Detection of *Encephalitozoon cuniculi* in the feline cataractous lens

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ABSTRACT

Purpose. Identification of *Encephalitozoon cuniculi* (*E. cuniculi*) as a possible causative agent for cataracts and uveitis in cats.

Methods. Within a 12 month study period, cats that were presented with focal anterior cortical or mature cataract and secondary uveitis underwent a complete ophthalmic examination, complete blood count, serum biochemistry, serologic tests for *E.cuniculi* and tests for *feline immunodeficiency virus* (*FIV), feline infectious peritonitis (FIP), feline leukemia virus (FeLV)* and *Toxoplasma gondii (T. gondii)*. PCR for DNA detection of *E. cuniculi* and *T. gondii* as well as cytologic examination of aqueous humor after paracentesis and phacoemulsified lens material were also performed. In addition histopathologic examination of the resected anterior lens capsule and attached lens epithelial cells was done. Serologic testing for antibodies against *E. cuniculi* was also performed in 100 ophthalmologically healthy cats.

Results. 11 (19 eyes) European shorthair cats with a median age of 3.5 years were included. 9/11 cats had bilateral cataracts, with 12/19 eyes having focal anterior cortical cataracts and 7/19 eyes having mature cataracts. In 14/19 eyes anterior uveitis was present. All cats had a positive antibody titer (1:80 – 1:10,000) for *E. cuniculi*. *E. cuniculi* DNA was detected by PCR and sequencing in 18/19 lenses and in 10/19 aqueous samples. Five tentative positive results were detected by cytologic examination. Spores were detected in 15/19 samples of lens material with histopathologic staining. Only 2/100 ophthalmologically healthy cats showed a positive antibody titer for *E. cuniculi*.

Conclusion. E. cuniculi is a cause of focal anterior cortical cataract and anterior uveitis in cats.

INTRODUCTION

Encephalitozoon cuniculi (E. cuniculi), an obligate intracellular, spore-forming, microsporidial parasite can infect many mammalian hosts, including humans.[1] Different diagnostic methods are described for the detection and identification of *E. cuniculi*. Transmission electron microscopy, light microscopy for cytologic and histopathologic examination, cell culture, serologic testing and polymerase chain reaction (PCR) testing are reported.[1] Three strains of *E. cuniculi* (strain I: rabbit strain; strain II: mouse strain; strain III: dog strain) have already been identified using PCR.[2]

E. cuniculi infection can be found in all organs, however the predominant affected sites are the kidneys, the central nervous system and the eyes.[3] Ocular manifestations have already been described in rabbits,[4-9] blue fox puppies,[10] mink,[11] cats[12, 13] and humans.[14-20] In rabbits, *E. cuniculi* causes cataract formation with lens capsule rupture and phacoclastic uveitis.[4-9] In blue fox puppies, severe cataract formation, inflammatory vascular lesions in the posterior ciliary arteries and necrotic retinal areas have been described.[10] Cataracts are seen as the ocular manifestations in mink.[11] One case of keratitis[12] and one case of cataract and uveitis[13] in cats have been published. Keratoconjunctivitis,[14] keratitis,[15-17] uveitis[18] and endophthalmitis[19, 20] are found in humans, particularly in immune-compromised patients.

In the last few years, cats with focal anterior cortical cataracts and therapy-resistant anterior uveitis were presented at our clinic. In many cases, the cause of the cataract and uveitis could not be determined. *E. cuniculi* causes cataracts with anterior lens capsule rupture in rabbits[4-8] and in one described case in the cat.[13] Based on these findings we hypothesized that *E. cuniculi* could be the causative agent of a focal anterior cortical cataract and uveitis in cats. To investigate our hypothesis, serologic testing in healthy patients as well as in cataract patients, PCR and cytology of

aqueous humor samples and phacoemulsified lens material as well as histopathologic examination of anterior lens capsules were performed.

MATERIAL AND METHODS

Eleven European shorthair cats (from a total number of 322 feline patients presented at the ophthalmology department of the Veterinary University in Vienna) with cataract and uveitis were included in the study during a one year study period (April 2009 – April 2010). Use of the cats for this study was approved by Institutional Animal Care and Use Committee of the Veterinary University in Vienna. The study was conducted in accordance with institutional guidelines and the knowledge of the owners.

Ophthalmic and physical examination and blood work

A complete ophthalmic examination before and after mydriasis with 0.5% tropicamide (Mydriaticum 'Agepha', Vienna, Austria) was performed, including direct and indirect ophthalmoscopy (Heine Video Omega 2C of Heine Optotechnik GmbH & CO KG, Hersching, Germany), slit lamp biomicroscopy (Kowa portable Slit-lamp SL-14 of CR Medical, Linz, Austria), fluorescein staining (Fluorescein Papier; Haag-Streit; Koeniz, Switzerland) and tonometry (Tono-Pen^{TM}XL Tonometer, Croma Pharma GmbH, Vienna, Austria). Photographic documentation (Kowa Genesis; Neumed, Harmannsdorf, Austria) was done after the ophthalmic examination. A physical examination, complete blood counts (CBC) and biochemical analysis were also performed.

