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PATHOMORPHOLOGICAL REACTIONS OF NEW ZEALAND WHITE RABBITS TO RECTAL ADMINISTRATION OF ENCEPHALITOZOON CUNICULI

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Encephalitozoonosis is caused by the protozoan parasite E. cuniculi. Previous studies suggested that the kidney and brain are the main target organs of spontaneous infection. To estimate whether a given number of spores influences the pathological pattern of infection, two different doses of inocula were given to rabbits per rectum. Brain, liver, kidney and lungs were evaluated histologically 90 days after infection and pretreatment with Fleet solution. The rabbits infected with 5×10^7 spores developed pathological changes in the liver and kidney. The brain and urinary bladder were also damaged. E. cuniculi was found immunohistochemically in tissue. Per rectum infection of rabbits appears to be a good model for the study of sexually transmitted diseases and enteric pathogens in people.

Key words: E. cuniculi, experimental model, pathology, immunohistochemistry

INTRODUCTION

Encephalitozoonisis is a disease caused by the obligate intracellular microsporidian protozoan E. cunciculi. Many animal species are susceptible to the disease, including rodents, lagomorphs,carnivores, and primates (Canning and Lom, 1986).

In immunocompetent animals, E. cuniculi induced infection is usually chronic, latent, and accompained by the formation of brain and kidney lesions (Didier et al., 1994). Immunodeficient hosts develop acute and lethal diseases (Koudela et al., 1993). Recently, Franzen et al. (1995) confirmed an occurrence of E. cuniculi infection in humans with acquired immunodeficiency syndrome (AIDS). Observations moreover suggest that the liver (Gordon et al. 1986), and brain (Weber et al., 1997) as well as the adrenal gland and heart (Mertens et al., 1997), are the prefered organs of E. cuniculi infection in AIDS patients. Current studies indicate that transmission of E. cuniculi between various hosts is feasible (Deplazes et al., 1996; Mathis et. al., 1997).

The objective of our report was to estimate the pathomorphological reactions of rabbits to rectal E. cuniculi infection.

MATERIAL AND METHODS

Rabbit isolate of E. cuniculi (kindly provided by Dr. Ditrich, Czech Republic) was grown in E6 cell culture. Spores were harvested at various passage levels, concentrated by centrifugation and resuspended in phosphate-buffered saline. Aliquots in suspension were counted in a haemocytometer just prior to the administration of the inocula.

Murine hyperimmune sera were obtained from 4-month-old, immunocompetent $C_{57}BL_6$ strain mice (Parasitological Institute, Košice). Mice were immunized intraperitoneally with 6 inoculations of 6×10^6 spores of E. cuniculi al three-day intervals. Sera were collected by decapitation 3 days after the last inoculation and stored at -20 ^{0}C until used for the IFAT (indirect immunofluorescent antibody test).

The IFAT was performed according to the method described by Chalupsky et al. (1973), using conjugates specific to the individual species of animals examined. Titres obtained by assaying the sera against the parasite ranged from 1024 to 2048.

Table 1 Results and design of experiment

Rabbit N°.	Inoculum (N° spores given in 1 ml PBS)	Pathological finding					
		nephritis	hepatitis	urocystitis	cholecystitis	encephalitis	pneumonia
Group A							
1	5×10^5	+	+	–	–	–	–
2	5×10^5	+	+	–	–	–	–
3	5×10^5	+	+	+	+	+	–
4	5×10^5	+	+	–	–	–	–
5	5×10^5	+	+	–	–	–	–
Group B							
1	5×10^7	+	+	+	+	–	–
2	5×10^7	+	+	+	+	–	–
3	5×10^7	+	+	+	+	+	–
4	5×10^7	+	+	–	–	–	++
5	5×10^7	+	+	–	–	+	–
Group C							
1	1 ml PBS	–	–	–	–	–	–
2	1 ml PBS	–	–	–	–	–	–

Twelve, 5-6-month-old New Zeland White rabbits were free of Coccidia, Pasteurella and E. cuniculi infections. Animals of both sexes were randomly divided into 3 groups and treated as shown in table 1. Animals of both groups A

and B were submitted to colonic enema (20 ml of Fleet formula) 90 minutes prior to infection as detailed by Fuentealba et al., (1992). Two control animals were neither treated with enema nor inoculum. Rabbits were killed 90 days after infection.

