Encephalitozoon cuniculi infection in Barbary striped grass mice (Lemniscomys barbarus)

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Summary

Encephalitozoon cuniculi is an obligate intracellular microsporidian parasite that commonly induces subclinical infections in rabbits, but occurs also in a range of other species, including various rodents, carnivores, humans and birds. The present report describes encephalitozoonosis in a group of captive Barbary striped grass mice (Lemniscomys barbarus) in a zoo collection. The aetiology was confirmed by immunohistochemistry and PCR with subsequent sequencing. The source of infection is not known.

Keywords: Encephalitozoonosis, microsporidia, Barbary striped grass mice (Lemniscomys barbarus)

Introduction

Encephalitozoon cuniculi (E. cuniculi) is a zoonotic, unicellular, spore forming parasite with an obligate intracellular development, belonging to the phylum Microsporidia, class Microsporea and order Microsporida¹–³. Microsporidia were originally thought to be protozoa but are now believed to be a sister group of fungi. The host range of E. cuniculi is wide⁴, but wild and domestic rabbits (Oryctolagus cuniculus) are most commonly and predominantly subclinically infected¹,². So far, four genotypes have been identified based on the number of 5'-GTTT-3' repeats in the ribosomal internal transcribed spacer (ITS) region of the rDNA gene; a “rabbit strain” (genotype I) identified in rabbits, wild birds, non-human primates and humans; a “mouse strain” (genotype II) and a “dog strain” (genotype III), both with broad host ranges that include rodents, birds, carnivores, primates, humans (genotype III), and a “human strain” (genotype IV) described in humans, cats and dogs⁵–⁷,⁴. Clinical disease is mainly seen in immunosuppressed individuals, but has also been reported in immunocompetent hosts including rodents, carnivores, humans and birds; it is usually associated with neurological signs and inflammatory lesions in brain and kidneys⁸,⁹. Infected animals intermittently shed infective spores with urine, sputum and/or faeces. Hosts are mainly infected via the oral route, i.e. by ingestion of water or food contaminated with microsporidian spores, via inhalation, or transplacentally⁹–¹¹. In the host, microsporidia develop by several cycles of merogony, followed by sporogony in the cytoplasm of infected cells. Previous studies have shown that in the early stage of infection, E. cuniculi infects macrophages as these phagocyte the parasites¹²,¹³. Macrophages are also responsible for their systemic spread, which occurs particularly in immunosuppressed hosts¹⁴. In addition, E. cuniculi infects a wide range of cells, such as vascular endothelial cells particularly in the brain, and tubular epithelial cells in the kidney¹⁵, leading to shedding via the urine¹⁶. Infectious spores use a coiled polar tube to enter host cells; this is ejected during invasion and penetrates the host cell, directly inserting the sporoplasm into the cytoplasm¹⁷–¹⁹.

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Case Report

A 2-week-old male Barbary striped grass mouse (*Lemniscomys barbarus*) was found dead in its enclosure in a zoological garden. The animal had not shown any clinical signs prior to death. The gross post mortem examination did not reveal any relevant macroscopic changes.

The histologic examination revealed multifocal inflammatory processes in brain, lung, spleen, liver, kidneys and pancreas, with a variable number of pseudocystic structures of up to 120 µm in diameter, or accumulations of individual, approximately 2 mm, acid fast, Gram-, and PAS-positive protozoan-like structures both within cells and extracellular (Fig. 1A, B). In the brain these structures were found within multifocal granulomatous infiltrates in cortex and brain stem. The infiltrates were generally located in close proximity to blood vessels and associated with focal microglial cell activation and accumulations (microglial nodules) (Fig. 1C, D). In addition, protozoan-like structures were also detected within the cytoplasm of numerous intact vascular endothelial cells (Fig. 1C, inset). In the cerebral cortex, the granulomatous inflammation was accompanied by diffuse perivascular parenchymal and meningeal lymphoplasmacellular infiltration. The spleen, lungs and kidneys also exhibited a (multi)focal granulomatous inflammation which contained these structures within macrophages (Fig. 2A). In the lungs, similar structures were also observed in endothelial cells, pneumocytes, bronchial epithelial cells and alveolar macrophages. The liver, heart, pancreas and kidneys displayed mild to moderate multifocal lymphoplasmacytic infiltrates with intracellular protozoan-like structures within macrophages, endothelial cells, and parenchymal cells, i.e. hepatocytes and renal tubular cells (Fig. 2B, C). Based on the type of inflammatory lesions and the morphology of these structures, *E. cuniculi* or *Toxoplasma gondii* infection was suspected.

