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ORIGINAL REPORT

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Retrospective study on *Encephalitozoon cuniculi* infections in 118 cat and 9 dog eyes

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Abstract

Purpose: This study aims to inform about the clinical image, diagnostic possibilities, and treatment options for cats and dogs diagnosed with ocular encephalitozoonosis.

Materials and Methods: Medical records of 7 dogs and 75 cats with ocular encephalitozoonosis presented at the Clinical Unit of Ophthalmology of the University of Veterinary Medicine Vienna between 08/2006 and 02/2022 have been compiled. Diagnosis was based on the presence of cataracts and positive antibody titer against *Encephalitozoon cuniculi*. Patient history, details on the characteristics of the cataracts, level of antibody titers, and the treatment regimen were summarized, as are the results of histological stainings of surgical samples to determine the presence of *Encephalitozoon cuniculi*, as well as PCR analyses to identify its sub-strains.

Results: Our analysis of the clinical data shows that focal cortical anterior cataracts often associated with anterior uveitis and lesions in the fundus are the most prevalent manifestation of ocular encephalitozoonosis in cats and dogs.

Conclusion: Serological testing and PCR analysis of lens material are the most important diagnostic tools. Timely phacoemulsification with adjuvant anti-inflammatory and antiparasitic medication is the most promising therapy for *Encephalitozoon cuniculi-induced* cataracts.

KEYWORDS

canine, cataract, eye, feline, lens, microsporidia

1 | INTRODUCTION

Microsporidiosis has gained increasing attention in the last decades. One of the most intensively studied pathogens of the phylum is *Encephalitozoon cuniculi*. The zoonotic parasite, first described in 1922 by Wright and Craighead,[1] is best known for infecting rabbits that clinically suffer from neurological symptoms, and renal and ocular diseases.[2] To date, *E. cuniculi* can be further differentiated into four strains that differ in the amount of 5′-GTTT-3′ repeats in the internally transcribed spacer region. Didier et al.[3] identified the genetic differences between *E. cuniculi* isolates by using PCR analysis. They suggested naming these strains according to their most frequent hosts: Strain type I is therefore called rabbit-strain, type II mouse-strain, and type III dog-strain.[3] In 2010, a new strain was found by PCR in a human patient who had received a renal transplant. Talabani et al.[4] called the isolate *E. cuniculi* strain type IV, now also referred to as the human strain. Although named after frequent hosts, the different strains do not show great host specificity, as is the case in humans, for example, where all strains have been detected.[5] Recently, *E. cuniculi* was recognized as a causative agent for cataracts in cats and dogs.[6-8] At present, a presumptive diagnosis of *E. cuniculi* infection in feline and canine eyes can be made based on clinical examination. This study aims to contribute to this growing area of research by exploring the clinical image, diagnostic possibilities, and treatment options of cats and dogs diagnosed with ocular encephalitozoonosis at the University of Veterinary Medicine Vienna between 08/2006 and 02/2022. A total of 75 feline and seven canine patients are included in the present study, representing the largest group of animals with clinical manifestations reported to date.

2 | MATERIALS AND METHODS

2.1 | Animals and data

All dogs and cats with an initial presentation between 08/2006 and 02/2022 with ocular encephalitozoonosis of one or both eyes are included in the current study. The same applies to ocular and serological samples sent to the institution by external ophthalmologists during this period.

The diagnosis was based on the presence of a cataract with concurrent positive antibody titer (and in one case without a serologic result, on the positive PCR result of lens material for *E. cuniculi*).

Wherever possible, information about the patients' age, sex, breed, origin, type of husbandry, and time of onset of symptoms were collected from the owner or were taken from the accompanying letters for external sample material. The patient history of four of the canine patients (D1, D2, D3, D7) has previously been described in greater detail in two case reports.[7,8]

The results of ocular examinations were taken from the medical records from the University of Veterinary Medicine Vienna or as described by external veterinarians, as well as from photographs taken during the consultations. Parameters recorded for the current study were the presence of lenticular abnormalities, anterior uveitis, and fundus changes. Additionally, cataract surgery or enucleation as well as therapies with fenbendazole were noted. Whenever possible, results from further diagnostic procedures after phacoemulsifications or enucleations were documented.

PCR analysis and sequencing, serological testing, cytology, and histopathological examinations (using Gram's and acid-fast trichrome (AFT) stain) were performed at the University of Veterinary Medicine Vienna. The exact methods of the different examinations are described by Benz et al.[6] Some serological samples were analyzed by the following external laboratories: LABOklin Labor für klinische Diagnostik GmbH & Co KG, IDEXX Vet Med Labor GmbH, In Vitro Labor für veterinärmedizinische Diagnostik und Hygiene GmbH and Vebio Laboratoire de Biologie Vétérinaire.

The cataracts were grouped into "focal cataracts" and "total cataracts." The focal cataracts were further categorized as cortical anterior, nuclear, and cortical posterior.

As a means of simplification, the eyes were classified as having anterior uveitis or not without grading severity. As soon as one or more of the following signs were present, the eye was classified as displaying anterior uveitis: keratic precipitates, cells or flare in the anterior chamber, swelling of the iris, or rubeosis iridis.

A follow-up study was undertaken for some of the patients that were reexamined after phacoemulsification and for some that were solely treated conservatively. Concerning the serological follow-up, feline patients with at least three titers measured at the University of Veterinary Medicine Vienna were included, but all titers documented for the dog patients were taken into account.

2.2 | Statistical analysis

The data was tabulated in Microsoft® Excel® for Microsoft 365 MSO (Version 2202 Build 16.0.14931.20806) and used to calculate abundances, means, and medians.

3 | RESULTS

The present evaluations include 75 cats with 118 affected eyes and seven dogs with nine affected eyes. Of these patients, 20/75 cats and 2/9 dogs were examined by ophthalmologists in clinics other than the Clinical Unit of Ophthalmology of the University of Veterinary Medicine Vienna.

3.1 | Cats

3.1.1 | Signalment and clinical findings

A total of 75 cats were included in the present study: 73 patients were European Shorthairs, one cat was a Russian Blue and one cat was described as a "Longhair" without breed specification; 2.7% ($n=2$) were entire male, 51.4% ($n=38$) male neutered and 45.9% ($n=34$) female neutered cats (information on the sex was not provided for one animal).

Concerning the type of husbandry, 59.4% ($n=41$) were able to go outdoors, 40.6% ($n=28$) were solely kept indoors and it was unknown for the rest. It was documented that 37 cats (15 indoor, 20 free roaming, and two cats with unknown type of husbandry) were originally from the streets, animal welfare organizations, or farms. The countries of origin were Austria in 54, Bosnia in one, Croatia in one, France in two, Germany in 12, Hungary in two, Switzerland in one case, and not provided for two cats.

The age at initial presentation ranged from 1.0 to 17.3 years, with a median of 5.2 years, and was unknown for one patient (Figure 1). Records of the onset of ocular symptoms as recalled by the owners existed for 51 cats (Figure 1), the youngest being 8 weeks old, and the oldest 14.3 years at the beginning of the disease with a median of 3.9 years.

A total of 118 eyes with cataractous changes were described as one cataractous eye was already enucleated and one phacoemulsified prior to the first presentation.

Unilateral cataracts were seen in 30 cats (40%) and bilateral cataracts in 45 cats (60%).

Of the lenticular opacifications described in more detail, 82/111 were focal and 29/111 total cataracts. Figure 2 and Figure 3 give details on the location of the focal cataracts and the distribution of bilateral cataracts, respectively. Some (37) of the focal cataracts were described as having a “vermiform process”, “tail” or “borehole-like path”, reaching posteriorly from a focal cortical anterior cataract. Figure 4A-1–E-2 show examples of eyes at the initial presentation.

Occurrence of anterior uveitis in conjunction with focal and total cataracts is depicted in Figure 5. No record of anterior uveitis was available for 24 cataractous eyes and none of the eyes without cataractous changes showed signs of uveitis.

At initial presentation, 18 eyes with cataracts and three eyes without cataracts showed fundic abnormalities. In these, changes included signs consistent with chorioretinitis, hyper- and hyporeflexia, gray infiltration, and vascular atrophy. However, fundoscopy was not always possible (e.g., when corneal and/or lenticular opacifications hindered the view of the posterior segment).

3.1.2 | Serology

The serological results from the first blood sampling of 74 cats ranged from <1:10 (probably a measurement error) to ≥1:10000, the most prevalent titers being 1:160–1:1280.

