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A R. Mohindra
*Henry Ford Health*

M. W. Lee
*Henry Ford Health*

G. Visvesvara

H. Moura

Ravi Parasuraman
*Henry Ford Health*

*See next page for additional authors*



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Case report

# Disseminated microsporidiosis in a renal transplant recipient

A.R. Mohindra
M.W. Lee
G. Visvesvara
H. Moura
R. Parasuraman
G.J. Leitch
L. Xiao
J. Yee
R. del Busto

**Key words:**
transplant; microsporidia; *Encephalitozoon cuniculi*; albendazole

**Abstract:** Disseminated microsporidiosis is diagnosed uncommonly in patients not infected with human immunodeficiency virus (HIV). We present a case of disseminated microsporidiosis in a renal transplant recipient who was seronegative for HIV. Chromotrope-based stains were positive for microsporidia in urine, stools, sputum, and conjunctival scrapings. Electron microscopy, immunofluorescence, polymerase chain reaction, and cultures of renal tissue identified the organism as *Encephalitozoon cuniculi*. The patient was treated with oral albendazole and topical fumagillin with clinical improvement. In addition, she underwent a transplant nephrectomy and immunosuppressive therapy was withdrawn. Follow-up samples were negative for microsporidia. However, the patient developed central nervous system manifestations and died. An autopsy brain tissue specimen demonstrated *E. cuniculi* by immunofluorescent staining. Disseminated microsporidiosis must be considered in the differential diagnosis of multiorgan involvement in renal allograft recipients.

The phylum microsporidia encompasses over 100 genera and 1000 species of protozoa, which are obligate intracellular, spore-forming organisms. Recently, microsporidia have gained an increasingly prominent role as opportunistic pathogens in the immunocompromised host. Enteric infections are common in patients infected with the human immunodeficiency virus (HIV), but they have also been reported in immunocompetent and immunocompromised hosts (1–3). Disseminated disease has also been appreciated in the HIV-positive patient (4–8), but it is extremely uncommon in the non-HIV-infected host (9). We present a case of disseminated microsporidiosis with *Encephalitozoon cuniculi* in an HIV-seronegative individual with end-stage renal disease following successful receipt of a renal allograft.

**Authors' affiliations:**

A.R. Mohindra[1],
M.W. Lee[2],
G. Visvesvara[3],
H. Moura[3],
R. Parasuraman[4],
G.J. Leitch[5],
L. Xiao[3],
J. Yee[4],
R. del Busto[6]

[1]Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan,

[2]Department of Pathology, Henry Ford Hospital, Detroit, Michigan,

[3]Division of Parasitic Diseases, Center for Disease Control and Prevention, Atlanta, Georgia,

[4]Division of Nephrology, Henry Ford Hospital, Detroit, Michigan,

[5]Department of Physiology, Morehouse School of Medicine, Atlanta, Georgia,

[6]Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan

**Correspondence to:**
Ramon del Busto
Division of Infectious Diseases
Henry Ford Hospital
CFP-1
2799 West Grand Blvd.
Detroit, MI 48202
Tel: (313) 916 2573
Fax: (313) 916 2993
e-mail: rbusto1@hfhs.org

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## Case report

A 45-year-old-female underwent a living-related donor kidney transplant in the Philippines before returning to her home in Canada. Her post-transplant course was complicated by two episodes of rejection that were treated with high-dose intravenous methylprednisolone and monoclonal anti-CD3 antibodies (muromonab-CD3). Eight weeks following engraftment, the patient developed an intermittent fever. A polymerase chain reaction (PCR)-based assay on blood for cytomegalovirus (CMV) was positive. She was treated for 2 weeks with intravenous ganciclovir; however, sustained defervescence did not occur. She was subsequently admitted for further evaluation and found to have bilateral keratoconjunctivitis, allograft tenderness, and an infiltrate on the chest radiograph.

