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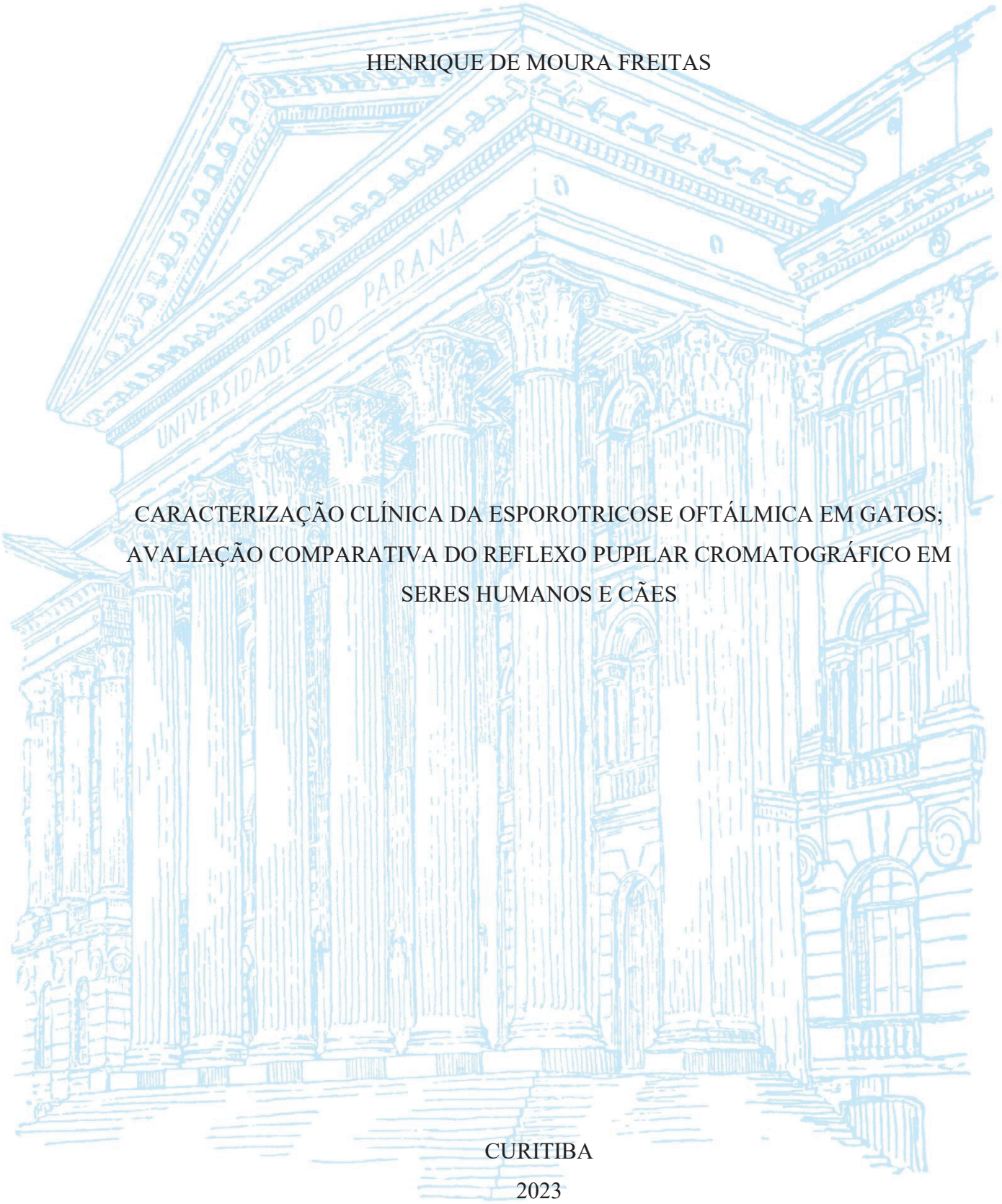
HENRIQUE DE MOURA FREITAS



CARACTERIZAÇÃO CLÍNICA DA ESPOROTRICOSE OFTÁLMICA EM GATOS; AVALIAÇÃO COMPARATIVA DO REFLEXO PUPILAR CROMATOGRÁFICO EM SERES HUMANOS E CÃES

CURITIBA

2023

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Orientador: Ph.D. Fabiano Montiani Ferreira

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08/03/2023 14:06:16.0
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Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

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THIAGO ALEGRE COELHO FERREIRA
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JOSÉ ADEMAR VILLANOVA JUNIOR
Avaliador Externo (PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ)

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10/03/2023 08:59:21.0
ROGERIO RIBAS LANGE
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Dedico essa tese aos meus pais, esposa e meus queridos filhos Noah e Miguel, sem vocês nada disso seria possível

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"Tudo tem o seu tempo determinado, e há tempo para todo o propósito debaixo do céu."

(Eclesiastes 3:1)

RESUMO

A presente tese compreende dois capítulos, o primeiro consiste de um artigo original a respeito da caracterização clínica de gatos diagnosticados com uma doença fúngica e importante zoonose denominada esporotricose. Esse capítulo aborda as principais lesões oftálmicas encontradas nesses animais, assim como as principais formas de tratamento para essas apresentações clínicas e uma possível forma de estadiamento das lesões encontradas, Tal trabalho se apresenta como uma possível referência para a comunidade científica para a inclusão da esporotricose oftálmica entre os possíveis diagnósticos diferenciais de blefaroconjuntivite em gatos. O segundo capítulo é um estudo comparativo entre seres humanos e animais onde é comparada a resposta de um teste denominado reflexo pupilar cromatográfico, que proporciona uma avaliação subjetiva da função da retina e do nervo óptico. O estudo compara as respostas ao estímulo luminoso com luzes vermelha e azul de comprimentos de onda específicos em pacientes humanos com retinose pigmentar e neuropatias ópticas isquêmicas ou hereditárias e cães previamente diagnosticados com atrofia progressiva de retina.

Palavras-chave: distrofias de retina; retinose pigmentar; pupilometria; esporotricose; conjuntivite em gatos.

ABSTRACT

This thesis comprises two chapters, the first consisting of an original article about the clinical characterization of cats diagnosed with a fungal disease and important zoonosis called sporotrichosis. This chapter addresses the main ophthalmic lesions found in these animals, as well as the main forms of treatment for these clinical presentations and a possible way of staging the lesions found. This work presents itself as a possible reference for the scientific community for the inclusion of ophthalmic sporotrichosis among the possible differential diagnoses of blepharoconjunctivitis in cats. The second chapter is a comparative study between humans and animals, where chromatographic pupillary methods are compared, which provides a subjective evaluation of retinal and optic nerve function. The study compares responses to light stimulation under red and blue light of specific wavelengths in human patients with retinitis pigmentosa and ischemic or hereditary optic neuropathies and dogs previously diagnosed with progressive retinal atrophy.

Key-words: retinal dystrophies; retinitis pigmentosa; pupillometry; sporotrichosis; feline conjunctivitis.

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SUMMARY

1. GENERAL INTRODUCTION

Comparative medicine is based on the similarity between human and animal physiology. From the genetic code to cellular functions, many phenomena are shared among the most diverse forms of life (Sengupta *et al.,* 2015). At the same time that adaptive features such as vision have facilitated the development of living things (Gehring, 2014), cellular changes and mutations in the genetic code have made possible the appearance of very similar diseases among animal species and their use as experimental models of human diseases (Petersen-Jones, 1998; Na *et al.,* 2021).

With the advent of animal domestication, this approximation between man and other species and the exploitation of the field by agriculture also allowed microorganisms such as *Sporothrix* spp. to leave the soil to colonize the skin of different animals, including humans (Lejeune and Kersting, 2010; Chakrabarti *et al*., 2015). These fungal species have adapted mostly to the body of the cat, an animal that has the habit of spending its claws on trees, contaminating them with the fungus present in organic matter. The first descriptions of this disease in humans refer to rural dwellers who became infected through thorns or contaminated organic matter, but it is a fact that nowadays this infection is predominantly zoonotic. (Rabello *et al.,* 2022). Moreover, the territorial behavior of the cat has promoted the spread of sporotrichosis in the feline species resulting from fights and territorial disputes, predominantly in cats with access to the street or semi-domesticated (Lopes-Bezerra *et al.,* 2018; Gremião *et al.,* 2021).

