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Opportunistic intestinal parasites in immunocompromised patients from a tertiary hospital in Monterrey, Mexico

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SUMMARY

Opportunistic parasites are still important agents causing morbidity and mortality in immunocompromised patients, particularly those living with HIV/AIDS. Few studies in Mexico have attempted to determine the prevalence of opportunistic intestinal parasites causing diarrhea in immunocompromised patients. A study was conducted to determine the intestinal parasites in HIV-positive and HIV-negative immunocompromised patients with diarrhea admitted to a tertiary care hospital in Monterrey, Mexico, from 2014 to 2015. Stool samples were examined for trophozoites, cysts, and eggs using the EGRoPe sedimentation-concentration technique and special techniques (modified Ziehl-Neelsen stain, modified trichrome stain). A total of 56 patients were included. The overall prevalence of intestinal parasitism was 64% (36/56); 22/36 patients were HIV-positive. Prevalence of opportunistic parasites was 69% in HIV-infected patients compared to 44% in HIV-negative patients (P=0.06). Microsporidia were the most frequently identified parasites (24/36, 67%), fol lowed by *Cryptosporidium* sp. (6/36, 17%), *Sarcocystis* sp. (4/36, 11%), *Cystoisospora belli* (3/36, 8%), and *Cyclospora cayetanensis* (1/36, 3%). Overall prevalence rates of microsporidiosis and cryptosporidiosis were 43% and 11%, respectively. Among HIV-infected patients, prevalence rates of microsporidiosis and cryptosporidiosis were 48% and 14%, respectively. We also report the first cases of intestinal sarcocystosis in Mexico, all in HIV-infected patients. In conclusion, microsporidia and coccidia are major parasitic agents causing diarrhea in immunocompromised patients, particularly HIV-infected patients.

Keywords: microsporidiosis, cryptosporidiosis, microsporidia, coccidia, HIV/AIDS, Mexico.

INTRODUCTION

Conditions, such as HIV/AIDS, use of immunosuppressive drugs, hematological malignancies, and several chronic diseases place patients at risk of opportunistic infections. Clinical outcomes in immunocompromised hosts have substantially improved over the past decade thanks to the widespread use of antiretroviral therapy (ART) and other medical advances [1-3]. However, diarrheal syndromes still represent a common complaint in HIV/AIDS patients, and it is expected that they gain relevance in an increasing number of immunocompromised individuals undergoing hematopoietic and solid organ transplants and immunosuppressive therapies [4, 5]. Several types of microorganisms may be implicated in the etiology of chronic diarrhea in immunocompromised human hosts but emerging opportunistic pathogens, such as coccidia and Microsporidia, play a major role.

Intestinal coccidia (*Cryptosporidium* spp., *Cystoisospora belli*, *Cyclospora cayetanensis*) and Micro-

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sporidia are obligate intracellular organisms that can infect healthy immunocompetent persons causing self-limited diarrhea, and immunocompromised hosts causing chronic/persistent diarrhea, malabsorption, dehydration, weight loss, and wasting [5-7]. Coccidia are Apicomplexan parasites that sexually reproduce in the intestinal epithelium. Transmission of coccidia occurs through fecal-oral route by ingesting food or water contaminated with oocysts [5]. Among intestinal coccidioses, cryptosporidiosis is the most clinically significant in immunocompromised patients. Cryptosporidiosis is an emerging opportunistic infection distributed throughout the world, with *C. hominis* and *C. parvum* being the main species reported [5, 6, 8]. Microsporidia is a phylum of fungi comprising at least 10 genera and 16 species implicated in human infections [9]. They have gained interest as emerging pathogens associated with HIV/AIDS pandemic since the 1980s and have been increasingly detected in other immunocompromised patients as well as immunocompetent persons [7]. Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are relevant causes of chronic watery diarrhea and wasting syndrome in immunocompromised patients. Microsporidia are transmitted via direct or indirect contact with contaminated water and food.

