Concurrent Parasitic Infections in a Renal Transplant Patient

To the Editor: Protozoan pathogens, including *Entamoeba histolytica*, *Giardia*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, and microsporidia such as *Enterocytozoon bieneusi*, are well-known agents of diarrhea and a major public health problem in developing countries. Infection with *Cyclospora cayetanensis* and *E. bieneusi* can occur in immunocompromised and immunocompetent persons. Severe diarrhea and weight loss along with anorexia, nausea, and low-grade fever occur in immunocompromised persons, particularly those with HIV/AIDS and transplant recipients who are taking immunosuppressive drugs (*1*,*2*). However, transient diarrhea occurs in immunocompetent persons, notably in travelers returning from countries with poor hygienic standards (*1*–*3*).

We report on a kidney transplant recipient who had uncontrollable diarrhea and weight loss in whom *C. cayetanensis* and *E. bieneusi* were detected in biopsy specimens; the diarrhea resolved after treatment with drugs that act specifically on these 2 parasites. The patient was a 55-year-old man from the Dominican Republic living in New York, NY, USA; he had a history of long-term diabetes, coronary disease, and alcoholism. He had undergone a cadaveric renal transplant 14 months earlier and had an uneventful posttransplant course. After returning from visiting family in the Dominican Republic, he sought treatment for acute, profuse watery diarrhea in early November, 2009. He had >10 watery bowel movements daily that were associated with a 20-lb weight loss. His symptoms persisted for 2 months, and he required 2 hospitalizations for the diarrhea.

Results of 4 repeat fecal specimen tests (routine diagnostic microscopy and culture) were negative for parasites. Colonoscopy findings were normal; because of evidence of leukocytes in the feces and elevated fecal fat level, however, he received empirically prescribed metronidazole. Because his diarrhea and weight loss persisted, an upper endoscopy was performed, which revealed the presence of microsporidia. He then received albendazole for 3 weeks without substantial benefit.

The biopsy specimens were sent to the Centers for Disease Control and Prevention (Atlanta, GA, USA) for further analysis. Biopsy slides were stained with hematoxylin and eosin and with Gram chromotrope (*4*) and examined by microscopy. The Gram chromotrope–stained slide revealed oval spores, pinkish-red in color, measuring ≈1 μm (*5*). These spores were supra nuclear in position and were consistent with *E. bieneusi* (Figure, panel A). The tissue sections were scraped from the slides, DNA was extracted, and conventional PCR was performed by using *E. bieneusi*–specific primers as described (*5*); the sizes of the amplified product in the tissue DNA specimen and in the *E. bieneusi* control specimen were identical (Figure, panel B), confirming the presence of *E. bieneusi*. On further microscopic examination of the Gram chromotrope and the hematoxylin and eosin–stained slides, oval bodies (8–10 μm) were seen. A few of these oval bodies exhibited 4 spindle-shaped structures which were identified provisionally as merozoites of a coccidian parasite (Figure, panel C). Others had morula-like internal structure (Figure, panel D). We hypothesized that the coccidian parasite could either be *C. cayetanensis* or *Cystoisospora hominis*. Because the parasites, in various stages, were just beneath the surface of the epithelium, rather than deep within the