Serology

In order to exclude other causes of uveitis antibody tests for feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP), and Toxoplasma gondii (T. gondii) (IgM

and IgG antibody test) and antigen testing feline leukemia virus (FeLV) were performed. An indirect immunofluorescence test (IIFT) was used to detect antibodies (IgG) against *E. cuniculi*. Serum dilutions from 1:20 to 1: 10,000 in twofold steps were applied to wells containing fixed spores on glass slides. Fluorescein isothiocyanate (FITC)-conjugated AffiniPure Goat Anti-Cat-IgG (Jackson ImmunoResearch Laboratories, Suffolk, United Kingdom) was used as the conjugate at a dilution of 1:50. Each IIFT run included a negative control. Positive control serum was obtained from a cat with an ocular *E. cuniculi* infection confirmed by PCR and sequencing using a previously published protocol.[13] Titers of 1:40 or higher were considered positive based on results from IIFT in rabbits infected with *E. cuniculi*.[21]

Cats with a positive antibody titer for *E. cuniculi* were treated with fenbendazole (Panacur®250mg Intervet, Vienna, Austria) at a dose of 20mg/kg once daily for 3 weeks. Cats with a positive antibody titer for *T. gondii* were treated with clindamycin (Antirobe®25mg, Pfizer, Vienna, Austria) at a dose of 10mg/kg twice daily for 3 weeks. All cats received uveitis treatment which consisted of dexamethasone-oxytetracycline ointment (Corti-Biciron®N, S&K Pharma, Rüdersdorf, Germany) three times daily.

Phacoemulsification

Before phacoemulsification cats received acepromazine (Vanastress® Vana GmbH, Vienna, Austria) at 0.1mg/kg and carprofen (Rimadyl® Pfizer, Vienna, Austria) at 4mg/kg IV. Thirty minutes before the operation phenylephrine (Neosynephrin-POS 5%® Ursapharma, Saarbrücken, Germany) solution was instilled in the eye with cataract. Anesthesia was induced with propofol (Propofol”Fresenius”1%®, Fresenius Kobi Austria, Graz, Austria) to effect and maintained with fentanyl (Fentanyl Janssen 0.5mg-Ampullen®, Janssen Cilang Pharma, Vienna, Austria) at 0,02mg/kg/h as well

as isoflurane in 100% oxygen. Before surgery an electroretinogramm (ERG) (Retinographics BPM-100 system; Eickemeyer. Tuttlingen, Germany) and ultrasound (Logiq 200; GE Ultrasound Europe, Solingen, Germany) of the eyes were performed. For surgery each cat was positioned in dorsal recumbency, with the head stabilized using a vacuum pillow under the operation microscope (Operationsmikroskop Opmi®CS-I; Zeiss, Oberkochen, Austria). The eyes were cleaned with 0.5% povidone iodine solution. Rocuronium (Esmeron® 10mg/ml, N.V. Organon, Oss, Netherland) at 0,6mg/kg a neuromuscular blocking agent, was injected intravenously. Afterwards the cats were ventilated by a standard small animal ventilator. An eyelid speculum was placed and anterior chamber paracentesis was performed at the 12 o'clock position. Aqueous humor (0.3-0.5ml) was aspirated with a 1ml syringe by use of a 25-gauge needle. Immediately after aspiration a corneal incision was made. Epinephrine (Suprarenin 1ml Ampullen® Sanofi Aventis, Frankfurt, Germany) and viscoelastic material (Acrivet Biovisc1.2%®, Acrivet, S&V Technologies AG, Henningsdorf, Germany) were injected into the anterior chamber and, after 1 minute, a continuous-tear curvilinear capsulorhexis was performed, when possible including the capsular opacity. The anterior lens capsule was collected for histopathologic examination. Subsequently, one-handed phacoemulsification and irrigation-aspiration were performed. Lens material from this procedure was collected for further investigation. In some cats a posterior capsulorhexis was performed because of a posterior capsular opacification. In these cases the posterior lens capsule was also collected for histopathology. In one eye of one cat, an intraocular lens (IOL)(AcriLyc, +53,5 Dpt, Acritec, S&V Technologies AG, Henningsdorf, Germany) was inserted into the capsular bag. The corneal incision was closed with simple corneal sutures (Polyglactin 910, Vicryl® 9/0 Johnson – Johnson Int.) and air was injected into the anterior chamber.

All cats were treated with dexamethasone-oxytetracycline ointment six times daily and carprofen (Rimadyl® Pfizer, Vienna Austria) at 2mg/kg once daily postoperatively. The cat which had an IOL implanted also received atropine ointment (Atropin 1%®, Allerheiligenapotheke, Vienna, Austria) once daily. The duration of treatment was dictated by the clinical findings.

PCR

PCR was performed on samples of aqueous humor and liquefied lens material, obtained by phacoemulsification. For DNA extraction the samples were centrifuged at 375 x *g* for 15 min. 50µl of the sediment was used for DNA isolation performed with a High Pure PCR Template Preparation Kit (Roche Diagnostics, Vienna, Austria). The procedure was performed starting with the lysis buffer according to the manufacturer's instructions.

Toxoplasma gondii PCR: Liquefied lens material and aqueous humor were assayed by PCR with the primers Toxo 529 for (5` CGC TGC AGG GAG GAA GAC GAA AGT TG 3`) and Toxo 529 ref (5` CGC TGC AGA CAC AGT GCA TCT GGA TT 3`) according to the protocol described by Homan[22] with minor changes. The reaction of 25µl contained 5µl of DNA, 10 pmol of each primer Toxo529for and Toxo529ref, 200µM of each dNTP, 0.625 U Go Taq® polymerase (Promega, Mannheim, Germany), 5µl 5x Green Go Taq® reaction buffer (including 1.5mM magnesium chloride; Promega, Mannheim, Germany). Thirty-five cycles were performed with denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min.