Laboratory studies revealed the following: leukocyte count 6900 per $mm^3$ (89% polymorphonuclear cells); hemoglobin 8.6 g/dL; platelets 169,000 per $mm^3$; blood urea nitrogen level 57 mg/dL; and serum creatinine level 2.9 mg/dL. Cultures of blood revealed neither growth of bacteria nor fungi. A PCR-based assay on blood for CMV was negative. Histologic sections of percutaneous renal biopsy tissue revealed intact normal-appearing glomeruli, a moderate tubulointerstitial infiltrate, and coccoid-appearing organisms within the cytoplasm of renal tubules. Chromotrope stains of specimens obtained from urine, stool, sputum, and conjunctival scrapings were performed as described previously (10). All of these specimens were positive for microsporidia (Fig. 1).

A treatment regimen consisting of albendazole, 400 mg by mouth twice daily, plus fumagillin eye drops was initiated and resulted in clinical improvement. In addition, the patient underwent an uncomplicated transplant allograft nephrectomy and all immunosuppressive medications were withdrawn. Impression smears of the nephrectomy specimen stained with the Gram-chromotrope stain (11) exhibited dark violaceous spores that varied in size from 1.8 to 2.6 μm. In addition, a large number of spores demonstrated posterior vacuolation and a midline stripe, characteristic of a microsporidial species. An indirect immunofluorescent study (12) of kidney impression smears demonstrated positive reactivity with anti-*E. cuniculi* serum at a dilution of 1:800 (Fig. 2). Analyses by PCR, culture, and electron microscopy of the renal tissue were performed as previously described (7, 11–15). The PCR analysis demonstrated the presence of four GTTT repeats in the internal transcriber spacer, which is characteristic of genotype III of *E. cuniculi* (15). Cell cultures, inoculated by renal specimens, revealed foci of infected cells after 2 weeks of incubation. Smears of culture-derived spores stained dark violet on Gram-chromotrope staining and reacted positively with *E. cuniculi* antiserum at a titer of $> 4096$. When reacted with the calcofluor white reagent, the spores revealed the characteristic bluish white fluorescence (Fig. 3). Transmission electron microscopy revealed the presence of an unseptated parasitophorous vacuole (PV) with parasites at different stages of development and mature smooth-walled spores (Fig. 4). Meronts were consistently found attached to the PV membrane. The spores demonstrated approximately five polar tube coils, a thin electron dense exospore, a thick electron-lucent endospore, and a thin cell

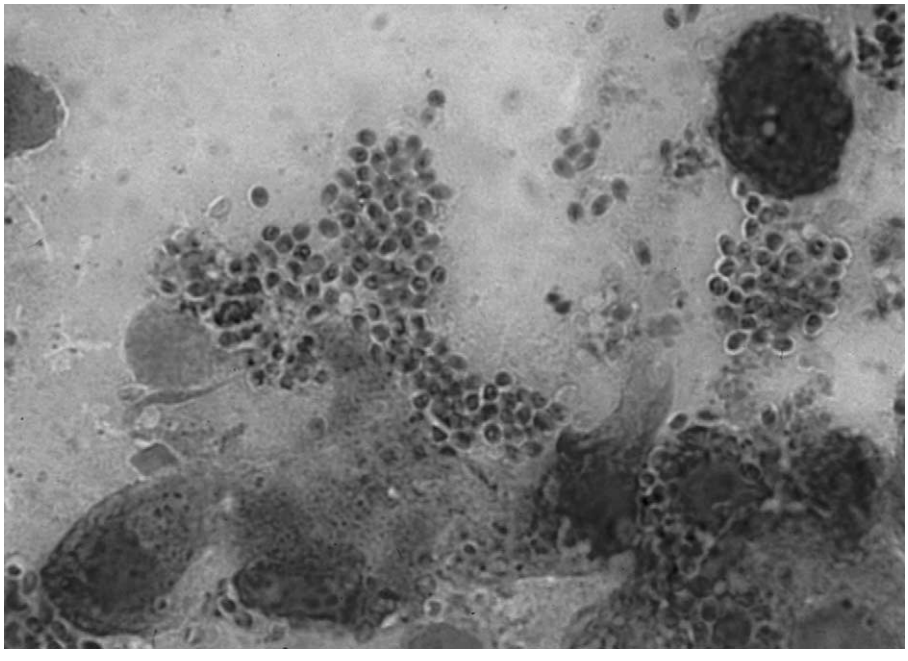

***Fig. 1.*** **Microsporidial spores from urinary sediment stained with the chromotrope technique. Magnification, × 400.**

