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SHORT COMMUNICATION

Occurrence of *Cryptosporidium* spp. in red foxes and brown bear in the Slovak Republic

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Abstract Wild animals can be involved in epidemiology of many important diseases and often act as reservoirs of pathogens which cause disease in domestic animals and humans. This paper aims the role of red fox (*Vulpes vulpes*) and brown bear (*Ursus arctos*) in the circulation of coccidian parasites from the genus *Cryptosporidium*. Cryptosporidiosis is known as an important enteric pathogen, clinical symptoms in particular in immune-compromised individuals range from mild to severe diarrhoea and dehydration, which could be fatal. Fecal samples from 62 red foxes shot during September 2010 to February 2011 and 63 brown bears collected during June 2010 to March 2011 in central and eastern Slovakia were examined for the qualitative determination of *Cryptosporidium* spp. antigens in faeces by sandwich ELISA kit. Overall, 38.7% (24/62) of faecal samples of red foxes and 55.6% (35/63) of faecal samples of brown bear were positive. Our preliminary results emphasize prevalence of *Cryptosporidium* spp. amongst brown bears and red foxes in Slovakia and highlight the potential risk for transmission of cryptosporidiosis to humans using the countryside for professional or recreational purposes.

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Introduction

The role of wildlife as reservoir of infectious agents that cause disease in domestic stock, pet, and captive animals and humans is still not fully understood. Published studies indicate that *Cryptosporidium* spp. are unlikely to be a threat to human public health, since isolates from foxes have been shown to be *Cryptosporidium canis* dog genotype, *C. canis* fox genotype and *Cryptosporidium muskrat* genotype (Zhou et al. 2004), which are rarely etiological agents of human cryptosporidiosis. Anyway, foxes frequently share their habitat with cervid species that can harbour cryptosporidial infections that may be of risk to public health (Hamnes et al. 2006; Robertson et al. 2006). Since foxes may hunt and feed on cervid species, mainly as scavengers, it is possible that foxes, too, could harbour cryptosporidial oocysts and provide a further route for their dissemination.

In literature, there is only one case of cryptosporidiosis described in free-ranging cub of black bear from WV, USA. Cryptosporidial oocystes have been documented in two captive Malayan sun bears (*Helarctos malayanus*) located in zoological parks in Taiwan (Wang and Liew 1990). In Slovakia, there are approximately 700–900 bears that are located mainly in the northern and central part of the country (including the areas of the National Park Low Tatras and Poloniny). Since bears as well as foxes may feed or scavenge on various small rodents or larger prey, there is a possibility of harbouring the same infections as their prey does and thus maintaining the infection and shedding cryptosporidial oocysts.

The most common sources for human infections are contaminated drinking and recreational water, household animals and infected people (Dillingham et al. 2002). Wild animals are generally regarded as playing only secondary role in transmission of infectious agents to either farm animals, species kept in captivity or pets, although the role of free-living mammals is inconsiderable regarding the circulation in nature. Therefore we decided to aim this study to red fox (*Vulpes vulpes*) and brown bear (*Ursus arctos*), both species living in the area of the National Park Low Tatras and Poloniny.

Materials and methods

Fecal samples from 62 red foxes shot during September 2010 to February 2011 and 63 brown bear collected by volunteers of the Slovak Wildlife Society during June 2010 to March 2011 in central and eastern Slovakia were examined by an in vitro immunoassay for the qualitative determination of *Cryptosporidium* antigen in faeces by sandwich ELISA kit (Cryptosporidium (Fecal) Diagnostic Automation, Inc. Calabasas). It is a double antibody (sandwich) ELISA using an anti-*Cryptosporidium* antibody to capture the antigen from the stool supernatant. A second anti-*Cryptosporidium* antibody is then added which sandwiches the captured antigen. This reaction is visualized by the addition of an anti-second antibody conjugated to peroxidase and the chromogen tetramethylbenzidine.The resulting blue colour development indicates the presence of *Cryptosporidium* antigens being bound by the anti-*Cryptosporidium* antibodies.

The absorbance was evaluated dually at 450/630 nm (ELISA Reader Opsys MR Thermo Labsystems). Positive samples were those with the absorbance equal or higher than 0.15 OD units. The absorbance lower than 0.15 OD units indicated that the sample does not contain detectable level of cryptosporidial antigen. The faecal smears were also analysed by modified Kinyoun's acid-fast stain (Garcia and Bruckner 1997), and examined using light microscope (Olympus BX41) at ×400 magnification.

Results and discussion

We examined faecal samples of 63 brown bear and 62 red foxes by Cryptosporidium Antigen Detection ELISA. The number of positive samples were in 35 brown bear (55.6%) and 24 red foxes (38.7%), respectively. Faecal smears were also evaluated by modified Kinyoun's acid-fast stain, results showed lower prevalence: 20.96% (13/62) of infected red foxes and 26.98% (17/63) of positive brown bears. The results of both methods used were in 85% relative conformity, the most matched was by OD values in the range of 0.426–1.056. The higher prevalence of *Cryptosporidium* spp. in brown bears, detected by means of both, serologic and microscopic methods, is evincible due to large home range of dominant male adults (20×30 km^2 and more), and following possibility of coincidental sampling of several faecal samples from the same individual.

Since *Cryptosporidium* spp. oocysts can be routinely found in small rodents, which are commonly consumed by foxes, finding of cryptosporidial oocysts in foxes is not that surprising. For instance, Hamnes et al. (2007) found relatively low but widespread prevalence of *Cryptosporidium* in fox samples. His results confirmed previous findings that also showed low (<10%) prevalence. Zhou et al. (2004) found 7.9% (6/76) prevalence of *Cryptosporidium* spp. in red foxes in MD, USA and Sturdee et al. (1999) similarly found 8.7% (2/23) prevalence in Warwickshire, England. The question is whether the oocysts are merely passing through the digestive tract of foxes or the foxes are actually infected and subsequently excrete the cryptosporidial oocysts.

Zhou et al. (2004) described the infection in foxes by *C. canis* fox genotype, *C. canis* dog genotype and *C. muskrat* genotype, what proves that foxes can be infected by a dog genotype as well as by its own fox genotype. Since *C. canis* dog genotype has already been found in humans (Fayer et al. 2001; Xiao et al. 2001), it is very important to consider the possibility of zoonotic potential of foxes in suburban areas.

Although foxes and bears do not represent a significant risk as a source of *Cryptosporidium* spp. contamination affecting humans, they might play the role as reservoirs of cryptosporidiosis. Therefore, it is important to monitor the prevalence of cryptosporidial infection in wild animals that can possibly directly or indirectly contact humans.

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