Encephalitozoon spp. PCR: A standard PCR with one primer pair and a nested PCR were performed. At first the samples were assayed according to the standard PCR protocol described by Guscetti with the primer pair MSP3 and MSP4A (300 bp). [24]

When the results were negative the samples were assayed by a nested PCR to increase the sensitivity. The protocol for the nested PCR was reported by Katzwinkel-Wladarsch with the outer primer pair MSP1 and MSP2A (367 bp) and the inner primer pair MSP3 and MSP4A. [23] The primer pair MSP3 and MSP4A (300 bp) amplifies a sequence including partial sequence of the 16S small subunit ribosomal RNA gene, the internal transcribed spacer (ITS) region and partial sequence of the 23S large subunit ribosomal RNA gene.[23]

According to the standard PCR protocol described by Guscetti[24] (with minor changes) the reaction mixture of 25µl contained 5 µl of DNA, 25pmol of each primer MSP3 (5` GGA ATT CAC ACC GCC CGT C(A,G)(C,T) TAT 3`) and MSP4A (5` CCA AGC TTA TGC TTA AGT (C,T)(A,C)A A(A,G)G GGT 3`), 250µM of each dNTP, 0.625U Go Taq® polymerase (Promega, Mannheim, Germany), and 5µl 5x Green Go Taq® reaction buffer (supplemented with magnesium chloride for a final concentration of 2.5mM; Promega, Mannheim, Germany). Thirty-five cycles were performed with denaturation at 94°C for 30 sec, annealing at 60°C for 1 min and extension at 72°C for 1 min. For the nested PCR the reaction mixture of 25µl contained 5 µl of DNA, 25pmol of each primer, 250µM of each dNTP, 0.625U Go Taq® polymerase (Promega, Mannheim, Germany), and 5µl 5x Green Go Taq® reaction buffer (supplemented with magnesium chloride for a final concentration of 2.5mM; Promega, Mannheim, Germany). Five microliters of DNA from the first round of nested PCR (with the primer pair MSP1 [5` TGA ATG (G,T)GT CCC TGT 3`] and MSP2A [5` TCA CTC GCC GCT ACT 3`]) were used in the second round of nested PCR (primer pair MSP3 [5` GGA ATT CAC ACC GCC CGT C(A,G)(C,T) TAT 3`] and MSP4A [5` CCA AGC TTA TGC TTA AGT (C,T)(A,C)A A(A,G)G GGT 3`]). Each of the two PCR setups consisted of 35 cycles with denaturation at 92°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 90 sec. [23]

Each PCR run contained a positive control and a non-template control. After agarose gel electrophoresis (2%) the PCR products were visualised with ethidium bromide under UV light.

The PCR-amplicons (in each cat one PCR-amplification of the lens) were sequenced for determination of species and genotype of *E. cuniculi*. PCR-amplicons were obtained for sequencing using Quantum Prep® PCR Kleen Spin Columns (Bio-Rad Laboratories, Vienna, Austria). The sequencing of the products was performed by AGOWA (Berlin, Germany). As negative control three normal lenses of cats, euthanized because of unrelated diseases, were used.

Cytology

Cytologic examination of aqueous humor samples and phacoemulsified lens material was performed. A cytospin preparation was prepared by spinning down an aliquot of 50-100µl on a microscopic slide. The staining was done using Hämaquick® (Romanowsky type stain; Biomed Labordiagnostik GesmbH, Oberschleißheim, Austria).

Histopathologic examination

The anterior lens capsules acquired during phacoemuslification of both eyes in eight cats, one eye in three cats and in two cases parts of the posterior capsule were submitted. In cat 11 the right eye was enucleated and the whole eye was examined histopathologically. The specimens were fixed in 4% neutral buffered formalin, embedded in paraffin wax and 2-3µm sections were stained with hematoxylin and eosin (H&E) and acid-fast trichrome (AFT) staining techniques.

To review the prevalence of *E. cuniculi* retrospectively records from submissions to the Institute of Pathology of the University of Veterinary Medicine Vienna were

examined. Of 244 feline eye cases between May 1996 and June 2009 all cases of anterior uveitis and/or cataract of undetermined cause were selected. The histological sections of 26 cases were evaluated for the presence of microsporidial parasites using H&E and AFT staining techniques.

Reexaminations

Complete ophthalmic examinations were performed one day, one week, one month and six months after phacoemulsification. The serologic test for *E. cuniculi* was repeated at the six month recheck examination. Photographs were taken to document the clinical findings.

Serologic testing of ophthalmologically healthy cats

Ophthalmic examination was performed on 100 cats from an animal health shelter in Vienna as well as cats presented with fractures of the limbs at the Clinic for Surgery and Ophthalmology at the Veterinary University in Vienna. If the cats were ophthalmologically healthy, further analysis was carried out. An IIFT was used for detection of antibodies (IgG) against *E. cuniculi*. All sera were initially screened at a dilution of 1:20 and 1:40. Subsequently positive sera were retested in dilutions from 1:20 to 1:10,000.

Statistical analysis

For statistical analysis, SPSS (Statistical Package for the Social Sciences) 14 was used. The Spearman test was applied for the correlation between the maturity of cataract, duration of cataract and the level of antibody titer.

RESULTS

In the present study 11 cats, six castrated males and five spayed females with a median age of 3.5 years (range 2 – 9 years) were included. Nine cats lived indoors and two were outdoor cats. Eight cats were originally acquired from farms and three cats from animal shelters. The mean time between the first recognized symptoms by the owner and presentation at our clinic was 1.2 years.

The description and duration of cataracts are summarized in Table 1. Nine (9/11) cats had bilateral cataracts, whereas in two (2/11) cats, only one eye was affected. In total, 20 eyes had cataracts, but in cat 4 the right eye had been enucleated previously hence only 19 eyes remained in the study. 12/19 eyes had an anterior capsular opacification with a focal anterior cortical cataract and a vermiform process into the nucleus (Fig. 1). 7/19 eyes had a mature cataract (Fig. 2). In 14 eyes anterior uveitis with aqueous flare and endothelial precipitates (Fig. 2) were found in addition to cataracts. Rubeosis iridis was seen in 7 eyes. One eye showed a negative ERG reading (left eye of cat 4). Despite the negative ERG result, the owner opted for phacoemulsification. In cat 11 the right eye was enucleated because of secondary glaucoma however it was included into the study.

