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A Microsporidian Isolated from an AIDS Patient Corresponds to *Encephalitozoon cuniculi* III, Originally Isolated from Domestic Dogs

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The ribosomal DNA internal transcribed spacer (ITS) region of a recently cultured human *Encephalitozoon cuniculi* isolate was analyzed by gene amplification and DNA sequencing. Restriction endonuclease digestion (*Fok*I) and double-stranded DNA heteroduplex mobility shift analysis were performed to determine their utility for strain differentiation. The human *E. cuniculi* isolate was identical to *E. cuniculi* III, which had been isolated only from domestic dogs until now. The patient providing the isolate owned a pet dog, but no microsporidia were detected in the pet's urine.

Microsporidia are obligate intracellular protozoan parasites (phylum Microspora). There are over 1,000 species of microsporidia in approximately 100 genera that infect invertebrates and vertebrates (2). Before the AIDS epidemic developed, reports of human infections with microsporidia were rare, but in the last 10 years, microsporidia have been shown to cause opportunistic infections in immunodeficient individuals and particularly in persons with AIDS (18). At least 10 species of microsporidia have been shown to infect humans. These include *Enterocytozoon bieneusi*, *Encephalitozoon* (*Septata*) *intestinalis*, *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Vittaforma corneae*, *Nosema connori*, *Nosema ocularum*, *Trachipleistophora hominis*, and species in the genera *Pleistophora* and *Microsporidium* (2, 9, 18). Comparative analysis of the microsporidian small-subunit rRNA gene (rDNA) is being done in an effort to define the sources of human microsporidial infections. *E. cuniculi* has been shown to infect a number of mammalian hosts (12–15), and there exist at least three strains of this species, on the basis of the number of 5′-GTTT-3′ repeats present in the internal transcribed spacer region of the rDNA (6, 7, 10). Strain I (three GTTT repeats) has been isolated and cultured primarily from rabbits and from one mouse. To date, strain II (two GTTT repeats) has been isolated from mice only, and strain III (four GTTT repeats) has been isolated from two domestic dogs only. While *E. cuniculi* infections of humans have been reported on the basis of morphology, only recently have cases been documented by biochemical and molecular criteria (3, 7, 11). The purpose of this report is to identify the strain of *E. cuniculi* isolated from an AIDS patient described by De Groote et al. (3).

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E. cuniculi I (from a rabbit), II (from a mouse), and III (from a dog); *E. hellem*; and *E. intestinalis* were grown in RK-13 cells and isolated as previously described (4–6). The human *E. cuniculi* isolate (CDC:V282) was from an AIDS patient described by De Groote et al. (3). Microsporidian DNA was extracted and amplified by PCR as previously described (5, 6, 17) using the primers int530f (5′-TGCAGTTAA AATGTCCGTAGT-3′) and int580r (5′-TTTCACTCGCCGC TACTCAG-3′), which are known to amplify the rDNA internal transcribed spacer region of *Encephalitozoon* species. The PCR product is approximately 1,000 bp long, which includes a large portion of the small-subunit rDNA, the entire intergenic region, and a small portion of the large-subunit rDNA. These primers did not amplify rDNA from tissue culture-derived *V. corneae* (formerly named *Nosema corneum*) or intestinal biopsy-derived *Enterocytozoon bieneusi*. The PCR products were purified and sequenced directly by the femtomole sequencing system (Promega, Madison, Wis.) as described previously (5, 6, 17). The DNA sequence of the amplified rDNA internal transcribed spacer region of the human *E. cuniculi* isolate was identical to that of *E. cuniculi* III, which was first isolated from two domestic dogs. This sequence is 5′-GGT**GTTTGTTTGTTTGTTT**GTAGGTAGTTTTGTGTT GTTGT-3′.

To corroborate these findings, the PCR products were digested overnight with the restriction endonuclease *Fok*I (New England Biolabs, Beverly, Mass.) as described previously (5, 6, 17). The *Fok*I restriction digest patterns (Fig. 1) showed that the human *E. cuniculi* isolate (Ec_h) generated a PCR-restriction fragment length polymorphism pattern that most closely resembled that of *E. cuniculi* III. The *E. cuniculi* strains can be distinguished when comparing distances of the two digest products in the region of the 234- and 194-bp DNA markers. The digest fragments of the three *E. cuniculi* strains differ by only 4 or 8 bp, and *Fok*I was the only restriction enzyme that we found that generated a discernible difference between these *E. cuniculi* strains (6).

