CASE REPORT

A new and highly divergent Enterocytozoon bieneusi genotype isolated from a renal transplant recipient

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Abstract (50 words): A 49-year-old renal transplant recipient was admitted to our hospital due to abundant liquid diarrhea and dehydration. Parasitological investigations including genotyping, led to the diagnosis of intestinal microsporidiosis due to a new and highly divergent ITS genotype of *Enterocytozoon bieneusi*. The potential route of transmission through horse stools is discussed.

Keywords: Microsporidia, *Enterocytozoon bieneusi*, immunocompromised host, zoonotic transmission, ITS, transplantation, horses
CASE REPORT

A 49-year-old man who received a renal transplant in November 1998 was admitted in December 2005 to the renal transplant unit at Nice University Hospital (France) because of abundant liquid diarrhea and dehydration leading to acute renal failure. His past medical history included chronic renal failure secondary to a polycystic kidney disease. The follow up after kidney transplantation was without any undercurrent problem. At the time of diarrhea, he was given prednisolone (5 mg once daily). Stool samples were performed and send to the laboratory for microbiological investigations. Bacterial and viral investigations were negative. However, several structures consistent with microsporidia spores were found after microscopic examination of both Uvitex and modified trichrome stains. The patient was successfully treated with nitazoxanide (1000 mg twice a day for 60 consecutive days) and the clinical symptoms disappeared. A stool sample performed after nitazoxanide withdrawal was negative for microsporidia. Enterocytozoon bieneusi was identified by a species-specific real-time PCR assay as described previously (6). Briefly, DNA was extracted using the QIAamp DNA stool kit (QIAGEN, Courtaboeuf, France) according to the manufacturer’s instructions. This E. bieneusi specific real-time PCR assay targeting the small subunit rRNA (SSU rRNA) was applied using a Rotor Gene 3000 platform (Corbett Life Science, Mortlake, Sydney, Australia). Specific detection of E. bieneusi was performed using primers Eb1 (CGACAGCTGTTGAGAATAC) and Eb5 (CAACGAATGACTTGACCCTGTAA). Detection of the 180-bp product was ensured by use of the specific TaqMan probe EbS2 (TGCTTATACTCAACGCGGAAAA). To provide further insight into the transmission route (zoonotic or human-to-human transmission) of E. bieneusi in our patient, the internal transcribed spacer ribosomal RNA region (ITS rRNA) region was amplified and sequenced with the primer set MSP3 (GGGAATCAGCCCGGGGATCTG) and MSP4B (CCAAGCTTATGCTTAAAGTCGCGG) (6). PCR products were purified and sequencing...
was performed using a BigDye terminator sequencing kit on an ABI PrismR 3130 genetic analyzer (Applied Biosystems). Nucleotide sequences were analyzed using Seqscape software (Applied Biosystems). Surprisingly, comparison of the nucleotides sequences of this isolate to GenBank database using the Blast algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed only a weak similarity with previously reported *E. bieneusi* genotypes. The most similar genotypes (94% homology, 241 bp) were genotypes “Horse 2” and “KB-5” (GenBank accession numbers GQ406054 and JF681179 respectively) that have been recently described in horses and in captive baboons respectively (9, 14). Importantly, a phylogenetic analysis of the ITS rRNA nucleotide sequences of our *E. bieneusi* isolate (named “MAY1”), together with all ITS rRNA sequences previously reported from humans or human and animal hosts, confirmed the close genetic relationship between our isolate and these highly divergent genotypes (Fig. 1). Epidemiological investigation by questioning spouse and patient revealed that the week before the symptoms appeared the patient worked in his farm where he breeds horses. Indeed he had to clean a well in which water was contaminated with horse stool. The main hypothesis is that our patient probably gets contaminated during that episode.

ITS rRNA nucleotide sequences of our *E. bieneusi* isolate has been deposited in GenBank database under accession number JN595887.

