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Identification of Emerging Parasites in HIV Patients Stool by Molecular Analysis

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Abstract

Infections of the gastrointestinal tract are common in people infected with acquired immunodeficiency syndrome (AIDS). The etiologic spectrum of enteric pathogens that affect to immunosupressed patients and cause diarrhea includes protozoan parasites as *Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli* and *Microsporidia* and non opportunistic, such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides* and *Strongyloides.* In Mexico there are not current researching studies that establish the association of parasites causing diarrhea in VIH infected individuals, for this reason the purpose of this study was to determine the frequency and importance of emerging parasites in individuals infected with the human immunodeficiency virus (HIV) and its correlation with the presence of gastrointestinal symptoms, stool samples from 100 VIH patients were collected and analyzed by cropoparasitoscopic method, Ziehl Neelsen staining and Polymerase Chain Reaction (PCR) to find opportunistic parasites. Sixty of the HIV patients (60%) had diarrhea at the time of sample collection. Emerging parasites were detected in 22% of samples, from these 59.1% (13/22) had diarrhea. *Cryptosporidium* spp. was detected in 7%, *Microsporidium* spp. were detected in 5% *I. belli* was detected in 1.0%, *G. intestinalis* was detected in 2%, and *C. cayetanensis* was found in 7%. 16% of the HIV patient samples were identified as being infected with other organisms, including Escherichia coli, *Entamoeba histolytica, Hymenolepis nana,* and *Iodameoba butschlii*.

This work emphasize the importance of knowing which are emerging and re-emerging parasites prevalent in HIV infected patients, in our community, the objective is to get better and more efficient management of these patients to increase the quality of life.

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1 Introduction

Infections of the gastrointestinal tract are common in people infected with acquired immunodeficiency syndrome (AIDS). The etiologic spectrum of enteric pathogens that affect to immunosupressed patients and cause diarrhea includes bacteria, parasites, fungi and viruses [1]. Within the diarrhea-causing parasites that infect these patients are opportunistic, as *Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli* and *Microsporidia* and non opportunistic, such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides* and *Strongyloides.* Chronic diarrhea has been reported in up to 50% of the patients infected with the causative pathogen of AIDS, human immunodeficiency virus (HIV), in developed countries and up to 90% of those in developing countries [2]. This condition often produces considerable weight loss and dehydration that exacerbates the patient's already weakened physical condition and complicates the overburdened physiological processes.

According to different researching studies HIV positive individuals are more susceptible to co-infections with *Cryptosporidium spp*. than HIV negative people, particularly younger males with poor personal hygiene habits [3] and other report says that *Isospora belli* was the predominant parasite followed by *Cryptosporidium spp*. and both were strongly associated with diarrhea [4].

In Mexico the VIH infection represents an important problem of public health, while transmission of blood-borne have ceased, the number of HIV-seropositive drug users, male sex working and homosexuals has increased, particularly in the northern of Mexico [5]. However there are not researching studies that establish the association of parasites causing diarrhea in VIH infected individuals, for this reason and considering that prevalence of parasites is very high in Mexico the study is focused to determine the presence of intestinal parasites in a group of VIH infected patients and know the association with diarrhea and other gastrointestinal symptoms.

2 Methods

2.1 Fecal Specimen Collection from HIV patients.

During the years 2010 and 2011, one hundred stool samples (3 consecutives) were obtained from adult patients (>17 years old), with diagnosis of HIV who were being treated at the Specialized Clinic of Mexico City, Mexico. Ninety five samples corresponding to male patients and just five belonged to female patients.

2.2 Parasitic Identification by Co-proparasitoscopic Analysis and Staining.

Fecal samples were stained with Lugol's iodine and examined under a light microscope in order to find cysts, oocysts and/or spores of parasites as previously described [6]. Liquid suspensions of fecal matter were stained with Ziehl Neelsen for viewing under an oil

immersion lens as previously described [7].

