The objective of this paper was to find out whether the immunosuppression caused by dexamethasone can have an effect on the activation of encephalitozoonosis in experimentally infected mice and, simultaneously, whether Encephalitozoon cuniculi itself has an immunosuppressive effect on the course of this infection. Immunocompetent mice of the ICR line suppressed by dexamethasone (1.6 mg.kg(-1)) were infected experimentally with microsporidium E. Cuniculi by intraperitoneal administration of 5 x 10(7) spores. During 42 d of the experiment, the values of differential white blood count, CD4(+) and CD8(+) T-lymphocytes, as well as CD 19(+) B-lymphocytes in blood were observed and recorded. The achieved results point to the fact that E. Cuniculi itself in the early stages of infection leads to a considerable decrease in the number of leukocytes and causes suppression of T-cell immune response. In combination with dexamethasone it also results in the suppression of both T and B-lymphocytes.
against IFAT by testing of 205 field samples from blue foxes. There was high agreement between the results of the ELISA and CIA (kappa=0.99), and the ELISA and IFAT (kappa=0.958). There was no significant statistical difference between the tests (p>0.05). It was concluded that the ELISA could be used to identify seropositive farmed blue foxes. The advantage of the ELISA lies in the potential of screening large numbers of animals with the goal of eradicating E. Cuniculi infection in the farms.

Authors: M Halanova, L Cislakova, A Valencakova, P Balent, J Adam, M Travnicek
Title: Serological screening of occurrence of antibodies to *Encephalitozoon cuniculi* in humans and animals in Eastern Slovakia
Full source: Annals of Agricultural and Environmental Medicine, 2003, Vol 10, Iss 1, pp 117-120
Author keywords: microsporidia; *Encephalitozoon cuniculi*; human and animal infection; IFA
KeyWords Plus: MURINE ENCEPHALITOZOONOSIS; MICROSPORIDIOSIS; INFECTION; RABBITS; PARASITE; PATIENT; MODEL
TGA/Book No.: 696AY
Discipline: Veterinary Science & Medicine
Document type: Article
Language: English
Address: Halanova M,Safarik Univ, Fac Med, Dept Epidemiol, Srobarova 2, Kosice 04180, SLOVAKIA
ISBN/ISSN: 1232-1966
Publisher: Inst Agricultural Medicine, Jazewskiego 2, PO Box 185, 20-950 Lublin, Poland
Abstract: Encephalitozoon cuniculi is one of the mammalian microsporidian pathogens that can affect a number of different species of animals as well as humans. The presence of specific serum antibodies to *Encephalitozoon cuniculi* was studied in a group of animals and humans from Eastern Slovakia by the indirect immunofluorescence of antibodies (IFA). 456 people, 571 rabbits, 457 mice, 193 dogs, 72 cats, and 59 sheep were examined. Specific anti-E. Cuniculi antibodies were found in 26 out of 456 human sera examined (5.7%). The highest occurrence of antimicrosporidial antibodies was found in the group of immunodeficiency patients -37.5%. In the group of animals, the highest positivity was observed in rabbits -41.7%, and in dogs -37.8. The relative high prevalence, especially in rabbits and dogs as potential sources of microsporidial infection for humans, indicates the importance of performing the screening examinations in animals with aim of reducing or halting the spread of this disease.