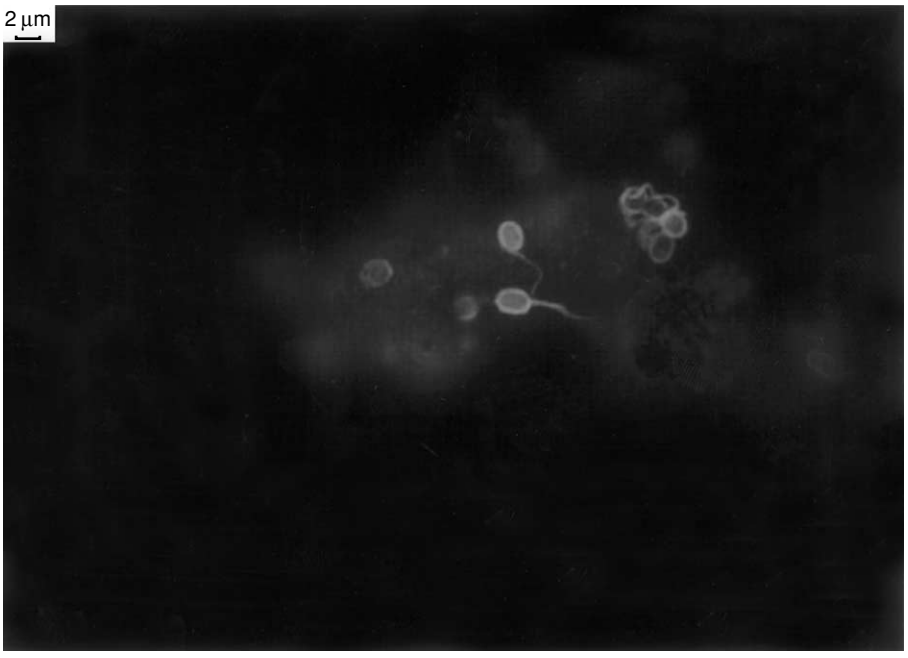


*Fig. 2.* **Impression smear of the nephrectomy specimen using immunofluorescence staining. Spore walls and extruded polar tubes are evident.**

