Histopathological aspects in the encephalitozoonosis of blue foxes puppies (*Alopex lagopus*)

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**SUMMARY.** The investigation were made on new born blue foxes puppies a fox farm where losses through mortality were registered during the first days of life. Fragments of the small intestine, kidneys, liver, lungs, myocardium and spleen were fixed in 10% formol aldehyde, introduced in paraffin, divided at 5 µm and coloured through the methods: MGG, PAS and HEA. The examination and microphotography were made at MC5 oc. 10, ob. 10, 20, 40 and 63 immersion. The histopathological examination proved the presence in the jejunum area enterocytes of the development stages characteristic to the species of *Encephalitozoon cuniculi* placed in parasite vacuoles. The same formations were identified in nephrocytes and in the alveolar epithelium. The infection of the blue foxes puppies with *Encephalitozoon cuniculi* is suggested to have taken place during intrauterine development, by transplacental transmission, determining the superacute evolution ending in death in the first days of life.

**Key words:** blue fox, *Encephalitozoon cuniculi*, cellular aggression, small intestine, kidneys.

**Introduction**

The encephalitozoonosis is known as a very serous zoonosis affecting people, carnivore, primates, some species of poultry and especially the rabbit, lab rodents, silver and especially blue foxes, having a systemic evolution. This evolution affects the nervous system, retina, kidneys, small intestine, liver, lungs, uterus, placenta etc. by intracellular development of parasites (6, 11, 12).

The placental transmission of parasites to the fox determines the appearance of clinical episodes in the new born in the first days of life, with an increased mortality (1).

The aim of these investigations is to reveal the cellular aggression of parasites on the small intestine and kidneys epithelium at the blue fox puppies and to draw attention on the diseases at animals and on the high risk of transmission the infection to people.

**Material and methods**

The histopathological investigations were made on 58 corpses of blue fox puppies aged 1-32 days, from a fox farm where important losses through mortality were registered. For diagnosis fragments of the small intestine, kidneys, liver, lungs, myocardium and spleen were drawn and fixed in 10% of formol aldehyde, introduced in paraffin, divided at 5 µm and coloured through the methods: MGG, PAS and HEA.

The examination and microphotography were made at MC5m oc. 10, ob. 10, 20, 40 and 63 immersion. The identification of parasites was made on the basis of the morphological dimensions and characters provided in the specialty literature.
Results and discussions

During the first days of life the cubs manifested bloatings and groans, followed by death. The presumptive diagnosis is that of “toxic syndrome”.

The histopathological examination of the jejunum mucous membrane showed the presence in enterocytes of some spheric formations of variable sizes, representing development stages of parasites (fig.1).

![Figure 1](image)

3 days old blue fox puppy naturally infected with *Encephalitozoon cuniculi*. The epithelium of the small intestine mucous membrane is parasited with stages of development placed in parasites vacuoles. MGG col. oc.10 x ob.10.

All the development stages of the parasite are in the enterocytes’ cytoplasm, placed in parasite vacuoles protecting them from the action of the cellular enzymes. Some of them are very small (2-4µm) with a dull, non-differential content, representing the stage of trophozoite (fig. 2).

The bigger spheric formations, that is the pseudocysts, are characterized by a softly moruled surface determined by many spheres densely placed and connected by a cytoplasmic trama. There is no own wall (fig. 3).

![Figure 2](image)

3 days old blue fox puppy naturally infected with *Encephalitozoon cuniculi*. The small intestine epithelium is destroyed by the existence of parasites stages in the enterocytes.
In the centre of the pseudocysts there is sporogenesis. At their margin there are schizogonic formations (schizonts with tachyzoits) from which spores develop. The round or oval spores with a diameter of 1.5-2.5 x 1.0-1.2 µm are isolated and have a polar filament appearing like a punctiform and shining fine structure. The spore’s mobility is given by the polar filament (15 µm), which goes outside and makes wavy movements. The parasite structures are slightly coloured with HEA and strongly coloured with PAS and MGG (fig. 4).

The parasited enterocytes suffer from the progressive degeneration once with the development from the stage of trophozoite to the one of pseudocyst. In the development stage of the pseudocyst, the enterocyte is very deformed, with the cytoplasm distroyed, with a pyknotic nucleus towards the peripheric area. By breaking the cellular membrane, the issued spores will invade the new epithelial cells (fig. 5).

