SHORT COMMUNICATION

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First report of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* infection of wild mice in Slovakia

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Abstract

Increased risk of zoonotic transmission of the potential human pathogenic species *Enterocytozoon bieneusi, Encephalitozoon intestinalis* and *Encephalitozoon cuniculi* was detected in wild immunocompetent mice (*Mus musculus musculus*; n=280). Analysis was conducted with the use of PMP1/PMP2 primers and SYBR Green RT-PCR. Using Real Time PCR and comparing the sequences with sequences in the GenBank, *E. bieneusi* was detected in 3 samples (1.07 %), *E. cuniculi* in 1 sample (0.35 %) and *E. intestinalis* in 1 sample (0.35 %). The results of this report document the low host specificity of detected microsporidia species, and imply the importance of synanthropic rodents as a potential source of human microsporidial infection.

Key words

mice, microsporidia, *Enterocytozoon bieneusi, Encephalitozoon intestinalis, Encephalitozoon cuniculi,* zoonotic potencial

INTRODUCTION

Microsporidia are obligate intracellular parasites infecting all major animal groups. Transmission is by the faecal-oral route, where the sources of infection are infected humans, animals or contaminated water and food [1]. Fourteen microsporidial species are considered to be pathogenic for humans, with *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem* being the most frequent [2]. The main localization of these parasites are the intestine enterocytes and, therefore, the most common clinical manifestation of infection is diarrhea. These opportunistic pathogens are becoming more important due to the increasing number of patients with HIV infection/AIDS, as well as other patients with compromised immune systems. In recent decades, the serological positivity of microsporidial infection was detected in a large number of immunocompetent individuals; it is therefore possible that the prevalence of this infection is high not only in humans but also in animals [3, 4, 5].

These data suggest that human pathogenic microsporidia circulate in the environment and support the idea that they are zoonotic, and should be considered as a potential threat to public health. This pilot study examines the occurrence of microsporidia in wild mice in the Slovak Republic.

MATERIALS AND METHOD

Study population – samples. 280 faecal samples were used in this study which were collected from mice trapped in five places in Slovakia (Košice city and its surroundings). Samples were collected in the period from September 2012 – October 2013.

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Molecular analysis – DNA isolation. Genomic DNA was extracted from 100 mg stool samples using the DNA Sorb-B Nucleic acid Extraction kit (AmpliSence, Russia), according to the manufacturer's instructions. Before extraction, the stools were homogenized and disrupted oocysts centifruged at 6,500 rpm for 90 seconds with the addition of 0.5-mm-glass beads, 1.0-mm-zircon beads and 300 µl lysis solution in homogenizer Precellys 24 (Bertin Technologies). Purified DNA was stored at -20 °C until use in real-time SYBR Green PCR.

Real-time SYBR Green PCR. For real-time SYBR Green amplification the procedure of Malčeková et al. (2013) was used [6], with the use of specific primer pair PMP1/PMP2 with annealing temperature 60°C [7].

PCR products were directly sequenced in both directions. Sequences were aligned and completed using Chromas Pro Programme and compared to known sequences in the National Centre for Biotechnology Information GenBank database.

RESULTS AND DISCUSSION

Information on the occurrence of microsporidia in wild rodents are rare. The first findings of the occurrence of *E. cuniculi* in wild rats in Japan and England were published in year 1986 by Canning and Lom [8]. Since that time, *E. cuniculi* has been described in many cases as a parasite of laboratory rodents, such as mice, rats, hamsters and guinea pigs [9, 10]. In 2002, Muller-Doblies et al. [11] isolated the murine genotype of *E. cuniculi* from wild rats (*Rattus norvegicus*) caught in Zurich, Switzerland; this means that even at present the prevalence of *E. cuniculi* is much larger than the published records suggest.

In 2007, a study was published by authors from Atlanta, USA, about the occurrence and transmission of original genotype Peru16 of *E. bieneusi* isolated from guinea pigs raised in households in Peru, South Amarica. This was compared to the isolates from the stools of children who

kept these guinea pigs. It was proved that both genotypes were identical. This indicates transmissive transfer of this species [12]. Reports of the occurrence of human pathogenic microsporidia in wild rodents were recorded in 2011 by Sak et al. [5]. The authors examined 289 house mice from eastern Europe (*Mus musculus musculus*) and house mice from western Europe (*M. m. domesticus*) from 74 sites across the Czech-German border. Three species of microsporidia were found: in 23 – *E. hellem*, in 42 mice – *E. cuniculi* and in 25 – *E. bieneusi*. This was the first report of natural infection of *E. hellem* in mice.

In the presented study, 280 faecal samples collected from mice trapped in five sites in East Slovakia were used. *E. bieneusi* was detected in 3 samples (1.07 %; Accession No. FR 729098.1), *E. cuniculi* in 1 sample (0.35 %; Accession No. EU 847243.1) and *E. intestinalis* in 1 sample (0.35 %; Accession No. EU 436735.1). The results of this study document the first detection of *E. bieneusi* and *E. intestinalis* species in mice in Slovakia. It also shows a low host specificity of detected microsporidial species, and that synanthropic rodents can be a potential source of microsporidial infection for humans.

Compared with a study from the Czech Republic, the positivity rate was not that high, but this certainly does not diminish the importance of this pilot study focused on a narrow area of Slovakia, and the significance of the wild mice as a source of microsporidial infection,

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