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Seroprevalence of antibodies to *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* in humans and animals

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ABSTRACT

The presence of antibodies against *Encephalitozoon cuniculi* (*E. cuniculi*) and *Encephalitozoon intestinalis* (*E. intestinalis*) was examined in 215 samples from humans and in 488 samples from five different species of domestic and companion animals in Slovakia. The 215 human samples and samples from 90 swine, 123 non-infected cattle (cattle), 24 cattle infected with bovine leukosis virus (BLV-positive cattle), 140 sheep and 111 dogs were examined by the enzyme-linked immunosorbent assay (ELISA). Samples with serum titres 1:200 or higher were considered as positive. Specific anti-*E. cuniculi* antibodies were found in humans (0.9%), swine (52%), cattle (2%), sheep (9%) and dogs (15%) except for the BLV-positive cattle at the titre of 1:200. The titre of 1:400 was detected only in humans (0.5%). The presence of specific anti-*E. intestinalis* antibodies at the titre of 1:200 was confirmed in humans (6%), swine (51%), cattle (11%), BLV-positive cattle (13%) and dogs (6%) but not in sheep. The anti-*E. intestinalis* antibodies reached the 1:400 in humans (1%), swine (4%) and BLV-positive cattle (17%). The presence of specific anti-*E. intestinalis* antibodies at the titre of 1:600 was observed only in one swine (1%). Significant differences were observed in animals at titres 1:200 and 1:400 (chi-squared test: $p < 0.0001$) for both pathogens and in humans only for *E. cuniculi* at the titre of 1:400 (chi-squared test: $p < 0.0075$).

1. Introduction

Microsporidia are obligate intracellular parasites that were recently reclassified from protozoa to fungi (Keeling and Doolittle, 1996; Keeling et al., 2000; Van de Peer et al., 2000; Keeling, 2003). Microsporidia are considered to be the cause of emerging infections in a wide range of animals and also in humans (Halánová et al., 2003; Bálent et al., 2004; Mathis et al., 2005; Valenčáková et al., 2006; Valenčáková et al., 2008; Santaniello et al., 2009).

The species *Encephalitozoon cuniculi* (*E. cuniculi*) and *Encephalitozoon intestinalis* (*E. intestinalis*) are the most spread microsporidian species in animals and humans and cause disease called encephalitozoonosis. In 1995, the zoonotic character of *Encephalitozoon* spp. was confirmed and microsporidia became the subject of considerable interest of both veterinary and human medicine (De Grotte et al., 1995). Long-lasting subclinical infections usually develop in immunocompetent adult hosts infected with microsporidia, while in immunodeficient or immunosuppressed hosts, such as patients with an acquired immunodeficiency syndrome (AIDS) or those with organ transplants, clinically significant and potentially lethal infections may occur. Acute and frequently lethal infections generally develop in hosts infected in the transplacental way (Valenčáková et al., 2003). Microsporidian spores appear to be relatively resistant under environmental conditions and species of microsporidia capable of infecting humans and animals have been identified in water sources. There is an increasing concern about water-borne transmission among farm animals (Didier et al., 2004).

Serological methods for the detection of microsporidia-specific antibodies include immunofluorescent antibody staining, complement fixation, enzyme-linked immunosorbent assay (ELISA) and western immunoblot assays (Garcia, 2002). These tests require the use of microsporidia antigens and therefore microsporidial antigens that could be grown in cell cultures were cultivated for the detection of antibodies in microsporidia species. Serological testing is the method commonly used for diagnosing microsporidial infections.

The aim of the present study was to evaluate the prevalence of specific anti-*E. cuniculi* and anti-*E. intestinalis* antibodies in randomly selected humans and animals.

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2. Material and methods

2.1. Humans

Altogether 215 immunosuppressed patients with various diagnoses were examined for the presence of anti-microsporidial antibodies. The patients were divided into three groups: group 1 – patients with bacterial infections (20), group 2 – patients with viral infections (123) and group 3 – gynaecological patients (72).

2.2. Animals

Serum samples were collected from 488 animals in Slovakia: 90 swine, 123 cattle, 24 cattle infected with bovine leukosis virus (BLV-positive cattle), 140 sheep and 111 dogs. They were examined for the presence of anti-microsporidial antibodies. All animals were apparently healthy.

2.3. E. cuniculi and E. intestinalis antigen

Mature spores of microsporidia grown *in vitro* in VERO E6 cells (green monkey kidney cells) were used as an antigen in serological tests (Valenčáková et al., 2002).

2.4. The enzyme-linked immunosorbent assay (ELISA)

Animal sera were examined by the modified ELISA described by Hollister and Canning (1987). The samples were diluted from 1:200 to 1:600. Anti-species immunoglobulin G labelled with horseradish peroxidase conjugate (SwARb/Px; Sigma, Germany) was used as the secondary antibody. Absorbance of samples was measured using a spectrophotometer Dynex Technologies 4.24 at the wavelength of 490 nm. The serum with absorbance at least 2.1-fold higher than that of the negative control serum was considered positive.

2.5. Statistical analysis

Statistical significance was determined using a GraphPad Prism test.

3. Results

In our study, we collected 215 samples of human sera and 488 samples of sera from different species of animals and examined them by ELISA for the presence of antibodies against *E. cuniculi* and *E. intestinalis*. The samples that gave a titre of 1:200 and higher were considered positive.

3.1. E. cuniculi

Two of 215 patients were positive at the titre of 1:200 (0.9%) and one at the titre of 1:400 (0.5%). Only one patient with bacterial infection was positive at the titre of 1:400 (5%) and two patients with viral infection at the titre of 1:200 (2%; Table 1).

Of 488 examined animals 79 were positive at the titre of 1:200 (16%). The prevalence of anti-*E. cuniculi* antibodies was the highest in swine (52%), namely 47 of 90 examined animals were positive at the titre of 1:200. Examination of dogs showed positivity in 17 of 111 animals (15%). We found 9% positivity in sheep, i.e. 12 of 140 sheep had anti-*E. cuniculi* antibodies. The prevalence of antibodies was the lowest in cattle (2%) as only three of 123 animals were proven positive at the titre of 1:200. No anti-*E. cuniculi* antibodies were detected in BLV-positive cattle (Table 2). None of the examined animals reacted positively to *E. cuniculi* at titres 1:400 or 1:600.

Table 1
Result of ELISA presenting antibodies against *E. cuniculi* and *E. intestinalis* in humans for the titres 1:200 and 1:400. The patients were divided into three groups: group 1 – patients with bacterial infections (20), group 2 – patients with virus infections (123) and group 3 – gynaecological patients (72).[a]

	Encephalitozoon cuniculi								*Encephalitozoon intestinalis*							
	Number of people	Positive 1:200		Negative 1:200		Positive 1:400		Negative 1:400	Number of people	Positive 1:200		Negative 1:200		Positive 1:400		Negative 1:400	
		n	%	*n*	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%
Group 1	20	–	–	20	100	1	5*	19	95	20	–	–	20	100	–	–	20	100
Group 2	123	2	2	121	98	–	–	123	100	123	7	6	116	94	3	2	120	98
Group 3	72	–	–	72	100	–	–	72	100	72	5	7	67	93	–	–	72	100
Total	215	2	0.9	213	99.1	1	0.5	214	99.5	215	12	6	203	94	3	1	212	99

[a] At the titre 1:600 all the samples were negative and omitted from this table.
* $p < 0.0075$.