Unfortunately, the laboratory diagnosis of emerging opportunistic pathogens is difficult requiring special methods and skilled microscopists. Coccidia and Microsporidia are thus infrequently recognized as causes of diarrheal illness in immunocompromised patients in developing countries. In Mexico, while etiological diagnosis of diarrheal syndromes is not routinely performed in several regions, some studies have attempted to determine the prevalence of coccidia and Microsporidia among immunocompromised patients. For instance, *Cryptosporidium* spp. have been reported in variable proportions (6%-82%) of immunocompromised adults and children from Mexico, and Microsporidia have been reported in 31%-62% [10-15, 17]. However, data on this topic are limited in northern Mexico. To the best of our knowledge, only a few studies on cryptosporidiosis and a case report of microsporidiosis exist in immunocompromised patients in this region [11, 18, 19]. Therefore, a study was conducted to determine the intestinal parasites in a group of HIV-positive and HIV-negative immunocompromised patients with diarrhea attending a hospital in Monterrey, Mexico.

PATIENTS AND METHODS

Study design and population

A prospective observational study was carried out from January 2014 to December 2015 at the Hospital Universitario Dr. José Eleuterio González, which is a 450-bed tertiary care teaching hospital in Monterrey, Nuevo Leon, northern Mexico. We included HIV-positive and HIV-negative immunocompromised patients aged 18 years or older who presented with diarrhea and were hospitalized in the internal medicine wards during this period. The local Ethics Committee approved the study.

Definitions

Diarrhea was defined as the passage of three or more loose or liquid stools per day. The immunocompromised population included patients with HIV/AIDS, chemotherapy, and hematological malignancies. HIV infection was diagnosed by means of immunoenzymatic assays, Western blot or HIV rapid tests, based on national guidelines. For purposes of this work, intestinal parasites, considered in a broad sense, included protozoa, helminths, and Microsporidia.

Stool sample collection and analysis

Fresh stool samples from patients were collected and sent to the parasitology section of the hospital laboratory for analysis of intestinal parasites. All collected stool specimens were fixed in 10% formalin and allowed to stand for 24 hours. First, we used the EGRoPe sedimentation-concentration technique for observation of trophozoites, cysts, and eggs [20]. A walnut-sized amount of feces was mixed with 10% formalin. Then, the solution was centrifuged at 2,500 rpm in conical tubes and supernatant material was removed. The precipitate was smeared on a slide with one drop of methylene blue, which is useful for visualization of internal morphology of common protozoa and examined under a light microscope. Second, a modified Ziehl-Neelsen acid-fast staining was used for the detection of coccidian oocysts [21]. Smears were stained with carbol fuchsin for 20 min, decolorized with 7% sulfuric acid, and counterstained with methylene blue. Slides were initially examined with 40x objective, and then at a magnification of 100x and 200x. Coccidia stained bright red against a blue background. Third, a modified trichrome stain (quick-hot Gram-chromotrope stain) was used to detect Microsporidia [22-24]. The formalin-fixed smears were dipped in a Coplin jar filled with a phenol-alcohol-fuchsin solution for 10 min. Then, they were decolorized with 0.5% sulfuric acid in ethanol, and stained with chromotrope 2R. The slides were first rinsed with acetic acid and then washed with 90% ethanol. The microsporidial spores stained dark violet/pink against a green background and were observed as small clusters at 40x, then under a 100x oil- immersion objective, and finally at 200x. Microsporidia's polar tubes were observed at the maximum magnification. Also, we performed Giemsa stain according to previous reports [23].

Statistical analysis

Data entry and analysis were performed using the statistical software Stata version IC 10 (StataCorp, College Station, TX, USA). We used descriptive statistics: relative frequencies for categorical variables, and the mean and standard deviation (SD) for age. The prevalence of intestinal parasitic infection was compared between HIV-positive and HIV-negative patients using Pearson's chi-squared (χ^2) test. *P* values<0.05 were regarded as statistically significant.

