ISOSPORIASIS IN VENEZUELAN ADULTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS: CLINICAL CHARACTERIZATION

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Abstract. A cross-sectional study was undertaken to determine the prevalence of isosporiasis and its clinical and laboratory pattern in Venezuelan patients infected with human immunodeficiency virus (HIV) (n = 397). At enrollment, they underwent a thorough clinical history and physical examination, and provided stool specimens for the identification of Isospora belli and other parasites. Isospora belli was identified in 56 subjects (14%) and diarrhea, either acute or chronic, was present in 98% of these cases (P < 0.001). Eosinophilia was strongly associated with isosporiasis (P = 0.01). It was also found that the presence of eosinophilia was more common in I. belli-infected patients without weight loss (P < 0.001). Twenty-six (81.25%) subjects with I. belli infection had CD4+ cell counts < 200 cells/mm³ (P = 0.03). In addition, the data and its description shows the association to be < 100 cells/mm³. This infection seems to be seasonal because the recovery of oocysts occurred mainly in months with significant rainfall. In fact, isosporiasis should be suspected in HIV-infected patients from tropical countries with diarrhea, weight loss, eosinophilia, and low CD4+ cell counts.

INTRODUCTION

Some immunodeficiency states predispose to severe and prolonged diarrhea from opportunistic parasites. Particularly, Isospora belli, a coccidian protozoa, is one of the most commonly identified causes of chronic diarrhea in patients with acquired immunodeficiency syndrome (AIDS). In some cases, parasitic diarrhea by such opportunistic agents such as I. belli may appear as the first manifestation of AIDS. This parasite is more commonly found in tropical and sub-tropical climates, and its prevalence varies according to different regions. It has been observed in 15% of patients with AIDS in Haiti, but in less than 2% of patients with the same condition in the United States.

Because chronic diarrhea in patients infected with human immunodeficiency virus (HIV) results in a significant morbidity and mortality, mainly caused by HIV wasting, the early detection and proper treatment of patients with AIDS and concomitant diarrhea is very important in preventing complications and prolonging a healthy life.

The efforts to prevent complications such as opportunistic infections due to HIV immunosuppression have resulted in the use of highly active anti-retroviral treatment (HAART). However, there are many countries of the world where patients have a limited access to this anti-retroviral therapy. In Venezuela, especially before the year 2000, few HIV-infected persons received HAART because the government did not recover the costs and the treatment was too expensive to be assumed by patients themselves. It is known that as a consequence of the absence of treatment, most of the patients reach advanced stages of immunosuppression and they are prone to acquire opportunistic infections.

Isosporiasis remains an important opportunistic infection in HIV-infected patients, especially in developing countries of Africa, Asia, and Latin America. However, few data exist on the clinical characterization and the epidemiologic aspects of this disease in these countries. Moreover, the prevalence of this infection is occasionally underestimated because not all HIV-infected patients are examined for the presence of this protozoan.

To provide some insight into the prevalence of isosporiasis in Latin America, we present data on its clinical and laboratory pattern among HIV-infected patients in Caracas, Venezuela.

MATERIALS AND METHODS

Study population. This cross-sectional study took place between July 1997 and August 2002. Consecutive patients with HIV infection referred from different hospitals and healthcare units to the Parasitology Department of the Faculty of Medicine of the Universidad Central de Venezuela in Caracas were included. These patients were referred to our laboratory as a part of their baseline investigations irrespective of their symptoms. Their informed consent was obtained and the Research Unit of the J. M. Vargas School of Medicine and the Consejo de Desarrollo Científico y Humanístico of the Universidad Central de Venezuela reviewed and approved the project and its ethical aspects.

Clinical evaluation. At enrollment, each participant provided a thorough clinical history and underwent a physical examination. The participants also provided results of laboratory tests, such as blood count determination, CD4+ lymphocyte counts, and viral load within six months of enrollment, when available. Information obtained via a questionnaire included demographic characteristics, current symptoms, previous opportunistic infections and treatments.