Brazilian states such as São Paulo, Rio de Janeiro, Rio Grande do Sul and Paraná have for many years been facing an epidemic of sporotrichosis. Besides the typical ulcerative and nodular skin lesions in cats and the lymphocutaneous lesions in humans, more and more extracutaneous lesions such as respiratory changes and conjunctivitis are related to this disease (Bonifaz *et al.,* 2007; Pereira *et al.,* 2014; Gremião *et al.,* 2015; Poester *et al.,* 2017;Yamagata *et al.,* 2017; Mothé *et al.,* 2021; Ramirez-Soto *et al.,* 2021; Arinelli *et al.,* 2022)

The first article of this thesis aims to identify the main ocular lesions in cats, highlighting the main forms of diagnosis and treatment.

At the same time that infectious diseases cause damage to public health, genetic and inherited diseases such as retinal degenerations and dystrophies and rarer diseases such as Leber's hereditary optic neuropathy cause visual loss and consequently a decrease in quality of life in humans and animals (Hartong *et al.,* 2006; Meyerson *et al.,* 2015; Ben-Yosef., 2022). Among the retinal dystrophies, retinitis pigmentosa (RP) in humans and progressive retinal atrophy (PRA), which predominates in purebred dogs, stand out. These two diseases require diagnosis through mechanisms that evaluate the ocular electrophysiology, the main method is an examination called

electroretinography (ERG). This exam evaluates the ocular electrophysiology through light stimuli that sensitize the retina and are captured by electrodes that record this electrical activity in graphs that are interpreted according to the adaptation to the environment, the duration and intensity of light stimuli, and can detect mainly pathological changes in photoreceptors called cones and rods (Petersen-Jones, 1998; Robson *et al.,* 2003; Brunel *et al.,* 2019)

One of the main difficulties of this exam is the cost and difficulty of execution, requiring a highly specialized professional for the best interpretation of the exam. Thus, subjective techniques have emerged as alternatives and easy to perform and interpret for the evaluation of the retina and optic nerve. Among these techniques is the evaluation of the pupillary light reflex through specific wavelengths of different intensities under red and blue light. This technique is called chromatographic pupillary reflex and makes it possible to access different pathways such as photoreceptors (cones and rods) and specific cells that are part of the ganglion cells belonging to the retina called intrinsically photosensitive retinal ganglion cells (ipRGCs) (Kardon *et al.,* 2011; Rukmini *et al.,* 2019).

Five different types of neurons are located in the retina of vertebrates: photoreceptors (cones and rods), which detect light, horizontal cells, bipolar cells, and amacrine cells, which function as interneurons, and ganglion cells that give rise to the optic nerve. These neurons are arranged in three nuclear layers that are separated by two other plexiform layers. The photoreceptors are located in the outer nuclear layer (outer retina), the interneurons in the inner nuclear layer and the ganglion cells and some amacrine cells in the ganglion cell layer (inner retina) (Sanes and Masland, 2015). IpRGCs represent about 0.2 - 1% of the total retinal ganglion cells and have a photosensitive pigment different from those found in the cones and rods called melanopsin. They are also related to several events such as the regulation of the sleep-wake cycle and the pupillary light reflex. (Markwell et al., 2010; Aranda and Schmidt., 2021)

The ganglion cells are the last cells to be degenerated in most retinal dystrophies and their death is related to the process of retinal remodeling secondary to the degeneration of photoreceptors and the vascular alterations triggered by this degenerative process and is evidenced only in the last stages of retinal degeneration (Garcia-Ayuso *et al.,* 2019). Evaluation of the chromatographic pupillary reflex makes it possible to differentiate whether the alteration comes from the outer retina or the inner retina (Kardon *et al.,* 2011). In Leber's hereditary optic neuropathy, ganglion cell death occurs exclusively as a result of apoptosis due to oxidative stress (Yu-Wai-Man *et al*., 2008). In glaucomatous optic neuropathy, first the inner retinal cells die (by apoptosis) and then the outer retinal cells die (by ischemia secondary to retinal microcirculation

changes) (Whiteman *et al.,* 2002), however, in the inner retina, ipRGCs are preserved up to 70% in most glaucomas (Gao *et al.,* 2022). These characteristics cause an absence of response under high brightness blue light (sensitizes mainly ipRGCs) and red light (sensitizes cones and rods) mainly in chronic glaucomas (most cases in dogs) and decreased response mainly under red light in cases of PRA in dogs and RP in humans.

The second article of this thesis discusses the application of the chromatographic pupillary reflex in humans and animals with neuropathies and retinal dystrophies demonstrating the applicability and functionality of this method in medicine and veterinary medicine.

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2. CHAPTER I - OCULAR LESIONS IN CATS DIAGNOSED WITH SYSTEMIC SPOROTRICHOSIS

2.1 ABSTRACT

Objectives: To describe the most common ocular lesions and demonstrate the frequency of ophthalmic involvement in a group of cats with systemic sporotrichosis.

Animals studied: Two hundred seventy-four cats with systemic sporotrichosis. The inclusion criteria were previous positive cytopathological, histopathological or fungal culture exams.

Procedures: In a prospective case-control study, two hundred seventy-four cats diagnosed with systemic sporotrichosis underwent ophthalmic evaluation and received treatment for systemic sporotrichosis. Of these animals, 63 had ocular abnormalities. Conjunctivitis was scored from 0 to 5. Diagnostic techniques utilized included fungal culture and cytopathological (10 eyes; 10 cats) and histopathological examination of the palpebral conjunctiva and eyes (6 eyes; 4 cats).

Results: Cytopathological examination of the conjunctiva, fungal culture and histopathologic exam proved to be important tests for the detection of *Sporothrix* sp. Five cats without evidence of ophthalmic abnormalities also had positive fungal cultures. The identified ocular lesions in animals with systemic sporotrichosis included increased serous discharge (79 eyes; 53 cats), blepharoconjunctivitis (33 eyes; 25 cats), conjunctivitis (39 eyes, 20 cats), blepharitis (9 eyes; 8 cats), uveitis (5 eyes; 3 cats) and lesions similar to Florida keratopathy-like lesions (2 eyes; 1 cat). Most eyes with conjunctivitis or blepharoconjunctivitis were characterized within score 4 (22 eyes ;30.56%).

Conclusion: Sporotrichosis should be considered in the differential diagnoses for conjunctivitis and blepharoconjunctivitis, especially in endemic areas. Fungal culture and cytopathology of ocular discharge and histopathological exams of the conjunctiva are important tools for diagnosing ophthalmic sporotrichosis.

Keywords: cats, conjunctivitis, blepharoconjunctivitis, sporotrichosis, conjunctival swabs, fungal culture

5.5 INTRODUCTION

Sporotrichosis is a subcutaneous mycosis caused by dimorphic fungi of the genus *Sporothrix* spp[1]. Currently, at least six clinically important species comprise the Sporothrix schenkii Complex[2]. In Brazil, the most important species is the *Sporothrix brasiliensis*[3,4,5,6]. This fungal complex has been isolated from a large variety of living organisms, including seaweed, insects, birds, reptiles, iguanas, camels, chimpanzees, dogs, armadillos, cattle, horses, donkeys, rodents, and humans[4]. However, the domestic cat (*Felis catus*) is the most susceptible species and is the primary cause of transmission in urban outbreaks[3]. Feline disease typically occurs from contamination of cutaneous wounds, especially bites and scratches from fights with other cats, with fungal propagules (usually spores) from organic matter or exudate, secretions, and aerosols[5].

The disease is zoonotic, and human sporotrichosis has been documented by contact with infected cats after bites and scratches or exudate contamination, and rarely with contaminated plants[4,7]. Human cases are usually lymphocutaneus, whereas cats mainly develop disseminated cutaneous disease. However, feline sporotrichosis can also cause extracutaneous manifestations such as respiratory or ocular disease and systemic signs such as lethargy and anorexia principally in immunocompromised animals[4].