Authors: F Guscetti, A Mathis, JM Hatt, P Deplazes
Title: Overt fatal and chronic subclinical *Encephalitozoon cuniculi* microsporidiosis in a colony of captive emperor tamarins (Saguinus imperator)
Full source: Journal of Medical Primatology, 2003, Vol 32, Iss 2, pp 111-119
Author keywords: histopathology; immunohistochemistry; isolation; microsporidia; polymerase chain reaction; serology
KeyWords Plus: MONKEYS SAIMIRI-SCIUREUS; ENTEROCYTOZOON-BIENEUSI; IDENTIFICATION; MACAQUES; PARASITE; RABBITS; STRAINS; DOGS
TGA/Book No.: 674XU
Discipline: Veterinary Science & Medicine
Document type: Article
Language: English
Address: Guscetti F, Stanford Univ, Sch Med, Div Radiat Biol, Dept Radiat Oncol, CCSR S, Room 1240, 269 Campus Dr, Palo Alto, CA 94304 USA
ISBN/ISSN: 0047-2565
Publisher: Blackwell Munksgaard, 35 Norre Sogade, PO Box 2148, Dk-1016 Copenhagen, Denmark
Abstract: The course of an infection with the microsporidian *Encephalitozoon cuniculi* in a colony of captive emperor tamarins (Saguinus imperator) is described. In two litters, the infection was associated with overt disease and death of all infants. Immunohistochemistry for E cuniculi showed generalized infections, and histopathologic evaluation revealed systemic vasculitis and disseminated mixed inflammatory cell infiltration with and without necrosis in several organs. Serologically, some of the juvenile animals presented with high titres for *Encephalitozoon*, while the...
adults had low titres. The E cuniculi 'dog strain' was identified by molecular means for the first time in Europe. The origin of the infection appeared to be a pair of breeding adults that originated from the US. Our data suggest that the organism persisted over years in the colony, and that subclinically infected animals most likely were involved in perpetuating the infection. Efforts should be made to ascertain if this microorganism is present in other captive populations of this endangered monkey species and to prevent its further spreading.

**Authors:** J Akerstedt  
**Title:** Humoral immune response in adult blue foxes (Alopex lagopus) after oral infection with *Encephalitozoon cuniculi* spores  
**Full source:** Veterinary Parasiology, 2003, Vol 113, Iss 3-4, pp 203-210  
**Author keywords:** Alopex lagopus; CIA; ELISA; *Encephalitozoon cuniculi*; experimental infection; fox; humoral immune response; microsporidia; western blot  
**KeyWords Plus:** NOSEMATOSIS; STRAINS; RABBITS  
**TGA/Book No.:** 677JW  
**Discipline:** Veterinary Science & Medicine  
**Document type:** Article  
**Language:** English  
**Address:** Akerstedt J,Natl Vet Inst,POB 8156 Dep,NO-0033 Oslo,NORWAY  
**ISBN/ISSN:** 0304-4017  
**Publisher:** Elsevier Science Bv,PO Box 211,1000 AE Amsterdam,Netherlands  
**Abstract:** Encephalitozoon cuniculi causes severe diseases in blue fox puppies. When pregnant vixens are infected, parasites are transmitted over the placenta to the unborn that subsequently develop encephalitozoonosis. Adult foxes themselves do not have signs of disease, but show antibody titres to E. Cuniculi. The purpose of the present study was to gain information on the immune response in adult foxes after experimental infection. Sixteen foxes were infected orally with E. Cuniculi spores, eight of them twice and 28 days apart. The two groups of animals showed elevated serological values in both the carbon immunoassay and in the ELISA. Elevated serological levels were recorded up to 1 year after the infection took place. The control group (n = 8) remained serologically negative throughout the trial. The results of the study showed that blue foxes could be seropositive for at least a year after oral infection with E. Cuniculi. (C) 2003 Elsevier Science B.V. All rights reserved.