Microsporidia are a diverse group of obligate intracellular parasites currently, classified as fungi (12). They infect a wide range of eukaryotic cells in numerous invertebrate and vertebrate hosts including humans, domestic and wild animals (12). *Enterocytozoon bieneusi* and the *Encephalitozoon* spp. are the major species infecting humans; *E. bieneusi* being the most prevalent (12, 15). Transmission mainly occurs through fecal-oral routes with sources of infection including other infected humans and animals, contaminated water and as illustrated recently food (1, 4). Microsporidia have emerged as an important cause of opportunistic
infection in patients with AIDS, being predominantly associated with wasting and diarrhea. However, recent studies have provided clear evidence that these infections are not restricted to AIDS and are also common in immunocompromised non-HIV-infected individuals as solid-organ transplant recipient (5, 7, 8). Moreover Sak et al. have recently provided new insights into the understanding of microsporidiosis highlighting a high prevalence of microsporidia in healthy subjects (10, 11). These findings suggest that the real distribution of microsporidiosis in humans is probably underestimated (1). Enterocytozoon bieneusi is probably the species in the genus with the most extensive genetic diversity (13). This genetic diversity of E. bieneusi relies on molecular methods; genetically distinct isolates having similar morphological characteristics (13). Presently, sequencing analysis of the ITS rRNA region is still considered as the gold standard for genotyping and epidemiological studies of E. bieneusi (13, 15). Until now, more than 90 E. bieneusi genotypes have been reported with new genotypes being regularly described (9, 13). It is now clear, that both host-adapted E. bieneusi genotypes with narrow host ranges and potentially zoonotic genotypes with wide host specificity have been identified (13). Analysis of nucleotide sequences of ITS rRNA provides valuable information about the transmission and pathogenic potential of E. bieneusi because it allows to determine the genotype in human and animal isolates. The most striking finding of our report is the discovery of a new and highly divergent E. bieneusi genotype in a human host. Indeed, near all the genotypes that have been shown to infect humans so far, belong to group 1 (3). To the best of our knowledge, human infections by E. bieneusi isolates that cluster outside the group 1 are exceptional and have been described in a single recent study performed in Central Africa (3). Taken together, E. bieneusi genotype “MAY1” and the one recently reported by Breton et al. in Gabon and Cameroon (genotype “CAF4”), are the only description of a highly divergent E. bieneusi genotype infecting a human host (3). Genotype “MAY1” groups together with the genotype
“Horse 2”, recently identified in an equid species (14). Interestingly, in our patient, symptoms began after contact with water contaminated with horse stool. Genetic similarity between our new genotype and the one described in an equid species, as well as the close contact with stool horses suggest a zoonotic transmission from horses to our patient. Unfortunately, stools from horses were not available for analysis.

In the present case, *E. bieneusi* infection appeared 7 years after transplantation. This finding is in agreement with previous data from literature with reported cases occurring from 19 days up to 15 years after kidney transplantation (7). At the time of diagnosis, the patient was given immunosuppressive therapy that was maintained while nitazoxanide was introduced. Both albendazole and fumagillin are the main drugs used to treat *E. bieneusi* infections (7). However, relapse is often observed after albendazole withdrawal and the efficacy of fumagillin is counterbalanced by its adverse effects, fumagillin exhibiting bone marrow toxicity leading to thrombocytopenia and neutropenia (1, 7). Here a complete recovery was obtained with nitazoxanide. This drug is not considered as the first-line therapy but has been already used with success in an HIV-infected patient (2).

In conclusion, we report a new and highly divergent genotype of *E. bieneusi* that is also the first description of an *E. bieneusi* genotype with close proximity with one recently described in horses (14). Whereas, the real prevalence of microsporidiosis in equid species has been poorly investigated yet, our data suggest that horses could act as potential sources of human microsporidia infections as suggested recently (14). These findings must be considered for the management of immunocompromised patients such as HIV-infected patients and solid-organ transplant recipients. In high-risk patients, giving advices such as avoiding close contact with animals and follow prophylactic measures will probably reduce the burden of this neglected disease.
REFERENCES


**FIG. 1.** Phylogenetic relationships among *Enterocytozoon bieneusi* genotype group 1 and all other genotypes reported and available in GenBank, as well as the nucleotide sequence identified in this study (MAY1), inferred by a neighbor-joining analysis of the ITS rRNA gene sequence, based on genetic distances calculated by the Kimura two-parameter model. Nucleotide sequence determined in this study (MAY1) is identified with a (●) before the
genotype name. Bootstrap values of less than 75% are not shown. The complete phylogenetic tree, including all *E. bieneusi* ITS genotypes published at the time of this manuscript is available upon request.