3.1.3 | Treatment

Based on the available data from the record, 53 cats received systemic treatment with fenbendazole (20–50 mg/kg SID for 3 weeks) at least once. No information was available for the remaining 22 patients.

Figure 6 presents an overview of the surgeries performed in 83 eyes of 57 cats including the usage of intraocular lens (IOL) implantations. The remaining 18 cats (35 eyes) solely received conservative treatment. In total, 37 of the surgeries were performed at the University of Veterinary Medicine Vienna, while 20 were executed elsewhere by referring veterinarians.

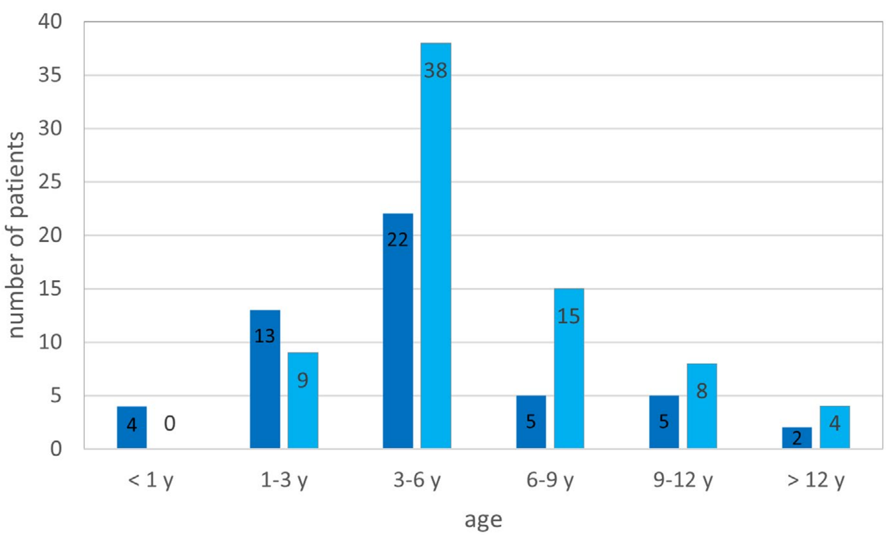

FIGURE 1 Age at the onset of symptoms as recalled by the cat owners ($n=51$; left columns) and age at initial presentation ($n=74$; right columns).

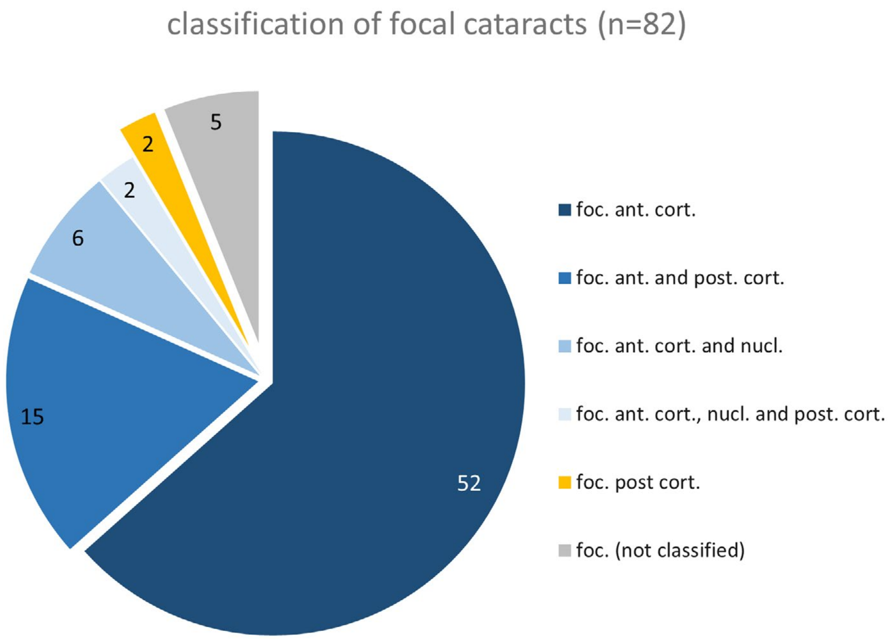

FIGURE 2 Further classification of the focal cataracts with total numbers. ant, anterior; cort, cortical; foc, focal; nucl, nuclear; post, posterior.

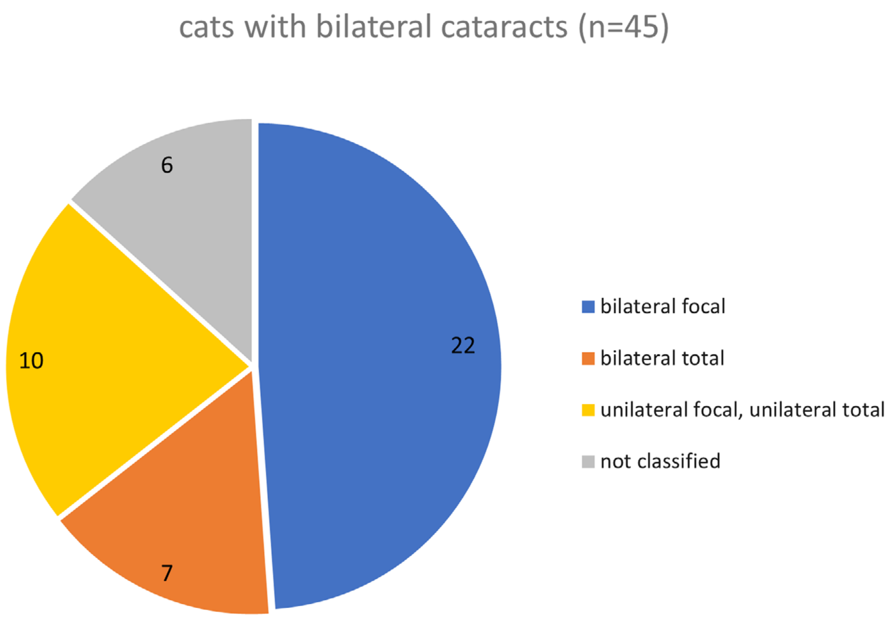

FIGURE 3 Distribution of types of cataracts in cats with bilateral lens changes in total numbers.

3.1.4 | PCR analysis

Liquified lens material retrieved at phacoemulsification or after enucleation was analyzed by PCR: 71 samples from 78 eyes from 54 cats (64 from either the left or right eye and seven mixed samples from both eyes) were positive for *E. cuniculi* (Figure 7). Sequencing revealed the presence of strain type II in 26/54 (48.1%), strain type IV in 25/54 (46.3%), and strain type III in 3/54 (5.6%) of the patients. Note that PCR products from only one eye for each cat were sequenced, as it was assumed that the same strain would be present in the other eye. In addition, aqueous humor from 11 cats (19 samples) was analyzed by PCR (Figure 7). Finally, seven urine samples were collected, of which only two samples were PCR positive.

3.1.5 | Cytology and histopathology

Cytological samples from liquified lens material ($n=17$) and from aqueous humor ($n=18$) were analyzed (Figure 7).

Parts of the anterior lens capsule from eyes that had been subjected to phacoemulsification ($n=62$) or enucleation ($n=10$) underwent histopathologic examination (Figure 7).

The 10 eyes described above were enucleated as the first choice of surgery.