2.3 PCR Amplification.

DNA was extracted from all fecal samples by use of the QIAamp™ DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. The DNA concentrations were measured by a spectrophotometer (Epoch; Biotek, Winooski, VT, USA), and then PCR was performed to amplify a fragment of SSU rRNA gen from *Cryptosporidium spp* [8], *Microsporidium* [9], *Isospora belli* [10], *Giardia intestinalis* [11] *and Cyclospora* [12] according to conditions summarized in table 1. Reaction were performed from 500 ng of template DNA in a reaction volume of 25 μL, consisting of 1× PCR amplification buffer with dATP, dGTP, dCTP, and dTTP (1.2 μM each), 0.5 ng each primer and 1.5 U of Taq DNA polymerase (Roche, Mannheim, Germany). The amplification was developed in a Maxi-gene thermal cycler (Axygen, Union City, CA, USA).

3 Main Results

3.1 HIV Patient Characteristics.

Of the 100 stool samples from the HIV adult patients, only five (5.0%) were from females and 95 (95%) were from males. Sixty of the HIV patients (60%) had diarrhea at the time of sample collection.

3.2 Parasite Identification by Co-roparasitoscopic Analysis and Staining.

Emerging parasites were detected in 22% (22/100) of the HIV patients, 59.1% (13/22) of whom had diarrhea. Among the 100 HIV patients, 7 were found to have *Cryptosporidium*, including 6 who had chronic diarrhea and one who had no diarrhea. Meanwhile, *Microsporidium* spp. was detected in 5 of the HIV patients, of which only one had diarrhea. *I. belli* was detected in only 1 HIV patient, and that patient had chronic diarrhea, and *G. intestinalis* was detected in only 2 HIV patients, of which only one had diarrhea. Finally, *C. cayetanensis* was detected in 7 of the HIV patients, 4 of whom had diarrhea and 3 of whom had no diarrhea (Table 2). The total data show that *Cryptosporidium* and *Cyclospora* were more frequent in the HIV patients with diarrhea. The rest of the studied parasites were detected at similar proportions between diarrhea and non-diarrhea cases. Among the HIV adult patients, *Cryptosporidium* spp. were detected in 7% (7/100), *Microsporidium* spp. were detected in 5% (5/100), *I. belli* was detected in 1.0% (1/100), *G. intestinalis* was detected in 2% (2/100), and *C. cayetanensis* was found in 7% (7/100). Sixteen (16%) of the HIV patient samples were identified as being infected with other microbes, including Escherichia coli, *Entamoeba histolytica*, *Hymenolepis nana*, and *Iodameoba butschlii*.

3.3 PCR Amplification.

The products of PCR are presented in Figure 2; we observed a 150-bp amplicon for *Cryptosporidium* spp., a 300-bp amplicon for *Microsporidium* spp., a 400-bp nested-PCR

amplicon for *I. belli*, a 298 bp amplicon from *G. intestinalis*, and a 300-bp nested-PCR amplicon for *C. cayetanensis*.

4 Labels of Figures and Tables

Table 1: Conditions of PCR reactions mix to amplify the SSU rRNA gene of emerging parasites.

Parasite	Primers	Tm	Cycles	Size of produced fragment
Cryptosporidium spp	JVAF (5′-ccaattacaaaaccaaaaagtcc-3′) JVAR (5′-atgacgggtaacggggaat-3′)	60 ºC	35	150bp
Microsporidium spp.,	V1 (5′-caccaggttgattctgcctgac-3′) and PMP2 (5′-cctctccggaaccaaaccctg-3′)	55 ºC	35	300bp
Isospora belli	Iso-18SF0 (5′-ctggttgatcctgccagta-3′) and Iso-28SR0 (5′-aaggctcaatcaagaacctccg-3′); Iso-18SF1 (5′-gatcctgccagtagtcat-3′) and Iso-28SR1 (5′-tgaagctaatccctcttcc-3′).	64ºC	35	500
G. intestinalis	P (5′-ggtggatcctgccggagcg-3′) and A (5′-gctctccggagtcgaac-3′)	50	35	298
C. cayetanensis	FIE (5′-tacccaatgaaaacagttt-3′) and R2B (5′-caggagaagccaaggtagg-3′); inner primers, F3E (5′-ccttccgcgcttcgctgcgt-3′) and R4B (5′-cgtcttcaaacccccctactg-3′).	53 ºC and 60 ºC	35 each one	500