To demonstrate another method for distinguishing among the *Encephalitozoon* species and for differentiating between the

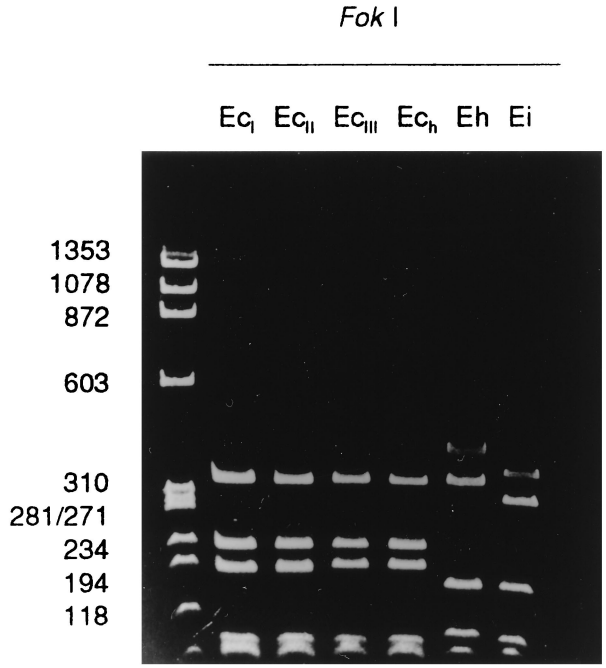

FIG. 1. PCR-restriction fragment length polymorphism of *E. cuniculi* I from a rabbit (Ec_I), *E. cuniculi* II from a mouse (Ec_{II}), *E. cuniculi* III from a domestic dog (Ec_{III}), the human isolate of *E. cuniculi* (Ec_h), *E. hellem* (Eh), and *E. intestinalis* (Ei). Ribosomal DNA was amplified by PCR from each microsporidian using the pan-*Encephalitozoon* primers, and the PCR products were digested with *Fok*I. DNA markers generated from ϕX174 digested with *Hae*III are shown in the first lane, and sizes are listed to the left in base pairs. Minor differences between the *E. cuniculi* strains, representing differences of 4 or 8 bp, can be discerned in the region between the 234- and 194-bp molecular markers.

strains of *E. cuniculi*, a slightly modified version of the double-stranded DNA heteroduplex mobility shift analysis method of Soto and Sukumar was performed as previously described (5, 6, 16). The first set of reactions (first panel in Fig. 2) shows that amplified rDNA of the human *E. cuniculi* isolate (Ec_h) differed from the rDNA PCR products of *E. hellem* (Eh) and *E. intestinalis* (Ei), as evidenced by the formation of heteroduplexes, as seen in the last two lanes. When amplified rDNA PCR products of the human *E. cuniculi* isolate were annealed with *E. cuniculi* I (Ec_I) or II (Ec_{II}), heteroduplexes also were generated, as shown in the second panel of Fig. 2, suggesting identity between these two organisms. No heteroduplexes were generated, however, if the amplified rDNA of the human *E. cuniculi* isolate was mixed with amplified rDNA from *E. cuniculi* III (Ec_{III}), shown in the last lane of Fig. 2. Controls were performed to demonstrate the fidelity of homoduplex formation and absence of heteroduplex formation by mixing the amplified rDNA products from two PCRs from the same microsporidian isolate (e.g., $Ec_I + Ec_I$). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (immunoblotting) of parasite proteins using murine polyclonal antisera against each *Encephalitozoon* species corroborated the results that the human *E. cuniculi* isolate appears to be identical to *E. cuniculi* III (data not shown).

Among the species of microsporidia presently known to infect humans, strain variations have been reported for *E. cuniculi* only. Ribosomal DNA sequences from numerous cultures of *E. hellem* isolated from humans in geographically diverse regions by Katiyar and coworkers and three isolated by Vossbrinck and coworkers were found to be identical to each other (10, 17). Hollister and colleagues, however, detected minor differences between their Wainwright isolate of *E. hellem* and the first *E. hellem* isolate based on Western blot and SDS-PAGE profiles (8), but the rDNA sequence data for this isolate have not been published. Three isolates of geographically diverse *E. intestinalis* were found to be identical to each other (5).