The primary ophthalmic manifestation of sporotrichosis in both cats and humans is granulomatous conjunctivitis, which has been poorly characterized to date [8,9,10,11]. Nevertheless, in most cases, dermatologic and respiratory manifestations are very severe, overlapping the more discrete ocular signs, the latter of which may become a lower treatment priority in some clinical situations. The main purpose of this study is to characterize the ocular lesions caused by sporotrichosis in cats with cutaneous lesions. Providing a clear clinical characterization of the ophthalmic manifestations of sporotrichosis will contribute to a better understanding of the disease pathogenesis and introduce the disease as an important differential diagnosis for conjunctivitis in cats living in endemic areas, something that is currently lacking in the literature. Additionally, knowing the spectrum of ophthalmic manifestations may result in better recognition of ophthalmic disease and improve directed treatment.

5.6 MATERIAL AND METHODS

2.3.1 Data collection and animal selection

This study was conducted at the Pontifical Catholic University of Paraná (PUCPR) and at the Federal University of Paraná (UFPR), between 2019 and 2020. The patients were seen at PUCPR. To assess the clinical and epidemiological profile of the studied population, a standard form was designed to record individual information of each cat with systemic sporotrichosis. Standardized questions consisted of sex, breed, reproductive status, habitat, possible contacting animals, affected owners, previous treatments, and exams. All procedures performed in the study were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for Use of Animals in Ophthalmic Vision and Research. PUCPR´s Research Ethics Committee (CEUA-PUCPR) approved the investigation under certificate #02060.

In a prospective case-control study, cats with ulcerative or nodular skin lesions, conjunctivitis, and nodules on the nasal bridge were forwarded by the zoonosis control center of the Curitiba to the attendance service in sporotrichosis of the veterinary school clinic of PUCPR. Only cats with a diagnosis of systemic sporotrichosis were included in the study, regardless of sex, breed, or age group. The inclusion criteria were a positive result for sporotrichosis on cytology, histopathology, and/or fungal culture from dermatologic or nasal lesions concomitantly with eye injuries, or from ophthalmic lesions alone. During the investigation period, 274 cats with systemic sporotrichosis were evaluated. The diagnosis was achieved after association of the following factors: anamnesis, clinical examination, visualization of fungal yeasts in the cytological or histopathological examination, and isolation and microbiological identification of *Sporothrix* spp. in a specific fungal culture medium. Sample collection was either of exudate from skin lesions by swab (for the fungal culture and cytological exam) or imprint (for the cytological exam) and skin biopsy in nodular lesions (for the histopathological exam). Cats with ocular or respiratory injuries without dermatologic lesions were tested by conjunctival or nasal swabs or histopathological exam (palpebral conjunctiva) to confirm the diagnosis of ocular and respiratory sporotrichosis.

2.3.2 Clinical Classification of Cutaneous and Ophthalmic Abnormalities

All cats underwent detailed clinical examination. Cutaneous lesions were classified as 1) fixed cutaneous (a single lesion, generally restricted to the fungal inoculation point), 2) lymphocutaneous (skin lesions were contiguous to the lymphatic pathway and associated with lymphangitis and regional lymphadenitis), or 3) disseminated cutaneous (multifocal or generalized ulcerative-gummy lesions). Ophthalmic and respiratory abnormalities were considered extracutaneous lesions, recorded following the airway examination, which sought to observe the presence of nodules or tumors on the nasal bridge, facial deformity, nodules in the nasal cavity, abnormal respiratory sounds, increased serous ocular discharge, sneezing, dyspnea, and tachypnea. In addition, all selected cats underwent ophthalmic evaluation, with neuro-ophthalmic evaluation through direct and consensual pupillary light reflex, dazzle reflex, and menace response performed with a 3.5V Finoff transilluminator with a halogen lamp (Welch Allyn®, Skaneateles Falls, NY, USA). Slit-lamp evaluation (Hawk-Eye, Dioptrix, L`Union, France) and corneal staining with fluorescein and lissamine green strips (Drogavet; Curitiba-PR, Brazil), tonometry using the Tono-pen tonometer (Mentor Ophthalmics Inc, Norwell, Massachusetts, USA), and funduscopy by indirect ophthalmoscopy (Welch Allyn®, Skaneateles Falls, NY, USA) after pharmacological mydriasis with 1% tropicamide (Mydriacyl®, Novartis®, São Paulo, SP, Brazil) were also performed.

Any lesion of the eyes or adnexa was considered as an ophthalmic disease. Signs of conjunctivitis were conjunctival hyperemia (mild, moderate, or severe), chemosis, presence of conjunctival granuloma with or without increased serous ocular discharge, and the presence of lymphoid follicles. Conjunctivitis with or without blepharitis (blepharoconjunctivitis) was staged from 0 to 5 according to HARTMANN et al. (2010)[12] (Table 1).

Table 1: Staging of sporotrichotic granulomatous conjunctivitis/ blepharoconjunctivitis by global score.

Score	Conjunctivitis/ blepharoconjunctivitis classification
0	No signs of conjunctivitis.
1	Mild conjunctival hyperemia associated with mild chemosis with or without epiphora.
2	Moderate conjunctival hyperemia and chemosis, with or without epiphora.
3	Conjunctival hyperemia and intense chemosis, with the onset of nodular or granulomatous appearance with or without epiphora.
4	Intense conjunctival hyperemia and chemosis, with an appearance of a clear nodular or conjunctival granuloma with or without epiphora or association with lymphoid follicles.
5	Severe conjunctivitis with tumoral appearance and eyelid margin deformation.

2.3.3 Diagnostic Testing

Samples were carefully collected from the inferior conjunctival fornix of the left eye of the twenty animals under manual restraint (10 with ocular abnormalities and 10 without ocular lesions) using conjunctival swabs for cytological examination. After collection, the sample was distributed on a glass slide, stained by the Romanowski method with rapid Panopticon (Laborclin – Pinhais – PR), and analyzed under an optical microscope with 40X and 100X objectives for yeast counting and evaluation of the inflammatory infiltrate.

A second swab of the left eye was collected from the same animals (10 with ocular abnormalities, and 10 without ocular abnormalities) for fungal culture. Swab material was incubated in Sabouraud dextrose agar with chloramphenicol (0.05g/L) for 5 to 7 days at 25ºC to visualize the mycelial phase, followed by incubation in brain-heart infusion agar at 37ºC to identify the yeasts. Identification of the mycelial phase was determined by the color change of the colonies and the observation of this phase under optical microscopy using the acetate tape prep method and staining with cotton-blue lactophenol. Diagnosis was confirmed by observing septate hyphae with small ovoid conidia in a 100X objective lens[13]. A small number of animals was selected from the cytological examination and fungal culture because of the difficult handling of the sick cats and staff training.

Among those 10 animals tested with ocular abnormalities, 2 animals with a conjunctivitis score of 4/5 were subjected to an incisional biopsy. Using a 2 mm biopsy punch, the right eye lower eyelid conjunctiva was collected following an intramuscular application of dexmedetomidine (10 mcg/kg), ketamine (3 mg/kg), methadone (0.3 mg/kg) and midazolam (0.15 mg/kg) in the semi-tendinous muscle of the right thigh. Two other animals with a score of 4 were submitted to bilateral postmortem enucleation, with biopsy material stored in 10% formalin, embedded in paraffin, and then submitted to serial sections of 5µm with a microtome. The slides were stained with periodic acid-Schiff stain (PAS) and hematoxylin-eosin (H&E)[14] and evaluated by optical microscopy using 40X and 100X objectives. Unfortunately, only 14 cats (5.11%) with ocular abnormalities were tested by laboratory techniques due to financial constraints by the Coronavirus disease 19 pandemic (COVID-19).

2.3.4 Treatment

All cats with a definitive systemic sporotrichosis diagnosis were treated with oral itraconazole every 24 hours at a dose of 50 mg/animal in cats weighing 1 to 3 kg or 100 mg/animal in cats weighing >3 kg and oral potassium iodide (400 mg/ml) at a dose of 2.5 to 5mg/kg every 24 hours. Animals diagnosed with severe conjunctivitis (scored 4-5), received in addition to previous treatments a single application of 0.5 ml intralesional amphotericin b at a concentration of 5mg/ml by insulin needle under the same tranquilization protocol as used for biopsy.

2.3.5 Statistical Analyzes

Descriptive statistics were performed with StatView (SAS Institute, Cary, New Jersey). Specificity, predictive value, and sensitivity calculations were performed using GraphpadQuickCalcs statistical software (Graphpad Software Inc., La Jolla, CA).

