ENCEPHALITOZOOON CUNICULI INFECTION IN RABBITS AND LABORATORY MICE IN EASTERN SLOVAKIA

PAVOL BÁLENT, MONIKA HALÁNOVÁ*, TATIANA SEDLÁKOVÁ, ALEXANDRA VALENČÁKOVÁ AND LÝDIA ČISLÁKOVÁ*

Department of Biology, University of Veterinary Medicine, 041 81 Košice, Slovakia
*Department of Epidemiology, Faculty of Medicine, P. J. Šafárik University, 041 80 Košice, Slovakia
e-mail: balent@uvm.sk

Received for publication May 06, 2003.

Abstract

In order to detect the presence of antibodies to Encephalitozoon cuniculi antigens, five different laboratory mouse colonies of ICR and C57BL/6 types as well as three breeding colonies of domestic rabbits and a colony of wild rabbits were examined. IFAT and ELISA were used as serological diagnostic methods. Out of 132 laboratory mice examined, 20 (15.2%) were serologically positive to E. cuniculi. Positive mice were detected in two out of five colonies. Serum specific antibodies were also detected in 16 (21.33%) individuals out of 75 rabbits coming from three different rabbit colonies examined by the IFA test, and in 21 (44.7%) wild rabbits out of 47 examined by the ELISA. Our results have confirmed the dissemination of the parasite in breeding colonies of laboratory animals and rabbits in our territory. Several preventive measures to improve the health of susceptible animal breeding colonies have been suggested.

Key words: rabbits, laboratory mice, Encephalitozoon cuniculi, epidemiology.

Encephalitozoon cuniculi is a strict intracellular mammalian parasite systematically classified as a separate phylum of Microspora (7). The absence of mitochondria results in the complete dependence of microsporidia on the energy metabolism of a host cell. E. cuniculi is relatively small with the dimension of 1.5 x 2.5 µm. A mature organism is a spore covered by a protein and chitin coating. The host cell is infected in the process of germination in which the spore extrudes its protoplasm through the everted polar filament into its cytoplasm.

The causative agent of the disease can be characterised by a relatively wide spectrum of susceptible mammalian hosts in the orders of Lagomorpha, Rodentia, Carnivora, and Primates. Encephalitozoonosis is most frequently developed after the oro-faecal or oro-urinal transmission; however, the vertical transmission of the infection is also quite common in carnivores and rodents (5). In the individuals with physiological immune system conditions, it is manifested as a chronic asymptomatic infection. The diagnostics of a clinically apparent disease demonstrates the suppression or deficiency of cellular immunity of the host organism (6).

Based on the biological data and data concerning E. cuniculi dissemination, the parasite can be concluded as a cause of serious infection of laboratory mouse colonies that deteriorates the results achieved in the examination of these animals. Neither economic consequences can be neglected, as encephalitozoonosis in farm rabbits leads to direct losses in profitability by the decrease in slaughter weight by 13% (10). All these facts have lead us to an attempt to determine the prevalence of microsporidioses in mice and rabbit breeding colonies in Slovakia on the grounds of reactions of specific antibodies in selected serological tests.

Material and Methods

Animals. Rabbits. The sera from 42 laboratory rabbits of two breeds (New Zealand White and Great Chinchilla) coming from two separate breeding colonies; from 33 slaughter rabbits of the Hyla breed out of a private farm in eastern Slovakia, and from 47 wild rabbits intended for reintroduction to the wilderness (ÚZ UVL Rozhanovce) were used for examination. Blood samples were taken by a marginal auricular venal puncture. After coagulation and subsequent centrifugation, the acquired sera were congealed and stored at the temperature of −20°C until their use in a serological tests.

Mice. The sera from 132 laboratory mice of two species (ICR and C57BL/6) coming from five different laboratory colonies (Table 1) were examined. Blood samples were taken by a cardiac puncture and processed in the identical way as in case of rabbit sera.