Stool evaluation. Participants were asked to bring not less than three stool samples on three different days (one sample per day on three different days). All participants were informed about the techniques for sample collection, transport and preservation. Fecal specimens were processed immediately after they were received. Each sample was divided into four portions for different coprologic analysis: 1) a direct method using saline and lugol solution for the diagnosis of protozoa; 2) a Kato-Katz analysis for the detection of helminths; 3) a Baermann technique for identification of Strongyloides stercoralis; and 4) Kinyoun staining for coccidian parasites. Participants were divided into two groups: HIV-infected patients with isosporiasis and all the remaining HIV-infected patients without evidence of other parasitic infections.
Definitions. A patient was considered infected with *I. belli* if this parasite was detected in one or more stool specimens. A stool was considered positive for *I. belli* if typical oocysts 20−30 μm long by 10−20 μm wide containing one or two sporoblasts were observed with any of the different techniques. Diarrhea was defined as three or more unformed stools in a 24-hour period. A diarrheic episode was defined as acute when it was present for less than three weeks and as chronic if its duration was more than three weeks. Weight loss was considered significant when patients referred lost of more than 10% of their baseline body weight within the period associated to diarrhea. To measure eosinophilia, two variables were used: absolute eosinophils count and relative eosinophilia. For the absolute counts, patients were divided into two groups using 500 cells/mm³ as the breakpoint. Relative eosinophilia was defined as more than four eosinophils in 100 leukocytes from peripheral blood. The relative value was considered to be due to the presence of leukopenia in most of the patients. When one of these variables was used the other one was not. Both were analyzed as explanatory variables, but only relative eosinophilia was used as a confounder for other explanatory variables.

Statistical analysis. All numerical variables were summarized using mean and 95% confidence intervals (CIs) if normally distributed or geometric mean if not normally distributed. Median and interquartile range (IQR) were used for those variables that included 0 and were not normally distributed. Categorical data were presented as proportions. Numerical data was also summarized into categories. The Student’s *t*-test was used to compare the means of continuous variables. Categorical variables were compared using Fisher’s exact test or the chi-square test.

Univariate and multivariate logistic regression analysis were performed to calculate odds ratios (ORs) and likelihood ratio tests with *I. belli* infection as the main outcome. All variables, even other parasite infections, were included in the crude analysis. Only those that were statistically significant in the crude analysis were analyzed in the multivariate model. They were also checked for interactions (effect modification). The general significance level was set at a *P* value < 0.05. All statistical analysis was performed using Stata 7.0 (Stata Corporation, College Station, TX).

RESULTS

Descriptive analysis. Three hundred ninety-seven HIV-infected patients that came to our clinic referred mostly from other health care services were enrolled in the study. There were 346 men and 51 women. The ages ranged from 17 to 63 years, with a geometric mean of 35.8 years (95% CI = 33.8−35.8). Most of the patients came from urban communities; only 11 (2.8%) came from rural areas. There were 122 (30%) patients with acute diarrhea, 178 (45%) with chronic diarrhea, and 97 (24%) without diarrhea. An evaluation of T lymphocyte populations for both study groups revealed that 218 (55%) had CD4⁺ cell counts with a geometric mean of 97 cells/mm³ (95% CI = 81.3−115.9, range = 1−1,000). Different parasites were detected in 274 cases (69%). The most commonly found were *Blastocystis hominis* (27.5%), *Cryptosporidium parvum* (15%), *I. belli* (14%), *S. stercoralis* (11.1%), and *Entamoeba histolytica* (10.3%).

The presence of oocysts of *I. belli* was documented in 56 (14%) of 397 patients. There were 50 men and 6 women infected with this parasite. The geometric mean age for the patients infected with *I. belli* was 34.7 years (95% CI = 33.9−35.6). Only 6 (11%) of these cases came from rural areas.

Acute *I. belli* diarrhea cases were studied according to the month of appearance of the disease. Chronic cases were not included due to the difficulty in determining with precision when the diarrhea began. The percentage of laboratory specimens positive for *I. belli* oocysts in these patients with acute diarrhea ranged from 81% during the rainiest months of the year (April, May, and June) to 19% during the driest months of the year (December and January) (Figure 1).

Some of the demographic characteristics of both *I. belli*-infected and non-infected patients are shown in Table 1. No association was noted between isosporiasis and age or sex. Either acute or chronic diarrhea was present in 98% (55 of 56) of the *I. belli*-infected patients: 17 (30%) had acute diarrhea, 38 (68%) had chronic diarrhea (Figure 2), and only one patient (2%) was an asymptomatic cyst carrier. There was no significant statistical association between acute and chronic diarrhea. Other common symptoms associated with isosporiasis were vomiting (13 of 56, 23%) (*P* < 0.001), abdominal pain (18 of 56, 33%) (*P* = 0.03), and weight loss of at least 10% (36 of 56, 64%) (*P* < 0.001).

Physical examination among patients with isosporiasis showed hepatomegaly in 6 (13%) of 47 patients and splenomegaly in 3 (6%) of 47. There was no statistical association between these signs and isosporiasis. Of 56 patients infected with *I. belli*, 9 (17%) took trimethoprim-sulfamethoxazole (TMP/SMZ) as prophylaxis for *Pneumocystis carinii* or toxoplasmosis at doses of one double-strength tablet (160 mg of TMP and 800 mg of SMZ) three times a week.