2.4 RESULTS

Between 2018 and 2020, 274 cats with sporotrichosis were evaluated at SVC- PUCPR for ophthalmic signs. Of these, seven animals (2.55%) were Siamese, three (1.09%) were Persian and 264 (96.35%) were mixed breed. Two hundred and twenty-one (80.65%) were males and 53 (19.35%) were females. One hundred and eighty-three cats (66.79%) were intact and 91 (33.21%) were spayed or neutered. The mean age of the population was 34.02 months, with a minimum age of three and a maximum of 156 months.

Ophthalmic lesions were found in sixty-three out of 274 cats (22.99%) definitively diagnosed with systemic sporotrichosis (Table 2). Among those cats diagnosed with ophthalmic signs, 62 (98.41%) were mixed breed and one cat (1.59%) was a Persian. Forty-three (68.25%) were males and 20 (31.75%) were females, 10 cats (15.87%) were castrated and 53 (84.13%) were intact. The average age was 27.32 ± 15.82 months, with a minimum age of three months and a maximum of 144 months.

Disseminated cutaneous sporotrichoses were identified in 211 animals (77.01%). Forty-four cats (16.06%) had focal cutaneous lesions, five cats (1.82%) had lymphocutaneous disease, and 148 (54.01%) had extracutaneous lesions (respiratory or ophthalmic). Among cats with ophthalmic lesions, disseminated sporotrichosis was documented in 60 animals (95.24%), and 61 animals (96.83%) had respiratory signs compared to only two (3.17%) with exclusively

ophthalmic abnormalities. Ophthalmic lesions included increased serous ocular discharge in 53 animals (84.13%; 79 eyes) of which 27 had unilateral signs (50.94%) and 26 had bilateral signs (49.06%), blepharoconjunctivitis in 25 (39.68%; 33 eyes) of which 17 had unilateral signs (68%) and 8 had bilateral signs (32%), conjunctivitis in 20 animals (31.74%; 39 eyes) of which 11 had unilateral signs (55%) and 9 had bilateral signs (45%), blepharitis without conjunctivitis in 8 animals (12.70%; 9 eyes) of which 7 had unilateral signs (87.5%) and one had bilateral signs (12.5%), uveitis in 3 animals (4.76%; 5 eyes) one with unilateral signs (33.33%) and 2 with bilateral signs (66.66%) and Florida keratopathy-like lesions in one cat (1.59%; 2 eyes) with bilateral signs. (Table 2). Animals with uveitis had diffuse corneal edema, an absence of a menace response and two animals had a positive diagnosis of feline leukemia virus (FeLV) by serological examination. Conjunctivitis and blepharoconjunctivitis were classified by score according to Table 3 and illustrated in Figure 1.

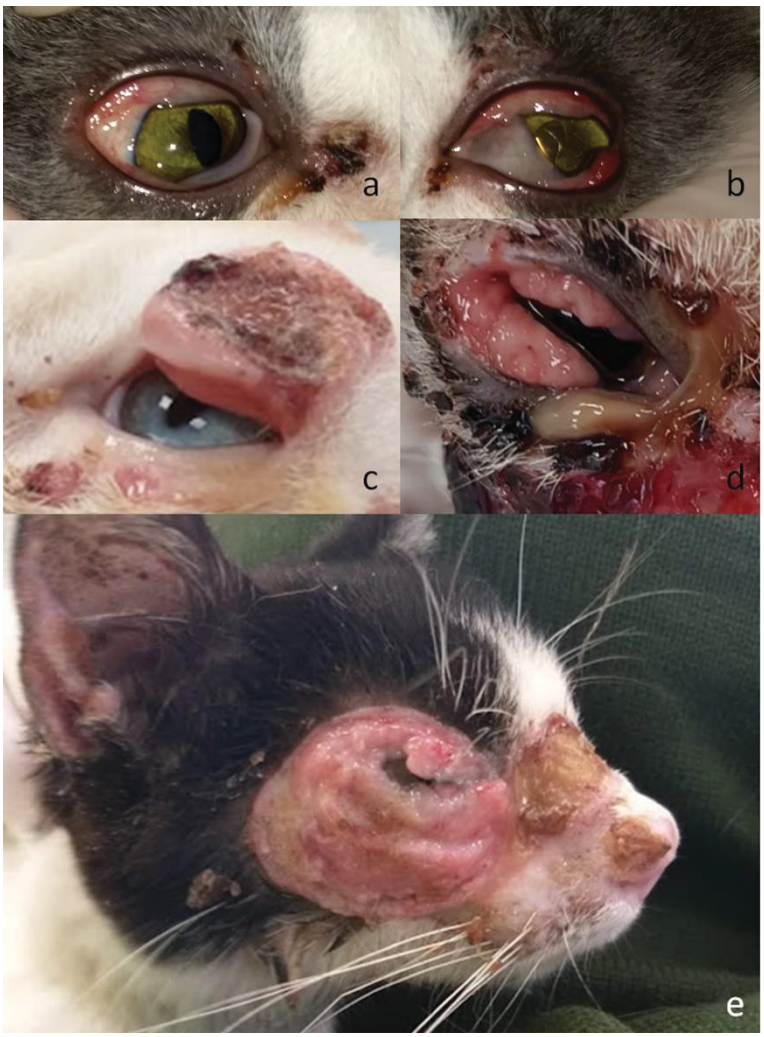

Figure 1 –Ophthalmic findings - A – Conjunctivitis score 1 (Patient 10). Note the epiphora with mild conjunctival hyperemia and chemosis. B – Conjunctivitis score 2. Marked hyperemia associated with chemosis and epiphora

(Patient 10). C – Conjunctivitis score 3 is similar to a score of 2 but associated with blepharitis. Note the severe eyelid swelling and the nodular conjunctiva appearance (Patient 01). D – Conjunctivitis score 4. Note the presence of blepharitis, mainly in the lower eyelid and nasal corner, extending to part of the upper eyelid, a large amount of purulent secretion in the nasal corner and intense chemosis with granuloma formation, in addition to epiphora. Lymphoid follicles were present, denoting a conjunctival score of 4 from 3. (Patient 02). E – Conjunctivitis score 5. Note the disruption of inability to fully visualize the eyelid margin along with the tumoral appearance, compromising the assessment of the ocular surface (Patient 21).

Table 2- Main clinical features and type of diagnostic test searching for sporotrichosis performed.

Cat Identification	Age (months)	Sex	Breed	Reproductive status	Skin lesion	Ocular condition	Respiratory signs	Positive fungal culture	Rssponse to treatment
Patient 01	96	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin and eye	Responsive
Patient 02	24	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 03	24	female	Mixed breed	Entire	Disseminated cutaneous	Serous discharge	Yes	Skin	Not responsive
Patient 04	48	female	Mixed breed	Entire	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 05	7	female	Mixed breed	Entire	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 06	36	male	Mixed breed	Neutered	None	Serous discharge	Yes	Eye and nasal discharge	Not responsive
Patient 07	24	female	Mixed breed	Entire	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 08	24	male	Mixed breed	Entire	Disseminated cutaneous	Serous discharge	Yes	Skin	Not responsive
Patient 09	72	female	Mixed breed	Entire	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin and eye	Not responsive
Patient 10	36	male	Mixed breed	Neutered	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin and eye	Responsive
Patient 11	12	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin and eye	Responsive
Patient 12	21	male	Mixed breed	Neutered	Disseminated cutaneous	Serous discharge	Yes	Skin and eye	Responsive
Patient 13	36	male	Persa	Entire	Disseminated cutaneous	Serous discharge and Florida keratopathy-like lesions	Yes	Skin and eye	Responsive
Patient 14	24	male	Mixed breed	Entire	None	Blepharoconjunctivitis and serous discharge	No	Eye	Not responsive
Patient 15	12	female	Mixed breed	Neutered	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Not responsive
Patient 16	6	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patiente 17	36	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 18	3	male	Mixed breed	Entire	Disseminated cutaneous	Uveitis	Yes	Skin	Not responsive
Patient 19	12	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin and eye	Responsive
Patient 20	30	male	Mixed breed	Neutered	Disseminated cutaneous	Serous discharge	Yes	Skin	Responsive
Patient 21	28	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis	Yes	Skin	Responsive
Patient 22	36	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 23	34	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Responsive
Patient 24	32	female	Mixed breed	Entire	Disseminated cutaneous	Blepharitis, uveitis and serous discharge	Yes	Skin	Responsive
Patient 25	32	female	Mixed breed	Neutered	Disseminated cutaneous	Serous discharge	Yes	Skin	Responsive
Patient 26	36	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 27	36	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 28	24	female	Mixed breed	Neutered	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 29	18	male	Mixed breed	Entire	Disseminated cutaneous	Serous discharge	Yes	Skin	Not responsive
Patient 30	36	male	Mixed breed	Neutered	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 31	30	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive

