

**SEROLOGICAL SCREENING OF OCCURRENCE OF ANTIBODIES TO
ENCEPHALITOOZON CUNICULI IN HUMANS AND ANIMALS IN EASTERN
SLOVAKIA**

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Abstract: *Encephalitozoon cuniculi* is one of the mammalian microsporidian pathogens that can affect a number of different species of animals as well as humans. The presence of specific serum antibodies to *Encephalitozoon cuniculi* was studied in a group of animals and humans from Eastern Slovakia by the indirect immunofluorescence of antibodies (IFA). 456 people, 571 rabbits, 457 mice, 193 dogs, 72 cats, and 59 sheep were examined. Specific anti-*E. cuniculi* antibodies were found in 26 out of 456 human sera examined (5.7%). The highest occurrence of antimicrosporidial antibodies was found in the group of immunodeficiency patients – 37.5%. In the group of animals, the highest positivity was observed in rabbits – 41.7%, and in dogs – 37.8. The relative high prevalence, especially in rabbits and dogs as potential sources of microsporidial infection for humans, indicates the importance of performing the screening examinations in animals with aim of reducing or halting the spread of this disease.

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INTRODUCTION

Microsporidia are ubiquitous obligate intracellular spore-forming, small pathogens that have caused significant agricultural losses and interference with biomedical research. To date, more than 1200 species belonging to 143 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts, including man [24]. Microsporidia are characterised by the production of resistant spores that vary in size, depending on the species.

The first mammalian microsporidial infection was reported in 1922 by Wright and Craighead in rabbits [25]. In humans, the first case was described by Magarinos Torres in 1927 [19], but generally microsporidia were not routinely diagnosed. Their importance has increased only in the past two decades, in addition to the consequence of its more frequent detection in the relatively healthy populations of the susceptible hosts, as well as up to the beginning of the human immunodeficiency virus (HIV) pandemic, because clinically manifested forms of this

disease occurs only under the influence of different immunosuppressive factors [17].

Natural ways of infection transmission are not known for certain. It is believed that the disease is spread horizontally in breeding with larger concentrations of animals by the oro-fecal route, but above all, along the oro-urinal pathway [21]. Vertical, transplacental transmission of infection may also play a key role in the epidemiology and pathogenesis, especially in carnivores and rodents [10, 14]. In humans, the fact that some microsporidian species as *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi* and *Encephalitozoon hellem* have a zoonotic character, is very important for spreading this microorganisms among the human population.

The aim of this work was to study the occurrence of the specific antimicrosporidial (anti-*Encephalitozoon cuniculi*) antibodies in humans and also in animals as the potential sources of microsporidial infection in Eastern Slovakia.

MATERIALS AND METHODS

456 people were examined for the presence of antibodies against *Encephalitozoon cuniculi*. The groups of examined people were selected as follows: 24 immunodeficiency patients, 57 gynaecological patients, 4 HIV positive patients, 9 soldiers - whipper-ins, 22 dog breeders, 92 forestry workers, 98 employees of a slaughterhouse, and 150 blood donors. All the examined people living in Eastern Slovakia.

For the presence of antimicrosporidial antibodies there were examined: 571 rabbits, 457 mice, 193 dogs, 72 cats, and 59 sheep, also from Eastern Slovakia.

As antigen, the spores of *Encephalitozoon cuniculi* were used. The spores were grown in E6 cells (Vero green monkey kidney cell), cultivated in modified RPMI 1640 medium supplemented with 5% foetal calf serum and an addition of antibiotics and antimycotics. After the bursting of the infected cells, mature spores were released into the media. The organisms were collected from culture supernatants after centrifugation at $450 \times g$ for 20 minutes. Sediment was re-suspended in non-buffered Percoll (density 1.30 g/ml, pH 8.8) and following centrifugation at $750 \times g$ for 20 minutes, separated from the spores. Subsequently, the organisms were processed according to Koudela *et al.* (1993) [11].

The indirect immunofluorescence of antibodies (IFA) was used as the diagnostic method to determine specific anti-*Encephalitozoon cuniculi* antibodies. The method was performed according to Chalupský *et al.* (1973) [1]. A fresh suspension of *E. cuniculi* from the tissue culture was placed in each well of a slide. The slides were air-dried for 24 h, then fixed in absolute acetone for 15 min and again air-dried. Sera were serially diluted, beginning at 1:16 and ending at 1:1024. Each of the wells on the slide was covered with 10 μ l diluted serum, and slides incubated for 30 min at 37°C in a moist chamber. The slides were then washed twice in distilled water and PBS at 10 min intervals. Following air-drying, the wells were

Table 1. Serological positivity to microsporidian *Encephalitozoon cuniculi* in human.