Laboratory tests showed relative eosinophilia in 22 (45%) of 48 persons having both *I. belli* infection and available laboratory results. The association between eosinophilia and isosporiasis was statistically significant (*P* = 0.01) when we compared the proportion of people with eosinophilia infected with *I. belli* with the rest of the patients (*P* = 0.01) (Table 1). The values of relative eosinophils in patients with isosporiasis ranged from 0% to 61%, with a median of 4% (IQR = 1.5−7). In the control group, the median was 1.0 (IQR = 0−5, range = 0−61%). We also found an association between weight loss and eosinophilia in isosporiasis cases: 59% (10 of 17) of the isosporiasis cases without weight loss had eosino-
philia compared with 21% (40 of 192) of the control patients without weight loss ($P = 0.001$). Furthermore, in $I.\ belli$-infected patients without relative eosinophilia, 73% (19 of 26) had weight loss in comparison with only 19% (35 of 187) of the patients without $I.\ belli$ infection and without weight loss ($P < 0.001$). There were patients infected with $S.\ stercoralis$ in both $I.\ belli$-infected and non-infected individuals. Because $S.\ stercoralis$ was the parasite most associated with eosinophilia in our study population, we calculated the median eosinophilia of $I.\ belli$-infected patients compared with a median of 2.5% ($IQR < 5\%$) 22/48 (45.83%) 70/257 (27.24%) 0.01 2.3 1.2–4.2 0.011

HAART†

<table>
<thead>
<tr>
<th>Type of Diarrhea</th>
<th>Isospora belli group (n = 56)</th>
<th>Control group (n = 341)</th>
<th>Chi-square $P$</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
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<tr>
<td>&lt;5%</td>
<td>26/48 (54.17%)</td>
<td>187/257 (72.76%)</td>
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<td></td>
</tr>
<tr>
<td>≥5%</td>
<td>22/48 (45.83%)</td>
<td>70/257 (27.24%)</td>
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<td></td>
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<tr>
<td>HAART†</td>
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</tr>
<tr>
<td>Yes</td>
<td>48/56 (85.71%)</td>
<td>285/341 (83.6%)</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>8/56 (14.29%)</td>
<td>56/341 (16.4%)</td>
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</tbody>
</table>

* OR = odds ratio, CI = confidence interval; HAART = highly active anti-retroviral treatment. $P$ values in bold are statistically significant.
† Patients taking at least one anti-retroviral drug.

Laboratory characteristics of the stool samples with $I.\ belli$ oocysts showed that 38 (67.9%) of 56 $I.\ belli$-infected patients had no other parasitic disease, while 18 (32.1%) of 56 $I.\ belli$-infected cases had mixed infections. The most common co-infections were $B.\ hominis$ in 10 (18%) patients with isosporiasis, $S.\ stercoralis$ in 8 (14%), and $C.\ parvum$ in 3 (5%).

Information about the characteristics of the stools was reported for 48 patients with isosporiasis. Visible blood was observed in two samples and mucus was observed in 40 (83%) specimens. The parasitic burden mean was three oocysts per microscopic field (40×). There was no association between the daily number of bowel evacuations and the parasitic burden. Forty-two (78%) patients had watery and abundant stools, 4 (7%) had stools of decreased consistency, and in 8 (14.28%) we could not determine the consistency of the stool.

The proportion of patients taking HAART was low: 3 (5%) of 56 patients infected with $I.\ belli$ and 47 (14%) of 341 patients in the other group were taking at least three antiretroviral drugs.

Univariate analysis. Univariate analysis showed that the presence of diarrhea ($OR = 21.6, P = 0.003$), weight loss ($OR = 5.4, P < 0.001$), vomiting ($OR = 5.9, P < 0.001$), relative eosinophilia ($OR = 2.3, P = 0.01$), and CD4$^+$ cell counts < 200 cells/mm$^3$ ($OR = 2.7, P = 0.035$) were significantly predictive for the risk of isosporiasis (Table 1). However, there was no significant association between having isosporiasis and co-infection with another parasite.
Multivariate analysis. In multivariate analysis, infection with *I. belli* was used as the main outcome and diarrhea, weight loss, vomiting, relative and absolute eosinophilia, and CD4+ cell count were analyzed as explanatory variables. The presence of diarrhea remained an independent predictor of isosporiasis (OR = 11.9, *P* < 0.001) after adjusting for weight loss, eosinophilia, mixed infection with *C. parvum*, and vomiting. In the model designed using weight loss as explanatory variable, the CD4+ cell count was also included as an adjusting variable. It was found that the risk of infection with *I. belli* was 5.5-fold higher in patients with weight loss in comparison with patients without weight loss. There was an effect modification between weight loss and eosinophilia. Those infected with *I. belli* with eosinophil levels < 5% had an increased risk (25-fold) of having more than 10% weight loss (OR = 24.6, *P* < 0.001). When eosinophilia was used as explanatory variable, there was a significant association between relative eosinophilia (OR = 9, *P* = 0.004) or absolute eosinophilia (OR = 6.5, *P* = 0.02) and isosporiasis in patients without weight loss. Once adjusted by other variables, the association between vomiting or isosporiasis or CD4+ cell count and isosporiasis no longer had statistical significance (Table 2).