Table 2: Positive samples to parasites and its relation with diarrhea. Frequency of intestinal parasites in stool samples from HIV patients.

Samples from HIV patients			
N=100	Female=5/100 (5%)	Male=95/100 (95%)	
Parasite	With diarrhea 60/100 (60%)	Without diarrhea 36/100 (36%)	total
Cryptosporidium spp.	6/7 (85.7%)	1/7 (14.28%)	7 (7%)
Microsporidium spp.	1/5 (20%)	4/5 (80%)	5 (5%)
Isospora belli	1/1 (100%)	0	1 (1%)
Giardia intestinalis	1/2 (50%)	½ (50%)	2 (2%)
Cyclospora cayetanensis	4/7 (57.14%)	3/7 (42.85%)	7 (7%)
Total emerging parasites	13/100 (13%)	9/100 (9%)	22(22%)
Other parasites (E. coli, E. histolytica, H. nana or I. butschili)	0	16/100 (16%)	16 (16%)

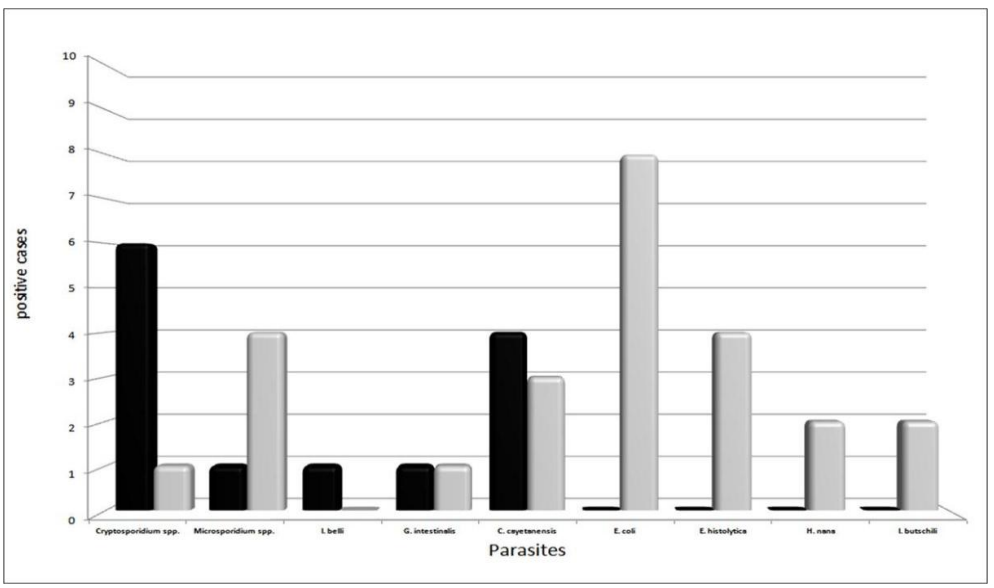

Figure 1: Positive cases of parasites in HIV stool samples with diarrhea ♦ and without diarrhea.

