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Short communication

First case report of infection caused by *Encephalitozoon intestinalis* in a domestic cat and a patient with AIDS

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ABSTRACT

Microsporidia are eukaryotic, intracellular obligate parasites that infect invertebrate and vertebrate animals, and have emerged as important opportunistic parasites in AIDS patients. We used light microscopy to detect microsporidial spores in stool samples of a domestic cat confirmed as *Encephalitozoon intestinalis* by PCR, owned by an AIDS patient with chronic diarrhea and *E. intestinalis* infection. Cats can be considered hosts of *E. intestinalis*.

1. Introduction

Microsporidia are eukaryotic obligate intracellular spore-forming parasites. More than 1200 species of the phylum Microsporidia were classified into approximately 100 genera that were recently reclassified from protozoa to fungi (Snowden and Shadduck, 1999; Hirt et al., 1999; Weiss, 2003). Microsporidia are ubiquitous in nature, with infections being described in both invertebrate and vertebrate animals, including insects, fish, and mammals (Snowden and Shadduck, 1999).

Microsporidia have emerged as important opportunistic parasites in patients with human immunodeficiency virus (HIV) infection (Weber et al., 1994). The species *Encephalitozoon intestinalis* is the second most common microsporidia isolated from patients with acquired immunodeficiency syndrome (Weber et al., 1994). The clinical syndromes associated with *E. intestinalis* infection are diarrhea, malabsorption, cholangitis, cholecystitis, sinusitis, bronchitis, conjunctivitis, nephritis and intestinal perforation (Soule et al., 1997). This pathogen has also been identified in immunocompetent individuals (Van Gool et al., 1997 and Raynaud et al., 1998).

The first identification of *E. intestinalis* in mammals other than humans was reported in the feces of donkeys, dogs, pigs, cows and goats collected during an epidemiological survey in two rural villages in Mexico (Bornay-Llinares et al., 1998). Other authors described infections in swine (Valenčáková et al., 2006), in fecal samples of gorillas in Uganda (Graczyk et al., 2002) and in the brain of foxes in Ireland (Murphy et al., 2007). *E. intestinalis* has been detected in birds, namely in pigeons and in domestic geese (Haro et al., 2005; Slodkowicz-Kowalska et al., 2006).

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To our knowledge, reports of microsporidial infections in cats as a potential reservoir of human infection caused by *E intestinalis* were not found in the scientific literature.

In this study, PCR-based and microscopic methods were used to detect microsporidial spores in stool samples of a cat having close contact with an AIDS patient with chronic diarrhea and *E. intestinalis* infection.

2. Materials and methods

2.1. Sources and collection of specimens

This study included one adult HIV-infected individual with diagnosis of AIDS and chronic diarrhea. An upper gastrointestinal endoscopy with biopsy specimen collection and a stool analysis for parasites were performed. After confirmation of *E. intestinalis* infection in the patient, two stools samples were collected from the asymptomatic healthy cat (age = 5 years) representing his animal companion.

2.2. Light microscopy

For identification of microsporidia, fecal smears from the patient and the cat were stained with the Gram-chromotrope technique as previously described (Moura et al., 1996). At least 50 fields of each stained smear were examined by light microscope under oil immersion at a magnification of 1000×.

Biopsy specimens from the duodenum of the patient were fixed in formaldehyde, paraffin embedded and stained with Giemsa and hematoxilin–eosin (Garcia, 2002). Other biopsy specimens were placed in Karnovsky fixative, embedded in polybedaraldite and stained with Azur II (Garcia, 2002). A biopsy sample was also stored in saline solution at −20°.

2.3. PCR amplification and sequencing

DNA purification protocol from freeze biopsy specimens and feces was performed according to our previous report (Velásquez et al., 1996).

In order to determine the presence of *E. intestinalis*, the PCR protocol was carried out essentially as previously described (da Silva et al., 1997). The primers used were SINTF1 (5′-TTTCGAGTGTAAAGGAGTCGA-3′) and SINTR (5′-CCGTCCTCGTTCTCCTGCCCG-3′), corresponding to nucleotides 362-382 and 861-881 respectively of the small subunit rRNA (SSU-rRNA) gene of *E. intestinalis* (GenBank Accession number U09929). The reaction mixture was modified by employing 10× buffer containing $(NH4)_2SO_4$, and by the addition of 400 ng/μl of bovine serum albumin. Cycling conditions were as follows: an initial cycle at 80 °C for 5 min, 36 cycles with a denaturation step at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s, with a 9 min final extension at 72 °C.

Agarose gel electrophoresis and ethidium bromide staining were used to visualise amplification products under UV transillumination.

The amplicons obtained from samples of the patient and his cat were purified from 1% agarose gels with the centrifugal filter device Ultrafree®-DA (Millipore) and sequenced using a Hitachi 3130XL Genetic Analizer (Applied Biosystems) with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequence similarity was analyzed using the Blast program of the National Center for Biotechnology Information.

