiasis also tends to be more severe in comparison with the immunocompetent patient. There are cases reported of infections with *C. belli* in patients with Hodgkin´s disease,[14] with non-Hodgkin lymphoproliferative disease,[19] in adults with T cell leukemia associated with type I human virus of T cells[20] and in patients with acute lymphoblastic leukemia.[21]

The diarrhea due to an infection with *C. belli* in individuals with AIDS is normally a secretory-type diarrhea, which, besides the febrile episode and the significant weight loss, leads to a very severe dehydration that requires hospitalization. Despite these findings, intestinal lesions caused by *C. belli* and the response to the treatment are similar in immunosuppressed and immunocompetent patients.[5]

There have been described cases of extraintestinal cystoisosporiasis in patients with AIDS.[22,23] This type of episode can be present as a history of dysphagia, nauseas, vomiting and profuse diarrhea, with a significant weight loss and in association with opportunistic infections like *Pneumocystis carinii*, cytomegalovirus and candidiasis.[22] During autopsies it has been determined the presence of severe cachexia, erythematous and hemorrhagic foci in small intestine, ulcerations up to 5 mm in diameter, atrophy of the brush border and hypertrophy of the lymphatic mesenteric ganglia.[5]

The drug, for both treatment and profilaxis, is the trimethoprim-sulfamethoxazole (TMP-SMX). Ciprofloxacine is the alternative in those cases in which the TMP-SMX is contraindicated (hypersensitivity, severe hematological disorders, infants younger than 2 months old, among others). In a randomized controlled trial, 22 patients with AIDS and infection with *C. belli* were assigned 160 and 800 mg of TMP-SMX, respectively or, 500 mg of ciprofloxacine twice a day during 7 days.[24] Those who responded clinically and microbiologically later received prophylaxis during 10 weeks (one dose 3 days a week). The diarrheic episode was resolved in 10 of 12 patients treated with ciprofloxacine. Only one of the patients treated with TMP-SMX continued excreting *Cystoisospora*, even on day 7 post-treatment, but without diarrheic symptoms, and with 3 additional days of treatment, there were no more oocysts detected in the feces. Another option is pyrimethamine associated with folic acid during 14 days, followed by prophylactic doses.[25]

Cryptosporidium spp

Tyzzer was the first to establish the genre *Cryptosporidium*, as well as to acknowledge the existence of various species through studies with *C. muris* from mice.[26] After the discovery of *Cryptosporidium,* nearly 50 years passed during which the parasite was constantly confused with *coccidia* from the *Sarcocystis*genre. Such confusion between both genres was because many of the oocysts of *Sarcocystis*spp.have a thin wall that frequently breaks and frees sporocysts, each one with four sporozoites, such as the oocysts of *Cryptosporidium* that harbor in their interior four sporozoites.[27] In the last years, various molecular characterizations of *Cryptosporidium* have contributed to elucidate the confused taxonomy of this protozoan, as well as to validate the existence of multiple species. As a result there are *C. andersoni* in cattle, *C. canis* in dogs, *C. felis* in cats, *C. hominis* in humans, *C. baileyi* in chickens and other birds, *C. galli* in birds, *C. meleagridis*in birds and humans, *C. molnari* in fish, *C. muris*in rodents and other mammals, *C. parvum* in ruminants and humans, *C. wrairi*

in guinea pigs, *C. saurophilum and C. serpentis* in lizards and snakes.[26-32]

The infectious form of *Cryptosporidium* corresponds to the oocyst, a resistance element of the parasite which permits the dissemination of the infection. The oocyst presents a wall that can be either thin or thick, which is related with different ways of sporogonic development and infection.[27]

Most recent efforts regarding research on cryptosporidiosis are directed mainly towards the ecological characterization of the parasite, its infection sources, and the associated risk factors. For this purpose, resources have been used such as molecular instruments, identification of metabolic pathways typical of *Cryptosporidium* for the development of therapeutic drugs, and the clarification of the immune response during the infection.[33] Thanks to these efforts, the complete sequencing of the genome was made easier for *Cryptosporidium parvum, Cryptosporidium hominis* and *Cryptosporidium muris.*

