Disseminated infection with a new genovar of *Encephalitozoon cuniculi* in a renal transplant recipient

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Conflict of interest: the authors declare that they have no conflicting interests in relation to this work.

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Abstract

Disseminated microsporidiosis is a life-threatening opportunistic infection. Here, we report about a previously undescribed genovar of *Encephalitozoon cuniculi* causing disseminated infection in a non-HIV-infected renal transplant recipient. Disseminated microsporidiosis must be considered in the differential diagnosis of chronic fever in renal allograft recipients even without urinary symptoms.
CASE REPORT

A 38-year-old woman with end-stage renal disease due to IgA nephropathy received a renal transplant. Her immunosuppressive therapy consisted of thymoglobuline, mycophenolate mofetil (MMF), and cyclosporine A (cyA).

Four weeks posttransplantation, the patient presented with intermittent fever. The clinical examination was unremarkable. No travel history was known. Laboratory examination showed a white blood cell count of $16.4 \times 10^9 \text{ l}^{-1}$ with a C-reactive protein of 6 mg/L (N < 6). Graft biopsy and magnetic resonance imaging (MRI) provided no evidence for rejection and no vascular or urologic complication. Urine, blood, stool and sputum cultures showed no fungal or bacterial growth. A PCR-based assay on blood for cytomegalovirus (CMV) was positive (5.15 log). The patient was successfully treated with ganciclovir: the fever resolved and she was discharged to home after a 4-day hospitalization.

Two weeks later, she developed fever (38°C), cough, non-specific abdominal pain and anorexia without transit troubles and was readmitted to our hospital. Initial investigations included full blood examination, demonstrating nonregenerative anemia and leucopenia without inflammatory syndrome. Renal function revealed a serum creatinine level of 86 µmol/L (N: 44 – 80). CMV DNA was undetectable in blood. Urine contained numerous cells but cultures showed no fungal or bacterial growth. Thoracic and sinus computed tomography scans did not reveal any lesions and brain MRI was unremarkable. No tubercle bacillus was detected on 3 successive sputa.

Stools were repeatedly negative for microsporidia, Cryptosporidium sp. and other parasites. After a 1-month hospitalization, all drugs (excepting MMF and cyA) were stopped to eliminate toxic etiology. At that time, many spores of microsporidia were
detected in urine (Figure 1), kidney biopsy and sputum smears by using Uvitex 2B staining (15). No spore of microsporidia was found in stools, duodenal biopsy and cerebrospinal fluid (CSF). No culture of the organism was performed. There was no evidence of microsporidia in the feces of patient’s dog; unfortunately, its urine and serum could not be sampled. The patient was given albendazole 400 mg twice daily for four weeks and 400 mg daily until her CD4 cell count raised up to 100/mm$^3$ (9 months). MMF was switched to azathioprine. This treatment led to clinical improvement, including resolution of fever after 5 days of treatment and reduction of abdominal pain after 2 weeks of treatment. Serum creatinine level decreased to 63 µmol/L. However, rare microsporidian spores continued to be shed in urine. Complete clearance of spores was observed only 5 months and a half after treatment initiation.

**Molecular specific identification.** DNA was extracted from the patient’s specimens (urines, kidney biopsy, sputum, CSF, stools and duodenal biopsy) by using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions, after an initial 30-min incubation step with 10 U lyticase at 37°C (Sigma Aldrich, Saint Quentin Fallavier, France). Species diagnosis was made by amplifying a 938-bp fragment of *E. cuniculi* small subunit rRNA gene by using 5'-GTGGTCTGCCCTGTGGGT-3' and 5'-CCCTCACAGCAGGCAGAAGC-3' primers (13). Amplification was performed on an 9700 PCR system (Applied Biosystems, Foster City, California) in a 50-µl volume containing 2mM MgCl$_2$, 1x Applied Biosystems Gold Buffer, 200 µM of each dNTP, 0.4 µM of each primer, 2 U of Applied Biosystems AmpliTaq Gold, and 10 µl of extracted DNA. After 9 min at 95°C, amplification consisted of 38 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s, followed by a 5-min terminal extension.
step at 72°C. The presence of *E. cuniculi* DNA was evidenced by this specific PCR on urines, sputum and renal biopsy specimens. *E. cuniculi* DNA was undetectable in duodenal biopsy, stools, CSF and blood specimens, in accordance with the absence of microsporidian spores at microscopic observation in these specimens. *E. cuniculi* DNA became undetectable in urine only 5 months and a half after the initiation of treatment with albendazole.