Encephalitozoon cuniculi antigen. Mature spores of microsporidia grown in vitro in VERO E6 green
monkey kidney cells were used as an antigen in serological tests. Permanently infected cells were cultivated in the modified RPMI 1640 medium supplemented with 5% foetal bovine serum and with the addition of antibiotics (STMC, PNC, AFTC). After centrifugation (400 g), spores were isolated from the supernatant of cellular medium, then rinsed in the solution of PBS, counted in a haemocytometer, and finally re-suspended in PBS to the required concentration (1 x 10^6 spores/ml).

**Serological tests.** IFAT. The indirect immunofluorescence antibody test was performed according to Chalupský et al. (1). A fresh suspension of *E. cuniculi* spores 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 1:4096. Each of the wells was covered with 10 µl of diluted serum, and the slides were 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 covered with 10 µl of swine anti-mouse (or anti-rabbit) immunoglobulin fluorescein isothiocyanate conjugate (SEVAC Praha, Czech Republic) diluted 1:160. After 30 min incubation at 37°C the slides were washed and air-dried. Then they were counter stained with Evans blue and mounted under cover slips in buffered glycerine. The serological reaction was evaluated microscopically based on the spore fluorescence in the field of vision of a ZEISS JENA microscope. The animals whose sera reacted at the titre 64 and higher were considered positive.

ELISA. The sera of wild rabbits were examined by the modified ELISA described by Hollister and Canning in 1987 (4). Hundred microlitres of *E. cuniculi* spores in coating buffer (1 x 10^6 spores/ml) was added to each well of ELISA plates. The plates were incubated overnight at 4°C and then they were dried and fixed with 1:1 mixture of acetone and methanol for 10 min. After blocking the unbound sites with 5% new-born calf serum (NBCS) in PBS for one hour at 37°C, the plates were washed with 0.05% Tween 20 in PBS (T-PBS) three times. The sera were diluted at 1:400 – 1:3200 in 2% NBCS in PBS, and incubated for one hour at 37°C. Then the plates were washed with T-PBS and incubated with swine anti-rabbit immunoglobulin peroxidase conjugate (SIGMA, Germany) for one hour at 37°C. After washing, an enzymatic colour reaction was generated using orthophenylenediamine substrate (OPD). The reaction was stopped after 10 min with 100 µl of 2M H_2SO_4. Sample absorbency was measured by a spectrophotometer Multiscan MCC/340 (Fa Labsystems) at the wavelength of 490 nm. The serum with the absorbency of a minimum 2.1 times higher than the absorbency of a negative control serum was considered positive.

**Results**

Out of 132 laboratory mice examined by the IFAT, 20 (15.2%) showed the presence of *E. cuniculi* antibodies. Positive samples reacted at the titres between 64 and 2 048. Serologically positive mice were detected in 2 out of 5 examined colonies (Table 1). Of the total number of 75 sera of laboratory rabbits and rabbits from private breeding examined by the IFA test, 16 (21.3%) were positive at the titre 64 and higher (Table 2). In the group of wild rabbits examined by the ELISA, the occurrence of *E. cuniculi* antibodies was observed in 21 samples (44.7%) (Table 3). Using of both tests, the serum specific antibodies were detected in 37 rabbits (30.3%).

### Table 1
Results of IFA test presenting *E. cuniculi* antibodies in laboratory mice

<table>
<thead>
<tr>
<th>Laboratory mice</th>
<th>Number of animals Examined</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony Species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A C57BL/6</td>
<td>21</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>B ICR</td>
<td>50</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>C C57BL/6</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>D C57BL/6</td>
<td>21</td>
<td>19 (90.5)</td>
</tr>
<tr>
<td>E C57BL/6</td>
<td>20</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>20 (15.2%)</td>
</tr>
</tbody>
</table>