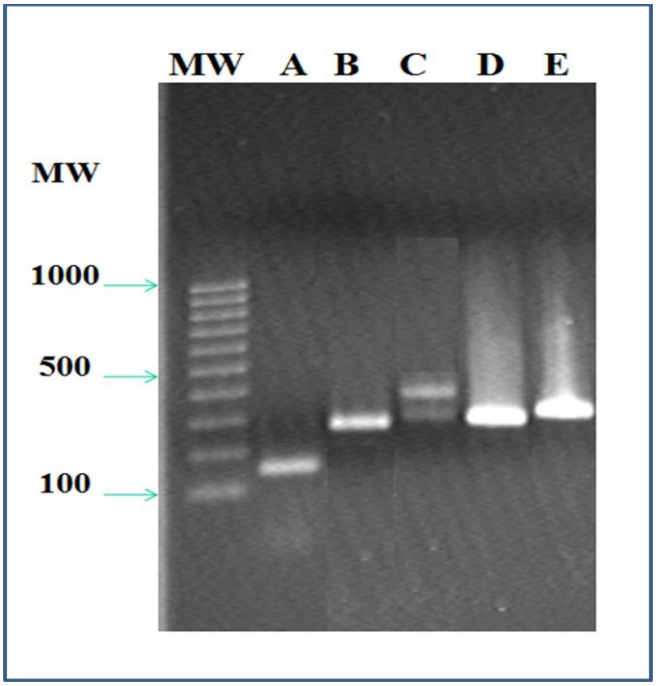

Figure 2: 2% Agarose gel electrophoresis of PCR stained with ethidium bromide. (MW) 100bp ladder. A) *Cryptosporidium* spp; an amplicon of 150bp is observed. B) A band of 300bp corresponding to *Microsporidium* spp. C) A 400bp fragment of *Isospora belli*. D) *Giardia intestinalis* amplified a fragment of 298bp and E) Nested-PCR of *Cyclospora cayetanensis* allowed to obtain a 300bp fragment.

5 Discussion

This work emphasize the importance of knowing which are emerging and re-emerging parasites prevalent in HIV infected patients, in our community, this information it is important in public health to get better and more efficient management of these patients and to increase the quality of life.

The parasites associated with diarrhea were *Cryptosporidium* spp. and *Cyclospora cayetanensis*. These findings indicated that the presence of emerging parasites is not always associated with diarrhea and gastrointestinal symptoms. Diarrhea is one of the most common gastrointestinal symptoms in HIV-infected individuals and can be caused by a multitude of pathogens, including bacteria and viruses, or physiologic disruptions resulting from the stringent regimen of pharmacologic agents, however, we did not notice any trend in positive results for HIV patients according to the length of disease.

Estimates of *Cryptosporidium* spp. [13] frequency in HIV patients have ranged from 16–20% around the world [14-15]. In our study, the Faust staining method had the highest diagnostic efficiency for *Cryptosporidium* spp. of the three staining methods, but was less efficient than the molecular PCR analysis. The data from our study agree with previous reports of *Microsporidium* spp. cases accounting for 10–27% of HIV patients [16-17]. The exceedingly small size of Microsporidia spores (1–3 μM) requires light microscopy with oil immersion and staining, which complicates diagnosis based solely upon

microscopic analysis and can lead to false negative results, this is important because some studies suggest the coexistence of both zoonotic and anthroponotic route of transmission. The frequency of *Isospora* spp. in HIV patients in the US and Europe has been reported as 15% and 18.2%, respectively [18-19]. *Giardia* spp. have been reported in 8–50% of HIV patients worldwide [20], while the rates of *Cyclospora* spp. reported among HIV patients worldwide have been relatively low (3-4.5%) [21], the frequency in our HIV study population was even lower. PCR will especially benefit diagnosis of parasitic infections. To date, only two studies in the literature have reported on the incidence of emerging parasites in stool samples of patients with ALL or another kind of cancer. All these data are similar to reported in other geographical areas [22]

Since prevalence data for the emerging parasites examined in our study are currently not available for the country-wide population of Mexico, the results presented herein provide novel and important insights into the current profile of emerging parasites in HIV patients from a large metropolitan area of Mexico (Mexico City) and its impact in the quality of life of these people.

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