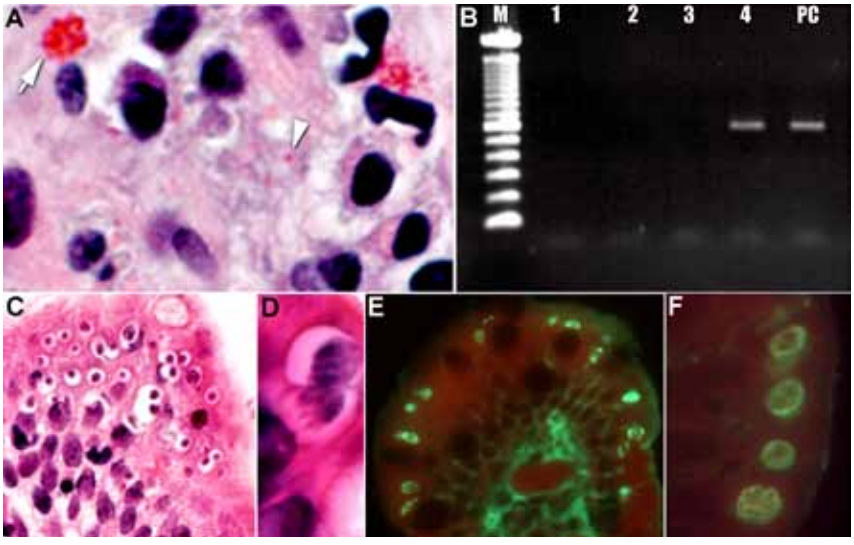

Figure. Tissue specimens from a kidney transplant recipient with concurrent parasitic infections after traveling to the Dominican Republic. A) Tissue section stained with Gram chromotrope. Note the apical location of a cluster of *Enterocytozoon bieneusi* spores at arrow (original magnification ×1,000) and single spore at arrowhead. B) Agarose gel showing PCR amplification of *E. bieneusi* 18S rDNA in the scraped section, as in panel A (M, 100-bp ladder; lane 1, DNA lysate diluted 1:5; lane 2, 1:10, lane 3, 1: 50 and lane 4, 1:100 of DNA lysate; lane 5 PC, positive control specimen). C) Tissue section stained with hematoxylin and eosin, demonstrating numerous sites in which *Cyclospora* spores are in developing stages (original magnification ×100). D) Higher power image of *Cyclospora* spores, showing the developing meronts (original magnification ×1,200). E) Immunofluorescent reactivity (dots in periphery) of the various life cycle stages of *Cyclospora* with a positive anti-*Cyclospora* serum sample (original magnification ×200). F) Note the bright fluorescence of the various parasite stages just below the apical (luminal) surface of the epithelial cells (original magnification ×1,000). A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/7/12-0926-F1.htm).

epithelium, we believed this organism to be a *Cyclospora* sp. rather than a *Cystoisospora* sp. We searched the serum bank of the Division of Parasitic Diseases, Centers for Disease Control and Prevention, and identified a serum sample from a person with a case of *C. cayetanensis* cyclosporiasis. An indirect immunofluorescence test was performed by using this serum on a deparaffinized section of the tissue biopsy specimen. Different stages of the coccidian organism were labeled brightly and produced apple-green fluorescence against a red counterstain (Eriochrome Black T), indicating that the parasite could possibly be a *Cyclospora* sp. (Figure, panels E, F). We considered that the *Cyclospora*-positive serum sample obtained from this particular patient may not be species-specific, since he might have also been infected with *Cystoisospora*. Therefore, we performed a real-time PCR assay that can distinguish *C. cayetanensis* from other coccidian parasites to identify the parasite definitively (*3*). DNA recovered from tissue in paraffin sections was successfully amplified and detected with this assay (data not shown), confirming the presence of *C. cayetanensis*.

The patient's illness was treated with albendazole for *E. bieneusi* infection and with trimethoprim and sulfamethoxazole for *C. cayetanensis* infection. The patient's diarrhea subsided after 1 week, and several subsequent fecal samples were negative for microsporidia spores and *Cyclospora* oocysts. His immunosuppressive medications were reduced, and he remained diarrhea-free for the following 3-year period of April 2010 to April 2013.

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Vaccinia Virus in Household Environment during Bovine Vaccinia Outbreak, Brazil

To the Editor: Several exanthematic vaccinia virus (VACV) outbreaks have affected dairy cattle and rural workers in Brazil and Asia, and have caused economic losses and affected health services (*1–3*). VACV, the prototype of the genus *Orthopoxvirus* (OPV), exhibits serologic cross-reactivity with other OPV species and was used during the smallpox eradication campaign (*1*). Several VACV strains have been isolated during bovine vaccinia outbreaks in Brazil and have been characterized by molecular and biologic methods (*3,4*). Bovine vaccinia infections in humans are frequently related to occupational contact with sick animals during milking but have never been shown to be associated with fomites or indoor environments (*1,3*).

In August 2011, a bovine vaccinia outbreak was reported in Carangola County, Minas Gerais State, Brazil. During this outbreak, several farms were affected, and the outbreak involved humans and dairy cattle. A 41-year-old man (patient 1) who worked on a farm (20°36′30.7″S, 42°17′53.9″W) was hospitalized. He had painful lesions on the hands, high fever, lymphadenopathy, malaise, and fainting episodes. This patient reported recent contact with sick animals on the farm during milking.

At the same time, a 57-year-old man (patient 2), the owner of the farm, had a lesion on the right hand. This infection was also related to occupational exposure. Some days after the appearance of the hand lesion, this patient presumably inoculated himself at the site of an abrasion he had recently received on his nose. This resulted in