Blood and serologic testing

Complete blood counts were within normal limits in all cats. Biochemical analysis showed slightly elevated creatinine values in four (4/11) cats. The results of antibody tests for *E. cuniculi*, *T. gondii*, FIV and FIP as well as the antigen tests for FeLV are listed in Table 2. A positive antibody titer for *E. cuniculi* was detected in all cats. The titer ranged from 1:80 ≥ 1:10,000. IgG and IgM antibodies for *T. gondii* were determined in all cats. All cats tested negatively for IgM antibodies. However, 6 cats showed a positive IgG antibody titer. All cats tested negatively for FIP and FeLV. 8/11 cats were negative for FIV; the remaining 3 cats were not tested. Only 2 of the 100

ophthalmologically healthy cats tested had a positive antibody titer (1:640; 1:80). The remaining 98 cats had a negative antibody titer for *E. cuniculi*.

PCR

The PCR results for *Encephalitozoon spp.* and *Toxoplasma gondii* DNA in lens material and aqueous humor are summarized in Table 3. DNA from *Encephalitozoon spp*. was detected using a standard PCR in 18/19 lens samples and in 7/19 aqueous humor samples. With nested PCR 10/19 aqueous humor samples were positive (3 cases were negative on the left and right side with both PCRs). In cat 11 standard and nested PCR of the lens was negative, but in this case the PCR of the lens samples was performed after storage in formalin and paraffin embedding. With DNA sequencing strain II was identified in 7 cats and an unknown strain (5'-GTTT-`3 repeated 5 times) was determined in 4 cats. The unknown strain was submitted to GenBank (accession number: GU735480). DNA from *T. gondii* could not be detected in lens material or in aqueous humor from any cat by means of PCR. DNA of *E. cuniculi* could not be detected in the three normal lenses.

Histopathology

From the pieces of anterior lens capsule delivered for histopathology (18 cases) 11/18 cases were positive, in 3/18 cases *E. cuniculi* could not be definitively identified, and 4/18 cases were negative. Microsporidia were found with AFT staining techniques in liquified lens material adjacent to the lens capsule or within lens epithelium (Fig. 3). In H&E sections the parasites were not visible, but in most of the cases gray-blue stained fine granulated material was suspicious for *E. cuniculi* in the AFT positive areas (Fig. 4). The lens epithelium was hyperplastic or metaplastic. In some pieces the surface of the capsule was covered by some lymphocytes, plasma

cells and macrophages. In the two posterior lens capsules microsporidia were evident as well (Fig. 5). The globe of the enucleated eye (cat 11 - Fig. 6) showed a deep stromal keratitis, a severe lympho-plasmacytic infiltration of the anterior uvea and some small perivascular infiltrates within the retina. On Descemet´s membrane precipitates of lymphocytes, plasma cells and macrophages could be found. The lens fibres beneath the anterior pole were liquefied and the lens capsule seemed to be ruptured in this area. Adjacent to the lens capsule rupture reactive inflammation was not obvious. Microsporidia were not detected using AFT and immunhistochemistry staining.

The retrospective histological examination of 26 feline eyes with uveitis and /or cataract identified 4 eyes that were positive. In one additional eye *E. cuniculi* organisms could not be definitively identified because of the bad state of tissue preservation. In the 5 eyes, intralenticular organisms consistent with microsporidia were visualized. They appeared as ovoid organisms of 1-2µm without internal structures, except a vacuole in some of them. The bodies were apparent with AFT-staining and not visible in H&E-sections usually. Just in a few high grade cases pale gray-blue structures could be seen. In all of the cases the ocular lesions were quite similar: lympho-plasmacytic infiltration of iris and ciliary body with lympho-plasmacytic precipitates on the corneal endothelium and on the surface of the iris and ciliary body. Small perivascular lympho-plasmacytic infiltrates in the retina were a further feature. The retina was not atrophic. In 2 eyes a fibrovascular membrane covered the iris surface. The lenses showed signs of cataract with liquefaction of lens fibers, deposition of calcium salts, epithelial hyperplasia with lens epithelial cell migration to the posterior capsule and fibrous metaplasia. The parasites were found within liquefied lens fibers adjacent to the anterior lens capsule. In 3 eyes they were found in close proximity to the posterior lens capsule as well. In all cases the lens capsule

seemed intact with the exception of one eye. In this eye a ruptured posterior capsule was covered by a thick rim of fibrovascular tissue. The ends of the capsule were embedded in this tissue and microsporidia could be found within this tissue but not outside.

Cytology

With cytology *E. cuniculi* spores were detected in 2/19 lens material samples (Fig. 7) and in 3/19 aqueous humor samples.

Reexaminations

All reexaminations were documented with photographs (Fig. 8). After 6 months signs of uveitis were no longer present in any eye. 17/19 eyes showed a positive menace response. In cat 4 a preoperative existing atrophic retina was the reason for loss of vision. Retinal folds were seen in 4/19 eyes, which did not change over time (Fig. 9). The main post operative complication were corneal erosions in 10/19 eyes. Postoperative hypertension occured in 4/19 eyes. One eye developed bullous keratopathy. Six months after the operation antibody titer testing for *E. cuniculi* was repeated. The results are summarized in table 2. The titer had increased in three cats, remained the same in one cat and decreased in seven cats. There was no correlation between maturity of the cataract, the duration of cataract and the level of the *E. cuniculi* antibody titer.

DISCUSSION

In this report *E. cuniculi* was found as a causative agent of cataract and uveitis in 19 eyes of 11 cats. Serology and PCR were the most effective diagnostic tools. All eyes

were successfully treated with a combination of medical treatment and phacoemulsification.