**DISCUSSION**

An overall *I. belli* prevalence of 14% was found in 397 HIV-infected patients. These data demonstrate that isosporiasis is a relatively common infection among HIV-infected patients in Caracas, even when they come from urban areas. Compared with other tropical countries, we found that this value is higher than the one reported by Cimerman and others8 in Brazil (2%), or by Punpoowung and others9 in Thailand (4.54%). However in India, Prasad and others10 reported a prevalence of 31%. In contrast, Navin and others11 detected no infections with *I. belli* in a large series of 602 participants infected with HIV in the United States (Atlanta, GA), probably because this infection is more commonly found in tropical and sub-tropical climates.1 Another possibility might be due to the use of TMP/SMZ as prophylaxis for *P. carinii* in HIV patients in developed countries during the pre-HAART era. This therapy has proven to also be effective in the prevention of infection with *Isospora*.411,12

Nevertheless, we found cases of infection with *I. belli* in patients taking TMP/SMZ in a prophylactic regimen for *P. carinii*. Nevertheless, since compliance with this treatment was not assessed in this study, we cannot exclude poor compliance or inadequate treatment as an explanation for the prevalence of *I. belli*-associated diarrhea. It is also possible that patients living in the tropics are more exposed to infective oocysts and that prophylaxis with one (double-strength) tablet of TMP/SMZ every three days may not be adequate for individuals from endemic regions. Some investigators have proposed higher doses of TMP/SMZ (two double-strength tablets, 3 times a week) as a better prophylaxis for treatment of infection with this parasite.13

We found that isosporiasis seems to be a seasonal infection. Throughout the study period, recovery of *I. belli* oocysts in patients with acute diarrhea occurred mainly in months with significant rainfall. Similar findings have been reported for cryptosporidiosis in Brazil.14

Clinically, the distinctive findings of the infection were both watery acute or chronic diarrhea, with a frequency of five or six times a day, accompanied by significant weight loss. Although it has been reported that chronic diarrhea is more common than acute diarrhea in patients with isosporiasis,1 in our study there was a significant proportion of patients with acute diarrhea. One explanation for this could be that these patients were seen immediately after the first symptoms, had access to an appropriate health care units, and/or had access to a specialized laboratory for the diagnosis of parasitic infections.

Although not statistically significant, we observed splenomegaly or hepatomegaly in a few patients, suggesting that these might be clinical signs of advanced HIV infection or a manifestation of other opportunistic infections or associated diseases.

Eosinophilia was strongly associated with isosporiasis. This observation, previously reported by others, suggests an aller-

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**Table 2**

Multivariate analysis of the population studied*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>Likelihood ratio test <em>P</em></th>
<th>OR ignoring interaction</th>
<th>95% CI</th>
<th>Likelihood ratio test <em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>11.9</td>
<td>1.6–90</td>
<td><strong>&lt;0.001</strong></td>
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<tr>
<td>No</td>
<td>1</td>
<td>NA</td>
<td></td>
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<tr>
<td>Type of diarrhea</td>
<td></td>
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<tr>
<td>Acute</td>
<td>1.0</td>
<td>NA</td>
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<tr>
<td>Chronic</td>
<td>1.9</td>
<td>0.6–5.8</td>
<td>0.262</td>
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<td>CD4+ cells/mm³</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>1.0</td>
<td>NA</td>
<td></td>
<td></td>
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<tr>
<td>≥200</td>
<td>0.7</td>
<td>0.2–2.2</td>
<td>0.567</td>
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<tr>
<td>Weight loss</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Eosinophils &lt;5%</td>
<td>24.6</td>
<td>5.7–105.5</td>
<td><strong>&lt;0.001</strong></td>
<td>1.0</td>
<td>2.0–15.2</td>
<td><strong>0.001</strong></td>
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<tr>
<td>Eosinophils ≥5%</td>
<td>1.3</td>
<td>0.3–5.4</td>
<td>0.734</td>
<td>5.5</td>
<td>0.8–6.0</td>
<td>0.131</td>
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<td>Relative eosinophilia</td>
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<td></td>
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<tr>
<td>In those without weight loss</td>
<td>9.0</td>
<td>1.9–42.3</td>
<td><strong>0.004</strong></td>
<td>1.0</td>
<td></td>
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<tr>
<td>In those with weight loss</td>
<td>0.7</td>
<td>0.2–2.9</td>
<td>0.579</td>
<td>2.2</td>
<td>0.4–2.3</td>
<td>0.860</td>
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<td>Absolute eosinophilia</td>
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<td></td>
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<tr>
<td>In those without weight loss</td>
<td>4.1</td>
<td>1.3–12.8</td>
<td><strong>0.020</strong></td>
<td>1.0</td>
<td></td>
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<tr>
<td>In those with weight loss</td>
<td>0.1</td>
<td>0.01–0.9</td>
<td><strong>0.011</strong></td>
<td>1.1</td>
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</table>