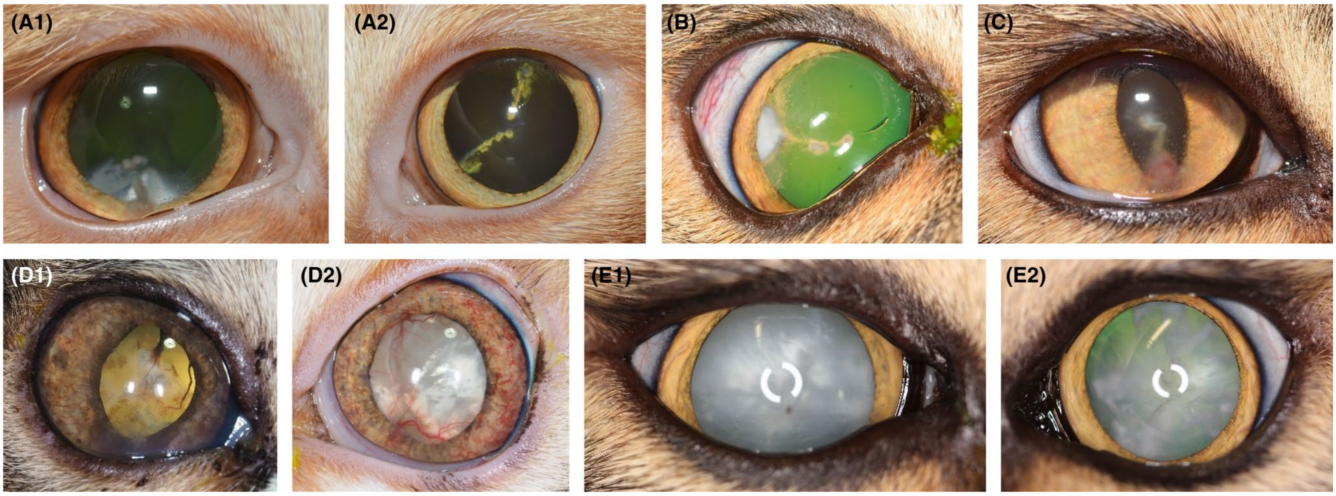

FIGURE 4 (A–E) Photographs taken at initial presentation. (A-1) 3.6-year-old male neutered European Shorthair; OD focal cortical anterior and posterior cataract, keratic precipitates, generalized rubeosis iridis. (A-2) 3.6-year-old male neutered European Shorthair; OS focal cortical anterior cataract. (B) 8.5-year-old female neutered European Shorthair; OD focal cortical anterior cataract with vermiform process reaching posteriorly, keratic precipitates, ingrowth from iris vessels to the anterior lens capsule. (C) 8.5-year-old female neutered European Shorthair; OD focal cortical anterior cataract with vermiform process reaching posteriorly, keratic precipitates, ingrowth from iris vessels to the anterior lens capsule. (D-1) 11-year-old female neutered European Shorthair; OD hypermature cataract with almost complete lens resorption, vessel on anterior lens surface, anterior uveitis. (D-2) 11-year-old female neutered European Shorthair; OS hypermature cataract, very prominent iris vessels that extend onto the anterior lens surface. (E-1) 3-year-old female neutered European Shorthair; OD hypermature cataract with pigment on anterior lens capsule, trace flare, keratic precipitates.

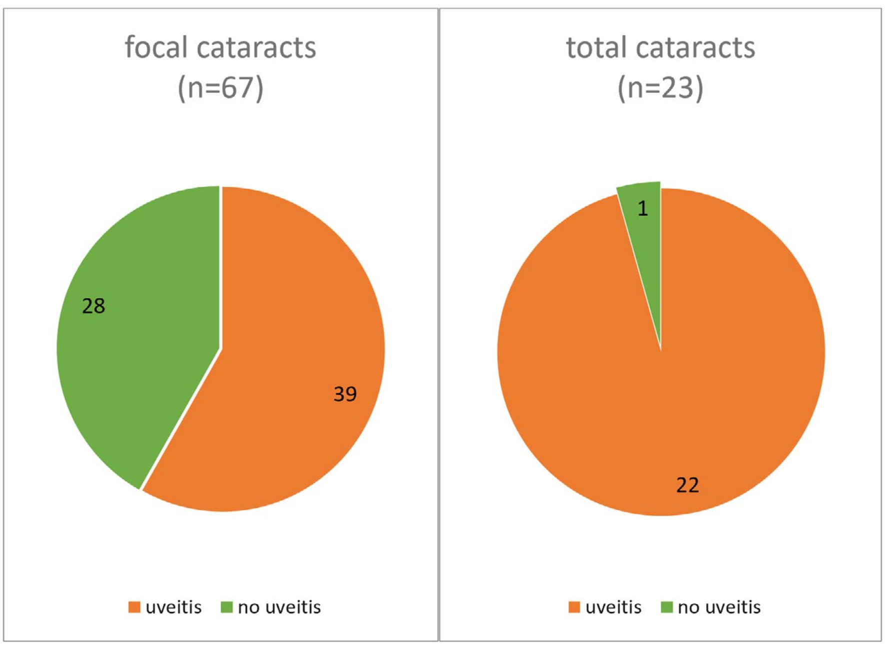

FIGURE 5 Occurrence of secondary anterior uveitis in eyes with focal cataracts (left) and total cataracts (right diagram) in absolute numbers.

In three cases, phacoemulsification was followed by a complicated course of disease requiring enucleation 3.58 years, 4 years, and 7 months after the first surgery. After phacoemulsification, microsporidia could be detected with AFT staining in two of these cases, while one was only suspicious for *E. cuniculi* spores. In the enucleated globes, the remaining lens capsule was negative in both eyes with previous positive results, and suspicious for microsporidia in the remaining eye. Details on the pathohistological examinations of these globes are given later in the section on "Clinical and Diagnostic Findings in the Later Course of Disease."

Retinal pathologies were detected during pathohistological examination of six of the 13 eyes that were enucleated in total, including minor to moderate lymphoplasmacytic infiltrates of retina and the choroid, fibrin effusion in the subretinal space, and atrophy of the outer retinal layers and the ganglion cell layer.

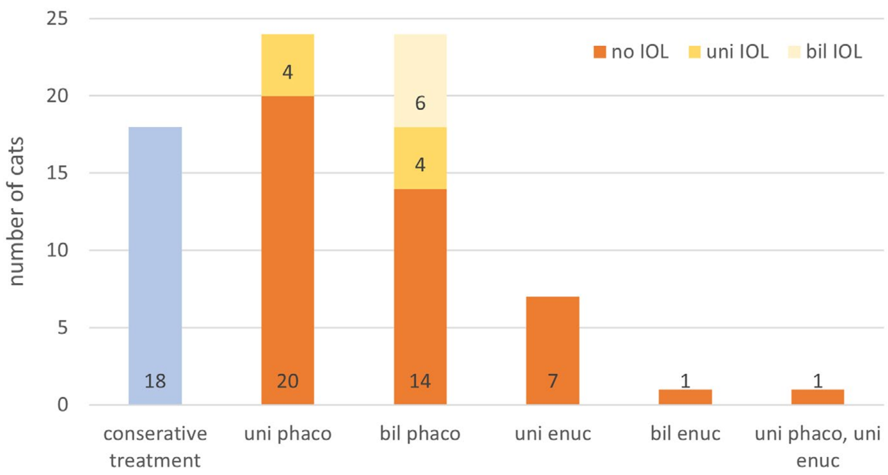

FIGURE 6 Treatment of the 75 feline patients. bil, bilateral; enuc, enucleation; IOL, intraocular lens; phaco, phacoemulsification; uni, unilateral.

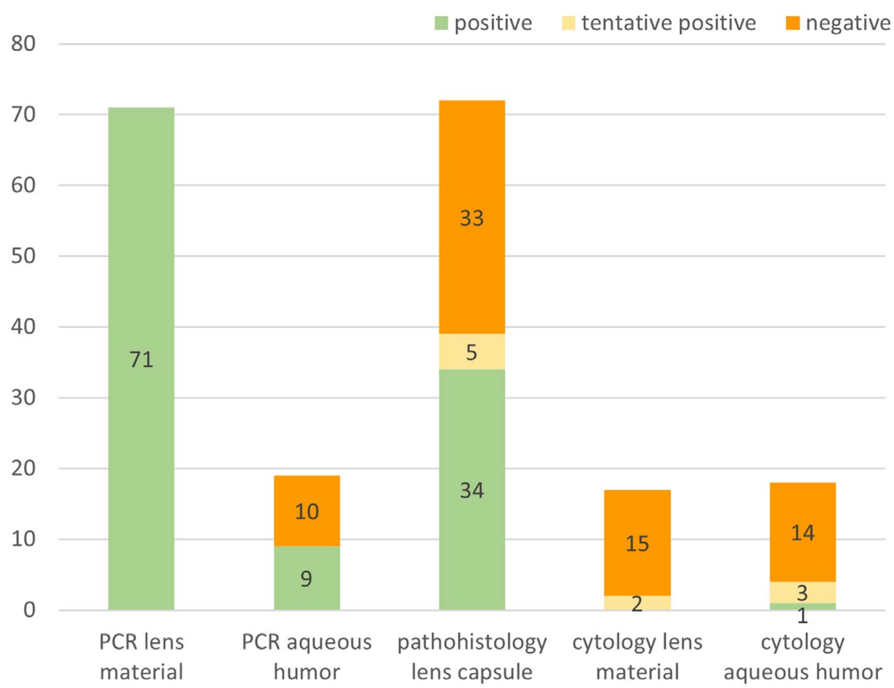

FIGURE 7 Methods for *E. cuniculi* detection after phacoemulsification or enucleation with total numbers of positive, tentative positive, and negative results.