Patient 32	30	male	Mixed breed	Entire	Disseminated cutaneous	Blepharitis and serous discharge	Yes	Skin	Responsive
Patient 33	36	male	Mixed breed	Neutered	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Responsive
Patient 34	12	male	Mixed breed	Entire	Disseminated cutaneous	Uveitis	Yes	Skin	Not responsive
Patient 35	8	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 36	48	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 37	48	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 38	48	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 39	18	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 40	18	female	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 41	24	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 42	24	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Not responsive
Patient 43	20	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis	Yes	Skin	Responsive
Patient 44	24	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Not responsive
Patient 45	36	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 46	36	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 47	18	female	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 48	36	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 49	8	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 50	24	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 51	36	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 52	30	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Not responsive
Patient 53	24	male	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 54	12	female	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Not responsive
Patient 55	36	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 56	8	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Responsive
Patient 57	48	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 58	24	female	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Not responsive
Patient 59	12	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 60	18	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis	Yes	Skin	Not responsive
Patient 61	12	female	Mixed breed	Entire	Disseminated cutaneous	Blepharoconjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 62	8	male	Mixed breed	Entire	Disseminated cutaneous	Conjunctivitis and serous discharge	Yes	Skin	Responsive
Patient 63	12	Female	Mixed breed	Neutered	None	Conjunctivitis and serous discharge	No	Eye	Not responsive

Table 3 – Number of eyes categorized by conjunctivitis/ blepharoconjunctivitis and respective clinical scores**.**

Score	Number of eyes
01	11 (15.27%)
02	13 (18.06%)
03	10 (13.89%)
04	22 (30.56%)
05	16 (22.22%)
Total	72

Of the 63 animals, 47 (74.60%) had clinical improvement with any of the proposed treatments. Twenty-two animals (34.92%) received an intralesional amphotericin injection. All animals with blepharoconjunctivitis or conjunctivitis that showed skin improvement during the treatment also showed improvement in ophthalmic lesions (Figure 2).

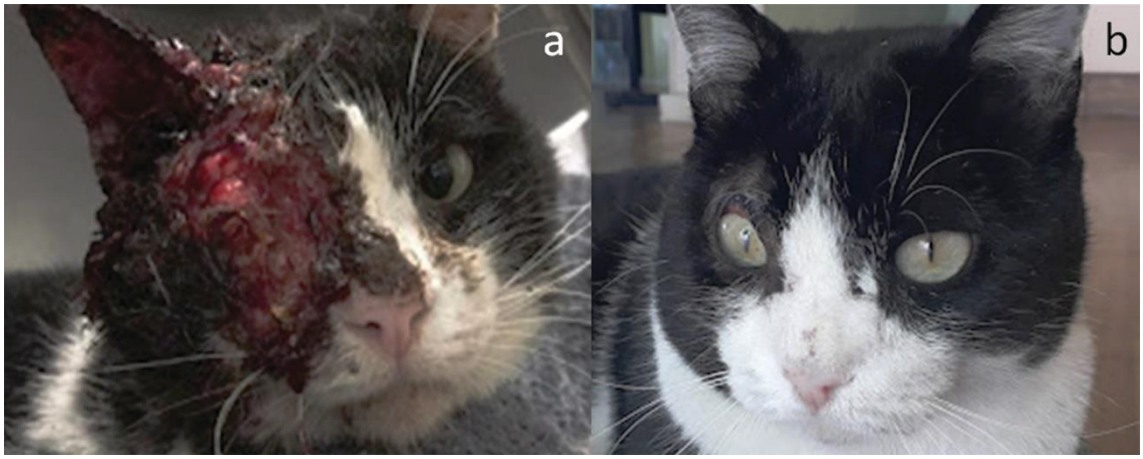

Figure 2 – Treatment response - Evolution of ophthalmic sporotrichosis after treatment with itraconazole, potassium iodide and amphotericin b. A – Blepharoconjunctivitis score 5 before treatment. B - Same animal after 6 months of treatment showing slight change to the palpebral fissure in the right eye compared to the normal left eye, including cicatricial elevation of the superior eyelid. (Patient 15).

Not all cats had laboratory testing of ophthalmic tissues for sporotrichosis, but in all samples collected from the conjunctiva of cats that were diagnosed with sporotrichosis and had ocular involvement had a positive fungal culture. Five conjunctival swabs of the ocular discharge in 10 affected cats (50%) were positive for sporotrichosis on cytology (Patients 01, 10, 14, 19, 63; Table 2) (Figure 3). Of the 10 cats without ocular lesions, 5 (50%) of the samples collected had fungal growth in culture and one sample (10%) had a positive cytological exam. Histologically, the organisms appeared as small, pink oval or dot-like yeasts in periodic acid-Schiff stain (PAS), lying in vacuoles often grouped within macrophages, but were also detected free in the conjunctival parenchyma. Numerous yeasts were identified in the palpebral conjunctival samples from all evaluated animals (Patients 09, 14, 44 and 63; Table 2) (Figure 3), whereas fungal organisms were not detected in other ocular structures.

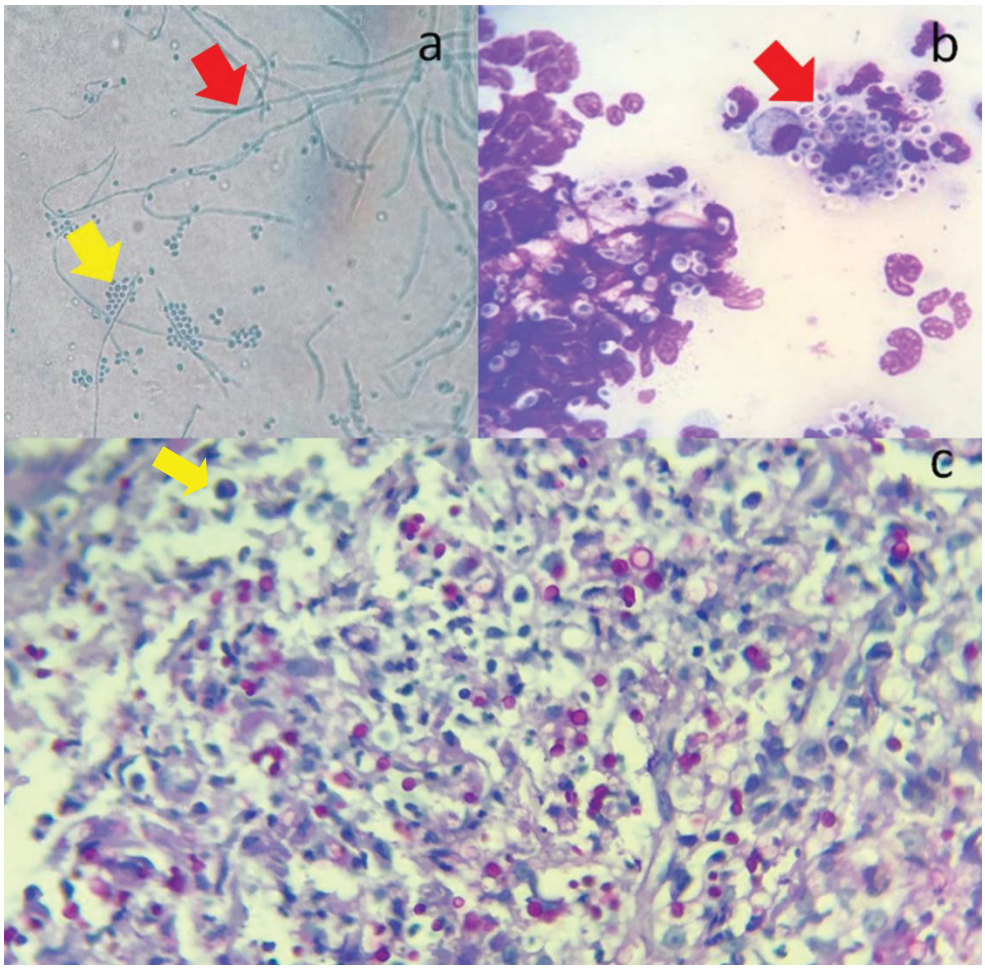

Figure 3 – Laboratory findings - A - Microscopic image (1000X magnification) of positive fungal culture and sample staining with cotton blue lactophenol. Note hyphae (red arrow) and fungal yeasts (yellow arrow). B – Conjunctival swab cytology. Note large amounts of pleomorphic yeasts interspersed with a pyogranulomatous inflammatory infiltrate (red arrow) (1000X rapid panoptic). C - Palpebral conjunctiva histopathology. Representative photomicrography showing the presence of pyogranulomatous inflammatory infiltrate, with epithelioid macrophages and numerous small, 2 to 6 µm diameter, dot-like to oval yeasts (yellow arrow) consistent with *Sporothrix schenckii* can be observed (PAS 1000X).