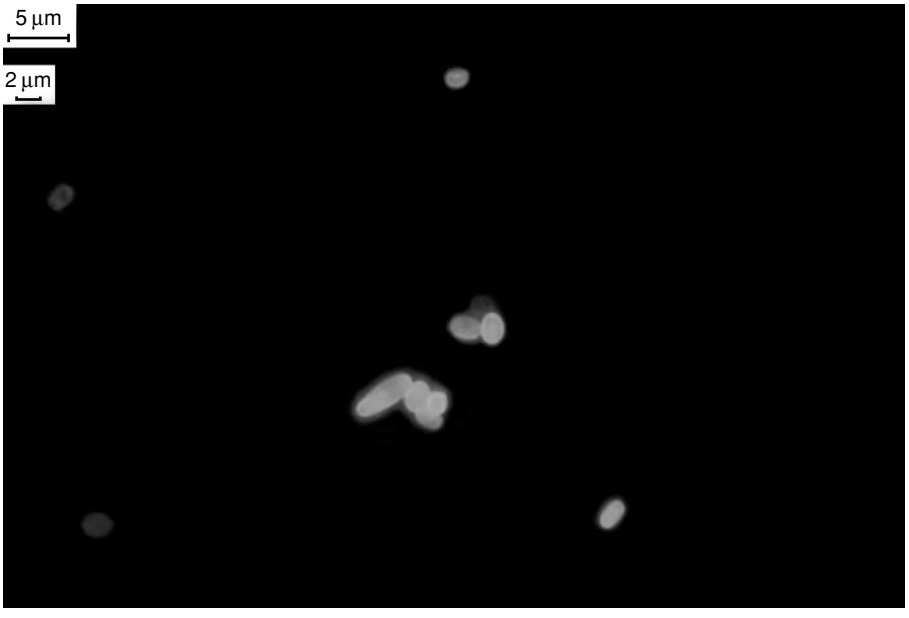


*Fig. 3.* **Smear of the culture supernatant stained with calcofluor white reagent.**

membrane surrounding the spore contents. All these features were consistent with the morphological identification of an *Encephalitozoon* species.

Treatment resulted in clinical improvement and subsequent evaluation of the urine, stool, sputum, and conjunctival scrapings using the chromotrope stain proved negative for microsporidia. Unfortunately, the patient developed thrombocytopenia and albendazole and fumagillin were discontinued after 4 weeks of therapy. During post-transplant week 24, the patient experienced a generalized seizure that led to a brain magnetic resonance imaging (MRI) revealing multiple, high-signal areas on T2 and diffusion images, with no significant mass effect. The post-gadolinium study demonstrated diffuse leptomeningeal enhancement. A brain biopsy

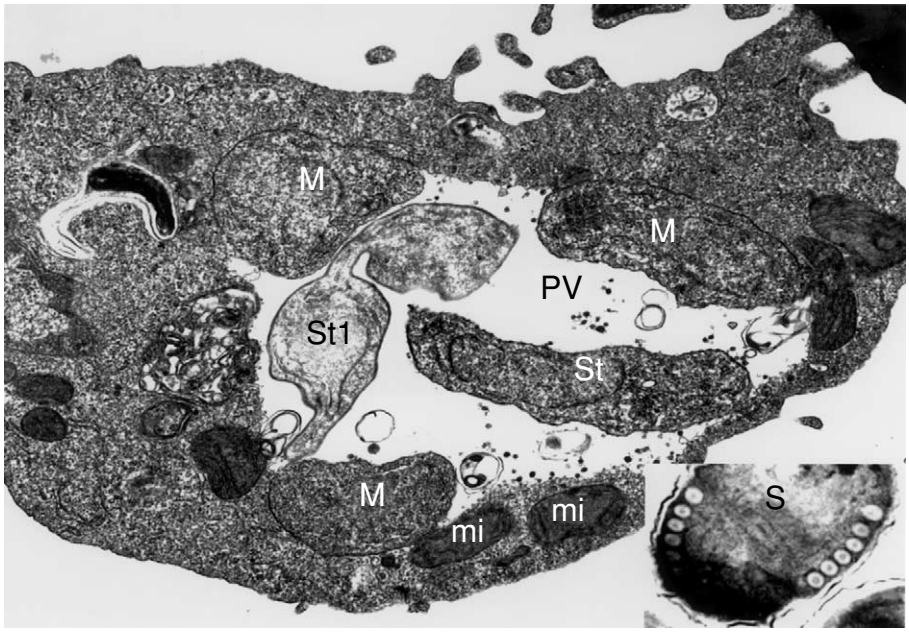


***Fig. 4.*** **Ultrastructure of *Encephalitozoon cuniculi* within an E6 monkey kidney cell demonstrating the parasitophorous vacuole (PV) containing meronts (M) and sporont (ST). Sporont (ST1) is undergoing cytokinesis. Note that the E6 cell mitochondria (mi) are closely associated with the parasite stages. *Inset*, a fully developed spore demonstrating the polar tube coils is shown.**

was attempted, but the procedure was aborted because of intraoperative hemorrhage. The patient expired 26 weeks after transplantation and an autopsy brain tissue specimen demonstrated *E. cuniculi* by immunofluorescent staining.

