Molecular Diagnosis of an *Enterocytozoon bieneusi* Human Genotype C Infection in a Moderately Immunosuppressed Human Immunodeficiency Virus-Seronegative Liver-Transplant Recipient with Severe Chronic Diarrhea

Microsporidia are intracellular parasites affecting the whole animal kingdom (11). The species most often encountered in humans is *Enterocytozoon bieneusi*. Intestinal *E. bieneusi* infections have been reported in several hundred human immunodeficiency virus (HIV) patients, while *E. bieneusi* infections in HIV-negative patients are extremely rare (11). We report what is to our knowledge the first finding of an intestinal infection caused by *E. bieneusi* human genotype C (based on analysis of the ribosomal DNA [rDNA] internal transcribed spacer [ITS] sequence) diagnosed by PCR in a liver-transplant recipient.

A 36-year-old HIV-negative woman receiving tacrolimus as a single immunosuppressive treatment after liver transplantation 5 years ago developed watery, unbloody diarrhea. Seven months after the onset of her symptoms, she was admitted for evaluation of chronic diarrhea, with 20 to 25 bowel movements per day. Several stool samples were negative for bacterial, viral, and parasitic pathogens. Two stool smears stained by the modified trichrome technique (Weber's chromotrope-based stain) (12) yielded abundant microsporidian spores. Fresh stool smears were analyzed by PCR, which allowed for the identification of the microsporidian species as *E. bieneusi* (5). PCR findings were verified by DNA sequencing of the amplified gene products. Upon albendazole therapy (800 mg/day) for 6 weeks, the frequency of bowel movements diminished dramatically to 2 to 3 per day, while stool samples obtained after 2 and 4 weeks showed no reduction in microsporidial spores and remained PCR positive.

*E. bieneusi* infections are extremely rare in HIV-negative patients, affecting mainly otherwise immunocompromised patients (11). Only five cases of human intestinal *E. bieneusi* infection in solid organ recipients have been reported (Table 1). In all cases, diarrhea started more than 18 months after transplantation, indicating that intestinal *E. bieneusi* infection might be expected only after a prolonged period of immunosuppression. The extent of immunosuppression, however, may not be severe, since our patient developed intestinal microsporidiosis despite a moderate immunosuppressive therapy of tacrolimus without cortisone. Moreover, the number of CD4-positive T-helper cells per microliter was above 200 in all solid organ recipients with intestinal microsporidiosis.

Currently, no curative therapy for *E. bieneusi* infection exists. Albendazole, which is effective against microsporidia other than *E. bieneusi*, seems to alleviate diarrhea in *E. bieneusi*-infected patients without clearing the infection (11). In the three successfully treated transplant patients with *E. bieneusi* infection, the discontinuation of the immunosuppressive therapy was probably what improved the patients’ health conditions (3, 6).

The diagnosis of infections caused by *E. bieneusi* has been markedly improved by the use of Weber’s chromotrope-based stain (12). However, there are still problems in finding microsporidial spores in human feces using both light microscopy and PCR (9). For species identification, both transmission electron microscopy and PCR are considered “gold standards”

### TABLE 1. Intestinal *E. bieneusi* infections in solid organ recipients reported in the literature

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Type of transplant</th>
<th>Immunosuppressive treatment</th>
<th>Time to onset of symptoms posttransplant (yrs)</th>
<th>Level of rejection</th>
<th>CD4 count (cells/μl)</th>
<th>Duration of symptoms (mos, unless stated otherwise)</th>
<th>Therapy</th>
<th>Microbiological clearance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>M</td>
<td>Heart-lung</td>
<td>ATG, methylprednisone,</td>
<td>3</td>
<td>Severe</td>
<td>328</td>
<td>8</td>
<td>Albendazole&lt;sup&gt;+&lt;/sup&gt;</td>
<td>no (2 yrs until death)</td>
<td>3, 7, 8</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>M</td>
<td>Heart</td>
<td>Cyclosporine, azathioprine,</td>
<td>4.5</td>
<td>Moderate</td>
<td>406</td>
<td>3 yrs</td>
<td>Metronidazole&lt;sup&gt;+&lt;/sup&gt;</td>
<td>no</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>M</td>
<td>Kidney</td>
<td>Prednisone, cyclosporine,</td>
<td>12</td>
<td>NR&lt;sup&gt;*&lt;/sup&gt;</td>
<td>549</td>
<td>3</td>
<td>Albendazole&lt;sup&gt;+&lt;/sup&gt;, stop of MMF</td>
<td>yes</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>M</td>
<td>Kidney</td>
<td>Prednisone, cyclosporine,</td>
<td>2</td>
<td>NR&lt;sup&gt;*&lt;/sup&gt;</td>
<td>278</td>
<td>NR</td>
<td>Metronidazole&lt;sup&gt;+&lt;/sup&gt;</td>
<td>yes</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>F</td>
<td>Kidney</td>
<td>Tacrolimus, prednisone,</td>
<td>1.5</td>
<td>None</td>
<td>350</td>
<td>1.5</td>
<td>Albendazole&lt;sup&gt;+&lt;/sup&gt;, metronidazole&lt;sup&gt;+&lt;/sup&gt;, stop of MMF, decrease of tacrolimus</td>
<td>yes</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>F</td>
<td>Liver</td>
<td>Tacrolimus</td>
<td>5</td>
<td>None</td>
<td>NR&lt;sup&gt;*&lt;/sup&gt;</td>
<td>9</td>
<td>Albendazole&lt;sup&gt;+&lt;/sup&gt;</td>
<td>no (7 mos)</td>
<td>Present case</td>
</tr>
</tbody>
</table>

<sup>a</sup> M, male; F, female.
<sup>b</sup> MMF, mycophenolate mofetil.
<sup>c</sup> NR, not reported.
<sup>d</sup> Initially 800 mg/day and then increased to 1,200 mg/day.
<sup>e</sup> 1,500 mg/day.
<sup>f</sup> Initially 400 mg/day for 15 days and then increased to 800 mg/day for 3 weeks.
<sup>g</sup> 400 mg/day for 2 weeks.
<sup>h</sup> 1,500 mg/day for 2 weeks.
<sup>i</sup> 800 mg/day for 3 weeks (two courses).
In solid organ recipients *E. bieneusi* was identified by at least one of the gold standard methods mentioned above in three patients who were recipients for kidney transplants and in another one who received a heart-lung transplant. PCR was used only for the three kidney recipients and for our liver-transplant patient.

The detection of *E. bieneusi* spores in pig fecal samples (2) raised the question of whether or not animals may serve as reservoirs for this microsporidian species. Breitenmoser et al. (1) demonstrated that *E. bieneusi* genotypes from animal origins were different from those originated from humans by comparing rDNA ITS sequences. To date, four different human-derived *E. bieneusi* genotypes, named A, B, C, and D, are known (1, 10). In our patient the underlying *E. bieneusi* strain was identified as genotype C, which has been found previously only in HIV-positive patients (1, 10).

In conclusion, we report the first case of an *E. bieneusi* human genotype C infection in a liver-transplant patient diagnosed by molecular methods. Intestinal *E. bieneusi* infection might become a medical problem in solid organ transplant recipients, especially after a long time of immunosuppressive therapy. The degree of immunosuppression, however, does not seem to have to be as severe as for HIV patients to cause disease. The discontinuation of immunosuppressive therapy might be one option to achieve a medical cure, while albendazole may lead to the alleviation of symptoms without eradicating the pathogen.

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REFERENCES


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