**Authors:** JC PattersonKane, P Caplazi, F Rurangirwa, RR Tramontin, K Wolfsdorf  
**Title:** *Encephalitozoon cuniculi* placentitis and abortion in a Quarterhorse mare  
**Full source:** Journal of Veterinary Diagnostic Investigation, 2003, Vol 15, Iss 1, pp 57-59  
**TGA/Book No.:** 667VU  
**Discipline:** Veterinary Science & Medicine  
**Document type:** Article  
**Language:** English  
**Address:** Patterson-Kane JC,Univ London Royal Vet Coll,Dept Pathol & Infect Dis,Hawkshead Lane,Hatfield AL9 7TA,Herts,ENGLAND  
**ISBN/ISSN:** 1040-6387  
**Publisher:** Amer Assoc Veterinary Laboratory Diagnosticians Inc,PO Box 1522,Turlock,CA 95381,USA  
**Abstract:** *Encephalitozoon cuniculi* is a microsporidian parasite, which has rarely been reported to cause placentitis in animals. A late-term aborted fetus and placenta from a Quarterhorse were presented to the Livestock Disease Diagnostic Center, University of Kentucky, for diagnostic examination. There was a necrotizing placentitis, with distension of many chorionic epithelial cells by intracytoplasmic vacuoles containing 1-2-mum-diameter, elongated, gram-positive organisms. The organisms were identified as E. Cuniculi by electron microscopy and by polymerase chain reaction using primers to microsporidial ribosomal DNA. Joints of the fetus were swollen, with gross and microscopic lesions of synovitis; however, E. Cuniculi DNA was not detected.
Authors: PJR Baneux, F Pognan
Title: In utero transmission of Encephalitozoon cuniculi strain type I in rabbits
Full source: Laboratory Animals, 2003, Vol 37, Iss 2, pp 132-138
Author keywords: Encephalitozoon cuniculi; rabbits; vertical transmission; transplacental infection
KeyWords Plus: MICROSPORIDIA; DIAGNOSIS; IDENTIFICATION; INFECTION; SEQUENCES
TGA/Book No.: 667QY
Discipline: Veterinary Science & Medicine
Document type: Article
Language: English
Address: Baneux PJR,Pfizer Global R&D,Comparat Med,BP 159,F-37401 Amboise,FRANCE
ISBN/ISSN: 0023-6772
Publisher: Royal Soc Medicine Press Ltd,1 Wimpole Street,London W1M 8AE,England
Abstract: Pregnant rabbits were serologically diagnosed as having been infected with Encephalitozoon cuniculi. At necropsy at 28 days of gestation, does, placentas and fetuses were found to be infected with E. Cuniculi strain type I as evidenced by using the nested-polymerase chain reaction (PCR) technique, thereby confirming vertical transplacental transmission.

Authors: FM HarcourtBrown, HKR Holloway
Title: Encephalitozoon cuniculi in pet rabbits
Full source: Veterinary Record, 2003, Vol 152, Iss 14, pp 427+
KeyWords Plus: PHACOCLASTIC UVEITIS; INFECTION
TGA/Book No.: 673BK
Discipline: Veterinary Science & Medicine
Document type: Article
Language: English
Address: Harcourt-Brown FM,30 Crab Lane,Harrogate HG1 3BE,N Yorkshire,ENGLAND
ISBN/ISSN: 0042-4900
Publisher: British Veterinary Assoc,7 Mansfield St,London W1M 0at,England
Abstract: The results of a serological test for Encephalitozoon cuniculi in 125 pet rabbits are reviewed, together with follow-up studies of clinical cases. Blood samples were taken from 38 asymptomatic rabbits and 87 rabbits showing neurological, renal or ocular signs suggestive of encephalitozoonosis. In the asymptomatic group, six of 26 (23 per cent) apparently healthy rabbits, sampled as part of a health screen, were seropositive; of the remaining 12 asymptomatic rabbits, sampled because they lived with seropositive companions, eight (66 per cent) were seropositive. Fifty-eight of the rabbits with clinical disease showed neurological signs, including head tilt, seizures, ataxia and swaying; three of them also showed renal signs and two showed ocular signs, and these five rabbits were all seropositive. Head tilt was the most common neurological sign in 21 of 23 (91 per cent) of the seropositive cases. All nine rabbits with ocular lesions were seropositive. In follow-up studies of clinical cases, the rabbits showed variable responses to treatment with albendazole, fenbendazole, antibiotics or corticosteroids, and some cases recovered without treatment.

Authors: J Akerstedt, CMO Kapel
Title: Survey for Encephalitozoon cuniculi in arctic foxes (Alopex lagopus) in Greenland
Author keywords: Alopex lagopus; arctic fox; Encephalitozoon cuniculi; Greenland; microsporidia; parasitic zoonosis; survey
KeyWords Plus: BLUE FOXES; RABBITS; IDENTIFICATION; NOSEMATOSIS; ANTIBODIES; DIAGNOSIS; STRAINS
TGA/Book No.: 661FL
Discipline: Veterinary Science & Medicine
Document type: Article
Language: English
Address: Akerstedt J,Natl Vet Inst,POB 8156 DEP,NO-0033 Oslo,NORWAY
ISBN/ISSN: 0090-3558
Publisher: Wildlife Disease Assn, Inc,810 East 10Th St,Lawrence,KS 66044-8897,USA
Abstract: Wild arctic foxes (Alopex lagopus) from Greenland were tested for antibodies to Encephalitozoon cuniculi with an enzyme-linked immunosorbent assay and a carbon immunoassay. Of 230 tested foxes none was seropositive. This finding contrasts with observations from other arctic areas and absence of rodents in the diet of these arctic foxes is the most likely explanation for absence of E. Cuniculi.