Animals with a positive fungal culture demonstrated a high conjunctival cytological specificity (100%, with a confidence interval between 47.82% and 100%) but a low sensitivity (40%, with a confidence interval between 16.34% and 67.71%) for sporotrichosis compared to fungal culture (golden standard). Consequently, the positive predictive value was 100% and the negative value was 35.71% with a confidence interval between 26.87% and 45.65% ($P < 0.05$).

2.5 DISCUSSION

Sporotrichosis is a common fungal infection in Brazil, especially in the south and southeast regions that are the most affected by urban disease epidemics[4]. In Curitiba, since 2014, there have been a large number of sporotrichosis cases in domestic cats, as well as humans, and is

considered an important zoonotic disease[15]. This disease usually affects semi-domiciled cats, with street access, through injuries caused by scratches, bites, or contact with lesion exudates rich in fungal elements, especially after fights[5,16]. As would be expected, sporotrichosis most often affects male non-neutered cats, situation that agreed with the present work.

We cannot rule out that *Sporothrix* spp. could be an environmental contaminant in some cases, as suggested by tear culture results (15 of 20 positive cultures), especially in cats with disseminated respiratory and cutaneous conditions, representing a possibility of contagion between animals and a dermatozoonotic form of the disease.

The majority of the evaluated cats had severe disseminated skin conditions with extensive ulcerous, exudative lesions often surmounted by scabs. Such lesions probably result from self-trauma and auto grooming habits with the presence of fungus in the claws and the oral cavity, in addition to dissemination via hematogenous or lymphatic routes[17,18]. The auto grooming habit of licking their limbs and then passing them over the face, possibly contributes to ophthalmic contamination by spreading the fungus over the ocular surface[19]. This may happen mainly due to the presence of cutaneous-disseminated infections with areas of extensive ulcerations and exudate on the face.

In the present study, of the 63 animals with ophthalmic involvement, at least mild signs (e.g. exclusively increased serous ocular discharge) were observed. Severe cases of granulomatous conjunctivitis and blepharoconjunctivitis were observed in 71.43% of the affected cats. The presence of general mucosal lesions, including conjunctival granulomas, was observed in 34.9% of cats in previous work[18]. Nonetheless, that study did not separate the mucocutaneous lesions by site of involvement, such as conjunctival injuries. Our study was performed by both ophthalmologists and dermatologists, more precise lesion identification was likely achieved.

One cat had increased serous ocular discharge concomitant to a respiratory condition. However, no evidence of systemic or dermatological alterations was observed, and two cats had exclusively ophthalmic abnormalities characterized by granulomatous conjunctivitis scored 4/5. A recent study described three cats with granulomatous conjunctivitis and increased serous ocular discharge secondary to sporotrichosis, without presenting systemic symptoms or skin lesions [11].

Systemic signs most frequently observed in cats with sporotrichosis involve the respiratory system[18], 96.83% of affected cats showed respiratory changes. There appears to be an

association of conjunctivitis, blepharoconjunctivitis, and increased serous ocular discharge with severe respiratory and disseminated conditions. Some cats presented the fungus in the conjunctiva even without ocular lesions. Furthermore, the proximity of the nasal cavity with the lacrimal system and their communication through the nasolacrimal duct could also contribute to the disease spreading from the airways to the ocular adnexa. Increased serous ocular discharge could also be explained in most cases by the intimacy of the nasal cavity with the lacrimal system; some animals can have nodules that extend into the nasal cavity[20] and may obliterate the nasolacrimal duct causing decreased tear outflow rather than epiphora. Additionally, ocular discharge could be a contributor in system spread via transmission to the nasal cavity during self-grooming, however further studies are needed.

Conjunctivitis was the second most common ocular lesion in cats with sporotrichosis, the majority of which were severe with a score of 4/5. However, conjunctivitis has been reported to be responsive to conventional sporotrichosis treatments, such as the use of itraconazole and potassium iodide or intralesional amphotericin B injection [21,22,23]. Scoring conjunctivitis in a similar manner may facilitate monitoring the evolution of the severity from the beginning of the therapy, and it can help to assess its efficacy of new medications in future studies.

It is important to consider other conjunctival conditions and comorbidities that were not investigated, such as chlamydiosis or herpesvirus, which could cause lesions similar to those mentioned in the present study. Infections associated with the feline respiratory complex, such as the aforementioned viral and bacterial infections, are considered the main causes of conjunctivitis in cats[24,25]. These infections were not tested in the present work because of financial restrictions, however, future studies of molecular biology will be performed to elucidate this bias. Despite the low number of samples of ocular discharge and conjunctiva collected for laboratory examination, 22.9% of cats with systemic sporotrichosis had ocular lesions with increased serous ocular discharge, blepharoconjunctivitis, and conjunctivitis being the main lesions found. Nonetheless, the present study suggests that sporotrichosis should be considered as a cause of conjunctivitis in endemic areas.

Sporotrichosis also seems to be an important cause of blepharitis and blepharoconjunctivitis, often appearing similar to other infectious diseases of the eyelids such as dermatophytosis and feline demodicosis, immune-mediated diseases such as pemphigus foliaceous, and neoplastic conditions such as squamous cell carcinoma[26,27]. The latter usually

causes expansive, ulcerated lesions in the periocular region and over the eyelids, and may present as deforming facial lesions, potentially appearing similar to the lesions caused by cutaneous-disseminated sporotrichosis disease. Lesions like these were observed in the cats studied. Thus, histopathological analysis and fungal culture are fundamental for this differentiation and to prove the presence of the fungus invading these tissues.

Three animals were diagnosed with uveitis, two of which were positive for FeLV. There is evidence that retroviral infections modify the immune response and may worsen the prognosis of cats with sporotrichosis[28]. In addition to FeLV, poor care and low quality of life may contribute to immunodeficiency and thus increased risk of infections such as sporotrichosis [4,17]. FeLV itself should be considered an important cause of uveitis in cats[28,29]. Few animals were tested for FeLV in the present study, and considering that histopathological evaluation did not reveal yeasts or fungal elements in the uveal tract, it is not possible to rule out other causes such as FeLV as the reason for uveitis. Additionally, eyes with uveitis had diffuse and dense corneal edema which made the assessment of intraocular structures very difficult. Intraocular sporotrichosis is not a typical presentation, understandably so considering that it is a mycotic infection of implantation and its dissemination is most often through the subcutaneous and lymphatic routes, causing cellulitis, lymphangitis, and lymphadenitis, affecting the skin, eyelids, and conjunctiva. In humans, there is some evidence that sporotrichosis may be associated with endophthalmitis and posterior uveitis through the hematogenous route, however, there are no similar studies in cats[30]. Other fungal infections such as cryptococcosis reach the retina and posterior uvea through the breakdown of the blood-aqueous barrier caused by vasculitis secondary to the fungal infection[31].