The examined animal originated from a group of approximately 20 Barbary striped grass mice that were housed in a savanna to grassland-like vivarium exhibit. Their diet consisted mainly of a mixture of seeds, vege-

**Figure 1:** Animal 1. Brain, cortex; granulomatous infiltrates with intralesional protozoan-like structures. **A.** Pseudocystic structures (PS) are present within cells, and there is evidence of microglial nodule formation (arrow) and microglial activation (arrowheads). HE stain. **B.** The protozoan-like structures are Gram-positive and are found arranged in the large pseudocystic structures (arrows) or, individually and as smaller groups, within macrophages (arrowheads) in the granulomatous infiltrates. Gram stain. **C.** The protozoan-like structures react strongly with a polyclonal rabbit anti- *E. cuniculi* antibody. Inset: Protozoan-like structures are also seen within vascular endothelial cells (arrowheads). **D.** The granulomatous infiltrate is mainly comprised of Iba-1+ macrophages/microglial cells (*+:* pseudocystic structures). The adjacent parenchyma exhibits activated, Iba-1+ microglial cells (arrowheads). **C, D.** Polyclonal rabbit anti- *E. cuniculi* antibody (C) and polyclonal rabbit anti-Iba-1 antibody (D), horseradish peroxidase method (Envision; Dako); antibody binding visualised with diaminobenzidine, haematoxylin counterstain. **A-D.** Bars = 10 µm.
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Table and fruits, to a lesser extent also of living insects. The mice were not in direct contact with other animals. In order to assess the relevance of the infection for the colony, two further, healthy mice, a male (animal 2) and a female (animal 3) of approximately 13 weeks, were electively euthanised and examined; samples were taken for histology and transmission electron microscopy (TEM). The histological examination of both animals revealed multifocal, predominantly mononuclear inflammatory processes. In animal 2, these were restricted to multifocal periportal lymphoplasmacytic infiltrates in the liver, with rare intralesional protozoan-like structures within Kupffer cells and hepatocytes (Fig. 2D). Animal 3 exhibited similar changes in the liver and focal lymphoplasmacellular infiltrates in the renal pelvis, with a few similar structures in intact tubular epithelial cells, partially associated with inflammatory infiltration. However, similar to animal 1, it also showed a marked granulomatous encephalitis with numerous protozoan-like structures. Again, cortex and brain stem were affected most intensely. The ultrastructural examination of the cortex revealed intralesional protozoan-like spores with an electron dense spore wall (exospore), chitinous radiolucent endospores, and a characteristic coiled polar filament (polar tube) arranged in rows with coils (Fig. 3), features consistent with *E. cuniculi*.

Immunohistochemistry (IHC) was performed on paraffin sections of lesions in all three animals, using protocols routinely applied for the detection of *T. gondii, Neospora caninum* or *E. cuniculi* and to stain macrophages and microglial cells (Iba-1+), and appropriate positive and negative controls. Briefly, a polyclonal rabbit anti-*T. gondii* (Neomarkers, Fremont, USA), a non-commercial mouse monoclonal anti-*N. caninum*, a non-commercial polyclonal rabbit anti-*E. cuniculi* and a polyclonal rabbit anti-Iba-1 (Wako Chemicals GmbH, Neuss, Germany) antibody were used, following previously published protocols.

The intralesional protozoan-like structures were strongly labelled with the antibody against *E. cuniculi* in all three animals (Fig. 1C, 2); they also reacted with the anti-*T. gondii* antibody, which is not specific for *T. gondii*, but known to also react with other apicomplexans, including *N. caninum, Isospora* spp. or *Eimeria* spp.