Authors: MA Lallo, MJ dosSantos, EF Bondan

Title: Experimental Encephalitozoon cuniculi infection in dexamethasone-immunosuppressed mice

Full source: Revista de Saude Publica, 2002, Vol 36, Iss 5, pp 621-626 (Selectively Covered)

Author keywords: immunocompromised host; encephalitozoon cuniculi; encephalitozoosis; animal models, disease; mice; inbred Balb C; dexamethasone immunosuppressed

KeyWords Plus: MICROSPORIDIOSIS; IMMUNOCOMPETENT; IMMUNODEFICIENT; MECHANISMS; CELLS

TGA/Book No.: 630UJ

Discipline: Veterinary Science & Medicine

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Language: Portuguese

Address: Lallo MA, Rua Batataes, 525 Apto 152, BR-01423010 Sao Paulo, SP, BRAZIL

ISBN/ISSN: 0034-8910

Publisher: Revista De Saude Publica, Faculdade Saude Publ da USP, AV Dr Maldo 715, 01255 Sao Paulo, Brazil

Abstract: Objective Microsporidian Encephalitozoon cuniculi has been recognized as an opportunistic pathogen in immunosuppressed individuals, such as AIDS patients. The objective of the study was to develop pharmacologically immunosuppressed animals as a model of the natural occurring E. Cuniculi infection.

Methods Distinct groups of adult Balb-C mice were immunosuppressed with different doses of dexamethasone (Dx, 3 or 5 mg/kg/day, intraperitoneal route - IP) and inoculated with E. Cuniculi spores by IP route intraperitoneally. Control groups (inoculated animals but non-immunosuppressed and non-inoculated animals but immunosuppressed) were also used. The spores of E. Cuniculi were previously cultivated in MDCK cells. The animals were sacrificed and necropsied at 7, 14, 21, 28 and 35 days post-inoculation. Tissue fragments were collected and processed for light microscopy studies, using Gram-chromotrope and hematoxylin-eosin staining techniques.

Results In all immunosuppressed and inoculated inoculated immunosuppressed mice, specially in those that received 5 mg/kg/day of dexamethasone, the most prominent necropsy findings were hepatomegaly and splenomegaly. The experimental inoculation resulted in a disseminated non-lethal infection, characterized by granulomatous lesions in several organs (liver lungs, kidneys, gut and brain) but notably in the hepatic tissue. Spores of E. Cuniculi were only seen in few animals treated with 5 mg/kg/day of Dx at 35 days post-infection.

Conclusions Microsporidiosis in Dx-immunosuppressed mice provides a useful model for studies of the microsporidial infection, resembling that one naturally occurring in immunodeficient individuals with AIDS.

Authors: E Keeble

Title: Encephalitozoon cuniculi in rabbits

Full source: Veterinary Record, 2002, Vol 151, Iss 22, pp 680

TGA/Book No.: 626TH

Discipline: Veterinary Science & Medicine

Document type: Letter

Language: English

Address: Keeble E, Royal Dick Sch Vet Studies, Hosp Small Anim, Easter Bush Vet Ctr, Roslin EH25 9RG, Midlothian, SCOTLAND