RESULTS

A total of 56 immunocompromised patients presenting with diarrhea were included in the study (Table 1). The mean age was 41 years old (SD 13, range 18-79). Thirty-eight patients (68%) were male and 18 patients (32%) were female. HIV infection was recorded in 29 patients (52%). Among all study patients, 36 (64%) were infected with at least one intestinal parasite; 22 of them were HIV-positive and had CD4 cell counts <200/μL. Microsporidia and protozoa were more prevalent than helminths. Microsporidia were the most frequently identified intestinal parasites (24/36, 67%) (Figure 1 and Figure 2), followed by *Cryptosporidium* spp. (6/36, 17%) (Figure 3), *Sarcocystis* spp. (4/36, 11%), *C. belli* (3/36, 8%) (Figure 4), and *C. cayetanensis* (1/36, 3%) (Figure 5). Among all immunocompromised patients, prevalence rates of microsporidiosis and cryptosporidiosis were 43% and 11%, respectively. Among HIV-infected patients, prevalence rates of microsporidiosis and

Table 1 - Intestinal parasitic infections in immunocompromised patients with diarrhea admitted to the hospital.[a]

Feature[b]	*All patients (n = 56)*	*HIV-positive patients (n = 29)*	*HIV-negative patients (n = 27)*	*P value*[c]
Age (years), mean (SD)	41 (13)	37 (10)	45 (14)	
Sex (male)	38 (68%)	19 (66%)	19 (70%)	
Any parasite	36 (64%)	22 (76%)	14 (52%)	0.06
Two parasites[d]	4	4		
Any opportunistic parasite[e]	32 (57%)	20 (69%)	12 (44%)	0.06
Any protozoa	13	10	3	
Cryptosporidium sp.	6	4	2	
Sarcocystis sp.	4	4		
Cystoisospora belli	3	3		
Cyclospora cayetanensis	1		1	
Entamoeba histolytica	1	1		
Strongyloides stercoralis	1		1	
Microsporidia	24	14	10	

[a]Intestinal parasites, considered in a broad sense, included protozoa, helminths, and Microsporidia.
[b]Variables are number (%) of patients, unless otherwise indicated.
[c]Pearson's χ^2 test.
[d]Stool examination of four HIV-infected patients showed *C. belli* + *Sarcocystis* sp. (2), *C. belli* + Microsporidia (1), and Microsporidia + *E. histolytica* (1).
[e]Opportunistic parasites include coccidia (*Cryptosporidium*, *C. belli*) and Microsporidia.

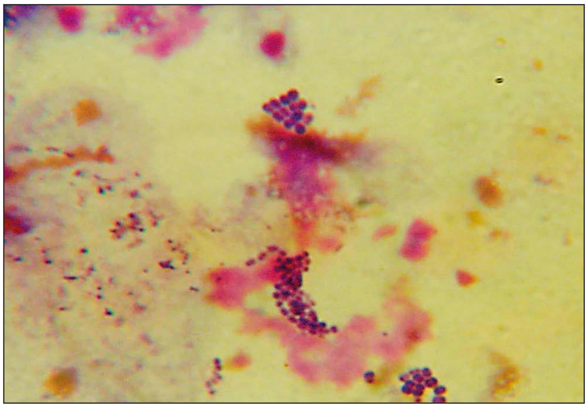

Figure 1 - Clusters of microsporidial spores seen in a Giemsa-stained smear (magnification, ×1,000). Bacteria, yeast cells, and debris are visualized as stained shades of pink and red.

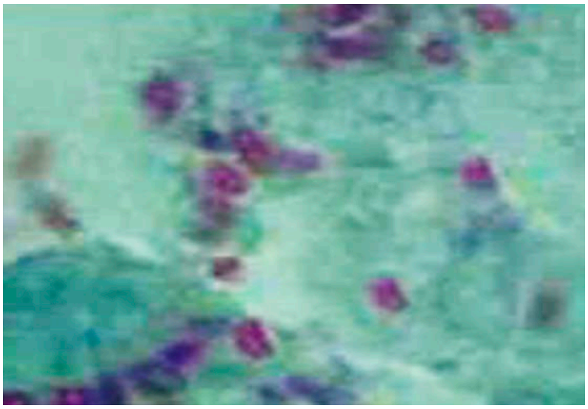

Figure 2 - Modified trichrome staining of a stool specimen containing microsporidial spores (magnification, ×2,000).

