

## ANTIBODIES TO ENCEPHALITOOZON CUNICULI AND RENAL LESIONS IN MICE

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*The kidneys of twenty seropositive mice were examined histologically in order to study the relationship between titre of antibodies to Encephalitoozon cuniculi and morphological structure. Sera of these animals reacted at dilutions between 1:64 and 1:2048. Histopathological changes were seen in 80% of E. cuniculi infected animals. Microscopically the lesions were divided into immunolymphoid type (50%), granulomatous type (5%) and fibrous type (25%). Chronic interstitial nephritis was seen mainly at higher antibody titres.*

*Key words: mice, antibodies, Encephalitoozon cuniculi, renal lesions*

### INTRODUCTION

*Encephalitoozon cuniculi* Levaditi, Nicolau et Schoen, 1923 is a frequent microsporidian parasite of laboratory animals. Rabbits, mice and rats are frequently infected (Chalupsky et al., 1979).

*E. cuniculi* has the potential to affect multiple organs including the brain, kidney, liver, heart, and lungs (Shadduck and Pakes, 1971). The kidney appears to be a target organ in *E. cuniculi* infection and chronic interstitial nephritis regularly occurs in animals experimentally (Shadduck et al., 1979, Mohn et al., 1982a) and naturally infected with this parasite (Levkut et al., 1997, Waller, 1977). However, *E. cuniculi* infection under normal immunological conditions produces only chronic asymptomatic lesions of organs.

The results of serological examination confirm the widespread occurrence of antibodies to this encephalitoozon in laboratory animals (Levkut et al. 1996). It can be supposed that the presence of antibodies actually means that the microsporidian has been present in the animals examined (Chalupsky et al., 1979) and highly positive animals react up to serum dilution of 1:512 and higher by IFAT (Levkut et al., 1996).

The purpose of the work was to study the relationship between titre of antibodies present and structure of kidney lesions in animals serologically positive.

#### MATERIAL AND METHODS

*Animals.* The animals used in our study were over eight weeks old laboratory-bred mice from different murine strains housed in rooms for experimental purposes and kept under conventional conditions (C57 bl/6, ICR).

*Sera.* Blood was taken by cardiac puncture and the sera obtained from the blood samples were frozen and maintained at -20°C until used. Sera of twenty seropositive animals were obtained from several sources of Slovak laboratory animal centres.

*Antigen.* *E. cuniculi* organisms isolated from mice were grown in "E6" cells (vero green monkey kidney cells). The cells were cultivated in modified RPMI 1640 medium with an addition of 5% foetal calf serum. The spores freshly collected from the culture supernatants according to Koudela et al., (1993) were used as the antigen for IFAT serological examination.

*Immunofluorescence.* Sera were tested by indirect immunofluorescence for the presence of antibodies to *E. cuniculi*. The animals whose sera reacted at a dilution of 1:64 or higher were considered to be positive. The titres were expressed as the highest serum dilution giving bright staining of the spore body as described previously (Chalupsky et al., 1973). The conjugate used was a fluorescein-conjugated swine anti-mouse globulin (Institute of Sera and Vaccines, Prague, Czech Republic).

*Histopathology.* The preparation and microscopical evaluation was carried out on six histological sections taken from two localities in each kidney and processed in the standard manner, i. e. fixed in 10% neutral formalin and embedded in paraffin. Sections of 5-6 µm thickness were stained with the haematoxylin-eosin and Giemsa stains.

*Classification of lesions.* Lesions were classified into immunolymphoid type (dominant cells: plasma cells and lymphocytes), granulomatous (dominant cells: macrophages, epithelioid cells) and fibrous (dominant cells: lymphocytes and fibrocytes).

#### RESULTS

The results of serological examination carried out on the twenty seropositive mice are given in Table 1. These animals were seropositive to *E. cuniculi* infection out of 132 animals tested. The samples reacted at dilutions between 1:64 and 1:2048 (Figure 1).