Tissue samples were taken from the brain, kidney, lung, liver and urinary bladder during necropsy. The material collected was 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 haematoxylin and eosin.

Formalin-fixed, paraffin-embedded material was used for imnunohistochemical ctrudy. The indirect streptavidin - perohidase method was applied. Briefly, undigested paraffin sections were incubated for 18 hours at 4^0C with mouse anti-E. cuniculi hyperimmune sera 1.1000 diluted in our laboratory (Štefkovič et al., 1997). They were then incubated with commercial anti-mouse IgG antibody (BioGENEX, San Ramon, USA) diluted in tris-buffered saline and finally with streptavidin-peroxidase complex. The immunological reactions were identified by diamino-benzidine. Sections were counter-stained with Mayer 's haematoxylin.

To control the specificity of the technique, tissue sections were examined with the following modifications; omission of the first antiserum, omission of the secondary antibody, omission of the streptavidin peroxidase complex, and omission of the diaminobenzidine. All tests gave negative reactions. Tissue samples with and without E. cuniculi were always used as positive and negative controls of the streptavidin peroxidase method.

RESULTS

In both groups A and B of rabbits, mild hepatomegaly and randomly scattered white spots of 1-2 mm in size on the kidney surface were the most significant gross lesions at necropsy. One rabbit of group A had a dilated urinary bladder with numerous small ulcers on the mucous surface. Dilation of both urinary bladder and gall bladder was found in four animals of group B. Dilated urinary bladders contained a dense, white, creamy mass with admixture of sandy material. Similar greenish-white substance was found in dilated gall bladders.

Histologically, the kidneys showed focal fibrosis (Figure 1) and granulomatous inflammation. The inflammatory reaction consisted of lymphocytes, a few macrophages and occasional eosinophils. The areas of granulomatous inflammation were localized around the arterial walls of the kidney. Sometimes, the occurennce of sclerotic glomeruli was recorded. Liver involvement was characterized by necrosis of individual hepatocytes with accentuated eosinophilia of the cytoplasm and pyknosis of nuclei. Inflammatory foci in the periportal areas was composed of plasma cells with an admixture of eosinophils. (Figure 2). Two rabbits of group B had microgranulomas in the brain. The granulomas were composed of macrophages and lymphocytes without marked tissue necrosis. Perivascular cuffs were observed mainly around the granulomas. (Figure 3). Extensive damage to urinary bladders was present as ulcers and massive desquamation of epithelial cells on the mucous surface.

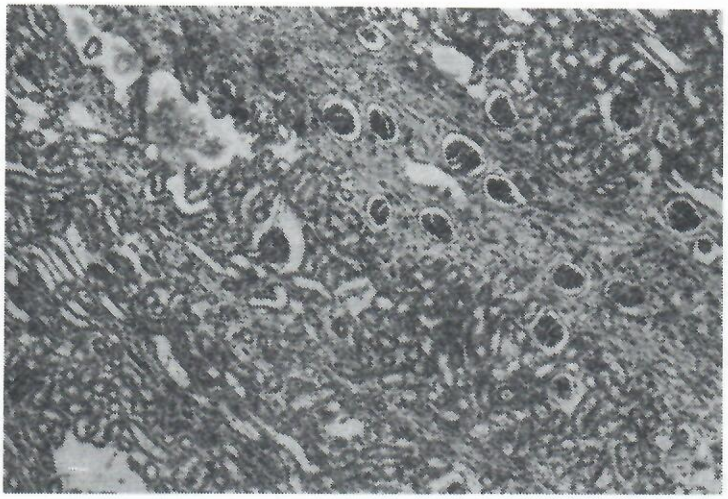

Figure 1. Kidney. Strip of fibrous tissue in kidney parenchyma (H & E, original magnif.160)