3.1.6 | Follow-up

Serology

Consistent individual serological follow up was available for 13 cats. Figure 8 shows the titers measured at the University of Veterinary Medicine Vienna only.

In total (pre- and postoperatively), 133 serum samples were investigated, of which 107 were analyzed at the University of Veterinary Medicine Vienna and the remaining 26 samples by external laboratories (Figure 9).

Ophthalmological findings

Follow-up for the 18 cats (35 eyes) that were not operated on was possible for three cats for a maximum period of 2 years: all three showed focal cortical anterior cataracts that extended in size in two patients, while the chorioretinitic scar found in one cat remained stationary.

Of the 36 cats receiving surgical treatment at the University of Veterinary Medicine Vienna, follow-up for up to 9 months postoperatively was conducted for 27 patients with 45 cataractous and nine non-cataractous eyes (42 eyes were treated with phacoemulsification, two were enucleated and one cataract was not operated on).

Pre-surgical cataract progression was observed in four eyes that were later phacoemulsified and in two eyes that were not operated on. Evaluation of cataract progression was not possible in the remaining eyes as the surgery was performed directly after initial presentation.

Development of posterior capsular opacification (PCO) of varying degrees occurred in 20 of the 42 phacoemulsified eyes included in the follow-up study.

Signs of anterior uveitis were found in at least one examination of 34 of the 45 cataractous eyes. Two of those were enucleated and the remaining 32 underwent

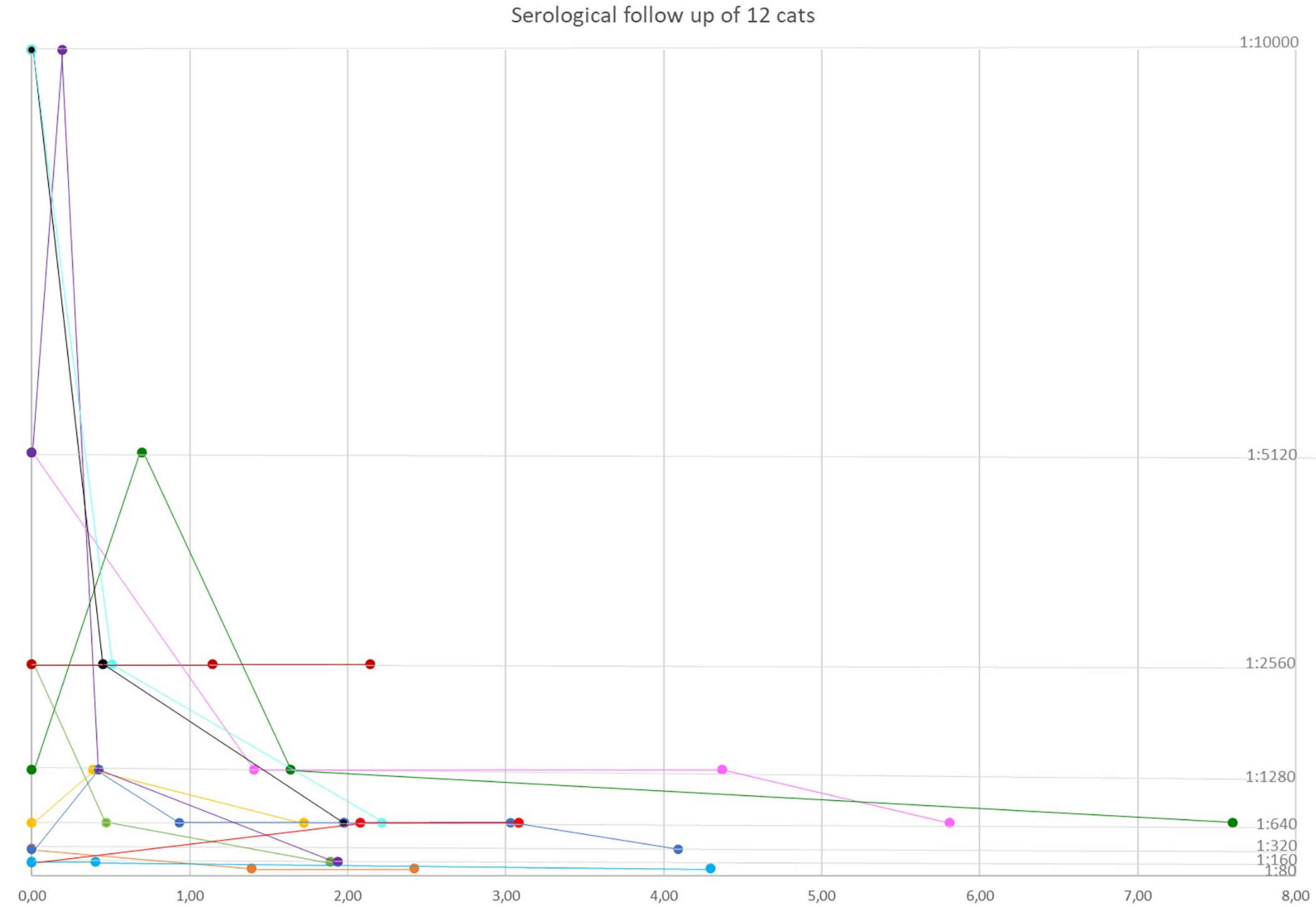

FIGURE 8 Serological follow up from 13 cats (*x*-axis, time since initial presentation in years, *y*-axis, serological titer).

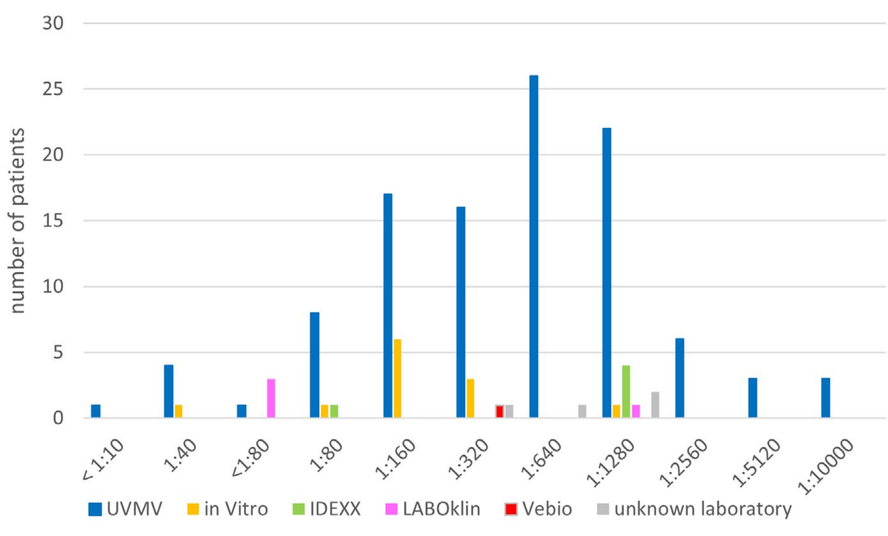

FIGURE 9 Sum of pre- and postoperative titers evaluated by the University of Veterinary Medicine Vienna and external laboratories. IDEXX, IDEXX Vet Med Labor GmbH; In Vitro, In Vitro-Labor für veterinärmedizinische Diagnostik und Hygiene GmbH; LABOklin, LABOklin Labor für klinische Diagnostik GmbH & Co KG, UVMV, Veterinary Medicine Vienna; Vebio, Vebio Laboratoire de Biologie Vétérinaire.

phacoemulsification. The anterior uveitis subsided in 20 of the 32 phacoemulsified eyes, persisted for up to 8 years post-operatively in 11 cats, and was not known for one cat. Figure 10A–C-2 shows examples of postoperative outcomes.

Fundic abnormalities were either diagnosed before surgery if fundoscopy was possible, or after the cataractous lens was removed (Figure 11A,B). Taking all cats and examinations together, 36/118 eyes had or used to have a cataract, and 8/30 otherwise healthy eyes developed fundic lesions. The remaining eyes either never displayed fundic lesions or the fundus was not, or only insufficiently accessible for evaluation. In addition to the appearances described at first presentation, pigment migration, fundus atrophia, retinal folds, retinal detachment, and a hyperreflective halo surrounding the optic disc were found. Progression of lesions occurred in 13 cases—one in an eye without a cataract and the remaining 12 after phacoemulsification of a cataractous lens.

Clinical and diagnostic findings in the later course of disease

As described above, three eyes were enucleated after persistence of ocular symptoms after phacoemulsification.