### Table 2
Results of IFA test presenting *E. cuniculi* antibodies in rabbits

<table>
<thead>
<tr>
<th>Rabbits Breed</th>
<th>Specification</th>
<th>Number of animals Examined Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand White</td>
<td>Laboratory</td>
<td>18</td>
</tr>
<tr>
<td>Great Chinchilla</td>
<td>Laboratory</td>
<td>24</td>
</tr>
<tr>
<td>Hyla Slaughter</td>
<td>Slaughter</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>75</td>
</tr>
</tbody>
</table>
Discussion

Encephalitozoonosis is currently ranked among protozoal diseases that can be detected in the breeding colonies of laboratory animals and rabbits. The danger of the infection rests in its hidden latent course in immunocompetent individuals with only an occasional manifestation of clinical symptoms of a neurological syndrome. In case of transplacental disease transmission there is a major problem with the breeding of an adequate number of viable young as the disease is frequently mortal. Moreover, as a zoonosis and opportune infection, it is a constant health risk for these persons who come into contact with latently infected individuals.

The original information on the prevalence of encephalitozoonosis in laboratory animals has been obtained on the basic of histological examination. Robinson (8) discovered that in 21% out of 280 examined rabbits typical histological changes caused by E. cuniculi were observed, and after six months there was 54% of individuals infected in the same laboratory colony. Based on histological examinations, the infection prevalence of 20 to 50% in mice was also reported by Shadduck and Pakes (9).

The first significant serological examinations were conducted by Chalupský et al. (2) in several breeding colonies of laboratory animals in the Czech Republic. By the application of the IFAT they discovered the following prevalence of the infection: 20—95% in rabbits (positive in 7 out of 12 breeding colonies), 29—85% in guinea pigs (4 positive out of 6), 14—80% in hamsters (4 positive out of 5), 15—30% in rats (2 positive out of 4), 0% in mice. According to the authors, the negative serological findings in laboratory mouse colonies resulted from an inadequate method used for their examinations.

As it was later shown, the IFA methodology on its own was not a problem in the diagnosis of encephalitozoonosis in mice. El Naas et al. (3) compared the serological results with histological kidney alterations in 20 positive and over 100 negative laboratory mice. They concluded that in as many as 80% E. cuniculi serologically positive individuals, histopathological changes in the renal tissue characteristic of encephalitozoonosis were observed, whereas no histological damage could be recorded in the negative animals.

Our results proved the allegations concerning the ubiquitous microsporidia of E. cuniculi in the environment to be correct and confirmed its presence in laboratory mouse and rabbit colonies on the territory of the Eastern Slovakia. In one particular case of mouse colony (C57BL/6 species marked D), and all three positive rabbit breeding colonies (marked F, H, and I), the endemic dissemination of the E. cuniculi parasite can be proved. The examination results in the rabbits fully correspond to the above-mentioned findings of other authors. The transmission of encephalitozoonosis in individual animal populations is facilitated by the contamination of the environment through the spores that eluted into the urine of afflicted animals.

In order to prevent the encephalitozoonosis in animal breeding colonies several organisational and health measures are to be taken. Firstly, it is the maintenance of veterinary and hygiene principles, such as the cleanliness of animal feeding and keeping, regular performance of efficient DDD, regular examination of breeding colonies for the presence of antibodies and selection of serologically negative individuals for further breeding and reproduction. An important role is played by the intentional development of animal immunity system competence and function; it can be achieved by the reduction of stress in breeding facilities, good-quality and well-balanced nourishment with the minimisation of sudden changes in daily feeding regimes.

Acknowledgments: This study was supported by the Slovak Grant Committee VEGA of the Ministry of Education and Science of the Slovak Republic No. 1/0580/03 and No. 1/9269/02.

References


10. Vávra J., Chalupský J.: *Encephalitozoon cuniculi* as a contaminant in the breeding of laboratory animals and rabbits. Research report, VI-1-9/1 SPZV, Charles University, Prague 1980, p. 84.