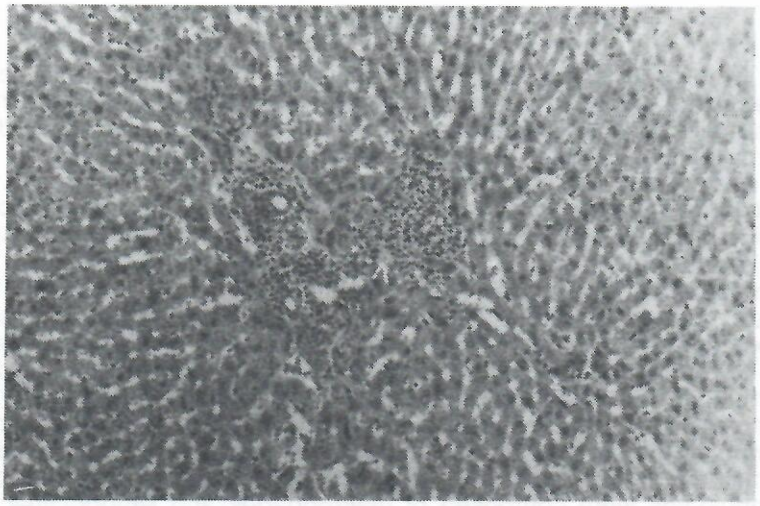

Figure 2. Liver. Hepatitis - lymphocytic infiltration in periportal areas with an admixture of eosinophils (H & E, original magnif. 160 x)

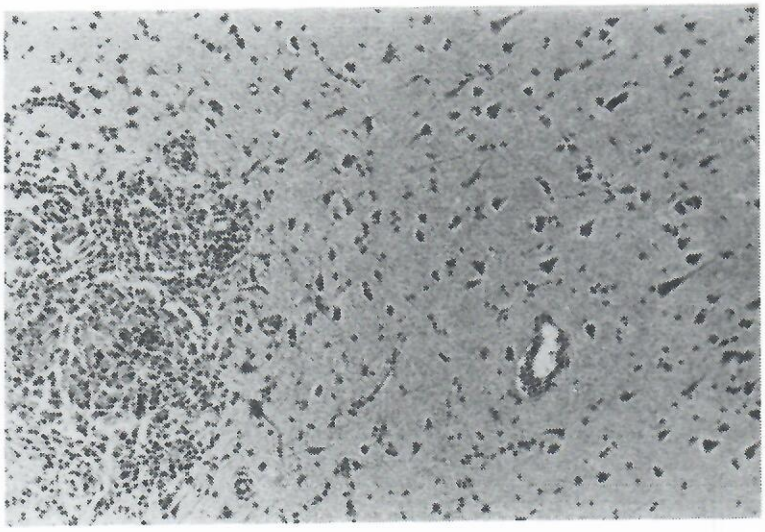

Figure 3. Brain. Granulomatous encephalitis with perivascular cuffing. (H & E, original magnif. 160 x)

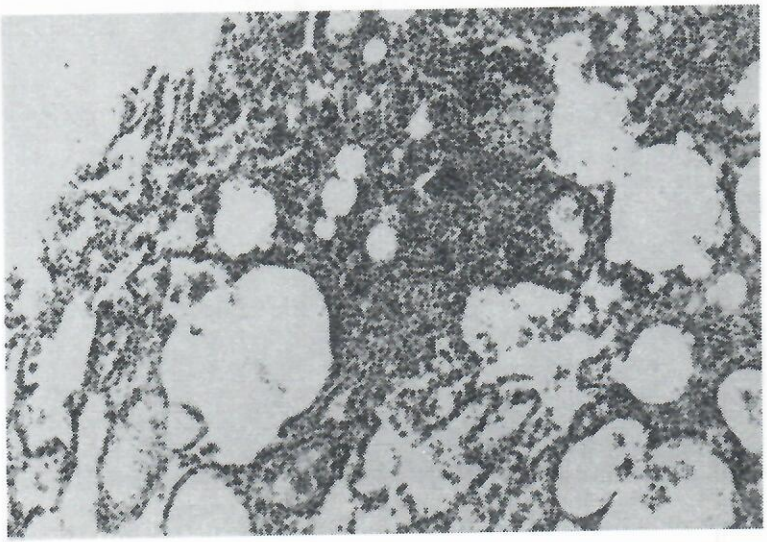

Figure 4. Lung. Lymphocytic infiltration (H & E, original magnif. 160)