Cryptosporidiosis is an infection distributed worldwide and it is among the most important emergent diarrheic diseases of the group of infections transmitted by contamination of food or water. Many of the cases are sporadic, but nearly 10% are epidemic-type episodes originated by the consumption of contaminated water and food.[24]

Cryptosporidiosis is responsible of high morbidity and mortality among patients infected with HIV. Within this group of people, those who have diarrhea elevate the prevalence up to 14% and 24% in developed and developing countries respectively.[34]

In a study conducted with 275 patients with AIDS stage disease and with chronic diarrhea, it was determined that the causal agent in 15,6% of the cases was *Cryptosporidium* spp. Out of that group, 33,3% were homosexual persons, and 10,6% used intravenous drugs. A 30% of the 15,6% infected patients with *Cryptosporidium* spp., also suffered of extraintestinal cryptosporidiosis.[35]

In 1982, the Center for Disease Control in Atlanta documented the role of *C. parvum* as the responsible for the infection of 21 patients with AIDS and 12 immunocompetents. These persons were exposed to *C. parvum* of bovine origin. Animal-human transmission has been observed, particularly from cattle to veterinarians, or towards inhabitants of rural zones, even from rabbits in laboratory context, towards investigators.[36] A series of studies point out that transmission of human isolates is contagious for a variety of mammals.[37] The infection of tourists has been described, mainly while travelling to developing countries, of workers in health centers and of personnel and children in daycares.[38,39,40] The infection in human beings with isolates from non-mammal animals has not been studied extensively.

Cryptosporidium is most studied of the *coccidia* associated with diarrhea; therefore, it is the one for which more information is available regarding its pathology and immunology. It infects mainly the small intestine. Regardless of the species, infection with *Cryptosporidium* spp. manifests itself as a diarrheic episode in 90% of cases. The immunocompetent patient ends up controlling the infection; it is characterized as an aqueous diarrhea, acute and self-limiting, that lasts from 5 to 10 days. However, in patients with defects in immune cellular response (AIDS, malnutrition, leukemia, etc.), *Cryptosporidium* frequently causes a chronic diarrhea that could involve the biliary tract.[41]

Despite numerous investigations on animal models conducted with *Cryptosporidium pavrum*, the reach of these models to explain the human immunological response is limited. The clinical perspective in rodents is far from the human one, as the mice don't develop diarrhea after the infection. Non-human primates; although they could probably be the best model, since they mimic the human disease; they present many technical difficulties in comparison with the murine models. *Cryptosporidium hominis*, the principal causal agent of cryptosporidiosis, infects only human and gnotobiotic pigs, these makes the animal models used for its study very limited. To make the analysis even more difficult, the comparison that emerges between the animal and human models shows that the immunological response is very far from each other. For example, it looks like in the mouse, the production of IFN is associated with innate and primary responses,[42,43] while it is possible that in the human it is more associated with the memory response towards the parasite.[44]

Cryptosporidium spp. has been detected in mesenteric lymphatic nodules.[45]It is believed that these are also sites that trigger an adaptive immune response. In the infection by *Cryptosporidium*, the secretion of chemokines by the epithelial cells produces recruitment of activated T cells towards the own lamella, which come from the Peyer plates, via NLM and through circulation. Regarding the presence of antibodies, even though there have been found specific immune globulines subtype IgG, IgM, IgA and IgE in patients infected and convalescent, it has been proven that mice with B cell depletion were able to resolve the infection by *Cryptosporidium.* At any rate, the secretory IgA would collaborate in controlling the infection, by blocking the entrance of the luminal stages of the parasite.[45]

During the course of the infection the following are produced: IL-8, chemokine ligand 5 (CCL5 or RANTES), monocyte chemotactic protein-1 (MCP-1) and macrophagic inflammatory protein-2 a (MIP-2 a).[45] These substances have a chemotactic function. Different cellular types (T lymphocytes, *natural killer* cells or NK, dendritic cells, monocytes, neutrophils, eosinophils, and basophils) are attracted towards the site of the infection, due to specific interactions between molecules and their receptors. The cells that are recruited to the intestine in a higher quantity are lymphocytes, macrophages and neutrophils, although these last would not have a relevant function in controlling *Cryptosporidium* spp.[45] Nonetheless, what is determinant for the resolution of the diarrhea are the CD4+ lymphocytes, reason for which this pathology is exacerbated in patients with AIDS.