One feline eye was enucleated 3.58 years after phacoemulsification. Histopathology revealed an iridal amelanotic melanoma. No microsporidia were detectable by AFT stain in the parts of the lens capsule given and the PCR yielded a negative result for *E. cuniculi*.

The left eye of another cat was enucleated 4 years after phacoemulsification due to a secondary glaucoma (Figure 10C-2). A spindle cell sarcoma consistent with a feline post-traumatic ocular sarcoma was diagnosed, which completely penetrated the areas of the iris and retina, partially infiltrated the cornea, and focally grew through the sclera up to the outer surface of the eye. No microsporidia were detectable by AFT in the remnants of the lens capsule. Fine needle aspirates from lung, intestine, and kidney tissue taken 1 year after enucleation were all indicative of sarcoma. The owner opted for euthanasia.

The right eye of another cat was enucleated 7 months after phacoemulsification due to iris bombata formation. Single formations suspicious for *E. cuniculi* spores were present in AFT staining and the innermost retinal layer (axons of second neuron) appeared strongly vacuolated and loosened, which was seen as evidence of neuronal depletion.

In addition, two cats included in the study were euthanized and full body autopsy was available.

One patient was euthanized at the age of 12.58 years due to a reduced general health condition, pancytopenia, and azotemia with signs of chronic kidney insufficiency. The patient received bilateral phacoemulsification 8 years prior to its death. The *postmortem* examination revealed a small cataractous and calcified remainder of the lens in the posterior chamber of the left eye. In the right eye, a residue from the lens capsule and exudate was found in the posterior chamber. Partial retinal detachment was detected and fluid rich in protein was evident under the retina. The outer retinal layers showed degeneration and the ganglion cell layer was markedly thinner. The kidneys showed a low-grade interstitial nephritis and fatty nephrosis. Immunohistochemical testing for microsporidia was negative for the eyes, kidneys, liver, intestine, and spleen.

Another cat has euthanized due to an epithelial cell-dominated thymoma at the age of 8.83 years. The left eye, which was treated with phacoemulsification 3.75 years prior to its death, showed an effusion rich in protein near the ora ciliaris retinae, partial retinal detachment, and hypertrophy of the retinal pigment epithelium. The kidneys

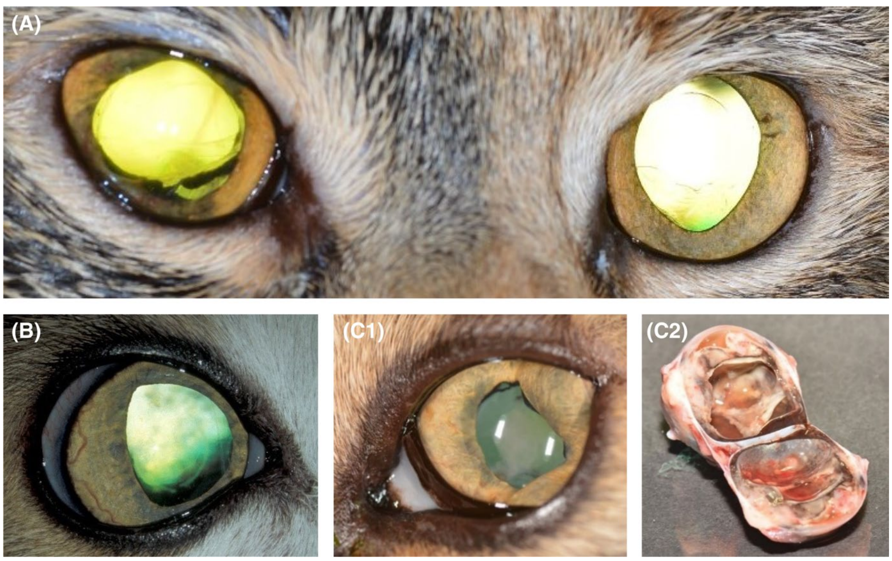

FIGURE 10 (A) 2.7-year-old female neutered European Shorthair; OU aphakic 7 months after bilateral phacoemulsification; OD slight discoria, pigment on the anterior lens capsule at the 4–7 o'clock position, slight rubeosis iridis; OS rhexis of anterior capsule visible. (B) 6.6-year-old male neutered European Shorthair, OD 2.5 years after phacoemulsification; aphakic eye with dyscoric pupil due to posterior synechia. (C-1) 6.8-year-old female neutered European Shorthair; OS 3.67 years post phacoemulsification; dyscoria, posterior synechiae with remaining anterior lens capsule, posterior capsular opacification and partial vitreal prolapse (same eye as in Figure 4E-2). (C-2) OS after encleation 4.33 years after phacoemulsification: Paramedian section of the globe with invasive growth of a sarcoma (same eye as in Figure 4E-2 and Figure 10C-1).

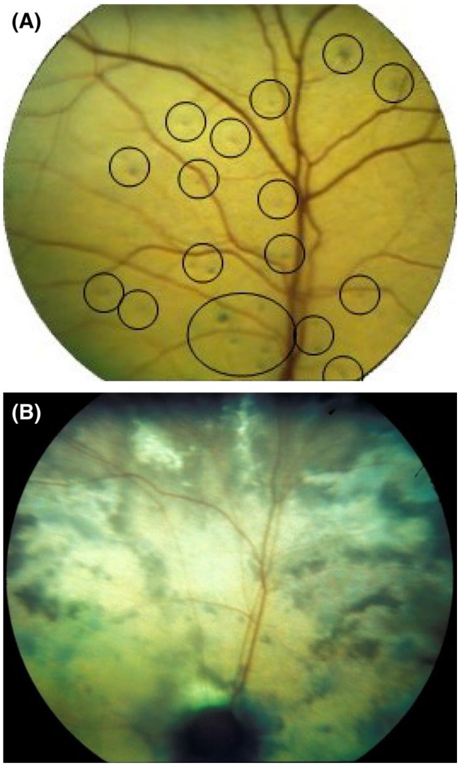

FIGURE 11 (A) 6-year-old female neutered European Shorthair; OD 20 months post phacoemulsification; multiple hyporeflective punctate areas (marked with black circles). (B) 6.5-year-old male neutered European Shorthair; OD 2.5 years post phacoemulsification; geographic changes and generalized hyperreflectivity, atrophy of the retinal vessels.

showed very discrete lymphoplasmacellular inflammatory lesions. Samples from the frontal brain, cerebellum, spinal cord, kidney, lung, liver, lymph node, spleen, and myocardium were excised for PCR analysis. The kidney sample tested positive for *E. cuniculi* strain type IV, which had previously been detected in the lens of the patient.

3.2 | Dogs

3.2.1 | Signalment and clinical findings

Table 1 summarizes the origins of the seven dogs, and the corresponding clinical information on the nine affected eyes included in this study. Briefly, all eyes harbored focal cataracts, and 7 of 9 eyes displayed uveitis. Figure 12A,B shows examples of anterior segments. Three dogs had fundic lesions at first presentation. D1 presented with bilateral punctiform hyporeflective areas, although only the right eye was cataractous. D3 showed several hyporeflective dots in the tapetal region in both eyes. During fundoscopy of D8, generalized small, punctate hemorrhages of the choroid were seen in the tapetal fundus of the right, otherwise healthy eye, and the cataractous left eye. *E. cuniculi* antibody titers ranged from 1:80 to 1:2560 at first presentation.

3.2.2 | Treatment

Medical treatment with fenbendazole at a varying dose between 25 and 55.5 mg/kg p.o. once daily for 3 weeks was documented for six patients (D1, D2, D3, D4, D5, D6). The remaining patient's systemic treatment status is unknown. Five eyes from four dogs (D1, D2, D4, D5) were phacoemulsified (Table 1).

3.2.3 | PCR results

Liquified lens material from the above four dogs retained during phacoemulsification revealed the presence of *E. cuniculi* strains II and III in one dog each, and strain IV in two dogs (Table 1). PCR analysis detected *E. cuniculi* DNA in aqueous humor from D5, but not D4.

3.2.4 | Cytology and histopathology

In contrast to the cats, no cytological samples from ocular specimens of the canine patients were examined. No *E. cuniculi* organisms were detected during pathohistological examination by Gram's or AFT stain in the three samples of lens capsules investigated (D1, D2, D4).

3.2.5 | Follow up

Serology and ophthalmological findings

Table 2 presents an overview of the follow-up serology and ophthalmological findings that were available for three dogs. Two dogs developed anterior and posterior capsular opacification/fibrosis post-cataract surgery (Figure 13).