Horizontal transmission of *E. cuniculi* - oral and nasal - are described from one species to another.[25, 26] But vertical transmission is presumed to play an important role in the mechanisms by which the microsporidium enters the lens in rabbits and mink.[6, 11, 27, 28] During embryologic development, in the first third of gestation the lens placode is formed followed a few days later by the lens capsule.[29] The presumption is that the organism can enter the lens during this time period.[4, 6] There are no studies describing the exact timepoint of infection with *E. cuniculi* during gestation in rabbits or in cats. But in one study *E. cuniculi* infected pregnant rabbits were euthanized at the 28th day of gestation. Brains, lungs, kidneys and placentas were investigated from fetuses and kidneys, brains and lungs from the pregnant rabbits. With nested PCR DNA of *E. cuniculi* could be found in all organs of fetuses and pregnant rabbits. The eye and the lens were not investigated in this study.[30] If there is already a decay of other organs at the 28th day of gestation, an infection of the lens is also possible during this time-period. In our study three litter mates of three affected cats had a positive *E.cuniculi* antibody titer too, but no signs of uveitis or cataract. In comparison companion animals of two affected cats showed a negative antibody titer. Thus the vertical route of infection also seems viable in cats, but further investigation is necessary.

We detected no sex predilection in our study. The study included 6 neutered males and 5 spayed females. The cat with cataract formation following *E. cuniculi* infection described in a previous case report was female.[13] In rabbits, cataract formation also appears to affect males and females equally.[6, 9]

In our investigation all age groups were represented with a median age of 3.5 years, but we saw a tendency for younger cats to be affected. The animals with *E. cuniculi*

induced cataracts in previous studies of rabbits (3 months – 5 years)[6, 7, 9], blue foxes (3 months)[10] and mink (5 months - 2.5 years)[11] also tended to be younger.

The initial site of cataract formation seems to be the lens epithelial cells, where the *E. cuniculi* spores were detected. The cataract was a focal anterior cortical cataract with a vermiform process into the nucleus. In most of the cases (14/19 eyes) the cataract was present together with a severe, therapy-resistant anterior uveitis with endothelial precipitates, aqueous flare and rubeosis iridis. Where clinical symptoms persisted for a longer timeperiod, the cortical cataract progressed into a mature cataract. In rabbits, the typical clinical symptoms are a white mass on the anterior lens capsule with subcapsular cataract and posterior synechia and dyscoria.[3, 4, 6-9] In most rabbits iridial hyperemia was also seen.[8, 9]

The cause for the uveitis could not be exactly determined in our cats. A lens capsule rupture was only found in two cats in our study in contrast to rabbits.[6, 9] In rabbits the anterior lens capsule (10.7µm)[31] is thinner than in cats (98µm)[32] and maybe this is the reason for more frequent ruptures in rabbits. Diffusion of toxin from the microsporidium through the lenticular capsule could be a reason for uveitis in cases with intact lens capsule.[4] In our histopathologic examinations we found the microsporidium only in the lens, however we could not exclude that the organism is present in other ocular tissues such as the uvea and induce uveitis.

In contrast to rabbit studies[6-9, 25, 33] we detected more cases of bilateral cataracts in our study. In blue foxes and mink unilateral and bilateral cataracts are described.[10, 11] Furthermore, in 4 eyes we found retinal folds which raises the question of whether *E. cuniculi* could be the cause of these folds. In the retrospective part of our study we determined perivascular lymphoplasmacytic infiltrates in the retina, but no microsporidial spores. We found no atrophic retina in any of the retrospective cases. In the litter of blue fox puppies the retina was described as having thickened, folded

and necrotic areas together with vascular changes. Spores were seen in the arterial walls after Gram staining, but not in the retina.[10]

Different methods have been used to detect *E. cuniculi* as the causative agent of cataract formation. To detect the antibody titer in rabbits immuno fluorescence assay (IFA) [8, 9, 20, 26] and an enzyme-linked immunosorbent assay (ELISA) test were performed.[7, 25, 26] Serology is an important diagnostic test to detect *E. cuniculi* infections in living rabbits.[34] A negative antibody titer for *E. cuniculi* excludes the microsporidium as a causative agent for a disease, however a positive antibody titer only indicates an infection.[21, 34] Therefore we wanted to establish the prevalence of *E. cuniculi* antibody titres in ophthalmologically healthy cats in Austria. We found a low prevalence of 2% in healthy cats in contrast to 23% in Slovakia (the cats were not ophthalmoscopically examined).[35] But in contrast all cats affected by cataract showed a positive antibody titer. Based on these results, a positive antibody titer in conjunction with clinical signs provides a strong indication for *E. cuniculi* infection in cats. Ocular lesions together with a positive antibody titer were also seen in rabbits.[25, 33] We could not show any correlation between the maturity of cataract and the level of the antibody titer in the present study. Six months after surgery the antibody titer decreased in seven cats, but was detectable in all patients. In rabbits a positive antibody titer was reported 13 months, 21 months and 36 months after infection.[28, 36] Therefore a very long or perhaps a lifelong positive seroprevalence could be possible in cats.

PCR is the most sensitive method to detect microsporidial DNA in lens material in rabbits.[3, 7, 8, 33] *E. cuniculi* DNA was found in 18/19 affected feline lens samples using PCR. In the enucleated eye of cat 11 no *E. cuniculi* DNA could be found. One reason may be the degradation of DNA due to storage in formalin. Another reason could be the long duration of infection and chronic inflammation.[37] As a negative control we

used three lenses of unaffected cats. These cats were euthanized because of unrelated causes, but showed no signs of cataract. *E. cuniculi* DNA could not be detected in any of these lenses with PCR. During sequencing the mouse strain was found in seven cats, as it has been found in blue foxes and mink[26] and an unknown strain was detected in the remaining cats. This indicates that the mouse seemes to be the reservoir for the infection in cats. Furthermore, in other studies the mouse strain was only found in Europe, however the rabbit strain has a worldwide distribution.[23] Questions about the seroprevalence of *E. cuniculi* in the mouse population in Austria and detection of *E. cuniculi* in cats with cataracts in other countries or continents arise and need to be addressed.