Pulmonary compromise is a rare complication of intestinal cryptosporidiosis, it has been described in patients that are

immunocompromised; most of them with advanced HIV/AIDS disease. Clinical manifestations of pulmonary cryptosporidiosis are unspecific and include chronic cough, fever and dyspnea as the most frequent symptoms, and it can be accompanied or not by radiologic signals.[46]

The patient with immunocompetence often resolves the infection by himself. In the immunocompromised patient, the severity of the symptoms and the development of the disease justify the use of therapeutic drugs. In the patient with AIDS, it is vital to reestablish, as far as possible, immunity, by means of highly active antiretroviral therapy, including HIV-protease inhibitors.[47]

Regarding an effective treatment, specific against *Cryptosporidium* spp., there is no determined drug that yet exists in the market.[24] Only the nitazoxanide, the paromomycin and some macrolides combined with other antibiotics, are considered moderately recommended, without them being considered curative. Nitazoxanide has been utilized in a dose of 500-1500 mg twice a day, during several weeks, even months. Paromomycin can be used on immunocompetent patients at a dose of 1500-2000 mg daily, during 7-14 days, to reduce symptoms, but without eradicating *Cryptosporidium*. In patients with AIDS, paromomycin appears to have no effect. Among the macrolides, various trials catalog the spiramycin, the clarithromycin, and the azithromycin, as drugs with doubtful efficiency in patients with AIDS. It is possible that Rifabutin, administered as prophylaxis against *Mycobacterium aviaum*, has a protective effect in a percentage of the cases of patients with AIDS.[24]

Cyclosporacayetanensis

In 1986, Soave and coworkers[48] described a diarrheic episode in four travelers returning from Mexico and Haiti, and they suggested that the causal agent was a new pathogen. Between 1991 and 1992, Ortega and colleagues[49] characterized this organism that during a long time generated controversy, and they defined it as a new species of *coccidian* capable of infecting the human being and belonging to the *Cyclospora* genre.

When a susceptible host consumes water or food contaminated with infectious oocysts, the sporozoites are freed to infect the intestinal epithelium of the duodenum and jejunum. After two cycles of asexual replication, type I y II meronts are formed; and this last form differentiates in sexual forms or gametocytes. The macrogametocyte is fertilized by the microgametocyte and a zygote is produced, from which the oocyst develops, it will then be excreted through feces in the form of non-sporulated oocysts.[50]

A great part of the information regarding the epidemiology of *Cyclospora* comes from tourists and countries where the protozoan is endemic, like Haiti, Guatemala, Peru and Nepal. Between 1996 and 1998 there were a series of epidemic diarrhea cases secondary to *C. cayetanensis* in the United States and Canada. In both countries the infection was attributed to the consumption of imported raspberries from Guatemala.[51-53]Researchdone after this situations suggested that Guatemala is a country where the infection by *Cyclosporacayetanensis* is endemic.[54,55]

The infection by *Cyclospora*is characterized by anorexia, nausea, flatulence, fatigue, abdominal pain, diarrhea, low grade fever and weight loss.[56-60] Clinical presentation varies slightly between non-endemic and endemic areas, where asymptomatic infections are more frequent. The clinical symptoms are more severe in children. In the endemic regions, the episodes tend to be more favorable each time as the patient advances in his life and the duration of the infection is shorter. In the elderly, as in children, the symptoms become more severe again.[53,56,61]