Group	Number of examined	Positive	
		N	%
Immunodeficiency patients	24	9	35.7
Gynaecological patients	57	10	17.5
HIV positive patients	4	-	-
Soldiers - whipper-ins	9	2	22.2
Dog breeders	22	-	-
Forestry workers	92	-	-
Employees of slaughterhouse	98	5	5.1
Blood donors	150	-	-
Total	456	26	5.7

Table 2. Serological positivity to microsporidian *Encephalitozoon cuniculi* in animals.

Species	Number of examined	Positive	
		N	%
Rabbits	571	238	41.7
Mice	457	73	16
Dogs	193	73	37.8
Cats	72	17	23.6
Sheep	59	8	13.6
Total	1352	409	30.25

covered with 10 μ l of rabbit anti-human (or anti-rabbit, anti-mice, anti-dog, anti-cat, anti-sheep) immunoglobulin fluorescein isothiocyanate conjugate (SIGMA, USA) of 1:160 dilution. After 30 min at 37°C the slides were washed and air-dried. Then they were counter stained with Evans blue and coverslips mounted with buffered glycerine.

RESULTS

In the case of a positive immunological reaction to the presence of antibodies against *E. cuniculi*, the spores were shown as oval, fluorescent formations of $1.5 \mu\text{m} \times 2.5 \mu\text{m}$. The humans and animals whose sera reacted at the titre 1:64 and higher were considered positive.

Of the total number of 456 human sera examined, 26 were positive, which represented 5.7%. The highest occurrence (%) of anti-*E. cuniculi* antibodies was found in the group of immunodeficiency patients - 9 positive (37.5%). Positive specific antibodies were found in 17.5% gynaecological patients (10 positive), 5.1% employees of a slaughterhouse (5 positive), and 22.2% soldiers - whipper-ins (2 positive) also. No positive titres were observed in the group of dog breeders, forestry workers, HIV positive patients and blood donors (Tab. 1).

In the group of animals, the highest positivity was observed in rabbits - 238 positive (41.7%) and in dogs - 73 positive (37.8%). In the group of the cats, 23.6% positivity (17 positive), in mice 16% (73 positive), and in sheep 13.6% (8 positive) were observed (Tab.2).

DISCUSSIONS

One from the most frequent microsporidia is *Encephalitozoon cuniculi*, which infects a wide range of mammalian hosts, including humans [6, 22]. In Slovakia, animal encephalitozoonosis was first described in farmed [9] and laboratory rabbits [12] using IFA as diagnostic method. Later, this disease was reported in mice [7], dogs [18], goats [3], cows [8], and also in mouflons and fallow deer [20].

In humans, *E. cuniculi* is responsible for various pathologies, affecting the nervous system as well as the respiratory and digestive tracts, and can also cause infection of the kidneys, lymph nodes and adrenal glands [22, 23]. The portal of entry of this pathogen remains unknown. The faecal – oral route of infection (enteric infection), inoculation of spores into abrasions (ocular infection) or inhalation of spores may all play a part in the infective process [2].

In our study, 1352 serum samples coming from different species of animals, as potential sources of infection for humans, were examined for the presence of antibodies against *E. cuniculi*. The highest positivity was observed in the group of rabbits and dogs. This is very significant from the epidemiological aspect, because *E. cuniculi* is commonly found in animals and antibodies to this organism have been found in surveys of the human population. In 1995, three strains *Encephalitozoon cuniculi* were identified, but only two are human isolates. Strain I was originally isolated from rabbits [15] and also described in humans [4], and strain III originally isolated from dogs [16] was also identified in AIDS patients [6]. Strain II was isolated from mice [5], and also from blue foxes [13], but has not been identified so far in humans. Accumulating evidence indicates that human infection with *Encephalitozoon cuniculi* results from animal sources. Also, our serological surveys in human population suggest that the number of positive a specific anti-*E. cuniculi* antibodies are diagnosed in groups of people with immunodeficiency and people working with animals or animal products.

In conclusion, it can be stated that a relatively high prevalence, especially in rabbits and dogs as potential sources of microsporidial (*E. cuniculi*) infection for people, indicates the importance of performing screening examinations of animals with the aim of reducing or halting of the spread of this disease.

Further studies are needed to isolate and compare strains isolated from different hosts coming from this area.

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