ISBN/ISSN: 0042-4900

Publisher: British Veterinary Assoc, 7 Mansfield St, London W1M 0at, England
Microsporidia are obligate intracellular parasites of the phylum Microspora. To date, more than 1,200 species within 144 genera have been described, with 14 infecting humans. Currently, no effective treatment exists for human microsporidiosis. In this study, the biochemical properties of the aminopeptidases were investigated within several species of microsporidia. Aminopeptidase activity was detected in 3 species of microsporidia, Encephalitozoon cuniculi, E. Hellem, and Vittaforma corneae, using a fluorometric substrate assay. Each species exhibited distinct aminopeptidase properties. The cytosolic neutral aminopeptidase activities of the Encephalitozoon spp. Were characterized as preferentially cleaving leucine, whereas those of V. Corneae cleaved arginine. Native polyacrylamide gel electrophoresis estimated the molecular mass of E. Cuniculi, E. Hellem, and V. Corneae as 74, 72, and 79 kDa, respectively. Enzymatic activity was inhibited by bestatin and its analogue, nitrobestatin, indicating that the enzyme was an aminopeptidase for all species. Inhibition with the chelating agents ethylenediaminetetraacetic acid and 1,10-phenanthroline characterized the enzymes as metalloaminopeptidases. Subcellular fractionation of the 3 microsporidial species suggested that the enzyme activity was localized in the cytosolic fraction. Optimal enzyme activity was observed at pH 7.2 for all species. This is the first report of enzyme characterization from these 3 species of microsporidia.

Phacoemulsification was performed on a New Zealand White rabbit with slowly progressive unilateral phacoclastic uveitis and cataract formation. The irrigating solution with lenticular contents were centrifuged and examined cytologically using Weber's chromotrope-based stain. Microsporidial spores were observed and positively identified as Encephalitozoon cuniculi via polymerase chain reaction. More than 1 year following surgical therapy, the rabbit is visual and comfortable without medications.
First isolation and characterisation of *Encephalitozoon cuniculi* from a free-ranging rat (*Rattus norvegicus*)

The microsporidian species *Encephalitozoon cuniculi* can infect a wide variety of mammals including man. It is a common parasite in rabbits and several sporadic infections in laboratory rats have been described. Based on molecular data three *E. Cuniculi* strains have been identified. Here we describe the first in vitro propagation of *E. Cuniculi*, which was isolated from a free-ranging rat (*Rattus norvegicus*). The rat was one of three seropositive animals among 23 rats captured in the city of Zurich. The new isolate was further characterised as strain II ("mouse"-strain) based on the rDNA internal transcribed spacer sequence. Western blot analysis of this isolate revealed slight differences to other available strain 11 isolates originating from laboratory mice and farmed blue foxes. The new isolate caused disseminated infection in liver and lung upon oral inoculation of Brown Norway (BN) rats and was transmitted to sentinel rats. This rat-adapted isolate will be valuable to study the pathogenesis of *Encephalitozoon* infections in the rat model. (C) 2002 Elsevier Science B.V All rights reserved.

Comparison of the significance of CD4+ and CD8+ T lymphocytes in the protection of mice against *Encephalitozoon cuniculi* infection

The role of T lymphocyte subpopulations in the protection against intraperitoneal (i.p.) and peroral *Encephalitozoon cuniculi* infections was compared in adoptive-transfer experiments using severe combined immunodeficient mice. Whereas CD8+ T cell-depleted, but not CD4+ T cell-depleted, BALB/c splenocytes failed to protect the mice against i.p. infection, only SCID mice reconstituted with both CD4+ T lymphocyte- and CD8+ T lymphocyte-depleted splenocytes succumbed to peroral infection. The results indicate that whereas CD8+ T cells are critical for the protection against an i.p. *E. Cuniculi* infection, both CD4+ and CD8+ T lymphocyte subpopulations play a substantive protective role in a peroral infection, i.e., natural route of infection.

Serological examination of rabbits for *Encephalitozoon cuniculi* infection

The results indicate that whereas CD8+ T cells are critical for the protection against an i.p. *E. Cuniculi* infection, both CD4+ and CD8+ T lymphocyte subpopulations play a substantive protective role in a peroral infection, i.e., natural route of infection.
The ribosomal RNA (rRNA) gene region of the microsporidium Heterosporis anguillarum has been examined. Complete DNA sequence data (4060 bp, GenBank Accession No. AF402839) of the rRNA gene of H. anguillarum are presented for the small subunit gene (SSU rRNA: 1359 bp), the internal transcribed spacer (ITS: 37 bp), and the large subunit gene (LSU rRNA: 2664 bp). The secondary structures of the H. anguillarum SSU and LSU rRNA genes are constructed and described. This is the first complete sequence of an rRNA gene published for a fish-infecting microsporidian species. In the phylogenetic analysis, the sequences, including partial SSU rRNA, ITS, and partial LSU rRNA sequences of the fish-infecting microsporidia, were aligned and analysed. The taxonomic position of H. anguillarum as suggested by Lom et al. (2000; Dis Aquat Org 43:225-231) is confirmed in this paper.