Fundic abnormalities in the right eye of D2 became apparent in OD of D2 only after surgery. First, a hyperreflective halo was seen around the optic disc and focal hyporeflective lesions were found in the tapetal fundus and focal depigmentations in the non-tapetal fundus Figure 14A1–A2. The halo progressed continuously as did the changes in the non-tapetal fundus Figure 14B1–B2. Six years after phacoemulsification, the neuroretina appeared thinner and the fundic vessels were smaller in diameter. At the last examination 9 years post-surgery, generalized highly progressive tapetal hyperreflexia, thinner fundic vessels, and partial hyporeflexia of the tapetum were apparent, making the fundus appear radially striped.

TABLE 1 Signalment, results from the ophthalmologic examination and serological titers for *E. cuniculi* for the canine patients.

Dog Nr	Signalment	Age at onset of symptoms	Cataract	Fundus	Anterior uveitis	AB titer	Surgery, sequencing
D1	1-y-old m. n. mix from Hungary	7 months	OD *C. foc. cort. ant.*	OU pathologic	OD+ posterior synechia OD	1:160[a]	OD phaco: strain IV (human)
D2	1.2-y-old f. n. mix from Serbia	8 months	OD *C. foc. cort. ant.*	OU normal	OD+ posterior synechia OD	1:2560[a]	OD phaco: strain II (mouse)
D3	7.5-y-old m. n. German Shepard mix from Hungary	3 months	OD *C. foc. cort. ant.;* OS *C. foc. cort. ant. et post.*	OU pathologic	OD+ posterior synechia OU	1:160[b]	/
D4	8-month-old f. American Staffordshire Terrier from Hungary	4 months	OS *C. foc. cort. ant.*	OU normal	OS+ posterior synechia OS	1:80[a]	OS phaco: strain IV (human)
D5	2-y-old f. mix from Namibia	1,5 years	OU *C. foc. cort. ant.*	OU normal	OS+ posterior synechia OS	1:1280[c]	OU phaco: strain III (dog)
D6	4-month-old f. mix from Croatia	3 months	OS *C. foc. cort. ant.*	OU pathologic	OS+ posterior synechia OS	1:640[a]	/
D7	11-month-old f. n. mix from Mexico	2 months	OD *C. foc. cort. ant. et post.*	OU normal	OD	1:640[a]	/

Abbreviations: AB, antibody; f, female; m, male; n, neutered.

[a]University of Veterinary Medicine Vienna.

[b]In Vitro-Labor für veterinärmedizinische Diagnostik und Hygiene GmbH.

[c]LABOklin Labor für klinische Diagnostik GmbH & Co KG.

4 | DISCUSSION

As the cases published by Benz et al.,[6] Nell et al.,[7] and Neuber et al.[8] are included in the retrospective study, these publications were not cited.

4.1 | Ocular manifestation

4.1.1 | Age

In this study, ocular changes were mainly recognized in young cats up to the age of 6 years. Unfortunately, the animals often presented later in the course of the disease, and actual onset of cataract formation may not have been registered by all patient owners. In other studies, cats with *E. cuniculi*-induced cataracts were reported to be from under 2–15 years.[9]

Ocular problems in the dogs included in this study became evident in young animals, all of them being only up to 18 months old at the onset of symptoms. Although the number of dogs in this study is very low, this finding aligns with the literature, where lens changes were noted in 3-month-old blue foxes,[10] in 5-month-old minks,[11] and in mostly young rabbits.[12-15]

4.1.2 | Lens

Both unilateral and bilateral cataracts have been reported in a variety of animals, the former being more common in cats (this study,[9]), the latter more common in dogs (this study) and rabbits.[13,14,16,17]

The location described most often in the current study was a focal cortical anterior cataract in both cats and dogs. A vermiform process was noted in 37 feline eyes with focal cataracts, but the actual number of this characteristic of the cataracts may be higher, since in some cases the location and exact appearance of the lesion was not provided.

Other studies on cat eyes are limited in patient numbers but record bilateral focal anterior cortical cataracts, bilateral incipient peripheral cortical cataracts, and immature cataracts without further classification OU in one cat each.[9]

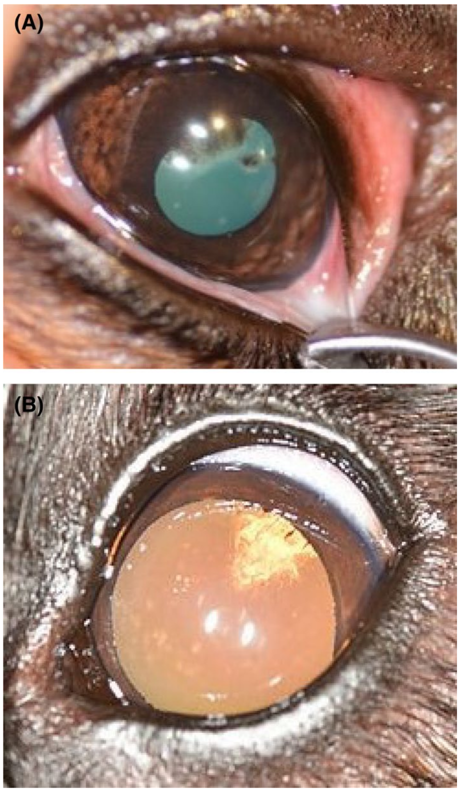

FIGURE 12 (A) D4; 8-month-old female intact American Staffordshire Terrier from Hungary; OS focal cortical anterior cataract, pigment dispersion on anterior lens capsule, posterior synechia. (B) D8; 2.2-year-old female intact mixed breed from Croatia; OS 22 months after initial presentation; focal cortical anterior cataract, posterior synechia.

Focal anterior subcapsular[16] and focal anterior cortical cataracts[12,18] associated with *E. cuniculi* have also been reported in rabbits. Unfortunately, no detailed information on the *E. cuniculi* cataracts in minks and blue foxes is available.[10,11] The solitary case of ocular encephalitozoonosis reported in a snow leopard manifested as a focal anterior cortical cataract OS and a mature cataract OD, which both progressed to hypermature cataracts.[19]

4.1.3 | Anterior uveitis

There are different theories to account for an inflammatory response to ocular infections with *E. cuniculi*. Firstly, the pathogen does not pose a problem when replicating in the segregated lens, only causing uveitis when the lens barrier is disrupted and lens proteins are released, which leads to a phacoclastic uveitis.[18] Secondly, antigens expressed by *E. cuniculi* have different modes of stimulating an inflammatory reaction in the eye, possibly by diffusion of antigens from the lens capsule,[15] or the presence of microorganisms in ocular structures other than the lens.

In support of the first hypothesis, mature or hypermature cataracts have been shown to cause phacolytic uveitis more often than immature or incipient cataracts, as the blood-aqueous barrier is impaired more in the advanced stages of cataracts.[20] Furthermore, lens capsule rupture is often seen in rabbits with cataracts, resulting in lens induced uveitis.[13,15,16,18,21] Rabbits have a thinner anterior lens capsule (10.7 µm,[22]), compared to dogs (42 µm) and cats (98 µm,[23]). In our study, of the nine canine eyes, seven (77.8%) had focal posterior synechia, which likely formed in order to seal a small defect in the lens capsule. In contrast, only 23/118 cat eyes (19.5%) had posterior synechia at the first presentation. It thus seems probable that disruption of the lens barrier is at least one factor contributing to the pathogenicity of *E. cuniculi* in the eye.

4.1.4 | Fundus

We found fundic lesions in 44 cat (36 cataractous and eight healthy) and eight (five cataractous and three healthy) dog eyes. However, the posterior segment of the eye could not be evaluated (at least before phacoemulsification) in the eyes with total cataracts, and a higher actual prevalence of pathological changes is likely. The rather high rate of fundic changes in cats in the present study suggests that *E. cuniculi* infection causes retinopathies in these animals. The focal hyporeflective dot-like lesions in both cats and dogs might be characteristic of an infection with *E. cuniculi* and can be interpreted as a sign of thickening of the ocular tissues overlying the tapetum lucidum due to fluid and/or cell accumulations in or under the retina. In support of this notion, lymphoplasmatic infiltrates and protein-rich fluid were found to be present in the retina and the subretinal space of cat globes.

