Amebiasis is an important human gastrointestinal infection affecting approximately 50 million people worldwide and accounts for greater than 100,000 deaths annually. The causative agent, Entamoeba histolytica, is an intestinal protozoan parasite unique among amoeba in its ability to disrupt the intestinal mucosa causing colitis and the capability for further hematogenous spread, producing potentially fatal abscesses. It appears to be extremely transmissible with an infectious dose as low as one viable cyst and is usually acquired from feocally contaminated food or water sources, with prevalence rates up to 6% in areas that have poor sanitation. It has been previously reported that stool protozoan carriage occurs at increased rates in men who have sex with men (MSM). Studies from East Asia have recognized E. histolytica as an emerging pathogen in MSM, where sexual activity, especially oro-anal contact, is suggested to be the mode of transmission. There have also been increasing reports from North America and Europe of invasive disease in the MSM population.

Although E. histolytica is known to be endemic in Australia, this has predominantly been recognized in the northern indigenous population. More recently there have been reports of amebiasis in MSM in larger Australian cities. A study from Sydney showed high rates of intestinal parasites in MSM, with 52.2% of stool specimens positive for protozoa, compared with 13% from non-MSM, of which E. histolytica had a prevalence rate of 0.24% in the MSM group. Another report of five cases of invasive amebiasis in MSM from Sydney further documents its existence in this at risk group.

Accurate prevalence and incidence data for E. histolytica are difficult to ascertain by microscopy alone, because it is morphologically indistinguishable, yet genetically distinct from non-pathogenic amoebic species; including Entamoeba dispar and Entamoeba moshkovskii. Improved methods of detection, with greater sensitivity and specificity include antigen and antibody detection and polymerase chain reaction. Serum antibody detection is most useful in patients with extra-intestinal disease and is able to detect antibodies specific for E. histolytica. Sensitivity of these tests is ~95% for patients with amoebic abscesses, 70–85% for patients with active amoebic colitis, and 10–20% for asymptomatic people passing cysts, with specificity of 95% generally reported, and E. histolytica-specific antibodies persisting for many years after exposure.

Epidemiological data on E. histolytica is important for public health awareness; this study therefore intends to further investigate the impact of E. histolytica on Sydney’s MSM community, by conducting a seroprevalence survey of two at-risk groups, comparing these to a selection of the general population. The St. Vincent’s Hospital pathology department is the principal referral hospital for human immunodeficiency virus (HIV) testing in Sydney. A total of 1,331 serum samples submitted to the microbiology department at St. Vincent’s Hospital between January 1, 2004 and December 31, 2008 were included in the study and were divided into three patient groups; high risk HIV-infected MSM, low risk HIV-uninfected MSM, and controls.

For the high risk HIV-infected MSM group, consecutive samples were selected from male patients attending several local general practices that specialize in MSM health, who tested positive for syphilis and were known to be HIV-infected. 429 samples were collected over the study period that matched these criteria. Patients co-infected with syphilis were thought to be at high risk on the basis of the likelihood that they have been participating in potentially higher risk sexual activity. On review of the literature, there was no published data suggesting that active syphilis increased the risk of developing amebiasis.

For the low risk HIV-uninfected MSM group, samples were selected from male patients attending the same local general practices specializing in MSM health, who tested positive for syphilis and were known to be HIV-uninfected. 446 samples were collected over the study period that met this criteria. The control group consisted of 456 random serum samples selected from male and female patients who attended several other general practices in the same suburbs as those from the MSM groups. Patients in the low risk and control groups were excluded if they were younger than 16 or known to be HIV-infected; there was no data on their syphilis status collected.

Serum samples from the study subjects were stored at –20°C until tested. Qualitative screening of serum immunoglobulin G (IgG) antibodies to E. histolytica were retrospectively performed using the commercial enzyme-linked immunosorbent
assay, *Entamoeba histolytica* Amebiasis kit, (Diagnostic Automation Inc., Calabasas, CA). The assay was performed in accordance with manufacturer’s instructions and the microwell plates were read on an LP 400 ELISA reader (Sano Diagnostics Pasteur Inc., Chaska, MN), set at a biochromatic reading of 450 to 620 nm. Samples reading greater than 0.4 optical density (OD) units were considered positive.