Microsporidia of the genus Encephalitozoon are emerging protozoal agents that mainly infect immunocompromised patients with AIDS. At present, disseminated infections with members of the genus Encephalitozoon can only be successfully treated with albendazole. As chitin is a basic component of the microsporidian spore, we evaluated, in vitro, the susceptibility of a human-derived strain of Encephalitozoon cuniculi to polyoxin D and nikkomycin Z, which are known competitive inhibitors of chitin synthetase enzymes. Using an in vitro assay, polyoxin D at 1, 10 and 100 μg/ml significantly reduced the number of parasitic foci on days 6, 9, and 15 post-infection. However, nikkomycin Z revealed a marked but lower reduction in the number of parasitic foci than polyoxin D. A significant reduction of parasitic foci was achieved for nikkomycin Z at 10 and 100 μg/ml up to day 9 post-infection. Polyoxin D was approximately tenfold more effective in our in vitro assay than nikkomycin Z.

Study on antibody prevalence to microsporidian Encephalitozoon cuniculi in dogs (Canis familiaris) using indirect immunofluorescence
Several species of mammals including the dog seem to be potential sources of encephalitozoonosis for animal as well as human hosts. The presence of specific serum antibodies to Encephalitozoon cuniculi was studied in a group of 178 dogs from Eastern Slovakia. The indirect fluorescence of antibodies was chosen as a diagnostic method. Entire cell corpuscular antigen of the in vitro grown microsporida and swine anti-dog FITC-conjugated immunoglobulin were used in the IFA test.

The animals whose sera reacted by immunofluorescence in a titre of at least 1: 20 were considered positive. Specific anti-E. cuniculi antibodies were found in 53 out of the 178 dogs investigated (29.8 %). Two thirds of the serum positive samples (37 out of 53) were in the lower titre range of 1: 20 to 1: 40. Sixteen dogs were seropositive at a dilution from 1: 80 to 1: 320. The other 125 dogs (70.2 %) showed seronegative reactions.

Our screening results indicated that the examination of canine sera for the presence of anti-E. Cuniculi antigens is of great importance especially in dogs with health problems. The IFA test is a very sensitive method for detection of early microsporidian infection in dogs and for indication of very small amounts of specific post-infectious antibodies.

The presence of antibodies against Encephalitozoon cuniculi in Angora goats was detected by the method of indirect immunofluorescence (IFAT). The animals reacting at the titre 1:64 and more were considered positive. Of the total number of 48 sera examined, 4 were positive at the titre 1:32 and 2 were positive at the titre 1:64. The occurrence of antibodies against E. Cuniculi indicates that one of the causes of disorders in the reproductive cycle in Angora goats may be microsporidia Encephalitozoon cuniculi, and that these animals may be potential sources of infection for people.
Microsporidia are obligate intracellular parasites infesting many animal groups (1). Lacking mitochondria and peroxysomes, these unicellular eukaryotes were first considered a deeply branching protist lineage (2) that diverged before the endosymbiotic event that led to mitochondria. The discovery of a gene for a mitochondrial-type chaperone (3-5) combined with molecular phylogenetic data (6-9) later implied that microsporidia are atypical fungi that lost mitochondria during evolution. Here we report the DNA sequences of the 11 chromosomes of the similar to 2.9-megabase (Mb) genome of Encephalitozoon cuniculi (1,997 potential protein-coding genes). Genome compaction is reflected by reduced intergenic spacers and by the shortness of most putative proteins relative to their eukaryote orthologues. The strong host dependence is illustrated by the lack of genes for some biosynthetic pathways and for the tricarboxylic acid cycle. Phylogenetic analysis lends substantial credit to the fungal affiliation of microsporidia. Because the E. Cuniculi genome contains genes related to some mitochondrial functions (for example, Fe-S cluster assembly), we hypothesize that microsporidia have retained a mitochondrion-derived organelle.