In blue fox cubs, considerable arterial lesions of the polyarteritis nodosa type were seen in the short and long posterior ciliary arteries, their uveal branches, and in intraretinal vessels. Additionally, *E. cuniculi* spores could be found in the arterial walls. The authors stated that the impairment of the vessels could either be directly caused by the microsporidia themselves or by an autoimmune response triggered by them.[10] In our study, pathological changes of the retinal layers and lymphoplasmacytic infiltration of the choroid were evident during histopathology in nine and two cat eyes respectively. Some of the severe lesions seen during fundoscopy were similar to those described by Cassotis et al.[24] in the context of intraocular metastasis by bronchogenic carcinomas in four cats. The authors described wedge-shaped discoloration in the tapetal fundus and subretinal serous exudation, as well as attenuation of retinal vessels. Fluorescein angiography and histopathology suggested that the lesions were

TABLE 2 Follow-up of three dogs.

		OD				OS				
Dog Nr.	Time-line	Cataract	Uveitis	Fundic lesions	Surg	Cataract	Uveitis	Fundic lesions	Surg	Titer/lab
D1	1st p	+	+	+	Phaco	-	-	+		1:160 UVMV
	2nd p 12 m post phaco	a, PCO	+	Blurry		-	-	+		1:160 UVMV
	3rd p 2y post phaco	a, PCO	+	Blurry		-	-	+		1:320 In Vitro
D2	1st p	+	+	-	Phaco	-	-	-		1:2560 UVMV
	2nd p 9 m post phaco	a, PCO	-	+		-	-	-		1:640 UVMV
	3rd p 2.58y post phaco	a	-	+/prog		-	-	+		1:640 UVMV
	4th p 4.25y post phaco	a	-	+		-	-	+		
	5th p 7y post phaco	a, PCO	-	+/prog		-	-	+		
	6th p 9y post phaco	a, PCO	-	+/prog		-	-	+		1:40 UVMV
D6	1st p	-	-	+		+	+	+		1:640 UVMV
	2nd p + 8 m	-	-	+		+	+	+		
	3rd p + 22 m	-	-	+		+	-	+		1:160 UVMV

Abbreviations: a, aphakic; In Vitro, In Vitro-Labor für veterinärmedizinische Diagnostik und Hygiene GmbH; p, presentation; PCO, posterior capsular opacification; phaco, phacoemulsification; prog, progressive; surg, surgery; UVMV, University of Veterinary Medicine Vienna, –, not present; +, present.

FIGURE 13 D2; 1.2-year-old female spayed mixed breed from Serbia; OD 6 years post phacoemulsification; fibrosis of anterior and posterior capsule (3–9 o'clock position) and posterior synechia at 4 o'clock position.

consistent with ischemic necrosis caused by blockades of the chorioretinal vessels by neoplastic cells. Although the causality is different in ocular encephalitozoonosis, immunocomplexes or the pathogens themselves may progressively harm the retinal and choroidal arterioles to the point of visible atrophy of vessels and fundus leading to a similar clinical appearance (Figure 11B). During histopathological examination of the cats' globes, no microorganisms could be detected in the retina. Nevertheless, it is possible that the organisms are not consistently located in ocular tissues, but are eliminated after irreversible damage is already done, as is seen in other organs affected by *E. cuniculi*.

4.1.5 | Concurrent ocular neoplasia

Ocular neoplasia was diagnosed after enucleation in two cat globes: one was an amelanotic melanoma of the anterior uvea, the other a spindle cell sarcoma.

Iris melanomas are the most prevalent primary intraocular tumors in cats but an amelanotic appearance is rarely observed.[25-27] Unfortunately, the patient was lost to follow-up after surgery and no information on development of metastasis was available.

Research reports that sarcoma development can precede traumatic events to the eye, but equally suggests that lens

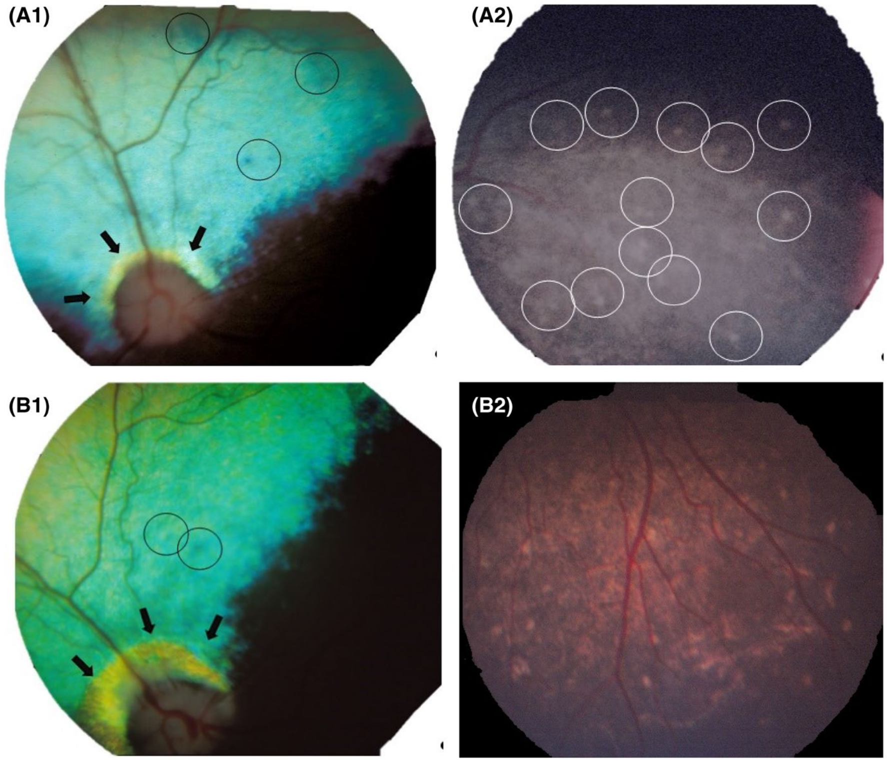

FIGURE 14 (A, B) Course of the funduscopic appearance of OD of D2, 1.2-year-old female spayed mixed breed from Serbia. (A-1) D2; OD 7 months post phacoemulsification; hyperreflective halo around optic disc (black arrows), singular hyporeflective lesions in the tapetal fundus (black circles). (A-2) D2; OD 7 months post phacoemulsification; small white dots in the non-tapetal fundus (white circles). (B-1) D2; OD 2.5 years post phacoemulsification; progression of halo around optic disc. (B-2) D2; OD 2.5 years post phacoemulsification; progression of focal depigmentations in the non-tapetal fundus.

impairment and chronic uveitis may be causal.[28-30] It can be assumed that the long-lasting uveitis present in the affected cat's eye was causative of the tumorous degeneration. Local recurrence after enucleation and metastasis of feline ocular sarcomas have also been reported in the past.[28,30] The patient eventually suffered from suspected sarcomas in the lung, intestine, and kidney which led to its euthanasia.

4.2 | Serology/prevalence

Indirect fluorescent antibody test (IFAT) has a sensitivity of 97.6% and a specificity of 100% for the detection of *E. cuniculi* antibodies in cats,[31] but there is no study evaluating the accuracy and diagnostic value of the test in dogs. We currently also lack established knowledge of cut-off values for positive results. In rabbits, a positive threshold titer of 1:40 has been used,[13,32] but a study with experimentally infected dogs revealed titers as low as 1:20, which may already be significant.[33]

A confounding factor with respect to *E. cuniculi* antibody titers is that laboratories external to the University of Veterinary Medicine Vienna apparently stopped the titration before the endpoint was reached, resulting in significantly lower reported titers for the same animals, compared to those found in our laboratory (Figure 9). These observations suggest that serological testing during the follow-up of a patient should be performed by the same laboratory and that titer levels from different testing sites should not be compared.

Worldwide studies on seroprevalences detected *E. cuniculi* antibody titers in 0%–26.8% of cats ((0/27),[34] (15/232–6%),[35] (34/127–26.8%),[36] (17/72–23.6%),[37] (18/295–6.1%)[38] and[6] (2/100–2%)). Between 0 and 48.5% of dogs were seropositive ((40/220–18.2%),[39] (73/193–37.8%),[37] (27/114–23.7% by IFAT, 78/367–21.3% by DAG),[40] (0/237),[41] (8/20–40%),[42] (27/125–21.6%),[43] (103/472–21.8%),[44] (33/68–48.5%)[45] and[46] (17/111–15.3%)). This would imply that dogs, although generally more frequently infected, are less prone to the development of ocular encephalitozoonosis according to our observations. Nevertheless, it must be considered that more studies were conducted with canine patients and that in total, almost twice the number of dogs has been tested in comparison to cats.