Infection by *Cyclospora* can be asymptomatic in endemic regions and immunocompetent patients. However, severe diarrhea has been reported in both cases. There are a few reports of fatal outcomes in cases of infection by *Cyclospora*.[62] In immunocompetent patients a moderate weight loss is experienced (3,5 kg. average), while the weight loss is much greater in the patient with AIDS (7,2 kg. average).[63,64]The incubation period lasts around 7 days.[57,58] Nonetheless, there is a difference regarding its duration between immunocompetents and patients with AIDS. While in the first group the disease persists for around 57 days, in patients of the second group it extends to around 199 days.[65,66] The frequency of bowel movements in immunocompetent patients with diarrhea by *Cyclospora* is arount 5 to 15 movements per day. Besides to the sudden loss of fluids, a poor absorption of D-xylose has been reported.[67]Biliary disease is also reported after the infection with *Cyclospora* with a higher frequency in the patient with AIDS.[66]Cholecystitis is another finding in patients with AIDS,[66,68] which presented as abdominal pain in the upper-right quadrant and elevation of the alkaline phosphatase.[60]

In both immunocompetentand immunocompromised patients, co-infections with Cyclospora, Cryptosporidium and other parasites have been described.[69] Moreover, Cyclosporaoocysts have been detected in samples of non-gastrointestinal origins. There are two reports of the presence of oocysts in sputum of HIV-positive patients, with a background of pulmonary tuberculosis,[70,71]this suggests that Cyclosporacould be considered an opportunistic pathogen.

Treatment with TMP-SMX, in various dosage schemes, has resulted effective in most cases to cure the diarrhea and to achieve the neutralization in the feces samples. Ciprofloxacin is the second choice treatment. The recurrence rate of this infection is higher when prophylactic treatment is not applied after treatment with the previously mentioned drugs.[24]

Diagnosis

Cystoisospora belli, Cryptosporidium spp. and *Cyclospora*cayetanensis can be detected in feces samples by very similar methods. In the three cases, the fixed and dyed

samples with Ziehl-Neelsen or Kinyoun techniques allow the observation of the oocysts with a color that goes from light pink to an intense red. In the case of *Cystoisospora belli*, the identification of anoocystthat is usually non-sporulatedand of 20-30 um in diameter, can also be done using the Giemsa stain and that produces neon-blue auto fluorescence, at a wave length of 330-380 nm.[72] In order to detect *Cryptosporidium* spp. in feces and other fluids, like sputum and biliary contents, fluorescence techniques have been developed with the use of specific antibodies, as well as other molecular techniques; however, acid-resistant stains continue to be the used commonly. The importance of these techniques is that, depending on their design, they help identify the species of *Cryptosporidium*, which is fundamental at an epidemiologic level, since there are anthroponotic and anthropozoonoticspecies.[24]

Conclusions

All *coccidiosis* associated to diarrhea in immunocompetent individuals are characterized by an acute diarrhea, normally self-limited. In immunocompromised patients, the disease can acquire severe forms, potentially fatal. People affected by the acquired immunodeficiency syndrome are particularly prone to suffer severe forms of cryptosporidiosis.

In regard to the approach for *coccidiosis* caused by *Cystoisospora belli* and *Cyclosporacayetanensis*, it does possess an adequate treatment scheme, especially relevant in the patient with AIDS. Up to date, what appears to generate the best response for the treatment of diarrhea by *Cryptosporidium*, is the nitazoxanide; unfortunately, an antiparasitic that is 100% effective against this agent still does not exist.

Opportunistic infections continue causing morbidity and mortality in patients with HIV all over the world, and the CD4+ count is strongly associated to the probability of progression of these diseases and death in these patients. Random and controlled trials and cohort studies, have documented that retroviral therapy reduces the incidence of opportunistic infections and, therefore, mortality.

It must be remembered that this group of protozoa require specific tests for its diagnostic. The intestinal *coccidia* can be distinguished with the Ziehl-Neelsentinction, one of the non-routinary tests that allow its identification and, that in many occasions, is not performed unless requested by the physician.

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