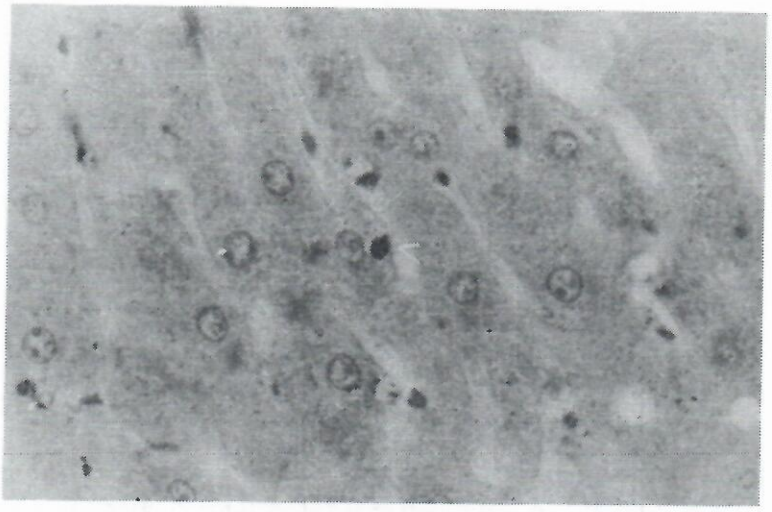

Figure 5. Liver. The cytoplasm of the hepatocyte contains a number of E. cuniculi. (immunohistochemical evidence of E. cuniculi, original magnif. 1000x).

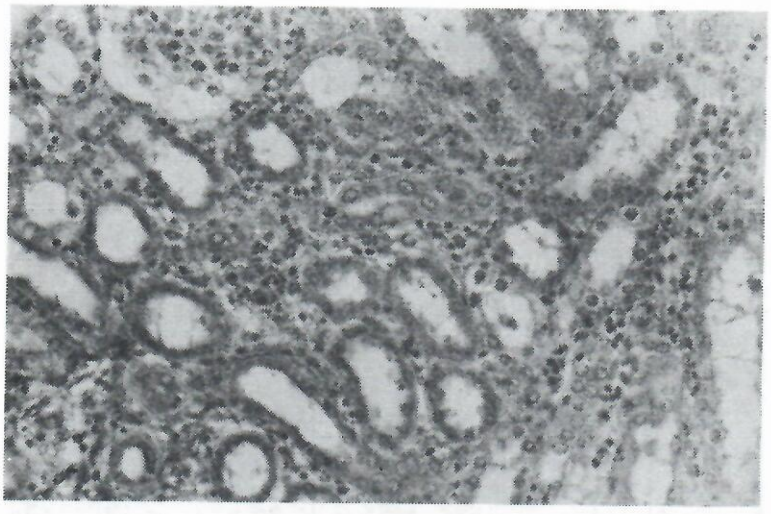

Figure 6. Kidney. A colony of parasites within the epithelium of a tubule. (immunohistochemical evidence of E. cuniculi, original magnif. 1000 x)

Submucous and muscular layers were diffusely infiltrated mainly by neutrophils, with a few lymphocytes and occasional macrophages. The lungs showed spots of lymphocytic infiltration randomly scattered in the parenchyma (Figure 4).

Immunohistochemically, E. cuniculi organisms were found in all of the organs tested with a predilection for the liver. The colonies of the parasites were observed intracellularly within a parasitophorous vacuole (Figure 5, 6). The controls were free of both lesions and E. cuniculi.