Using histopathologic examination we tried to identify spores in the lens epithelial cells. Because of the small size of the spores, detection is difficult with routine H & E staining, but specific staining methods such as AFT, Ziehl-Neelson, silver methenamine, Gram staining and Weber's chromotrope staining are more effective.[4, 10, 24, 38] In our study we identified the spores using AFT. The parasites were not clearly visible in H&E sections, but in most cases finely granulated material which stained gray-blue and looked suspiciously like spores were detected. Wolfer *et al.* found the spores in the lenses of rabbits with Gram and AFT, but not with PAS and H&E staining.[6] In the same study, neutrophils and macrophages were found in the area of lens capsule rupture surrounded by fibrous tissue with lymphocytes and plasma cells.[6] In our investigation we also found lymphocytes, plasmacytes and macrophages on the surface of the capsule, the typical cells for chronic inflammation. In rabbits no spores could be detected outside the lens.[6] The same result was found in four enucleated eyes of the retrospective part of this study. In the case report of a cat with keratitis, the spores could be seen between the stromal fibers in the cornea.[12]

With cytologic examination we found only 5 tentative positive results. Therefore cytology is not a useful diagnostic tool for detection of spores in aqueous humor and phacoemulsified lens material because of its low sensitivity. Cytologic examination was successfully used in other body fluids (duodenal aspirate, urine, bronchoalveolar lavage fluid, sputum and conjunctival smears) in humans.[38] Perhaps AFT-staining may increase diagnostic sensitivity.

Anterior uveitis is a common clinical phenomenon in cats. Many systemic diseases may cause this condition such as FIP, lymphosarcoma caused by FeLV, FIV*, T. gondii*, and many mycotic infections.[39] In order to investigate the presence of such diseases, a number of tests were undertaken. Only 6 cats had a positive IgG antibody titer for *T. gondii*. The IgM antibody titer for *T. gondii* was negative in all cats. Burney *et al.* described the use of PCR to detect *T. gondii* DNA in aqueous humor.[40] DNA could not be detected in the lens or in the aqueous humor in our cases. All other tests for the aforementioned systemic diseases were negative. Therefore, systemic diseases other than *E. cuniculi* infection were excluded as possible causes. Furthermore there was no history of trauma in any of the cats. Peiffer *et al.* described cases of idiopathic uveitis in cats.[41] Common findings were iris nodules with lymphocytes and plasma cells, iris neovascularisation, keratic precipitates, inflammatory debris on the lens capsule, cataract, vitreal debris and glaucoma. Despite investigation for infectious or neoplastic diseases the cause could not be determined. There seemed to be similarities to our cases and therefore *E. cuniculi* may also have been the infectious agent in these idiopathic cases.

The treatment aims for microsporidial infection are to eliminate the parasite, suppress inflammation and treat the clinical symptoms.[3] As in rabbits[7, 8] surgical removal of the lens is the treatment of choice. Two cats (cat 4 and cat 11) lost one eye because of

secondary glaucoma after conservative treatment of cataract and uveitis. The atrophic retina in cat 4 seemed to be the consequence of the chronic inflammation. Conservative treatments with both successful[42] and poor[6] outcomes have been reported in rabbits. Conservative treatment included systemic and topical administration of dexamethasone and oxytetracycline.[42]In addition to our study five cats were treated conservatively as owners declined operations. The treatment included systemic and topical corticosteroids and fenbendazole. The cataract and uveitis symptoms worsened during the observation period. The *E. cuniculi* antibody titer increased in two cats, remained equal in one cat and decreased in one cat. One cat was lost for follow up. Treatment with corticosteroids is controversial because of their immunosuppressive effects.[25, 34] For this reason we used topical corticosteroids and systemic anti-inflammatory treatment consisted of non-steroidals only in the clinical study. Furthermore, fenbendazole was used for the treatment of the microsporidial infection. Fenbendazole treatment is also recommended to prevent and treat *E. cuniculi* infections in rabbits.[43] Albendazole is used in cases of microsporidial infections in rabbits[7] and humans.[18-20]

The present study detected *E. cuniculi* as a causative agent for cataract formation and anterior uveitis in cats using different methods. Serology and PCR turned out to be the most sensitive diagnostic tools. Phacoemulsification to remove microsporidia, followed by specific treatment for *E. cuniculi*, as well as symptomatic treatment for uveitis, led to a comfortable and visual eye in most of the cats of the current study.

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Figures and Tables

Table 1: Cataract description and duration of cataract in each cat

Cat	Age years	Eye	Cataract	Duration
1	4	OD OS	Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification Mature cataract	1 year
2	2	OD OS	Mature cataract Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification	1 year
3	9	OU	Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification	6 months
4	2	OD OS	Secondary glaucoma because of cataract and uveitis. Enucleation before the study. Mature cataract with pigmentary deposits on the anterior lens capsule	3 years
5	4	OD OS	Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification No cataract	2 months
6	2	OD OS	Immature cataract with pigmentary deposits on the anterior lens capsule Mature cataract	2 years
7	3	OU	Immature anterior and posterior cortical cataract	1 week
8	4	OU	Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification	3 years
9	2	OD OS	No cataract Mature cataract	3 years
10	3	OU	Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification	4 months
11	4	OD OS	Secondary glaucoma because of cataract and uveitis. Enucleation during study period Focal anterior cortical cataract with vermiform process into nucleus; focal anterior capsular opacification	2 years