Figure 3
3 days old blue fox puppy naturally infected with *Encephalitozoon cuniculi*. Pseudocysts. PAS col. Oc. 10 x ob. 40.

Figure 4
3 days old blue fox puppie naturally infected with *Encephalitozoon cuniculi*. Pseudocysts with spores, enterocyte distroyed. MGG col. oc. 10 x ob 63 Imm.
The same formations were identified in the epithelium of the distal and collecting twisted tubes from nephron (fig. 6), in the moderate infections, and in the alveolar epithelium from lungs and liver in less serious infections. We mention that in the kidneys the lesions of the parasite epithelial cells were accompanied by infiltrations of the interstice with lymphocytic inflammatory cells. The nephron’s alterations may be connected with the polyuria and dehydration affecting sick animals (10).

The brain was not investigated, the encephalitozoonosis being a surprise in the histopathological investigation.

Encephalitozoon cuniculi produces a serious disease at the blue fox puppies. The infection of the foxes during pregnancy provides the transplacental passage of parasites, the colonization of the fetus and rendering it sick in the intrauterine period of time. The foxes do not show clinical signs, but serologically speaking they are and they stay seropositive for a year time after the oral infection with spores, providing the transplacental transmission of the disease to a new series of puppies (2).

Encephalitozoon cuniculi is considered to be of high risk for the AIDS sick people or other types of immunodeficiency. At many host animals the infection with this parasite has a subclinical evolution. At some carnivores the disease comes up at the new born, when the transmission is transplacental. At the blue fox (Alopex lagopus) and at the dog (Canis familiaris) the encephalitozoonosis may be serious. At the dog the disease was recorded in many countries, including in South Africa and USA, being a major zoonotic risk if we take into consideration.

Figure 5
3 days old blue fox puppy naturally infected with Encephalitozoon cuniculi. Pseudocysts with spores. MGG col.
Oc. 10 x ob. 63 Imm.

Figure 6
Encephalitozoon cuniculi: placed in the epithelium of the renal tubules.
HEA col.
Oc. 10 x ob.20
the relationship between the dog, as a pet, and human being (3).

The diagnosis of the disease is based on the clinical signals, the anatomo-pathological changes and the detection of circulating antibodies produced by Encephalitozoon cuniculi. The most frequent serologic tests in diagnosing this parasite are: the test of indirect immuno-fluorescence (IFAT), the carbon test (CIA) and ELISA test (2).

The prevalence of encephalitozoonosis varies, depending on the host species where it evolves and the geographic area. Thus, the prevalence of the disease at the human being is very high in Slovakia where, using the: the test of indirect immuno-fluorescence – IFA, anti-Encephalitozoon cuniculi antibodies were identified at 5.7% of the human subjects examined and 37.5% of the immunodeficient ones were seropositive; at the rabbits the incidence was of 41.7% and at the dog, of 37.8%. The relatively high prevalence of the disease at rabbits and dogs suggests a potential source of infection for people (7).

In the area of Campania, Italy, the prevalence of the disease at 2 year old rabbits was of 10.3%, and in the North – of over 90% at adults and 0% at young rabbits aged 79-80 days (5).

From a lot of 125 rabbits, 70% showed neurological, renal and ocular signs, suggestive for encephalitozoonosis and seropositive reactions, and from the asimptomatic ones, 23% were seropositive (8). At the pregnant females the placenta and the fetuses aged 28 days were infected with Encephalitozoon cuniculi, type 1 root, confirming the vertical, transplacental transmission of the disease (4). In Israel, the prevalence of encephalitozoonosis was of 60% at the clinically healthy horses and of 68-80% at the ones with neurological signs and colics (9).

Conclusions

Using the histopathologic investigations, the bowel and renal site of the species Encephalitozoon cuniculi was diagnosed at new born blue fox cubs aged 1-3 days with an incidence of 3.4% from the investigated cases.

In the small intestine and renal epithelium there were identified the development stages of parasites as trophozoites and pseudocytes with pores and in the interstice – infiltration with mononuclears.

The death of the blue fox cubs in the first days of life tends to be justified by the crossplacental transmission of the infection with Encephalitozoon cuniculi.

Bibliography


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