Post-mortem examination revealed macroscopic lesions in the kidney of several seropositive animals. They were characterized by small, cream-coloured or reddish, well-demarcated foci. These multifocal lesions were widely scattered under the capsule.



Table 1. Serological titre and type of kidney lesions in mice

No.	IFAT titre	Type of kidney lesion		
		Immunolymphoid	Granulomatous	Fibrous
1	64	-	-	-
2	256	+	-	-
3	256	+	-	-
4	2048	-	-	+
5	2048	+	-	-
6	64	+	-	-
7	2048	-	-	+
8	1024	+	-	-
9	64	-	-	-
10	64	-	-	-
11	1024	-	-	+
12	2048	+	-	-
13	2048	+	-	-
14	64	+	-	-
15	64	+	-	-
16	64	-	-	-
17	256	-	+	-
18	256	+	-	-
19	256	-	-	+
20	2048	-	-	+

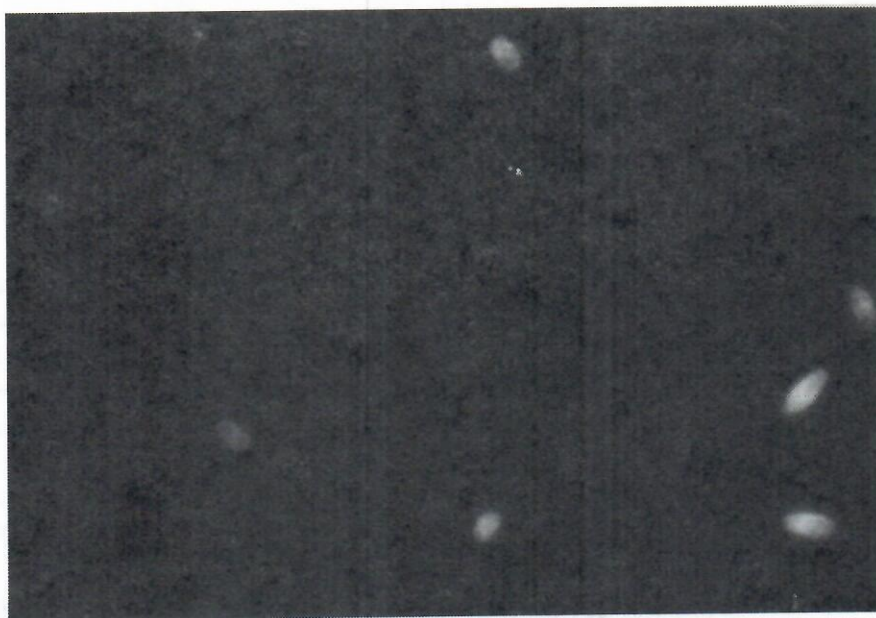


Figure 1. Positive reaction of examined mouse at dilution 1: 256(IFAT; x1000).

Histological changes were seen in 80% of *E. cuniculi*-infected animals. Microscopically, the lesions were divided into immunolymphoid type (ten cases) (Figure 2), and granulomatous type (one case), and fibrous type (five cases). Accumulation of inflammatory cells was often associated with blood vessels. In the immunolymphoid type formation of granuloma was seen in connection with blood vessels. Fibrotic tissue was observed at the junction of the cortex and medulla.