In the present study, these alterations were not evident in cats with sporotrichosis, although ophthalmoscopic evaluation was impaired due to severe blepharoconjunctivitis and corneal edema caused by uveitis. It is possible that in cats, *Sporothrix* spp. does not have tropism for choroid, retina, or optic nerve, or that infections within these tissues were subclinical. Retinal and optic nerve abnormalities such as chorioretinitis and optic neuritis may be associated with fungal infections such as cryptococcosis, blastomycosis, and histoplasmosis in humans and animals[31,32,33,34]. In humans, there are reports that sporotrichosis can also be related to similar clinical presentations, especially in immunocompromised patients[35].

Compared to cytology, all samples collected from animals with ophthalmic conditions had a positive fungal culture, suggesting that culture is a very reliable test in the presence of ocular

abnormalities, and has been recommended as the gold standard for sporotrichosis diagnosis[13]. Cytological examination and fungal culture are easy and inexpensive diagnostic tests for conjunctival sporotrichosis. Cytology demonstrated high specificity and low sensitivity compared to fungal culture for conjunctival sporotrichosis, with a high positive predictive value. Thus, although the visualization of *Sporothrix* spp. pleomorphic yeasts in the cytological examination of the conjunctiva are not common, its occurrence allows the presumptive diagnosis of ophthalmic sporotrichosis and helps direct therapy by ruling out other fungal infections such as cryptococcosis and histoplasmosis as differential diagnoses. Exfoliative cytology of the palpebral conjunctiva, commonly used for conjunctivitis diagnosis[11], could increase the sensitivity of the diagnosis, but this method was not used due in this investigation to the difficulty handling sick animals for collection of these samples using the cytological brush.

Limitations of the study include the low number of histopathological examinations, which were limited due to budget constraints and the difficulty of managing the anesthetic team to monitor patients. Biomolectular tests were not performed for the same reason. Some of these hospital visits were expensive and required a multidisciplinary approach. Future studies should broaden the data on histopathologic and biomolecular analyses.

Our results showed that sporotrichosis is an important differential diagnosis in conjunctivitis and blepharoconjunctivitis and should be investigated especially in the presence of concomitant respiratory and cutaneous alterations, particularly in endemic regions. Fungal culture, histopathological examination of the conjunctiva, or cytology are preponderant for confirmation of the diagnosis. In the absence of other tests with greater sensitivity, cytology by swab may be an alternative. Animals without ocular lesions may harbor the fungus in the conjunctiva; however, histopathological or molecular biology exams are essential for the understanding of the physiopathogenesis of ophthalmic sporotrichosis, thus avoiding the presence of contaminations or comorbidities that may influence the diagnosis of these ocular lesions.

2.6 INTEREST CONFLICTS

The authors declare that there are no conflicts of interest regarding this study.

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3 CHAPTER II - COMPARATIVE EVALUATION OF CHROMATIC PUPILLOMETRY IN DEGENERATIVE DISEASES OF THE RETINA AND OPTIC NERVE IN DOGS AND HUMANS

3.1 ABSTRACT

Chromatic pupillometry can be used for the evaluation of the retina and the optic nerve in humans and animals. The objective of this investigation is to report and compare the chromatic pupillary responses in humans and animals. Material and methods: Two groups of dogs were selected, the first with progressive retinal atrophy and the second a healthy control group and three groups of human patients, the first diagnosed with retinitis pigmentosa, the second with optic neuropathies and the third a healthy control group. All groups were tested using red (630 nm) and blue (480 nm) light and pupillary responses were analyzed in a dark-adapted environment. Results: Pupillary responses in both wavelengths were less intense in canine and human patients with retinal dystrophies. Human patients with optic neuropathy, showed a less intense response to blue light compared to the control group, however several factors may have influenced these results. Conclusion: The manual method of chromatographic pupillary evaluation might be considered a form of screening and subjective evaluation of neuropathies and retinal dystrophies; new methods must be developed for a more objective evaluation in humans.

Key words: retinal degenerations; retinopathies; progressive retinal atrophy; pupillometry; chromatographic methods**.**

3.2. INTRODUCTION

The retina is a light-sensitive neurosensory structure, made up of layers of modified neuros and photoreceptor cells (rods and cones). Cones are less light sensible cells responsible for color vision whereas rods are most sensitive to light and dark changes, shape and movement and contain only one type of light-sensitive pigment, rhodopsin[1,2].

The pupillary light reflex (PLR) is the constriction and subsequent dilation of the pupil through antagonistic actions of the pupillary sphincter and iris dilator muscles, after light stimulation and is directly related to the functioning of the optic pathway and the autonomic nervous system[3]. Its operation involves the functioning of the retina and its photoreceptor cells, optic nerve, optic chiasm and optic tracts, but its mere presence is not a good parameter of visual function. People and animals with retinal

diseases that lead to complete functional blindness may initially have normal PLR. The severity and location of the lesion greatly influences the presence of PLR[4].

Thus, some retinal and optic nerve diseases can directly interfere with the PLR response and consequently, the evaluation of this reflex becomes essential in any ophthalmological examination. Examples of such diseases are including glaucomatous neuropathy[5], Leber's hereditary optic neuropathy (LHON)[6], retinitis pigmentosa (RP)[7] in humans, progressive retinal atrophy (PRA)[8,9] and sudden acquired retinal degeneration syndrome (SARDS)[10,11] in animals.

In the beginning of the current century, it was discovered that specific ganglion cells of the retina were photosensitive[12] and were involved in stimulating the PLR through a pigment called melanopsin[13-16]. Light stimulation by means of wavelengths referring to red and blue light, for a certain period of time, manages to individualize the light response in cones and rods in contrast to the response of ganglion cells and, consequently, this evaluation can mean an important technique to segment the differential diagnoses in diseases of the retina and optic nerve. This clinical assessment is called chromatic pupil light reflex[17-21].

These special ganglion cells that express melanopsin were denominated intrinsically photosensitive retinal ganglion cells (ipRGCs) and make up about 0.2 – 1% of the total ganglion cells in humans and are especially stimulated by blue light[22,23]. The evaluation of retinal and optic nerve diseases by means of the pupillary reflex under chromatic pupillometry methods is very popular among veterinary ophthalmologists (REFERENCIAS de publicacção de veterinária) but not very well diffused among medical doctors that are ophthalmologists. It is a non-invasive, practical, and fast technique, which can differentiate conditions that reduce the quality of life in humans and animals, being potential causes of blindness in both species.

Different diseases such as retinitis pigmentosa (RP)[19-22], progressive retinal atrophy (PRA)[8,23], glaucoma[5], and ischemic or hereditary optic neuropathies[18,24] have already been evaluated using the chromatographic pupillary reflex, however, no study has focused on comparing this response in humans and animals, which is the main objective of the present work.

3.3 MATERIAL AND METHODS

3.3.1 Veterinary evaluation

Twelve dogs were examined by the Ophthalmology Service at the Teaching Hospital at the Federal University of Parana (UFPR). Six animals were previously diagnosed with PRA and six animals were healthy individuals, having normal vision and free of ocular disease. The inclusion criteria for the affected animal group were having nyctalopia and subnormal or absent electroretinographic response. No optic neuropathies were observed in dogs during the study due to low prevalence in this species.

Data records about the breed, age, sex, and the presence of ocular or systemic diseases and clinical history evidence have been recorded. All animals underwent a complete ophthalmological examination. Anterior segment examination was performed by slit-lamp biomicroscopy (Hawk-eye, Dioptrix, L'Union, France). Vision testing was performed by the ability to track falling cotton wool and the ability to negotiate an obstacle course in photopic and scotopic conditions.

After checking the presence or absence of a menace response, direct, consensual and dazzle light reflexes were performed using a 3.5 V Finoff halogen fiber optic transilluminator (Welch Allyn®, Skaneateles Falls, NY). Chromatic pupillometry was executed using a cPLRtester (VISION BIOMEDICAL SOLUTIONS, Apatin, Vojvodina, Serbia) and its response registered according to a printed pupil gauge varying from 1 to 12 mm^4. After 30 seconds of dark adaptation, the test was performed by positioning the instrument 3 cm in front of the eyes, the left eye and right were stimulated once a time with red light (630 nm) for 10 seconds with 10 seconds break between stimuli. After 30 seconds, the same eyes were stimulated with blue light (480 nm) for 10 seconds with 10 seconds break between stimuli. During the light stimulation, the pupillary diameter was evaluated using the printed pupil gauge. With each light stimulus, the contralateral eye was kept manually closed throughout the procedure.