3. Results

3.1. Case report

A 51-year-old male was admitted with vomiting, diarrhea of 6 months duration (10–15 stools/day) and weight loss (8 kg). He was homosexual, HIV positive treated with Efavirenz, Zidovudine, Lamivudine with a history of hepatitis C and diagnosis of acute intermittent porphyria. He had a previous admission presenting symptoms including vomiting, diarrhea and diagnosis of cystoisosporosis. On physical examination, as positive data he showed dehydration, muscular hypotrophy and right upper quadrant pain. Routine blood studies were within normal limits, while alkaline phosphatase level was 290 U/l (normal, 40–129 U/l). The CD4 count was 119 cells/mm^3 and the viral load level of 260,980 copies/mm^3. On day 6 of hospitalization the stool test revealed *Cystoisospora* oocysts and clindamycin was indicated. On day 15 the patient persisted with diarrhea but with fewer stools despite treatment with clindamycin. On day 20 a new stool examination identified *Cystoisospora* oocysts and structures compatible with microsporidia spores. Albendazole was indicated. On the day 22 of admission an upper gastrointestinal endoscopy was performed showing jasper duodenum. Biopsies were taken from the most distal portion of the duodenum. On day 35, after 15 days of treatment with albendazole, the patient presented fewer bowel movements per day but with persistent diarrhea. On day 37 the patient was discharged.

3.2. Light microscopy

Spores of microsporidia were identified in fecal specimens collected from the patient and the cat. The spores had a positive Gram stain when they were stained with the Gram-chromotrope technique. The size was approximately 2.0 μm × 0.9 μm, with bacillary aspect and the presence of vacuoles or diagonal band in at least some spores (Fig. 1).

Different stages of *E. intestinalis* were observed in biopsy specimens of the duodenum from the patient. They developed within parasitophorous vacuoles in the cytoplasm of epithelial cells and lamina propria of the small intestine. The spores were round or oval, purplish when stained with hematoxilin–eosin, blue stained with Giemsa and also blue and surrounded by white vacuoles when stained with Azur II. Septa could be observed between the vacuoles that surrounded the different stages (Fig. 2).

3.3. PCR amplification and sequencing

A *E. intestinalis* SSU-rRNA region of 520 base pairs (bp) was amplified using the species-specific primers SINTF1/SINTR. Amplicons of the right size had been

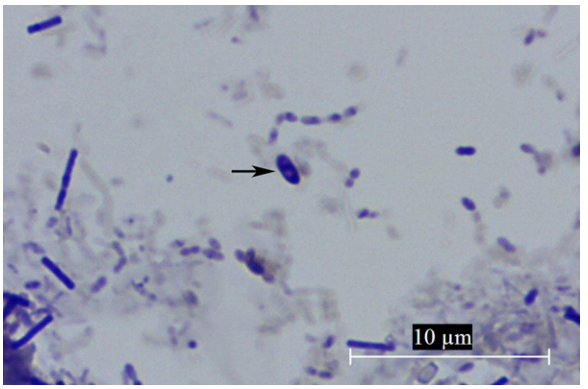

Fig. 1. Spore of Microsporidia in a stool sample of the domestic cat stained with Gram Chromotrope (magnification × 1000). The arrow indicates the organism.

obtained using as template DNA from frozen biopsy and stool specimens of the patient confirming the infection by *E. intestinalis*. When the DNA from feces of the domestic cat were analyzed by PCR, the amplification product of 520 bp was also obtained (Fig. 3).

The nucleotide sequences of the amplicons obtained from the biopsy and stool samples of the patient and the feces of the cat showed that all of them were 520 bp elements showing an identity of 100% with the region of the SSU-rRNA gene of *E. intestinalis* (GenBank Accession number U09929).

4. Discussion

In the present study, for the first time, both molecular and microscopic methods were used to detect microsporidial spores of *E. intestinalis* species in fecal samples of a healthy pet cat, as a potential reservoir of human infections. Several microsporidia species that are pathogenic for humans have been identified in domestic cats.

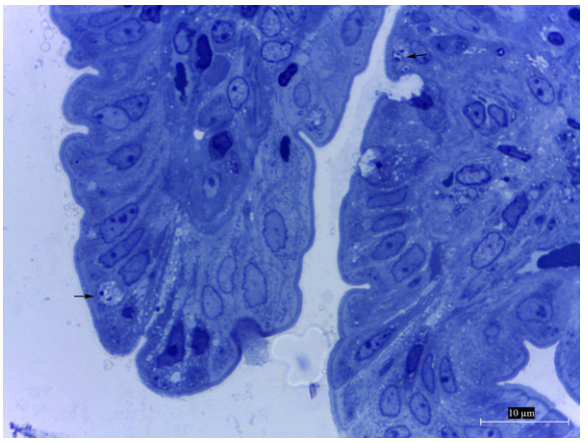

Fig. 2. Spores of Microsporidia in the epithelium of the duodenum from the AIDS patient. Light microscopy of an Azur II stained biopsy specimen (magnification × 1000). The arrows indicate the organisms in the parasitophorous vacuoles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