**Molecular subspecific typing.** Subspecific typing was made by PCR and sequence analysis of 403-bp fragment containing the internal transcribed spacer (ITS) of the rRNA genes, as previously described (1). PCR products were purified with the QIAquick PCR Purification kit (Qiagen) and sequenced on both strands with the PCR primers and the BigDye Terminator kit (Applied Biosystems) on an Applied Biosystems 3730 automated sequencer. Sequence analysis showed the presence of five repeats of 5'-GTTT-3' in the ITS region of all tested specimens collected from our patient (1 sputum, 1 renal biopsy and 4 urine samples), indicating that she was infected with a previously undescribed strain, which we propose to name type IV strain (Figure 2).

**Immunological methods.** By use of indirect immunofluorescence technique (IFAT) (16), a serum sample taken early after infection showed a moderately strong IgG antibody response against the spore wall of *E. cuniculi*, but no reaction was observed against the polar tube. After 3 months, the IgG titer against the spore wall increased 2 fold with an IgG positive response against the parasite polar tube.

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Several species of microsporidia can cause disease in humans. Most cases have been described in HIV-infected patients but microsporidia are being considered as emerging pathogens in transplant recipients (8). The most frequently recognized species in humans is *Enterocytozoon bieneusi*. It is mainly found in the upper gastrointestinal tract and associated with diarrheal illness. *Encephalitozoon* spp. infections are less frequently identified and are characterized by their potential to disseminate (4, 7). Disseminated microsporidiosis due to *Encephalitozoon* spp. have been described most commonly in patients with acquired immunodeficiency syndrome and only rarely in those with other forms of immunosuppression. To our knowledge, only 5 cases of *E. cuniculi* infections in non-HIV-infected immunocompromised patients have been reported in the literature, in addition to our case. Among the 6 cases, 5 occurred in transplant recipients. A sixth patient who presented with iris tumor caused by *E. cuniculi* infection had idiopathic CD4+ T lymphocytopenia (6). The clinical characteristics of our patient and of the other non-HIV-infected patients are reported in Table 1. All patients had severe immunosuppression that could facilitate *E. cuniculi* infection. Disseminated infection was described in 4 patients (4/6 patients). One patient only had respiratory distress and another one had ocular manifestation. The most commonly reported clinical manifestations of disseminated infection were keratoconjunctivitis, fever, abdominal pain and respiratory symptoms (cough, thoracic pain).

In all these cases, microsporidia were isolated in various body fluids or tissues including urine, sputum, stools, conjunctival scraping, brain and kidney biopsy. Urine specimens seem to be the most contributive samples. Indeed, five patients had positive urine specimen. The last patient data were not provided because diagnosis was made post mortem on the lung biopsy.
In our patient, microsporidian spores were isolated from urines, sputum and renal biopsy, and visualized microscopically. Species identification was confirmed by specific PCR. Sequence analysis of the ITS region was used to establish the *E. cuniculi* strain type on the basis of the number of 5'-GTTT-3' repeats. Three types of strains had previously been identified by ITS sequence analysis (types I, II and III; also named “rabbit strain”, “mouse strain” and “dog strain”, respectively) (3). *E. cuniculi* genotype III had been isolated in 2 non-HIV immunocompromised patients (10, 12, 14). *E. cuniculi* type I had been detected in the iris tumor biopsy of one patient (6). Our patient was infected with a newly discovered genotype.

Identification of the infecting species of microsporidia is determinant for treatment choice. Albendazole has demonstrated activity against *E. cuniculi* in vitro (2) and *E. intestinalis* in vivo in patients with AIDS (11). Four out of 6 non-HIV immunocompromised patients with *E. cuniculi* infection have been treated with albendazole, associated with fumagillin eye drops in 3 patients with ocular infection (Table 1). Clinical improvement was seen in all treated patients but relapses occurred after treatment interruption in 2 patients: one patient died from cerebral *E. cuniculi* infection 4 months after treatment (10) and the other one experienced a relapse 1 year after (5). In our patient, parasite shedding in urine decreased but did not cease completely until 5 months and a half after the beginning of treatment.