DISCUSSION

The present experiment involved dose-distinct rectal administration of E. cuniculi to New Zealand white rabbits. We observed pathological lesions compatible with encephalitozoonosis induced by per rectum instillation of E. cuniculi as described by Wicher (1991).

Both dosages of inocula used in this work induced changes commonly in the liver and kidney. This is not surprising, because E. cuniculi is known to infect some phagocytic cells e. g. monocytes (Weber et al., 1994). These phagocytic cells move this protozoan to susceptible tissues with a high blood flow such as the liver and kidney (Cox et al., 1979). The liver moreover is the target organ of E. cuniculi infection in people with AIDS. Similarly, the liver is the principal site of pathological lesions in rabbits after rectal administration of the parasite (Fuentealba et al., 1992).

A higher dose of inocula ($5x10^7$) caused serious damage to the urinary bladder in the majority of rabbits in group B. Injury to the urinary bladders was probably developed as the result of the massive outflow of parasites from the kidney to the outer environment via the urine. Cox et al. (1979) detected large numbers of spores i. e. 50 to more than 500 spores per ml in the urine of rabbits between 31-63 days after experimental infection. Only small numbers of spores were intermittently excreted up to day 98 of infection.

Only one rabbit of group A had the same lesion in the urinary bladder as was found in group B. No rabbit of group A showed brain lesions, while two rabbits of group B developed granulomas in the brain tissue. The occurrence of granulomas mirrors the presence of cell-mediated immune rections. Immunity, which is T-cell dependent, cytokine-mediated, and effected by macrophages (Didier, 1995) and/or antibodies, checks microsporidian infection (Hermanek et al. 1993). The discrepancy in our pathological findings could be explained as follows:

(1) It may be that only certain vegetative forms of E. cuniculi are infective;

(2) A higher dose of organisms may lead to the clonal abortion of immune system cells (Miller, 1993) which are responsible for control of microsporidiosis via T-dependent cell-mediated and/or antibody mediated immune reactions. Immunological suppression caused by a higher dose of E. cuniculi resulted in

the most serious damage to organs. The experiment did not clarify the mechanism of immunity depression. Additional experiments directed towards the effect of E. cuniculi doses on host immune response can improve our understanding of the differences in the pathological pattems of encephalitozoonosis.

CONCLUSION

Macroscopial and histological examination revealed that the main target organs in intrarectal E. cuniculi infection are the kidney and liver, whereas brain damage is rarely observed. Pathomorphological reactions in rectal E. cuniculi infection are characterised as lymhophocytic infiltration and/or granulomatous inflamation. As documented in this paper, rectal inoculation of E. cuniculi in the rabbit is a suitable model for the study of sexually transmitted diseases and microsporidial pathogenesis, mechanisms of resistance, and immunotherapy. This study also suggested that the pathomorphological reactions in encephalitozoonosis may strongly depend on the infectivity of E. cuniculi spores and/or host immune response.

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PATOMORFOLOŠKEPROMENEUSTANOVLJENE KOD NOVOZELANDSKOG BELOG KUNIĆA NAKON REKTALNE ADMINISTRACIJE E. CUNICULI

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SADRŽAJ

Prema ranijim istraživanjima encefalitozoonozisa,ciljni organi pri spontanoj infekciji kunića protozoom E. cuniculi su bubrezi i mozak. U cilju da se utvrdi da li broj spora utiče na razvoj patoloških promena pri veštačkoj infekciji, dve različite doze infekta su inokulirane kunićima per rectum. Mozak, jetra, bubrezi i pluća kunića su 90 dana nakon infekcije i tretmana rastvorom Fleet-a histološki pregledani. Patološke promene na jetri i bubrezima, kao i oštećenja moždanog tkiva i mokraćne bešike su ustanovljeni kod kunića koji su inficirani sporama u količini od 5x107. U tkivima je imunohistohemijski ustanovljeno prisustvo E. cuniculi. Veštačka infekcija kunića per rectum po svemu sudeći, predstavlja dobar eksperimentalni model za ispitivanje polnih zaraza i crevnih infekcija kod ljudi.