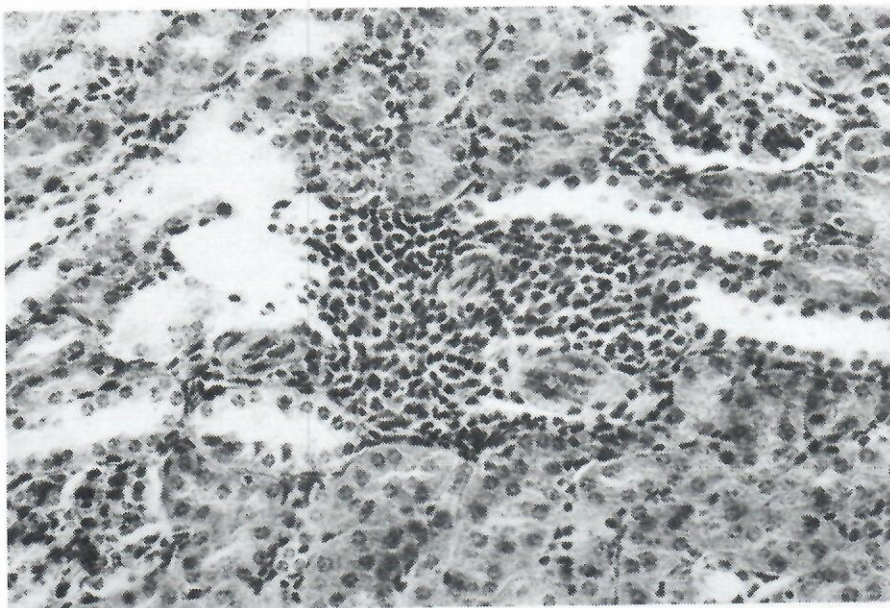


Figure 2. Lymphoid cell infiltration around the vessels (HE; x320).

#### DISCUSSION

Previous results have shown that a serological titre  $> 1:64$  usually represents infection with *E. cuniculi* (Chalupsky et al., 1979, Levkut et al., 1996). Positive serum titres to *E. cuniculi* ranged from 1: 64 to 1: 2048. The variation in titres indicates different humoral immune responses developed following natural infection of animals with *E. cuniculi*. Serologically positive animals did not display clinical abnormalities. Twenty percent of the seropositive mice did not show kidney lesions. These animals were positive only at a dilution of 1:64. It can be supposed that this titre represents the cornerstone between seronegative and seropositive and/or initial stage of *E. cuniculi* infection.

Kidney lesions in 80% of seropositive animals were divided according to the predominance of cells into three types. The most prevalent structured type



was immunolymphoid (50%). This type was found at different titres in seropositive animals. The majority of the lesions, which were observed in our study, appear to be a milder form of lesion (Innes and Stewart, 1991). This leads one to speculate whether the mild focal lesions could have remained dormant and later manifested themselves as chronic interstitial nephritis entities. In only one case of this structural type was *E. cuniculi* observed. Stewart et al. (1986) and Szabo and Shadduck (1988) observed plasmalymphocytic interstitial nephritis in their experiment in infected adult dogs, but did not find any parasites present in the organs examined. In addition, no parasites were noted in the blue fox vixens studied by Mohn et al. (1982b). The granulomatous type was recorded only in one seropositive animal. This type of lesion is rarely observed in the kidney of *E. cuniculi* infected animals (Stewart et al., 1988, Levkut et al., 1996). Evidence of chronic interstitial nephritis that is fibrous in type appeared in 25% of the kidney lesions. This kind of kidney lesion was mainly connected with a higher serological titre. The interstitial nephritis could be due either to persisting antigen or antigen/antibody complexes or possibly to the development of autoimmunity (McCluskey and Colvin, 1978).

The positive titre in mice and the presence of lesions in large numbers of animals shows a relationship between interstitial lesion and seropositivity. The study demonstrates that the most prevalent lesions in the kidney of infected mice are immunolymphoid in type and the results also showed that chronic interstitial nephritis is connected mainly with a higher titre of seropositivity that reflects changes in immunological responses of the animal to antigen.

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#### OŠTEĆENJE BUBREGA U ZAVISNOSTI OD TITRA ANTITELA PROTIV E. CUNICULI U MIŠEVA

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#### SADRŽAJ

U radu je ispitivan stepen oštećenja bubrega u miševa seropozitivnih na E. cuniculi. U ispitivanih miševa titar antitela se kretao od 1 : 64 do 1 : 2048. Histopatološke promene u bubrezima su nađene kod 80% inficiranih životinja. Stepenn oštećenja bubrega je bio u velikoj korelaciji sa titrom antitela u krvnom serumu inficiranih životinja.