Disseminated microsporidiosis must be considered in the differential diagnosis of chronic fever in renal allograft recipients even without urinary symptoms. Because of the broad range of infected sites and symptoms that microsporidia can cause in severely immunocompromised patients, the search for microsporidian spores in urine and not only stool specimens should be performed in cases of unexplained fever and
abdominal pain particularly if urines contain numerous cells and bacteriologic cultures remain sterile.


Figure legends.

Figure 1.
Patient's urine specimen showing reniform *Encephalitozoon* spores, as visualized by Uvitex 2B staining (x1000 magnification).

Figure 2.
Alignment of the ITS sequences of the 3 known *E. cuniculi* strain types with our patient's strain, which we propose to name type IV strain (GenBank accession Nr. HM045511).
**Table 1.** Characteristics of non-HIV-infected immunocompromised patients with *E. cuniculi* infection.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Graft / immunodepression</th>
<th>Clinical symptoms</th>
<th>Positive specimen for microsporidial spores</th>
<th>Method for species identification</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Clearance of spores</th>
<th>Strain type</th>
</tr>
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<tbody>
<tr>
<td>(10)</td>
<td>Canada 2002</td>
<td>Keratoconjunctivitis, fever, allograft tenderness</td>
<td>Urine, sputum, stools, conjunctival scraping, brain and kidney</td>
<td>TEM, IFA, PCR</td>
<td>Albendazole 800 mg/d 4 weeks and fumagillin eye drops</td>
<td>death</td>
<td>Relapse after clearance of spores</td>
<td>III</td>
</tr>
<tr>
<td>(5)</td>
<td>Mexico 2003</td>
<td>Fever, diarrhea, thoracic pain, ocular discomfort, abdominal pain</td>
<td>Urine, grafted kidney</td>
<td>TEM, IFA</td>
<td>Albendazole 400 mg/d 2 weeks and fumagillin eye drops</td>
<td>clinical improvement</td>
<td>Relapse after clearance of spores</td>
<td>NA</td>
</tr>
<tr>
<td>(9)</td>
<td>USA 2003</td>
<td>Bilateral keratoconjunctivitis, fever, graft tenderness</td>
<td>Urine, sputum, stools, conjunctival scraping, brain and kidney</td>
<td>TEM, PCR</td>
<td>none</td>
<td>death</td>
<td>no</td>
<td>NA</td>
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<tr>
<td>(12, 14)</td>
<td>USA 2004</td>
<td>Respiratory distress</td>
<td>Lung biopsy</td>
<td>TEM, PCR, DNA sequencing</td>
<td>none</td>
<td>death</td>
<td>no</td>
<td>III</td>
</tr>
<tr>
<td>Case</td>
<td>Country</td>
<td>Clinical Features</td>
<td>Imaging and Genetic Studies</td>
<td>Treatment</td>
<td>Duration</td>
<td>Outcome</td>
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<tr>
<td>(6)</td>
<td>Switzerland 2005</td>
<td>idiopathic CD4 lymphopenia iris tumor tumor biopsy, urine TEM, PCR</td>
<td>Albendazole 800 mg/d 4 weeks and fumagillin eye drops</td>
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<tr>
<td>This case</td>
<td>France 2008</td>
<td>Kidney / cyclosporine A and mycophenolate mofetil (replaced by azathioprine) fever, cough, abdominal pain urine, sputum, kidney biopsy PCR, DNA sequencing, IFA</td>
<td>Albendazole 800 mg/d 2 weeks and 400 mg/d 9 months</td>
<td></td>
<td>clinical improvement after 5 months and a half IV</td>
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</table>

TEM: transmission electron microscopy, IFA: indirect immunofluorescence assay, PCR: polymerase chain reaction, NA: not available
Figure 1. Talabani et al.
Figure 2. Talabani et al.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Strain I</td>
<td>TGTTGTTGTGGGATGGATGTTTGTTT--------------------GTGG</td>
</tr>
<tr>
<td>Strain II</td>
<td>TGTTGTTGTGGGATGGATGTTTGTTT--------------------GTGG</td>
</tr>
<tr>
<td>Strain III</td>
<td>TGTTGTTGTGGGATGGATGTTTGTTT--------------------GTGG</td>
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<tr>
<td>Our patient’s strain</td>
<td>TGTTGTTGTGGGATGGATGTTTGTTT--------------------GTGG</td>
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