By defining the sources of pathogens, it might be possible to reduce exposure and prevent infections. Microsporidia are increasingly reported to cause opportunistic infections in AIDS patients, and in many cases, the sources of these infections are unknown. *Enterocytozoon bieneusi*, *E. intestinalis*, *E. hellem*, *N.*

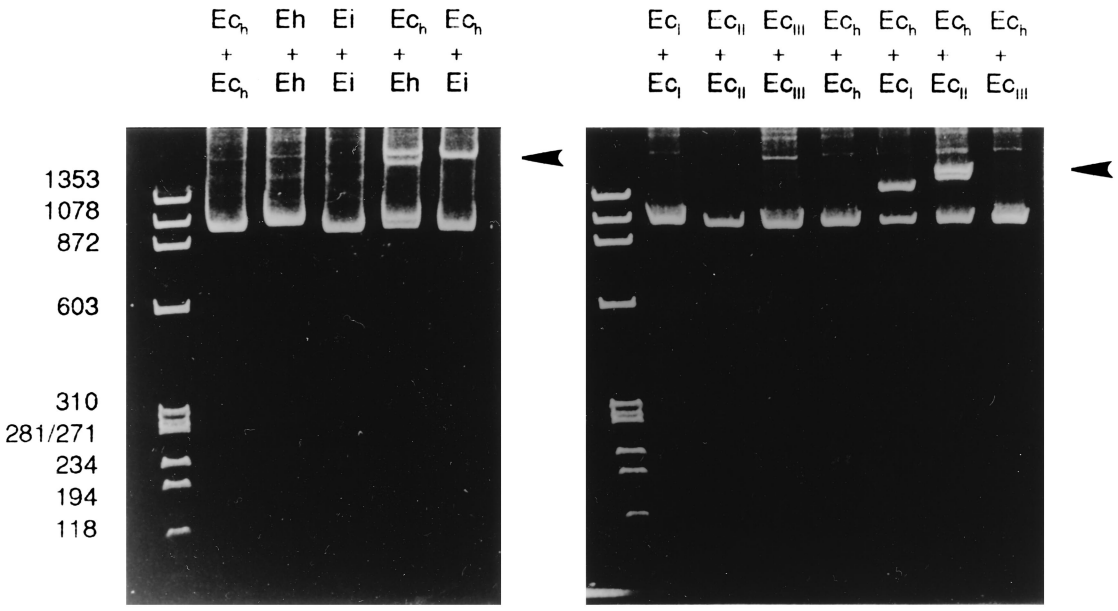

FIG. 2. Double-stranded DNA heteroduplex mobility shift analysis of microsporidia. Aliquots of PCR-amplified rDNA products were mixed as shown, denatured, and allowed to reanneal. Homoduplexes migrated to a position of approximately 1,000 bp. Heteroduplexes migrated more slowly (arrowheads) because of conformational changes in noncomplementary regions. DNA markers generated from ϕX174 digested with *Hae*III are shown in the first lane of each panel, and sizes are listed to the left in base pairs. See the legend to Fig. 1 for abbreviations.

ocularum, *T. hominis*, and *V. corneae* have been reported in humans only (18), although *E. hellem* was recently found to infect parakeets, as determined by Southern analysis (1). *Pleistophora* species typically infect fish, and the few case reports of human *Pleistophora* infections were in fishermen (18). *Nosema* species typically infect insects (18), but no direct proof exists that the human *Nosema* infections resulted from exposure to infected insects.

Too few isolates of *E. cuniculi* have been obtained and analyzed to assess host specificity or to determine if humans are at risk for becoming infected by exposure to dogs (or vice versa). The difference in the 5′-GTTT-3′ repeats among strains of *E. cuniculi*, however, provides a marker for monitoring the source and spread of these infections. These results suggest that additional *E. cuniculi* isolates or specimens need to be analyzed to help define the epidemiology of this microsporidian species and that pet dogs may need to be monitored in households with persons at risk for microsporidiosis.

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ADDENDUM

Since the submission of this paper, Deplazes et al. (3a) reported that *E. cuniculi* I, which was originally described in rabbits, has been found to infect humans with AIDS in Switzerland, further suggesting that *E. cuniculi* is a zoonotic parasite.

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