Identification of Emerging Parasites in HIV Patients Stool by Molecular Analysis

Leticia Eligio-García¹, Enedina Jiménez-Cardoso²a, Apolinar Cano-Estrada³, Adrian Cortés-Campos⁴

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 immunosuppressed 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 research 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 immunosuppressed 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.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Primers</th>
<th>Tm</th>
<th>Cycles</th>
<th>Size of produced fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>JVAF (5’-ccaattacaaaaaccaaaagtc-3’) and JVAR (5’-atgaagggtaacgggaat-3’)</td>
<td>60 °C</td>
<td>35</td>
<td>150bp</td>
</tr>
<tr>
<td><em>spp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microsporidium</em></td>
<td>V1 (5’-caccaggtgtgtgctgcgtg-3’) and PMP2 (5’-cctctccggaaccaacctg-3’)</td>
<td>55 °C</td>
<td>35</td>
<td>300bp</td>
</tr>
<tr>
<td><em>spp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Isospora belli</em></td>
<td>Iso-18SF0 (5’-ctggtgtgtatcctgcg-3’) and Iso-28SR0 (5’aaggtctcaatcagaacctc-3’); Iso-18SF1 (5’-gtccttcgctagtagtcat-3’) and Iso-28SR1 (5’-tgaagctaatccctc-3’).</td>
<td>64°C</td>
<td>35</td>
<td>500</td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
<td>P (5’-ggtaggtggtgcgagc-3’) and A (5’-gtctcggagtcac-3’)</td>
<td>50</td>
<td>35</td>
<td>298</td>
</tr>
<tr>
<td><em>C. cayetanensis</em></td>
<td>FIE (5’-taccaatgaaaacagttt-3’) and R2B (5’-caggagaagccaggtagg-3’); inner primers, F3E (5’-ctctccgctcgtcgtgtc-3’) and R4B (5’-cttcttcacccctcactg-3’).</td>
<td>53 °C</td>
<td>35 each one</td>
<td>500</td>
</tr>
</tbody>
</table>
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Table 2: Positive samples to parasites and its relation with diarrhea. Frequency of intestinal parasites in stool samples from HIV patients.

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

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