PREVALENCE OF ENTERIC PROTOZOA IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-POSITIVE AND HIV-NEGATIVE MEN WHO HAVE SEX WITH MEN FROM SYDNEY, AUSTRALIA

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Abstract. A prospective, comparative study of the prevalence of enteric protozoa was determined among human immunodeficiency virus (HIV)–positive and HIV-negative men who have sex with men (MSM) in Sydney, Australia. A total of 1,868 patients submitted stool specimens; 1,246 were from MSM (628 HIV positive and 618 HIV positive) and 622 from non-MSM were examined over a 36-month period. A total of 651 (52.2%) stool specimens from MSM were positive for protozoa compared with 85 (13%) from non-MSM. There was a significant difference in the prevalence of Blastocystis hominis, Endolimax nana, Entamoeba histolytica/dispar complex, Entamoeba hartmanni, Iodamoeba butschlii, and Enteromonas hominis detected between MSM and non-MSM (P < 0.001). The only notable difference between HIV-negative and HIV-positive MSM was that HIV-infected MSM were found to more likely have a Cryptosporidium parvum infection. Entamoeba histolytica was found in 3 patients, E. dispar in 25, and E. moshkovskii in 17, all of whom were MSM. When compared with a control group, MSM were significantly more likely to harbor intestinal protozoa and have multiple parasites present. The results of this study show high rates of enteric parasites persist in MSM and highlight the importance of testing for intestinal parasites in MSM. This is the first report of E. moshkovskii from MSM.

INTRODUCTION

High rates of enteric protozoan parasitism have been reported among men who have sex with men (MSM) in metropolitan areas throughout the world.1–3 Sexual activity has been shown to be the primary mode of transmission for several important parasitic diseases and has resulted in a significant prevalence of enteric parasitic infections among male homosexuals.4 Furthermore, the association between intracellular intestinal parasites and infection with human immunodeficiency virus (HIV) is well documented. Opportunistic parasites such as Cryptosporidium parvum and the microsporidia, are a major cause of morbidity and mortality in patients with acquired immunodeficiency syndrome worldwide.

Previous documented prevalence rates of enteric protozoa in MSM include 48.5% in the United States, 50.6% in mainland China, 57% in the United Kingdom, and 81% in a 128 Australian homosexual males.1,3–5 Intestinal parasitosis is an important health problem affecting people in both developing and developed regions of the world. Although this is usually self-limiting and almost invariably non-fatal, it results in significant morbidity. However no recent prevalence data on the presence of intestinal protozoa carriage exists in Australia from HIV-positive and HIV-negative MSM. Therefore, a prospective study investigating a cross-sectional cohort of HIV-positive and HIV-negative MSM was undertaken to determine the prevalence of intestinal protozoa carriage in each population in Sydney, Australia.

To date, most prevalence studies have only used microscopic detection of enteric parasites. Newer molecular methods offer highly sensitive and specific alternatives for detection of a variety of parasites from clinical samples including Dientamoeba fragilis, Entamoeba histolytica, E. dispar, and E. moshkovskii.6–8 Furthermore, molecular methods enable differentiation of morphologic identical species as in E. histolytica (pathogenic) from E. dispar (non-pathogenic).

Molecular methods were included in our prospective study because previous rates of infection with E. histolytica/E. dispar were 37% in Sydney, Australia.9 However, the true prevalence of pathogenic strains (E. histolytica) remains unknown. No studies to date have used molecular methods to determine the prevalence of E. moshkovskii in MSM populations.

MATERIALS AND METHODS

Specimens for examination for ova, cysts, and parasites were submitted to the Microbiology Department at St. Vincent’s Hospital in Sydney from March 2003 through February 2006. Consecutive stool samples were obtained from 1,246 MSM (628 HIV-positive patients and 618 HIV-negative patients) attending several general practices in the inner city of Sydney that specialize in gay men’s health issues. An additional 622 consecutive non-MSM stool specimens were obtained from alternative general practices from the same general location. All stool samples were submitted for patients with diarrhea. All patients were male. A single fecal sample was collected from each patient and divided into two vials: one with sodium acetate–acetate acid formalin (SAF) and one without preservatives. Permanent staining using a modified iron-hematoxylin stain (Fronine, Riverstone, New South Wales, Australia) that incorporates a carbol-fuschin staining step to detect coccidian parasites was performed on the SAF-fixed samples according to the manufacturer’s instructions. Samples were also stained for microsporidial spores using the Uvitex 2B stain.10 A formalin-ethyl acetate concentration technique was also used on the SAF-fixed portion of feces for the recovery of helminth and helminth ova as previously described.11

