SHORT COMMUNICATION

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The first case of *Enterocytozoon bieneusi* infection in Poland

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Abstract

Microsporidia are intracellular parasites that cause opportunistic infections in humans of various immunological status. Only a few case reports exist on microsporidial infection in solid organ transplant recipients worldwide. The presented study demonstrates the first case in Poland of *Enterocytozoon bieneusi* infection in a liver transplant patient. Parasites were diagnosed in stool samples using both modified trichrome staining and PCR.

Key words

Microsporidia, *Enterocytozoon bieneusi*, transplant recipient.

INTRODUCTION

Microsporidia, small intracellular parasites, are recognized worldwide as a cause of opportunistic infections in humans of various immunological status. Risk groups for the development of clinical microsporidiosis include persons with AIDS, organ transplant recipients, contact lens wearers, travellers, children and the elderly. Two intestinal species – *Enterocytozoon bieneusi* (infecting enterocytes) and *Encephalitozoon intestinalis* (infecting enterocytes, macrophages and endothelial cells, resulting in disseminated infection) – are responsible for the majority of human microsporidiosis cases. The primary clinical course of infection with these species is chronic diarrhea, fever, nausea and weight loss [1, 2, 3]. The epidemiological status of microsporidiosis in Poland is poorly understood, and no data exist on intestinal microsporidia infections in patients after organ transplantation. In the presented study we report the first case of *E. bieneusi* infection confirmed by molecular methods in a liver-transplant recipient in Poland.

MATERIALS AND METHOD

Faecal samples (n=180) from 60 organ transplant patients were obtained from the Gastroenterology, Hepatology and Immunology Clinic of the Children's Memorial Health Institute in Warsaw, Poland, between February – May 2011. Abdominal symptoms were manifested only by 4 persons. The faecal samples were collected from all the patients on three occasions, and tested for the presence of 2 intestinal microsporidia, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. All samples were diagnosed using polymerase chain reaction (PCR). Prior to the DNA extraction, 3 samples from each patient were mixed together in equal proportions. Total DNA was extracted from 200 µl of homogenized stool using QIAmp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions. Primers SINTF (5'-TATGAGAAGTGAGTTTTTTTC-3') and SINTR (5'-CCGTCCTCGTTCTCCTGCCCG-3') were used to amplify the 545 bp DNA fragment of SSU rDNA of *E. intestinalis* [4]. Primers EBIEF1 (5'-GAAACTTGTCCACTCCTTACG-3') and EBIER1 (5'-CCATGCACCACTCCTGCCATT-3') were used to amplify the 607 bp fragment of SSU rDNA of *E. bieneusi* [5]. Two sets of controls, i.e., positive and negative, were incorporated in each PCR.

RESULTS

Positive PCR results were obtained for one person (1/60= 1.7%). The PCR product was subsequently sequenced in both directions using EBIEF1 and EBIER1 primers. The DNA sequence alignments and phylogenetic analysis were conducted using MEGA version 4.0. The isolate was closely related to *Enterocytozoon* sp. originally isolated from a dog (97.39%) and showed 100% identity with *E. bieneusi* isolate obtained from a man (Fig. 1). Additionally, microscopical observation revealed the presence of microsporidial spores in a faecal smear stained by Chromotrop-2R technique and collected from this patient.

The patient, a 15-year-old girl, received a liver transplant in January 2011. Immunosuppressive therapy consisted of tacrolimus (0.13 mg/kg/day) and mycophenolate mofetil (25.6 mg/kg/day). She had history of cystic fibrosis (CF), hyperglycemia and HCV infection. She also had billiary complications treated by non-surgical methods such as balloon dilatation and stinting. The patient did not show diarrhea or any other abdominal manifestations during the period of faecal sample collections.

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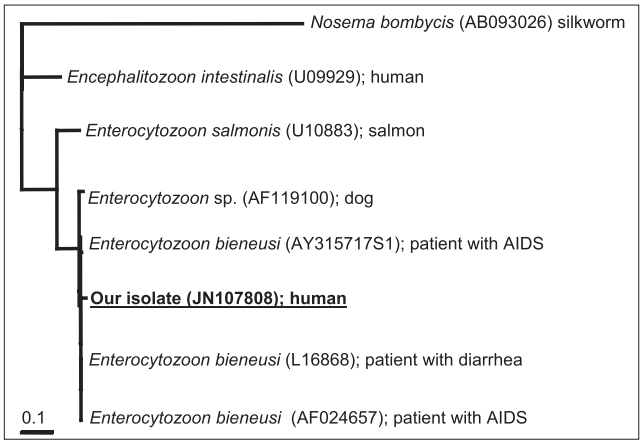

Figure 1. Phylogenetic tree of *Enterocytozoon* isolate and selected accessions from GenBank, based on the small subunit (SSU) rRNA gene fragment sequences. The phylogenetic tree was created using nucleotide sequence alignments utilizing the Kimura-2 parameter algorithm as a distance method, and neighbour-joining as the tree construction method. 0.1 on the scale bar indicates 1 nucleotide substitution per 100 sites. Sequence of *Nosema bombycis* was used as an out group.

DISCUSSION

To our knowledge, this is the first case of *E. bieneusi* infection confirmed by molecular methods in a liver-transplant patient in Poland. Only very limited information is available in Poland on human microsporidiosis, and to date only limited epidemiological investigations have been performed. In this preliminary study on the distribution of opportunistic parasites in immunocompetent and immunodeficient patients, 7 cases were detected of microsporidia infection in organ transplant recipient patients by microscopical observation of faecal smears using Chromotrop-2R technique [6]. However, these infections were not confirmed by molecular methods. In another study by Słodkowicz et. al. [7], 3 cases of microsporidiosis were identified in patients undergoing hemodialisis. By means of fluorescence *in situ* hybridization (FISH) technique, 2 cases of *E. cuniculi* infection were identified, and in one sample, co-infection of *E. cuniculi* and *E. intestinalis* was detected.

Additionally, the FISH technique was used in environmental studies on the prevalence of selected species of microsporidia in reservoir hosts and fresh food products. By means of multiplexed FISH, *E. bieneusi*, *E. hellem* and *E. intestinalis* spores were detected in samples originating from captive mammals housed in the zoological garden in Poznań, Poland, and in several species of birds in Poland [8, 9]. Spores of *Enterocytozoon* and *Encephalitozoon* were also detected in fresh berries, sprouts and green-leaved vegetables [10].

Based on a review of literature data, the reported cases of microsporidiosis in solid-organ-transplant and bone-marrow-transplant recipients are primarily associated with *E. bieneusi*. Infections with *E. intestinalis* and *E. cuniculi* are detected very rarely [11, 12].

CONCLUSION

Intestinal microsporidial infection can become a medical problem in solid-organ-transplant recipients, especially following long-term immunosuppressive therapy. For this reason, organ transplant recipients should be regarded as a risk group for infection with intestinal microsporidia and should be monitored for these parasites routinely, not only in cases of persistent diarrhea or other abdominal symptoms.

Nucleotide sequence Accession No. The isolate 259CZW was deposited in the GenBank, Accession No. JN107808.

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