Out of the 429 high risk HIV-infected MSM group, a total of 22 tested positive for *E. histolytica* antibodies. Of the 456 in the control group, only two tested positive and one tested positive from the 446 low risk HIV-uninfected MSM group. Demographics and results for the three groups are listed in Table 1. Statistical analysis was performed on categorical data with quantities derived from two-by-two contingency tables, using the χ<sup>2</sup> test and Fisher’s exact test for probability, with the results listed in Table 2. There was no statistical significance between the low risk HIV-uninfected MSM and control groups for having positive *E. histolytica* serology, with a relative risk (RR) of 0.51. The high risk HIV-infected MSM group had a significantly greater rate of seropositivity when compared with both the low risk HIV-uninfected MSM group, RR 22.87, and the control group, RR 11.69.

These results are similar to those from other seroprevalence studies from Taiwan. One hypothesis for the greater rates observed in HIV-infected MSM is that they develop more invasive disease than HIV-uninfected MSM, as the two groups should have similar colonization rates of *E. histolytica*. This is possibly caused by their immunosuppression, specifically deficiencies in cell mediated immunity, allowing for increased rates of invasion of the trophozoites through mucosal barriers. The role of CD4<sup>+</sup> T-cell function has previously been hypothesized as a contributing factor to disease progression in amoebic liver abscesses.

Once introduced into industrialized countries, there is potential for further spread and for the amoeba to become endemic in such groups as the MSM population where, through oro-anal sexual contact, it may soon be a common sexually transmitted disease. There is also the potential for further spread into non-MSM populations; as described in Tokyo, where there were increased rates of *E. histolytica* seropositivity in heterosexual women and in Canada with a report of a sexually transmitted cluster of amebiasis in heterosexuals and female homosexuals.

There were several limitations to this study that should be taken into account. Apart from their HIV status, very little was known about the demographics of our sample population.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics and results of groups tested for <em>Entamoeba histolytica</em> antibody&lt;sup&gt;a&lt;/sup&gt;</th>
<th>High risk MSM</th>
<th>Low risk MSM</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persons tested</td>
<td>429</td>
<td>446</td>
<td>456</td>
<td>1,331</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>36 (23–62)</td>
<td>42 (19–87)</td>
<td>41 (16–87)</td>
<td>40 (16–87)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>429</td>
<td>446</td>
<td>241</td>
<td>1,116</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>0</td>
<td>215</td>
<td>215</td>
</tr>
<tr>
<td>Persons with positive OD readings (&gt; 0.4 units)</td>
<td>22</td>
<td>1</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>% Positive</td>
<td>5.13</td>
<td>0.22</td>
<td>0.44</td>
<td>1.88</td>
</tr>
</tbody>
</table>

<sup>a</sup> MSM = men who have sex with men; OD = optical density.

### Table 2

<table>
<thead>
<tr>
<th>Summary of the relative risks when comparisons were made between the 3 groups&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RR</th>
<th>95% CI</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk MSM vs. low risk MSM</td>
<td>22.87</td>
<td>3.96–133.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>High risk MSM vs. controls</td>
<td>11.69</td>
<td>3.09–44.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low risk MSM vs. controls</td>
<td>0.51</td>
<td>0.07–3.90</td>
<td>0.51</td>
</tr>
</tbody>
</table>

<sup>a</sup> RR = relative risk; CI = confidence interval; MSM = men who have sex with men.

There was no information gathered regarding patients symptoms or underlying immunosuppression, travel history, ethnicity, or sexual behavior. Because we were sampling patients attending general practitioners, there may have been more immunosuppressed people in this data set leading to a selection bias toward increased rates of seropositivity. Furthermore, as *E. histolytica* occurs at very low rates in Sydney we may not have captured a true representation of seropositivity rates in this community with the small sample size used.

This is the first study to show that in a large industrialized western city such as Sydney, sexually active HIV-infected MSM are at greater risk of developing *E. histolytica* infection than HIV-uninfected MSM and the general population. This finding is in keeping with the growing body of evidence that HIV infection is associated with extra-intestinal amebiasis, which is especially important in MSM who are known to have increased carriage of intestinal parasites. It is therefore essential that physicians be aware of the possibility of amebiasis as an emerging infection in industrialized cities with large MSM populations and the need to adequately study and treat this at risk group if presenting with symptoms of amebiasis, preventing morbidity and further spread within MSM or into the greater community.

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Authors’ addresses: Rodney James, Joel Barratt, Deborah Marriott, John Harkness, and Damien Stark, Department of Microbiology, St. Vincent’s Hospital, Darlinghurst, New South Wales, Australia. E-mails: rod_s_james@yahoo.com.au, Joel.Barratt-1@uts.edu.au, d.marriott@stvincents.com.au, j.harkness@stvincents.com.au, and dstark@stvincents.com.au.

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