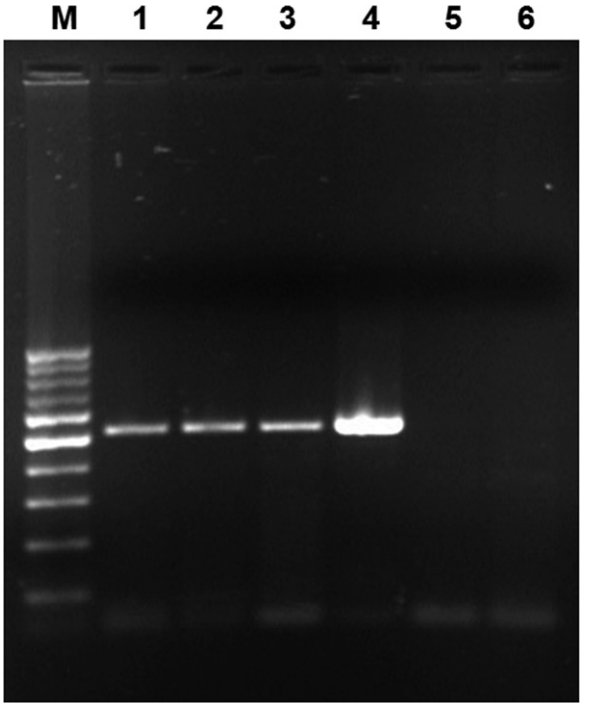

Fig. 3. Agarose gel electrophoresis of PCR products for the identification of *E. intestinalis*. Lane M, molecular size marker (100 bp ladder); lanes 1 and 2, DNA template of biopsy and stool specimens respectively from the patient; lane 3, DNA template from cat feces; lane 4, positive control corresponding to a plasmid containing the the SSU-rRNA gene of *E. intestinalis*; lane 5, human DNA used as negative control; and lane 6, reaction mixture.

Enterocytozoon bieneusi species was identified from feces in 1 of 12 cats using primers specifically designed in Switzerland (Mathis et al., 1999). In Portugal the presence of microsporidian spores in stool samples was determined in animals including cats and species identification was performed by PCR with species-specific primers for *E. bieneusi*, *E. intestinalis*, *Encephalitozoon cuniculi*, and *Encephalitozoon hellem* based in the SSU-rRNA gene followed by nucleotide sequencing. Isolates from cats were identified as *E. bieneusi* by PCR and confirmed by sequencing, while the other analyzed species were not detected (Matos et al., 2004). In another report, four cats were infected with the zoonotic genotype Type IV (synonym of PtEb III) and two cats were infected with genotypes PtEb IV and PtEb VIII in Portugal (Lobo et al., 2006). Genotype Type IV and genotype L were detected in 30 of 60 cats in Germany (Dengjel et al., 2001). In Colombia eight cats were infected with genotypes Type IV, Peru 10, WL11 (synonym of Peru 5) and genotype D-like (Santín et al., 2006). In Japan one cat was found infected with *E bieneusi* genotype IV (synonym of K) (Abe et al., 2009). Cats infected with genotypes Type IV (K, Peru2, BEB5, BEB5-var, CMITS1, PtEBIII) and WL11 (Peru5) were identified and these genotyped have also been reported in humans (Santín and Fayer, 2011).

Infections due to *E. cuniculi* species in cats were described. A case of generalized encephalitozoonosis and cerebellar hypoplasia in a young kitten was reported. Diagnosis was established by histopathological, immunohistochemical and molecular biological investigations

demonstrating characteristic lesions and DNA of *E. cuniculi* in tissue sections (Rebel-Bauder et al., 2011). In another report, a serological survey was conducted to determine the prevalence of IgG antibodies to spores of *E. cuniculi* in cats with and without a diagnosis of chronic kidney disease. Antibodies to *E. cuniculi* were found in 15 of the 232 analyzed samples, which included 4 of the 36 cats with chronic kidney disease (Hsu et al., 2010). *E. cuniculi* has three strains (I, II, and III also named "rabbit strain," "mouse strain," and "dog strain") with differences in their biology and epidemiology (Didier et al., 1995). *E. cuniculi* is not common in cats, but a potential role of domestic cats in transmission of the infectious agent cannot be excluded.

In our study we describe the *E. intestinalis* species infections in a cat and a patient with chronic diarrhea and AIDS using light microscopy in stools and duodenal specimens and by PCR-based methods. In contrast with *E. bieneusi* and *E. cuniculi* species that had different strain, *E. intestinalis* is a very homogenous species. No variation of the internal transcribed spacer of the rRNA sequences was detected in isolates of *E. intestinalis* (Didier et al., 1996; Liguory et al., 2000). We identified *E. intestinalis* species but we did not carried out additional studies in order to determine if the same strain was present in the cat and the patient.

Although we did not confirm the directionality of transmission between the cat and its owner, the identification of *E. intestinalis* spores in the cat allowed the recognition of a new host for this parasite. According to this, cats could be a possible source of *E. intestinalis* infection for humans, and this condition should be take in account in order to reduce and prevent human exposure, mainly in groups of people at risk of microsporidiosis.

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