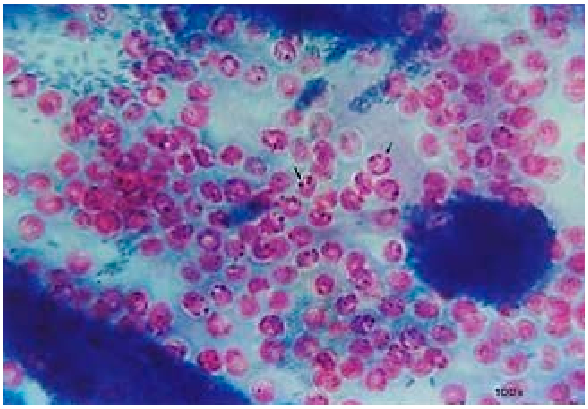

Figure 3 - *Cryptosporidium* oocysts detected by modified Ziehl-Neelsen staining (magnification, ×1,000).

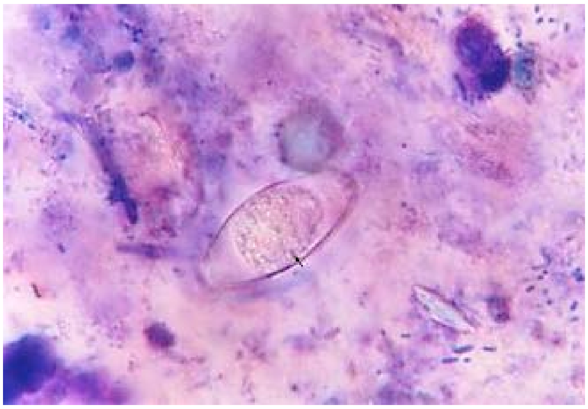

Figure 4 - *Cystoisospora belli* oocyst visualized by methylene blue staining (magnification, ×1,000).

cryptosporidiosis were 48% and 14%, respectively. Most parasites were more frequently detected in HIV-infected patients. With regard to non-opportunistic parasites, only one case of *Strongyloides stercoralis* infection and one case of *Entamoeba histolytica* infection were reported. Multiparasitism was reported in 4 individuals (11%), all of them HIV-infected, who presented *C. belli* and *Sarcocystis* sp. (2 patients), *C. belli* and Microsporidia (one patient), or Microsporidia and *E. histolytica* (one patient). We did not find a statistically significant difference in the overall prevalence of intestinal parasitic infection (P=0.06) or in the prevalence of opportunistic intestinal parasitic infection between HIV-positive and HIV-negative patients (P=0.06).

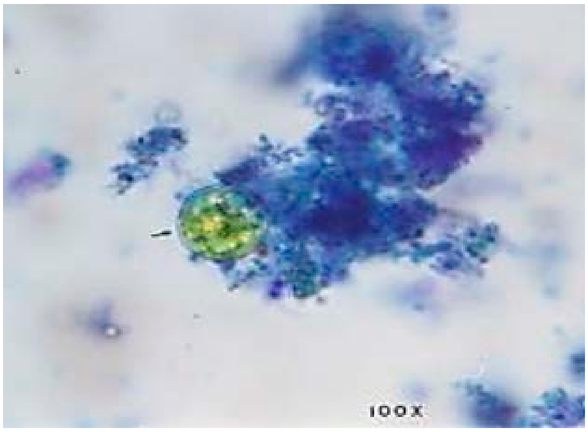

Figure 5 - *Cyclospora cayetanensis* oocyst detected by methylene blue staining (magnification, ×1,000).

DISCUSSION

We studied the parasites detected in fecal specimens from immunocompromised adults presenting with diarrhea to a hospital in Monterrey, Mexico. The overall prevalence of intestinal parasites was 64% in the immunocompromised population of this study. We found various intestinal parasites in the stool specimens, but opportunistic parasites accounted for the majority. Prevalence of opportunistic parasites was 69% in HIV-infected patients compared to 44% in HIV-negative patients, with a non-statistically significant difference (P=0.06). This finding suggests that both patient groups are similarly affected by opportunistic intestinal parasites. Nevertheless, the small sample size of this study limits the statistical power of testing.