Table 2: Summary of serologic screening tests

Cat	*E. cuniculi IgG Pre OP*	*E.cuniculi IgG Post Op*	*Toxoplamsa gondii IgG*	*Toxoplamsa gondii IgM*	*FIP IgG*	*FIV Ab*	*FeLV Ag*
1	1:320	1:1280	-	-	-	ND	-
2	1:1280	1:1280	1:4000	-	-	-	-
3	1:80	1:640	1:4000	-	-	-	-
4	1:320	1:160	-	-	-	-	-
5	1:280	1:160	1:4000	-	-	-	-
6	≥1:10000	1:2560	1:4000	-	-	ND	-
7	≥1:10000	1:2560	-	-	-	ND	-
8	1:2560	1:640	-	-	-	-	-
9	1:5120	1:1280	-	-	-	-	-
10	1:640	1:1280	-	-	-	-	-
11	1:320	1:160	-	-	-	-	-

Ab: Antibody; Ag: Antigen, ND: not done

Table 3: PCR, histopathology and cytology results

Cat	L *E. cuniculi*		L *Toxoplasma*		A *E. cuniculi*		A *Toxoplasma*		Histopathology		Cytology	
	PCR		PCR		PCR		PCR		H&E, AFT		Hämaquick®	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	L OU	A OU
1	**+**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	**+**
2	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**
3	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
4	**ND**	**+**	**ND**	**-**	**ND**	**+**	**ND**	**-**	**ND**	**+**	**-**	**-**
5	**+**	**nc**	**-**	**nc**	**-**	**nc**	**-**	**nc**	**+**	**nc**	**-**	**+**
6	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**+**	**-**
7	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**
8	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**-**
9	**nc**	**+**	**nc**	**-**	**nc**	**+**	**nc**	**-**	**nc**	**+**	**+**	**+**
10	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**-**	**-**
11	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**

L: lens; A: aqueous humour; ND: not performed nc: no cataract

Cat 11: OD enucleated, but samples for PCR were taken

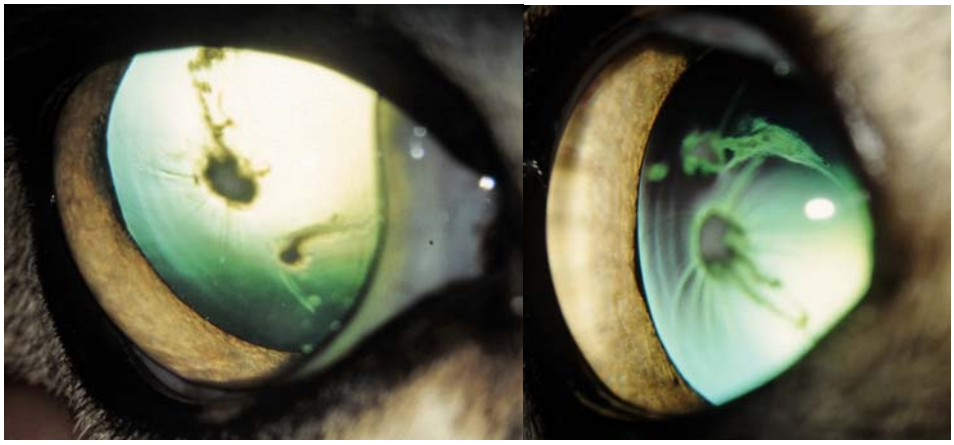


Fig. 1: Focal incipient anterior cortical cataract with capsular opacification and vermiform process into the nucleus in cat 2.

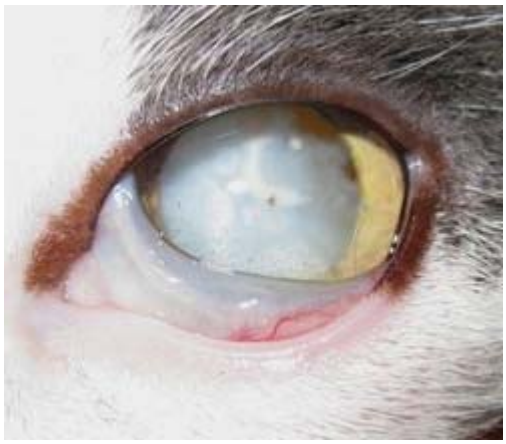


Fig. 2: Total mature cataract with endothelial precipitates in cat 3.

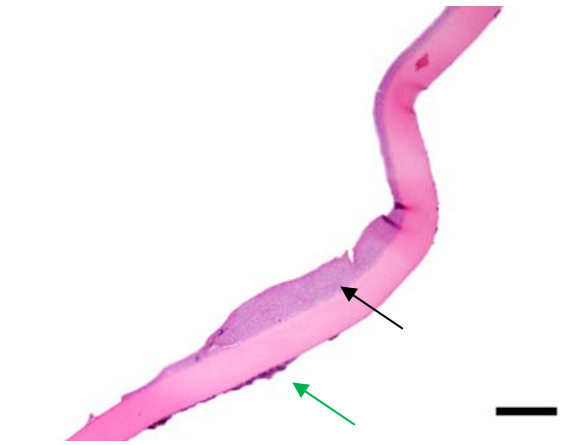


Fig. 3: Microsporidial spores (gray-blue stained fine granulated material – black arrow) in the lens epithelial cells or the liquefied lens material attached to the anterior lens capsule. At the surface of the capsule lymphocytes, plasma cells and macrophages were found (green arrow).(H&E, 10x; bar=150µm).