## Discussion

Intestinal microsporidiosis is a common infection of HIV-infected individuals and the vast majority of cases are caused by *Enterocytozoon bieneusi*. Multiorgan involvement with microsporidial species has also been reported. Sites affected have included the upper and lower respiratory tracts, the eye and its adnexae, genitourinary tract, hepatobiliary tract, musculoskeletal system, and the central nervous system (4–9). However, disseminated microsporidiosis has rarely been reported in patients who are not HIV-infected (9).

Among transplant recipients, only six cases of microsporidial infection have been reported in detail (16–21). Five patients were solid organ recipients who developed a chronic diarrheal illness (16–20) and a sixth case occurred in an individual who received an allogeneic bone marrow transplant with an infection limited to the lungs (21). None of these cases demonstrated multiorgan involvement. *E. cuniculi* has been identified in the kidney of an HIV-negative kidney-pancreas transplant recipient; however, no other clinical findings indicating systemic disease were reported (22).

In our patient, microsporidial dissemination was evident from samples of sputum, urine, stools, conjunctivae, brain, and kidney. Confirmatory studies using a variety of techniques identified the organism as *E. cuniculi*. In contrast to *E. bieneusi,* which usually induces infection limited to the gut and biliary tree, *E. cuniculi* frequently causes disseminated disease in HIV-infected individuals. Despite the presence of microsporidia in stool, our patient did not manifest diarrhea or other gastrointestinal manifestations. Notably, she incurred a prominent keratoconjunctivitis, a feature well appreciated as a presenting manifestation of disseminated *Encephalitozoon* infection (9). Factors that may have contributed to dissemination in our patient likely included the employment of high-dose glucocorticoid steroids and anti-CD3 antibodies during anti-rejection therapy. CMV infection may have further enhanced her susceptibility to the microsporidial infection.

The optimal therapy of microsporidiosis has not been clearly established. However, the benzimidazole derivative, albendazole, has been the most frequently utilized agent. Albendazole has demonstrated activity against microsporidia *in vitro* and is at least partially effective in HIV-infected patients afflicted by chronic sinusitis, lower respiratory infections, cerebral infections, urinary tract infections, myositis, and disseminated disease (5, 7, 9). Most of these patients have been infected by an *Encephalitozoon* species, particularly *E. cuniculi*, *E. hellem*, or *E. intestinalis*. A double-blind, placebo-controlled study of intestinal and extraintestinal infection attributable to *E. intestinalis* in eight HIV-infected subjects, demonstrated clinical benefit and clearance of the

parasite from stools of all patients treated with albendazole (23). However, the shedding of microsporidial spores in urine continued in five of the eight treated subjects. The therapy of *E. bieneusi* infestation by any agent has been less salutary. Although some AIDS patients with intestinal microsporidiosis have shown symptomatic improvement with albendazole, intestinal parasitosis persisted in all patients despite treatment (24). Other drugs associated with clinical improvement in small number of patients with diarrhea caused by *E. bieneusi* include thalidomide, furazolidone, and oral fumagillin (9).

Our patient was treated with albendazole, 400 mg twice a day for 4 weeks, plus fumagillin eye drops for keratoconjunctivitis. In addition, immunosuppression was abbreviated and eradication of microsporidium from urine, stool, sputum, and conjunctival scrapings was documented. Unfortunately, therapy was terminated due to the development of thrombocytopenia, a side effect common to both drugs. Despite the initial positive response to therapy, cerebral disease was proven at autopsy, with brain tissue harboring spores of *E. cuniculi*.

In conclusion, disseminated microsporidiosis, although rare, must be considered in the differential diagnosis of multiorgan infection in renal allograft recipients. Employing a systematic approach toward the diagnosis of microsporidia in non-HIV-infected patients, will, as we anticipate, increase the recognition of additional cases of this morbid and lethal illness. In addition, the presence of microsporidia may also reflect susceptibility to other intestinal parasites.

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