Stool specimens diagnosed with E. histolytica/E. dispar..
complex (by microscopy) underwent further testing using enzyme-linked immunosorbent assays (ELISAs) and polymerase chain reaction (PCR) to identify the species present. Antigen was detected in fresh stool samples with the TechLab E. histolytica II test kit (TechLab, Inc., Blacksburg, VA), and the Entamoeba Celisa Path kit (Cellabs Pty. Ltd., Sydney, New South Wales, Australia) according to the manufacturer’s instructions.

DNA extraction was performed on fresh or frozen (-20°C) feces with the QIAamp™ DNA stool mini kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. A PCR for E. histolytica, E. dispar, and E. moshkovskii was performed using the primers and amplification profiles previously described. The amplified PCR products were analyzed by electrophoresis on 1% Ready Agarose Gels (Bio-Rad, Marne la Coquette, France). When discordant results were obtained for repeated EIAs and PCR products, DNA sequencing was performed. The PCR products were purified using the QIAquick™ PCR Gel Extraction Kit (Qiagen) as per the manufacturer’s instructions and subsequently sequenced in both directions on an ABI Prism 3700 sequence (Applied Biosystems, Foster City, CA). Statistical analysis was performed on categorical data using the chi-square test.

RESULTS

Of the 1,868 patients who submitted specimens, 1,246 were from MSM (628 HIV positive and 618 HIV negative) and 622 were from non-MSM. A total of 363 (52.2%) stools from MSM were positive for protozoa compared with 85 (13%) from non-MSM (P < 0.001). Similar significant differences were observed in fecal carriage of Endolimax nana, E. histolytica/E. dispar complex, E. hartmanni, Iodamoeba butschlii, and Enteromonas hominis in MSM compared with non-MSM. There was no significant difference between MSM and non-MSM for fecal carriage of Chilomastix mesnili. A similar finding was also observed for the pathogenic enteric protozoa D. fragilis, Giardia intestinalis, and Cryptosporidium parvum. No helminth or helminth ova were detected in any of the samples.

Cryptosporidium species were more common in HIV-infected MSM than in HIV-negative MSM and the control group. Slight differences were observed in the prevalence of all other protozoa between the HIV-positive and HIV-negative groups. However, these differences were not statistically significant. No microsporidia was detected in any of the groups including the HIV-positive group of MSM. The MSM were significantly more likely to have multiple parasites in their stool compared with non-MSM (43.5% versus 8%; P < 0.001) (Table 2).

Entamoeba histolytica/E. dispar complex was detected in 54 samples by microscopy with differentiation into species possible in 45 patients by PCR (Table 3). Six samples failed to amplify any product, and three samples were not tested by PCR because of insufficient material for testing.

Enzyme-linked immunosorbent assays were performed on only 51 of the 54 samples because of insufficient material in the remaining three samples. All samples were negative by the TechLab E. histolytica II test kit, and three samples were positive for E. histolytica by the Entamoeba Celisa Path kit.

Fifteen of 25 sequences of the E. dispar amplicons showed a 99–100% similarity to E. dispar sequences in GenBank (e.g., accession no. Z49256), whereas three of three E. histolytica amplicons sequences showed a 99–100% similarity to E. histolytica sequences in GenBank (e.g., accession no. X56991). Fifteen of 17 E. moshkovskii amplicons showed 100% identity to E. moshkovskii sequences in GenBank (e.g., accession no. AF149906).

DISCUSSION

High rates of infection with enteric protozoa were detected in this study, predominantly in MSM. In addition, these patients were significantly more likely to harbor multiple parasites. These findings support earlier studies that showed high rates of intestinal parasitism in MSM throughout the world. Reports of intestinal parasitism in MSM through the world. Typical symptoms of protozoan infections include gastrointestinal symptoms such as diarrhea, abdominal pain, and sometimes fever. The prevalence of protozoan infections can vary significantly among different populations, and the methods used for detection can influence the results. In this study, the authors used a combination of microscopy and molecular techniques to identify the presence and species of protozoa in stool samples from MSM and non-MSM.