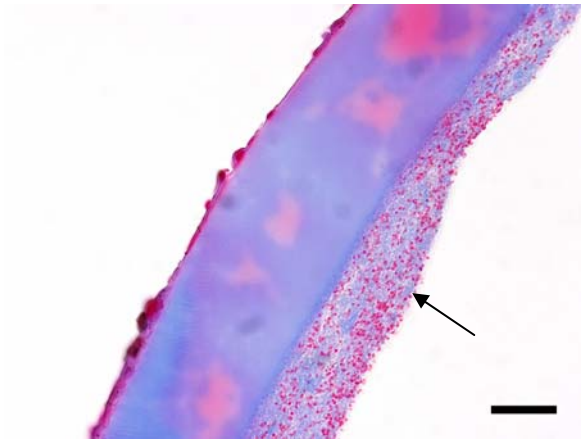


Fig. 4: Microspiridial spores (red stained fine ovoid organismens – arrow) attached to the anterior lens capsule with AFT staining (AFT, 40x; bar=40µm).

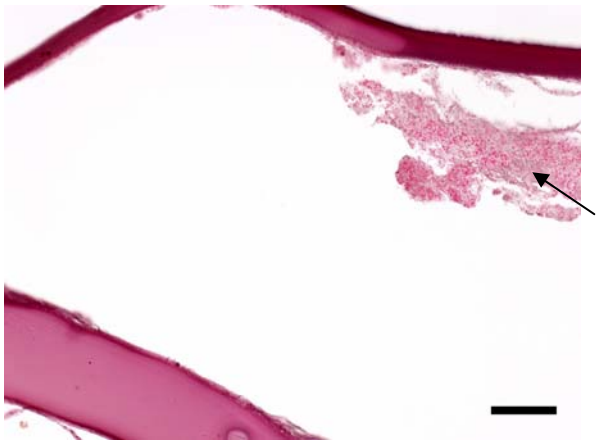


Fig. 5: Posterior lens capsule with attached microsporidial spores (arrow) with AFT staining (AFT, 20x; bar=80µm).

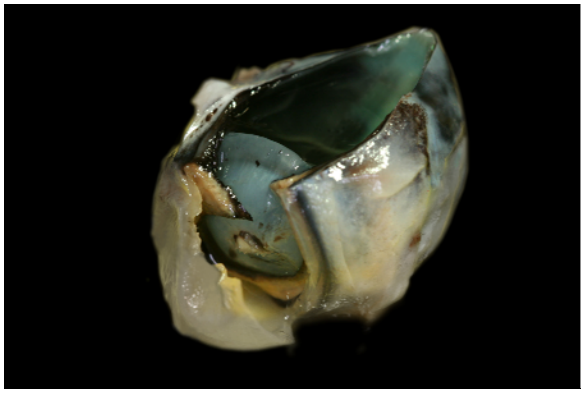


Fig. 6: Gross pathology of the right eye of cat 11. The anterior lens capsule rupture is surrounded by pigment.

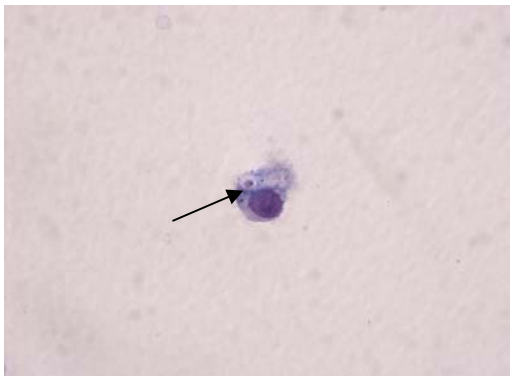
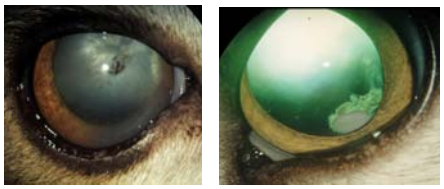
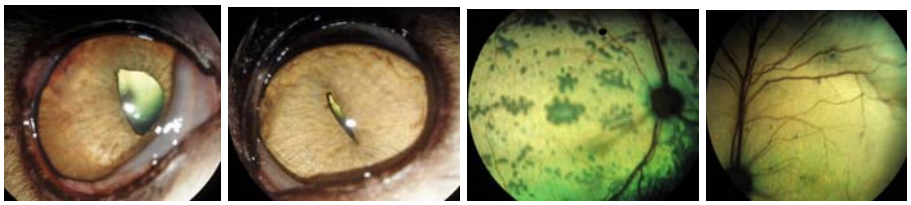


Fig. 7: Lens epithelial cell with microsporidial spore in the cytoplasma (100x).


preoperative


1 week post OP

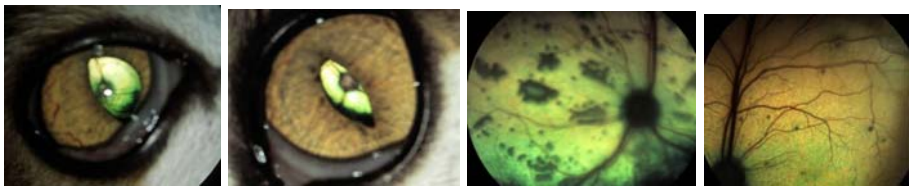

6 months post OP

Fig. 8: Photographic documentation of the pre and postoperative presentation.

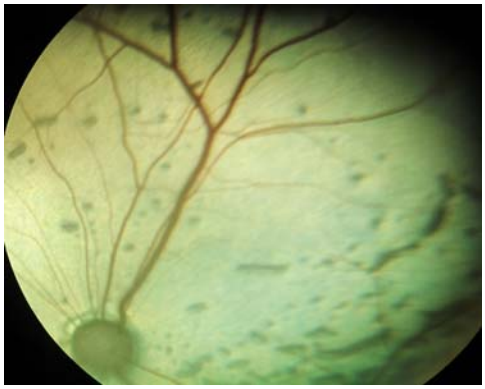


Fig. 9: Retinal folds in the left eye of cat 9.