The number of dogs included in the present study is limited, but they had lower antibody titers than the cats. In both species, antibody levels decreased after treatment, but persisted for several years, even though the supposed site of infection—the lens—was removed. Similarly, antibody persistence was detected in untreated rabbits over the entire study period of 38 weeks in naturally infected cases and over a year in parenterally infected animals.[47,48] Antibodies were detected in an experimentally infected dog that did not receive treatment for the entire trial of 370 days.[49] In a human patient with idiopathic $CD4^+$ T-lymphocytopenia and overt encephalitozoonosis caused by strain type I, antibodies against *E. cuniculi* were still found more than 21 months after first detection. The symptoms caused by an iris tumor and endophthalmitis improved with the person's treatment of oral albendazole (400 mg BID).[50]

An immunocompetent individual ocularly infected with *E. cuniculi* type I during a laboratory accident showed the highest antibody titer 20 months post infection, but it was still positive up to 6 years after infection, albeit at low levels, and without concurrent clinical signs. He was treated with albendazole for 5 months (400 mg daily) and flumagillin for 4 months (10 mg/mL).[51] Conversely, the American Staffordshire puppy described by Engelhardt et al.[52] with nephritic encephalitozoonosis caused by strain type I was seronegative 3 months after treatment with fenbendazole.

4.3 | Treatment

In the present study, the administration of systemic fenbendazole at a dosage between 20 and 55.5 mg/kg for 3 weeks was documented for a large share of cats and dogs. This treatment regimen eliminates and prevents *E. cuniculi* in rabbits.[32] However, rigorous clinical trials to determine dosage and duration in cats and dogs have not yet been undertaken.

One cat eye, which was enucleated 7 months following phacoemulsification, contained some material suspicious for *E. cuniculi* spores. However, no evidence of *E. cuniculi* presence could be detected in the other five feline globes available for further examinations after previous phacoemulsification (either because of enucleation or autopsy). This suggests that the surgery was successful in eliminating *E. cuniculi* from ocular structures.

4.4 | Pathogen detection—Cytology/histopathology/PCR

PCR of the lens material is more sensitive than cytology and histopathology, yielding 100% positive results. Only 10/21 samples from aqueous humor were PCR positive, suggesting that the spores are mostly situated, or are more highly concentrated, in the lens itself. Cytological analysis of the lens material and aqueous humor is least reliable and microsporidia could only be found in about half of the anterior lens capsules in cats and in none of the three canine samples during pathohistological examination. Thus, the diagnostic focus should be on PCR testing of the lens material in surgical cases.

Two of seven feline urine samples were positive for *E. cuniculi* by PCR, which is consistent with the observation of intermittent pathogen excretion in rabbits.[53] Botha et al.[54] conducted a study where dogs were orally infected with urine of seropositive individuals containing unknown numbers of *E. cuniculi* spores. In this instance, the dogs did not express clinical signs but developed positive antibody titers. Additionally, nephritis was observed during pathological examinations. Moreover, Jeklová et al.[55] successfully infected rabbits via an ocular route (administering eye drops with *E. cuniculi* spores into the conjunctival sac) and suggested that ocular infection may occur during territorial urine marking of these animals. Direct infection or contamination of food or water by urine is a possible source of transmission, but testing of the urine is not a reliable method to rule out infectivity of an individual.

E. cuniculi strain type II was identified by sequencing of PCR products in 26/54 cats, strain type III in 3/54 cats, and strain type IV in 25/54 cats. To the authors' knowledge, this is the first time *E. cuniculi* type III was documented in cats. In other studies, strain type II was most commonly detected in cats,[9,56,57,58] but the current study suggests that strain type IV is equally prevalent in feline patients.

E. cuniculi strains I, II and III have been previously isolated from dogs,[52,58,59] and we have now documented strain IV in two dogs from Hungary as well.

E. cuniculi strain type I was neither detected in cat nor dog patients in the present study. This suggests that horizontal transmission from rabbits does not play a role in the development of ocular encephalitozoonosis in cats and dogs.

4.5 | Transmission

Several mechanisms for the transmission of *E. cuniculi* have been proposed. Firstly, rodents can act as a reservoir for *E. cuniculi* in many parts of Europe,[34,60,61,62] and the ingestion of infected mice appears to be a logical infection pathway for cats. Of the 75 cats in our study, only one was purebred, whereas 37 feline patients were adopted from animal shelters, the streets, or farms. It is thus probable that most of the mother animals lived outdoors and could have been exposed to wild rodents. Interestingly, a study from Iceland detected *E. cuniculi* antibodies in wild arctic foxes, minks, and mice[60] while a study from Greenland found none of 230 foxes seropositive.[63] This discovery was attributed to the fact that the arctic foxes in Greenland, in contrast to those in Iceland, do not feed on rodents.

Secondly, intrauterine infection with the pathogen is a plausible cause for cataracts in cats, analogous to the situation observed in rabbits, where *E. cuniculi* was detected in the unopened eyes of up to 10-day-old kits.[64] These authors postulated that transmission is restricted to the period of lens development during embryogenesis, when the lens is supplied by the *tunica vasculosa lentis*. This vascular system becomes obsolete with the commencement of the production of aqueous humor, regresses in late fetal development, and is usually completed by 14 days postnatally in dogs.[65,66] Furthermore, the mature lens capsule was thought to be impenetrable to microsporidial spores.[12] However, in 2019, Jeklová et al.[67] found *E. cuniculi* spores in the lenses of 4-month-old rabbits after experimental oral infection. Nevertheless, the histological images differ considerably from those seen in clinical cases, as the spores in the experimental study were mostly extracellular, and no inflammatory reaction was present. Thus, whether or not transmission with subsequent ocular infection can occur between adults needs further study.

Since animals affected by encephalitozoonosis are often housed in large numbers, such as dogs in large breeding kennels,[59,68] or minks and blue foxes in captivity,[11,69] an interplay between horizontal and vertical pathogen transmission seems likely. Poor hygiene may also facilitate the outbreak of disease in these animals.

The degree and age of onset of intraocular inflammation seem to be linked to the thickness of the lens capsule. Anterior uveitis could be diagnosed more frequently and earlier in the canine than in the feline patients, correlating with the thinner lens capsule in dogs. Similarly, young rabbits already show extensive abscesses in the anterior chamber and other signs of anterior uveitis associated with lens capsule rupture.

In utero infection of the fetal lens is a probable pathway of *E. cuniculi* transmission in cases with ocular manifestation. As no *E. cuniculi* strain type I could be isolated in the current study, the infection of the mother animals through contact with or feeding on rodents, but not on rabbits, is likely in cats and dogs.

In summary, although more research is necessary, *E. cuniculi* is increasingly being recognized as a cause of cataracts in cats and dogs, and awareness of the pathognomonic clinical presentation should be raised for a probably underdiagnosed disease.

5 | SUMMARY AND CONCLUSION

The present cat and dog patient histories show that *E. cuniculi* induced cataracts progress without surgical intervention. Timely phacoemulsification with adjuvant anti-inflammatory and antiparasitic medication with fenbendazole is the most promising therapy, although sometimes enucleations after post-phacoemulsification complications were necessary. Further research is needed to evaluate more precisely the effects of fenbendazole on clinical improvement and pathogen and antibody reduction in cats and dogs.

Due to the characteristic appearance of the disease, a diagnosis can be based on signalment (age of patient and maternal contact with rodents), results from ophthalmological examinations, and positive antibody titers. Serological testing is an important non-invasive diagnostic tool, but is not as sensitive as PCR analysis, is subject to inter-laboratory variability, and yields a great range of antibody levels. Nevertheless, this is the first study to provide extensive serological follow-up in naturally occurring cases of *E. cuniculi* infections, revealing a steady decrease in antibody titer, although antibodies sometimes persisted for several years after lens removal.

The most typical appearance of ocular encephalitozoonosis in cats and dogs is a focal cortical anterior cataract which can progress to a total cataract. In addition, anterior uveitis and fundic lesions are commonly associated with infection with the pathogen. Progression of fundic changes could be seen even after phacoemulsification, suggesting that there must be other mechanisms by which *E. cuniculi* can damage ocular structures than by direct replication in the lens.

AUTHOR CONTRIBUTIONS

Sophie Hofmann-Wellenhof: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft. **Barbara Nell:** Project administration; resources; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data were retrieved from the universities´ digitally saved records. The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study used archived tissues, images, samples, and medical records of privately-owned animals and is a retrospective study. The pictures are property of the Veterinary

University of Vienna and the owners give permission for publication to the university.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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