### Table 1

<table>
<thead>
<tr>
<th>Parasite</th>
<th>MSM (n = 628)</th>
<th>MSM (n = 681)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential pathogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entamoeba histolytica/E. dispar complex†‡</td>
<td>34 (5.4)</td>
<td>20 (3.2)</td>
</tr>
<tr>
<td>Giardia intestinalis</td>
<td>17 (3)</td>
<td>28 (4.5)</td>
</tr>
<tr>
<td>Cryptosporidium species‡</td>
<td>2 (0.6)</td>
<td>14 (2.2)</td>
</tr>
<tr>
<td>Blastocystis hominis‡¶</td>
<td>135 (21)</td>
<td>111 (18)</td>
</tr>
<tr>
<td>Non-pathogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endolimax nana†</td>
<td>74 (12)</td>
<td>62 (10)</td>
</tr>
<tr>
<td>Entamoeba coli†</td>
<td>30 (5)</td>
<td>18 (2.9)</td>
</tr>
<tr>
<td>Entamoeba hartmanni‡</td>
<td>27 (4)</td>
<td>9 (1.4)</td>
</tr>
<tr>
<td>Iodamoeba butschlii</td>
<td>24 (4)</td>
<td>8 (0.3)</td>
</tr>
<tr>
<td>Enteromonas hominis‡¶</td>
<td>9 (1.4)</td>
<td>10 (1.6)</td>
</tr>
<tr>
<td>Chilomastix mesnili</td>
<td>6 (0.9)</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>Trichomonas hominis</td>
<td>0</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>Retortamonas hominis</td>
<td>0</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

* MSM = men who have sex with men; HIV = human immunodeficiency virus
† Entamoeba histolytica/disp complex isolates are composed of pathogenic E. histolytica and non-pathogenic E. dispar (further testing was performed to differentiate between the two species).
‡ Significant difference (P < 0.01) between MSM (HIV positive and HIV negative) and controls.
¶ Significant difference (P < 0.01) between HIV-positive MSM and the other two groups (MSM HIV negative and controls).
§ Pathogenicity is controversial.

### Table 2

<table>
<thead>
<tr>
<th>No. of protozoa</th>
<th>HIV negative</th>
<th>HIV positive</th>
<th>No. (%) controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170 (50.6)</td>
<td>151 (52.4)</td>
<td>78 (91.8)</td>
</tr>
<tr>
<td>2</td>
<td>93 (27.7)</td>
<td>89 (30.9)</td>
<td>5 (5.9)</td>
</tr>
<tr>
<td>3</td>
<td>86 (11.3)</td>
<td>29 (10.1)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>4</td>
<td>21 (6.5)</td>
<td>7 (2.4)</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>20 (3.2)</td>
<td>11 (3.8)</td>
<td>0</td>
</tr>
<tr>
<td>≥6</td>
<td>2 (0.6)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
</tbody>
</table>

* For definition of abbreviations, see Table 1.
introduction of highly active antiretroviral therapy (HAART) in developed countries, sexual risk behavior has increased among HIV-infected MSM.\textsuperscript{13} Our results do not show a corresponding increase in intestinal protozoa, which suggests that there are probably other factors involved in parasite acquisition and maintenance. Furthermore, eradication of intestinal protozoa is probably not attainable with safe-sex practices alone.

The genus 	extit{Entamoeba} comprises six species (\textit{E. histolytica}, \textit{E. dispar}, \textit{E. moshkovskii}, \textit{E. poleki}, \textit{E. coli}, and \textit{E. hartmanni}) that are present in the human intestinal lumen. Humans are the primary reservoirs and with the exception of \textit{E. histolytica} all of these parasites are considered non-pathogenic.\textsuperscript{14} This report highlights the usefulness of molecular methods in differentiating the morphologically identical 	extit{Entamoeba} complex, with \textit{E. dispar} and \textit{E. moshkovskii} being more common than \textit{E. histolytica}. \textit{Entamoeba moshkovskii} has not been previously detected in MSM. It is considered primarily a free-living amoeba and has rarely been shown to infect humans. Human isolates of \textit{E. moshkovskii} have been reported in patients from North America, Italy, South Africa, Bangladesh, India and Iran.\textsuperscript{8,15–18} Although previous studies have shown that \textit{E. moshkovskii} is not associated with disease, studies in India reported \textit{E. moshkovskii} in patients with dysentery.\textsuperscript{15–17}