![Figure 2: A-C. Animal 1. A. Spleen. Granulomatous splenitis with abundant protozoan-like structures within intralesional macrophages (arrows). B. Heart. Protozoan-like structures are found within numerous endothelial cells of small vessels (arrowhead). C. Kidney with several pseudocystic structures within intact tubular epithelial cells (arrowheads). The adjacent interstitium exhibits a focal lymphoplasmacellular infiltration (arrow). D. Animal 2. Liver. Protozoan-like structures are found within mononuclear infiltrates in portal areas and within scattered hepatocytes (arrow) and Kupffer cells (arrowhead). A-D. Polyclonal rabbit anti- *E. cuniculi* antibody, horseradish peroxidase method (Envison; Dako); antibody binding visualised with diaminobenzidine, haematoxylin counterstain. A-D. Bars = 10 µm.](image-url)
Polymerase chain reactions (PCR) specific for *T. gondii* (RE sequence)\textsuperscript{25}, *N. caninum* (Nc5 region)\textsuperscript{21} and *E. cuniculi* (small-subunit rRNA gene)\textsuperscript{26} were performed on fresh tissue samples of liver, kidney and brains of all three animals. These were negative for *T. gondii* and *N. caninum*, but positive for *E. cuniculi*. Sequences of the PCR products (Synergene, Zürich, Switzerland) were identical to *E. cuniculi* sequences deposited in GenBank and identified them as genotype III.

Twenty-one months after these examinations, another adult male Barbary striped grass mouse (animal 4) was found dead in the enclosure. The diagnostic gross post mortem examination revealed a substantially reduced body condition, splenomegaly and enlargement of the mesenteric lymph nodes. The histological examination identified a moderate multifocal granulomatous encephalitis affecting the cortex, and a diffuse granulomatous pneumonia and myocarditis, with pseudocystic protozoan-like structures in endothelial cells, respiratory epithelial cells and macrophages within the lesions. IHC confirmed the protozoa as *E. cuniculi*. Spleen and mesenteric lymph nodes exhibited follicular hyperplasia. Interestingly, neither microsporidia nor inflammatory processes were observed in any other organ.

**Discussion**

*E. cuniculi* is an obligate intracellular microsporidian organism that commonly causes subclinical and occasionally lethal infections in rabbits, but can also infect a wide range of hosts, including mice, rats, muskrats, guinea pigs, hamsters, ground shrews, goats, sheep, pigs, horses, domestic fowl, and captive foxes, domestic cats, a variety of other carnivores, non-human primates, and humans\textsuperscript{6,8,27–31}. *E. cuniculi* infections lead to a variety of different manifestations in different species. In rabbits, the species which appears to be infected most frequently, neurological, renal, and/or ocular disease are the most common clinical manifestations of encephalitozoonosis. Sudden death is also seen, mainly with fulminant infections, in association with lesions in brain or myocardium\textsuperscript{2,32,33}. In Barbary striped mice and other closely related rodents, encephalitozoonosis seems to take a more fulminant, fatal course\textsuperscript{34}. A group of Steppe lemmings (*Lagurus lagurus*) presented with weight loss, aggressive behaviour, conjunctivitis and paresis, death occurred within 48 hours. There were no gross changes, but the histological examination revealed a pyogranulomatous inflammatory reaction in brain and kidneys, with intralobular microsporidian spores. *E. cuniculi* genotype III was identified as the cause\textsuperscript{34}. In other, systematically more distant species, such as canids, transplacental infection is more common, therefore, the disease is most frequent in young animals and manifests as anorexia and stunted growth, ataxia, tremors, posterior weakness, and blindness, with progression to circling, aggressive behaviour and convulsions, and in some cases death\textsuperscript{10,28,35,36}. In the blue fox and in primates, besides inflammatory processes with intralobular organisms in brain, kidneys, lungs, liver, and placentas, vasculitis and perivasculitis is a prominent histological feature of *E. cuniculi* infection, indicating its affinity for blood vessels, i.e. vascular endothelial cells\textsuperscript{29,30,35–37}.

To our knowledge this is the first case of encephalitozoonosis in Barbary striped mice, confirming the wide host range of *E. cuniculi*. Affected mice showed no changes suggestive of immunosuppression or concomitant disease, they neither exhibited clinical signs prior to death or euthanasia; these findings suggest subclinical infection in the colony, occasionally leading to death in individual immunocompetent animals. In affected mice, infection was associated with a multifocal granu-
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Granulomatous inflammatory response, mainly in brain (3/4 animals), lungs (2/4) and kidneys (1/4), with evidence of an affinity of the microsporidia for endothelial cells in these organs. The granulomatous encephalitis mainly affected cortex and brain stem, indicating a predilection site similar to that in rabbits, and the nephritis was consistent, though less extensive. The pathological findings in the brain, such as endothelial cell infection and protozoan uptake by macrophages in association with substantial inflammatory processes, suggest that the brain might be the primary target organ and also the site of persistence of E. cuniculi in Barbary striped grass mice. Previous studies have identified the brain as a site where parasites persist for a longer time period than in other organs, e.g. the kidneys. Uptake of protozoa by macrophages/microglia from infected (and destroyed) endothelial cells might hinder elimination of spores and trigger their persistence in the brain. In experimentally infected rats, a decrease or resolution of granulomas in liver, lung or kidneys with time and during disease progression has been described; this was correlated to the dose of infection.