Microsporidia were detected in 24 patients (prevalence of 43% among all immunocompromised patients), 14 of them in HIV-positive patients (prevalence of 48%). Epidemiological studies in Mexico have determined a prevalence of microsporidiosis of 8% in two rural communities in Central Mexico, 31% in HIV-infected patients with diarrhea from Mexico City, and 62% among pediatric patients with leukemia or lymphoma from several regions [15, 17, 25]. As noted, prevalence rates are higher among immunocompromised populations. Single cases of microsporidiosis have been reported in two kidney transplant recipients from Mexico City; in a child with X-linked agammaglobulinemia from the state of Chihuahua, and a malnourished child with diarrhea from Mexico City [19, 26-28]. Overall, *E. bieneusi*, *E. intestinalis*, and *E. cuniculi* have been identified in Mexico. Among HIV-infected patients presenting with diarrhea, we found a higher prevalence rate of microsporidiosis (48%) in our hospital in Monterrey vs. the one reported in Mexico City (31%) [15]. Unfortunately, microsporidial genera and species were not identified in our study because the required techniques (immunofluorescence, electron microscopy, or molecular testing) were not available. A recent systematic review and meta-analysis of the prevalence of opportunistic parasites in HIV-infected people, including 131 studies, found variable prevalence rates of Microsporidia infection (0.7-81.3%) with substantial heterogeneity (I^2 96.7%, P<0.0001) [29]. Authors concluded that patients with diarrhea greatly contributed to the heterogeneity and had a statistically significant higher pooled prevalence of Microsporidia infection.

Coccidia were found in 12 patients, of whom 9 were HIV-positive. In our study, *Cryptosporidium* was the most frequent coccidia, as usually reported elsewhere. The prevalence of cryptosporidiosis was 11% among all immunocompromised patients and 14% among those with HIV infection. In Mexico, *Cryptosporidium* was first reported in the 1980s in children from San Luis Potosí state, and immunocompromised patients from Mexico City and Monterrey, including hospitalized patients and HIV-infected children. Cryptosporidiosis is endemic in both urban environments and rural communities in Mexico, with prevalence rates ranging from 3% to 51% in immunocompetent children [30-37]. The prevalence is higher (6%-82%) among immunocompromised patients, including HIV-infected adults and children, and malnourished children [10-15]. Clinical significance of *Cryptosporidium* infections is greater in immunocompromised individuals than in children with a normal immune status due to the severity of the disease in the first group [5, 6]. Infection in children is rather asymptomatic or self-limited.

The frequency of microsporidiosis was much higher than that of cryptosporidiosis (43% vs. 11%). This unusual predominance of Microsporidia over coccidia was also found by Gamboa et al. in an HIV-infected cohort in Mexico City, and by Jiménez-González et al. in a small group of children from central Mexican states with hematological malignancies [15, 17]. Nevertheless, *Cryptosporidium* is the quintessential opportunistic parasitic agent in most studies.

C. belli and *C. cayetanensis* were only found in three HIV-positive patients and one HIV-negative patient, respectively. Surprisingly, four cases of *Sarcocystis* spp. (formerly *Isospora hominis*) infection were found. According to our literature review, these seem to be the first reports of human intestinal sarcocystosis in Mexico. Sarcocystosis is an uncommonly reported parasitic infection that may cause intestinal and muscular illnesses in humans. *S. hominis* and *S. suihominis*, which are two recognized human species causing intestinal sarcocystosis, are acquired by the ingestion of mature cysts in undercooked or raw beef and pork, respectively. Epidemiology and clinical impact of sarcocystosis are largely unknown and reports continue to be rare [38, 39]. Unlike other coccidia, sarcocystosis is not considered an opportunistic infection and evidence linking sarcocystosis with AIDS is limited with only a few cases published globally [40-43]. Interestingly, two of these patients were coinfected by *Sarcocystis* sp. and *C. belli* [40, 41]. In our study, the four intestinal sarcocystosis cases occurred in HIV-infected patients, two of whom were coinfected with *C. belli*.

In conclusion, Microsporidia and coccidia are relevant parasitic agents causing diarrhea in immunocompromised patients, particularly HIV-infected patients, from Monterrey, Mexico. The vast majority of intestinal parasitic infections were caused by opportunistic agents. In HIV-infected patients (all of them with CD4 cell count <200/μL), these AIDS-defining illnesses are evidence of important gaps in HIV care. Among the coccidioses, we observed four cases of intestinal sarcocystosis, which seem to be the first reported in Mexico.

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Conflict of interest
The authors declare no conflicts of interest.

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