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Clinical symptoms and diagnosis of encephalitozoonosis in pet rabbits

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Abstract

Infections with *Encephalitozoon cuniculi* in rabbits are observed at increasing frequency and are known as opportunistic infections in immunocompromised humans. 191 pet rabbits with suspected encephalitozoonosis, presented at the Animal Hospital of the Veterinary University of Vienna (Austria), were included in this study. Rabbits were serologically examined for antibodies against *E. cuniculi* (144 positive out of 184 rabbits with suspected encephalitozoonosis compared to 14 positive out of 40 clinically healthy rabbits tested as part of a standard health check) and *Toxoplasma gondii* (8 positive out of 157). Of the 144 seropositive rabbits with clinical signs, 75% showed neurological symptoms, 14.6% demonstrated phacoclastic uveitis and 3.5% suffered from renal failure. 6.9% of the animals had combined symptoms. Vestibular disease dominated within the rabbits that showed neurological symptoms. Polymerase chain reaction (PCR) could not detect parasite DNA in urine or cerebrospinal fluid (CSF), but did so in 4 out of 5 samples of liquefied lens material in cases with phacoclastic uveitis due to lens capsule rupture. Additionally further diagnostic procedures, such as inspection of the external ear canal ($N = 69$), radiography of the tympanic bullae ($N = 65$) were performed to rule out differential diagnosis. 54.2% of the patients exhibiting neurological symptoms recovered within a few days, while 87.5% of the rabbits suffering from renal failure died or had to be euthanized.
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1. Introduction

Encephalitozoon cuniculi is an obligate intracellular microsporidium. The organism has a wide host

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distribution, but primarily affects rabbits. Nowadays *E. cuniculi* is also considered as an opportunistic pathogen in human patients with acquired immunodeficiency syndrome (AIDS) or other immunocompromised people (Zender et al., 1989; Deplazes et al., 1996; Wasson and Peper, 2000). In rabbits the central nervous system, kidneys and eyes are predilection sites. Histologically, lesions are most frequently recognized in the brain and kidney, consisting of focal nonsuppurative granulomatous meningoencephalitis and focal to segmental interstitial nephritis with variable degrees of fibrosis (Cox and Gallichio, 1978).

Serological testing is the commonly used method of diagnosing *E. cuniculi* infections. Nevertheless, the presence of antibodies only indicates chronic *E. cuniculi* infection but does not confirm the organism as a causative agent of disease. A definitive diagnosis in the living rabbit, however, is difficult. It is usually obtained by a combination of clinical–neurological/ophthalmological examination, serological verification and by ruling out differential diagnoses. Detection of *E. cuniculi* DNA by polymerase chain reaction (PCR) in secretions or body fluids of humans appears to be a sensitive and specific method to confirm the presence of the pathogen. However, PCR testing is not yet sufficiently evaluated for the detection of *E. cuniculi* DNA in rabbits with supposed encephalitozoonosis.

The aim of the study was to find out seroprevalences of rabbits with suspected encephalitozoonosis compared to asymptomatic rabbits. Furthermore different diagnostic procedures (particularly a PCR for the detection of parasite DNA in urine, CSF or liquefied lens material) for intravitam diagnosis of encpehalitozoonosis in naturally infected rabbits were assessed.

2. Materials and methods

2.1. Study population

191 pet rabbits showing symptoms of encephalitozoonosis were included in a clinical prospective study. The patients were presented to the Animal Hospital of the University of Veterinary Medicine (Vienna, Austria) between August 1999 and February 2006. Symptoms of encephalitozoonosis were termined as neurological symptoms (see below), signs of renal insufficiency (including polyuria, polydipsia, pollakisuria and azotaemia) and/or signs of phacoclastic uveitis (including cataract, uveitis and granuloma). Patients that showed one or more of the above-mentioned symptoms were included in the project.

2.2. Clinical examination

Each rabbit underwent a complete clinical examination, including a full history. In seropositive rabbits special attention was paid to clinical parameters such as general behaviour, body condition and hydration status. Food intake and course of disease were evaluated as well. Neurological symptoms of the group of seropositive rabbits were divided into three categories (vestibular disease, paresis/paralysis and miscellaneous). Signs of vestibular disease were classified as head tilt, ataxia, circling, rolling and spontaneous nystagmus. Head tilt in patients with vestibular disorders was divided into three categories, minor (less than 20°), moderate (20–45°) and high (higher than 45°).

2.3. Sampling and further diagnostic procedures

Blood was collected from the vena saphena lateralis of 184 affected rabbits. Serum samples of these animals were tested for the presence of specific antibodies against *E. cuniculi* and 157 samples were also tested for the antibodies against *T. gondii*. In both cases serological diagnosis was carried out by an indirect immunofluorescent antibody test (IFAT) using spores (*E. cuniculi*) or cell-culture derived tachyzoites (*T. gondii*) as antigen. Anti-rabbit IgG (heavy and light chains) fluorescein-conjugated IgG-fraction goat conjugate (Southern Biotechnology Ass. Inc., Birmingham, USA; PBS dilution 1:100) was used for the IFAT to detect antibodies against *E. cuniculi*. The positive threshold titre for *E. cuniculi* was 1:40 (Suter et al., 2001). Each serum was tested and titrated from 1:40 to 1:10 000 in two bold steps. Additionally, blood samples from 54 rabbits showing no symptoms of encephalitozoonosis were tested for the presence of antibodies against *E. cuniculi*. 40 of these 54 animals, including 10 from a breeding stock of laboratory rabbits were tested as part of a health check. The remaining 14 were tested because they lived in close contact to rabbits with a confirmed *E. cuniculi* infection.

In case of vestibular disease ($N = 69$), rabbits underwent an otoscopic examination of the external ear canal and, if possible, also the tympanic bullae were examined ($N = 65$) radiographically. A complete ophthalmic examination was performed in rabbits with signs of phacoclastic uveitis. 45 rabbits that died or had to be euthanized underwent post-mortem examination.

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