The source of infection in this colony of Barbary striped grass mice was not identified, as the first occurrence of the disease was not associated with any incident, introduction of animals, or changes in housing. The population is closed since it was first established several years back with founder animals from another zoological collection in Germany. Interestingly, the same E. cuniculi genotype (type III, “dog strain”) has previously been identified in the same zoo more than 20 years ago, albeit in a colony of emperor tamarins (Saguinus imperator). Any connection between the two infections remains highly speculative, since the mice were not kept at the zoo at this time and are now housed in a building more than 100 m distant from the building of the affected primates. However, wild mice cannot be excluded as a source of the agents as these are often latent, seropositive carriers. E. cuniculi spores are highly resistant in the environment and can survive several months under humid conditions, for transmission direct contact with infected individuals is not required, and water-borne infections might be possible, facilitating wide spreading of the parasites in facilities housing or breeding animals.

Due to the clinical and histological presentation of encephalitozoonosis in rodents, the disease is difficult to differentiate from those caused by other protozoan pathogens, such as T. gondii and N. caninum, of which rodents are natural intermediate hosts and reservoirs. The differential diagnosis in animals that exhibit protozoan-like structures within several organs would include T. gondii, N. caninum, Sarcocystis spp. and E. cuniculi. E. cuniculi spores and T. gondii or N. caninum bradyzoites can be differentiated by light microscopy based on their morphology: T. gondii and N. caninum bradyzoites are 2-4 x 6-8 μm in size, form small cysts (30-100 μm diameter), are Gram-negative, not birefringent and not acid fast; E. cuniculi spores are smaller (1.5-2.5 μm) and form large pseudocysts (up to 120 μm diameter), are Gram positive, birefringent and acid fast. Differentiation between T. gondii and N. caninum is only possible at ultrastructural level, by IHC or based on their genome. However, T. gondii antibodies tend to cross react with, for example, N. caninum and E. cuniculi, whereas antibodies against E. cuniculi show no cross-reactivity with T. gondii, N. caninum, or other protozoa. Therefore, a combined IHC and molecular approach might be required for definite identification of the causative agent.

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La source de l’infection n’est pas connue. L’immunohistochimie et PCR avec séquençage ultérieur. Le parasite microsporidien Encephalitozoon cuniculi est un parasite intracellulaire obligatoire qui induit généralement des infections subcliniques chez les lapins, mais survient également dans toute une gamme d’autres espèces, y compris divers rongeurs, carnivores, humains et oiseaux. Le présent article décrit un cas d’encephalitozoonose chez un groupe de rats rayés de Barbarie (Lemniscomys barbarus) dans un zoo. L’étiologie a été confirmée par immunohistochimie et PCR avec séquençage ultérieur. La source de l’infection n’est pas connue.

**Mots-clés:** Encéphalitozoonose, microsporidies, rat rayé de Barbarie (Lemniscomys barbarus)

**Parole chiave:** Encephalitozoonosis, microsporidia, rat striato (Lemniscomys barbarus)

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**Infection à Encephalitozoon cuniculi chez des rats rayés de Barbarie (Lemniscomys barbarus)**

Encephalitozoon cuniculi est un parasite microsporidien intracellulaire obligatoire qui induit généralement des infections subcliniques chez les lapins mais survient également dans toute une gamme d’autres espèces, y compris divers rongeurs, carnivores, humains et oiseaux. Le présent article décrit un cas d’encephalitozoonose chez un groupe de rats rayés de Barbarie (Lemniscomys barbarus) dans un zoo. L’étiologie a été confirmée par immunohistochimie et PCR avec séquençage ultérieur. La source de l’infection n’est pas connue.

**Mots-clés:** Encéphalitozoonose, microsporidies, rat rayé de Barbarie (Lemniscomys barbarus)

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**Literature**

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