Three samples were found to be positive by PCR for \textit{E. histolytica}. Because the patients had no recent history of overseas travel within the last five years, the species identity was confirmed by sequencing. It was presumed that the infections were locally acquired. The discovery of \textit{E. histolytica} within MSM is of great public health concern. This is highlighted by the experience in Japan where amebiasis has become endemic in homosexual males and causes significant morbidity and mortality with complications such as colitis and liver abscesses occurring more frequently in homosexual and bisexual men.\textsuperscript{14,19} A recent outbreak of sexually transmitted 	extit{Shigella sonnei} among homosexual males in the same inner city area of Sydney demonstrates how quickly a pathogen can be transmitted and become endemic in the homosexual community.\textsuperscript{20} A similar situation with \textit{E. histolytica} becoming endemic in the Australian gay community could occur and highlights the importance for continued surveillance.

The Cellabs kit, unlike the Techlabs kit, detected all three positive \textit{E. histolytica}-containing stool specimens despite the fact that both kits use monoclonal antibodies against Gal-specific lectin of \textit{E. histolytica} strains. Several studies have previously shown ELISA to be less sensitive than PCR in the detection of these parasites.\textsuperscript{21–23} Although the numbers of \textit{E. histolytica} detected were small (\textit{n} = 3), our results support previous conclusions that in countries with low endemicity for these parasites, molecular tests are more sensitive than ELISA kits.\textsuperscript{24–25}

Prevalence of \textit{B. hominis} was high, and it was the most common protozoa detected in all three groups. However, there is little knowledge of the basic biology of this organism. As a result, controversy surrounding its role as a pathogen still exists.\textsuperscript{26}

\textit{Giardia} was the most prevalent of the pathogenic protozoa detected in all groups. This finding suggests that this pathogen is not spread by the anal-oral route or the fecal-oral route. Such transmission is probably secondary to intermittent, sporadic shedding of cysts and that \textit{Giardia} is predominately a small bowel pathogen.\textsuperscript{27}

\textit{Dientamoeba fragilis} infection rates in MSM in this study were comparable to those found in non-MSM. Furthermore, HIV-positive MSM are not more susceptible to infection than HIV-negative patients. Results of our study are consistent with previous documented prevalence rates of approximately 1%, 1,12,28 The reason for these findings are unknown but are probably similar to those for \textit{Giardia} because both are pathogenic flagellates associated with acute and chronic gastrointestinal infections.\textsuperscript{29,30}

The prevalence of \textit{Cryptosporidium} and microsporidia have both decreased in HIV-positive infected individuals with the advent of HAART therapy.\textsuperscript{31,32} Both of these parasites tend to infect immunocompromised hosts with CD4+ cell counts < 100 cells/\mu L. The low prevalence of cryptosporidiosis and the absence of microsporidiosis in our study indicate a relatively healthy patient group with access to therapy and health care despite not knowing their immune status (CD4 count or whether patients were receiving HAART therapy). This study demonstrated the absence of helminths or helminth ova. Infectivity usually requires a period of egg maturation in the environment. This finding precludes transmission of these parasites by the fecal-oral route. Furthermore, Australia also has a relatively low level of helminth infections, as seen in other developed countries.

A weakness of our study was that only one stool specimen was examined. This may underestimate the true prevalence of parasitic infections because previous studies have reported higher numbers of \textit{E. histolytica}, \textit{G. intestinalis}, and \textit{D. fragilis} when additional stool samples are examined.\textsuperscript{3–9,33} However, this factor would probably influence both groups equally. Our study confirms the high rates of intestinal parasitism in MSM regardless of HIV status and is the first report of \textit{E. moshkovskii} in MSM.

Men who have sex with other men continue to be a high risk group for intestinal parasitic disease and should be routinely screened for intestinal protozoa. Furthermore, information and education about sexual practices may influence re-infection and transmission rates.

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