*OR = odds ratio; CI = confidence interval; NA = not applicable.
logic mechanism probably due to mucosal damage that could increase the absorption of possible allergens such parasitic antigens and alimentary antigens. This finding is also correlated with the frequent presence of mucus in the stool samples of the patients. The presence of mucoid stools associated with isosporiasis has been previously described and suggests an inflammatory process. Mucoid stools are also characteristics of the diarrheal episodes produced by invasive enteric pathogens such as Shigella, Yersinia, or Salmonella. However, the presence of blood that very often can be found associated with these bacteria was not a common finding in our patients. In future studies, it would be interesting to evaluate the presence of fecal markers of enteric inflammation such as fecal leukocytes and lactoferrin, as well as bacteriologic investigations.

In our study, there was also a relationship between weight loss and eosinophilia in I. belli-infected patients since it was found that the presence of eosinophilia was more common in those patients without weight loss and conversely, weight loss was more common in patients without eosinophilia. One explanation for these findings could be that in patients with significant weight loss there is more severe HIV disease and as a consequence, the alteration of the immunologic system could in some way affect the production of eosinophils. Substantial weight loss during the course of HIV infection has been ascribed to malnutrition. Some investigators have proposed that AIDS-associated malnutrition alters cellular immunocompetence by decreasing phagocytic function, antibody secretion, activity of the complement system, and the absolute number of functional lymphocytes. Thus, clinical interactions need to be more fully investigated.

The fact that CD4+ cell counts did not reach statistical significance in the multivariate analyses as a predictor for the risk of isosporiasis may be due to some missing values during the initial recruitment of the patients. Although most of the patients included in this study were in an advanced stage of the HIV infection, patients infected with Isospora had lower CD4+ cell counts compared with the control group. The mean CD4+ cell count for patients with isosporiasis was 64 cells/mm^3, which confirmed previous observations that I. belli usually occurs in patients with CD4+ cell counts < 100 cells/mm^3. Isosporiasis has been considered to be mainly a water-borne and a food-borne disease. Thus, the presence of other co-pathogens such as C. parvum or B. hominis that share a similar oral route of transmission could indicate a similar source of infection for all these parasites. However, the association between I. belli and S. stercoralis is interesting because the latter is transmitted by larval penetration of the skin. Although it has not been proved that I. belli can be sexually acquired, the association suggests that both parasites could share a fecal-oral transmission during sexual intercourse between men who have sex with other men. Others investigators also have found a similar association between both parasites. We found no clinical or laboratory differences when comparing patients infected only with I. belli with those with others associated co-pathogens.

In summary, isosporiasis should be suspected in HIV-infected patients from tropical countries with either acute or chronic watery diarrhea and weight loss. However, symptoms are non-specific and could be indicative of other infections, including their primary HIV infection. The presence of diarrhea is a common feature of AIDS and is also associated with other parasitic infections, such as cryptosporidiosis or microsporidiosis, which are known opportunistic infections of HIV-infected individuals. Nonetheless, in endemic regions, eosinophilia could be an important suggestive marker of I. belli infection, and physicians should have an increased level of suspicion for infection with Isospora in patients with increased levels of eosinophils in peripheral blood. This is especially true for HIV-infected persons with low CD4+ cell counts, who appear at greater risk of acquiring this parasitic infection. Studies are necessary to further define all of the risk factors for isosporiasis and to determine if it is possible to obtain better prophylaxis by increasing the dose of TMP/SMZ.

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