Indirect ophthalmoscopy was performed after inducing mydriasis using 1% tropicamide (Mydriacyl®, Novartis®, São Paulo, SP, Brazil) with a 3.5 V Finoff halogen fiber optic transilluminator (Welch Allyn®, Skaneateles Falls, NY) and a 20D lens (OPTOMED OY LTD., Finland).

A mini portable Ganzsfield (HMsERG model 1000, Ocuscience®, NV) was positioned at 1 cm from the corneal surface, within the eyes placed centrally in the palpebral fissure and the animals were evaluated under scotopic and photopic conditions. Electroretinography (ERG) was performed in affected

dogs using manual restraint without the use of anesthesia or sedation after pharmacological mydriasis with 1% tropicamide (Mydriacyl®, Novartis®, São Paulo, SP, Brazil) and 10% phenylephrine eye drops (Frumtost, São Paulo, SP, Brazil)[25].

Animals were placed in sternal recumbency. A topical corneal anesthetic was applied (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA), followed by the placement of an active corneal contact recording electrode (ERG-Jet, Fabrinal SA, La Chaux-de-Fonds, Switzerland), and hypodermic platinum needles (Model E2, Grass Technologies, Warwick, USA) were used as reference and ground electrodes, positioned in 2 cm from the lateral canthus and at the base of the neck, respectively. Electrode impedance was maintained at <5 kΩ, and bandpass was 0.3–300 Hz[25]. After 20 minutes of dark-adaptation a simplified scotopic ERG protocol consisting of a combined rod–cone response to a standard intensity (average of four flashes, 0.1 Hz, 0.47 log cds/m2) and a high-intensity (average of four flashes, 0.05 Hz, 1 log cds/m2) flashes under scotopic condition was performed in all animals.

A signed informed consent was obtained from the participant owners. All procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology's (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, and the institution´s own Animal Use Committee.

3.3.2 Medical evaluation

Six human patients with retinal degeneration (group 01), six human patients with optic neuropathy (group 02) and six healthy patients without retinal degeneration or optic nerve alterations (group 03) were evaluated by the Service of Ophthalmology of the Retina e Vítreo Clinic (Curitiba-PR, Brasil). All individuals from group 01 showed visual impairment and/or nyctalopia, fundus and electroretinographic alterations compatible with RP. Patients from group 02 have been ophthalmoscopically diagnosed as having optic neuropathy.

ERG was performed only in group 01 after pupillary dilation using 1% tropicamide eye drops (Mydriacyl, AlconTM, São Paulo, SP, Brazil) associated with 10% phenylephrine eye drops (Frumtost, São Paulo, SP, Brazil). An ocular electrophysical equipment RETI-port/scan 21 (Rolant Consult Stasche & Finger GmbH, Branderburg an der Havel, Germany) was used, and a dome Ganzfeld semi-automatic machine, in addition to electrode monopolar on the contact lens-shaped cornea, a ground and reference electrodes (DTL/ERGJet, Gold foil and HK-Loop electrodes). An ERG standard protocol was used[26]. A

topical corneal anesthetic was applied (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA) followed by the placement of an active corneal contact recording electrode (ERG-Jet, Fabrinal SA, La Chaux-de-Fonds, Switzerland), a reference and ground electrodes were positioned in 2 cm from the lateral canthus and forehead center, respectively. Electrode impedance was maintained at <5 kΩ. After 20-30 minutes of dark adaptation under scotopic conditions, a simplified scotopic ERG protocol consisting of a combined rod–cone response to a standard intensity (average of four flashes, 0.1 Hz, 0.47 log cds/m2) and a high-intensity (average of four flashes, 0.05 Hz, 1 log cds/m2) flashes was performed in all patients of group 01. The second and third groups were not performed the ERG exam. The same veterinary protocol was used to evaluate the chromatic pupillometry in the three groups[4].

Patient signalment such as gender, age, the onset of signs, presence of nyctalopia or blindness, ocular and fundus alterations, and familiar history were evaluated in all patients. A signed informed consent was obtained from the participants and the project was approved by the Medical Ethics Committee.

3.3.3 Statistical Analyzes

A Shapiro-Wilk test was used to access data normality. Student's t-Test was performed to compare the ratio B/R among the groups and Mann-Whitney test and Kruskal-Wallis test to compare pupillary diameters between groups. All analyses used a significance level of $p < 0.05$ and were performed using the Graphpad Quick Calcs statistical software (Graphpad Software Inc., La Jolla, CA). The right eye was chosen for statistical analysis to avoid duplication of information.

3.4 RESULTS

3.4.1 Clinical evaluation of dogs

A total of 12 dogs were evaluated by the Veterinary Ophthalmology Service of UFPR. Both groups had four females (66.66%) and two males (33.44%). The mean age of the affected group was 16.5 ± 14.33 months and of unaffected group was 33.3 ± 21.73 months. Six breeds were represented in this work (Table 1). All affected animals were previously diagnosed with PRA and showed severe visual impairment during the evaluation. ERG recordings showed extinguished rod-cone activity (flat line). Menace response, dazzle reflex, and cotton ball test were negative in all affected dogs in both eyes. These dogs also failed to avoid objects in scotopic and photopic conditions during the obstacle course

tests. Consensual and direct PLR were preserved in the youngest animals, but slower in the oldest dogs (Number 01 and 02).

Table 1 – Distribution of affected and unaffected animals according to breed, sex, age and disease.

Group	Animal ID	Breed	Sex	Age (Months)	Ocular disease
Affected	Number 01	German Spitz	Male	36	PRA OU
Affected	Number 02	German Spitz	Male	36	PRA OU
Affected	Number 03	German Spitz	Female	4	PRA OU
Affected	Number 04	German Spitz	Female	4	PRA OU
Affected	Number 05	German Spitz	Female	4	PRA OU
Affected	Number 06	German Spitz	Female	15	PRA OU
Control	Number 07	German Spitz	Female	16	None
Control	Number 08	Yorkshire Terrier	Female	7	None
Control	Number 09	Mixed Breed	Female	14	None
Control	Number 10	Labrador Retriever	Male	60	None
Control	Number 11	English Cocker Spaniel	Male	60	None

PRA = Progressive Retinal Atrophy; OU (*Oculus uterque* or both eyes).

Pupillometry results under red light were not different among the groups, nonetheless, the values under blue light and the ratio B/R OD of the affected group was significantly higher in affected dogs by Mann-Whitney test ($p<0.05$). The highest pupillometry values also were in the affected group (Tables 2 and 3), even the control group being represented by breeds of different sizes. All affected dogs were unresponsive to red light and responsive to blue light (Figure 1), although the response under blue light decreased with age.

Table 2 – Pupillary diameter of affected animals under blue and red light.

	Pupillary diameter (mm)						
Stimuli	Number 01	Number 02	Number 03	Number 04	Number 05	Number 06	Mean
Red OD	6.5	8	3.5	2.5	3.5	6	5.00 ± 1.96
Red OS	6.5	8	3.5	2.5	3.5	6	5.00 ± 1.96
Blue OD	3.5	4	1.5	1.5	1,5	3	2.5 ± 1.04
Blue OS	4	4.5	1.5	1.5	2	3	2.75 ± 1.18
B/R OS	0.62	0.56	0.43	0.60	0.57	0.50	0.55 ± 0.06
B/R OD	0.54	0.50	0.43	0.60	0.43	0.50	0.50 ± 0.06

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*).* B/R (ratio between the pupillary diameter under the blue light by the puppillary diameter under the red light).

Table 3 – Pupillary diameter of unaffected animals under blue and red light.

	Pupillary diameter (mm)						
Stimuli	Number 07	Number 08	Number 09	Number 10	Number 11	Number 12	Mean
Red OD	3.5	4	4	6	5	3	4.25 ± 0.99
Red OS	3.5	4	4	6	5	3	4.25 ± 0.99
Blue OD	1	1.5	2	1.5	2	1	1.5 ± 0.41
Blue OS	1	2	2	2	2	1	1.67 ± 0.47
B/R OS	0.29	0.50	0.50	0.33	0.40	0.33	0.39 ± 0.08
B/R OD	0.29	0.38	0.50	0.25	0.40	0.33	0.36 ± 0.08

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*).* B/R (ratio between the pupillary diameter under the blue light by the puppillary diameter under the red light).

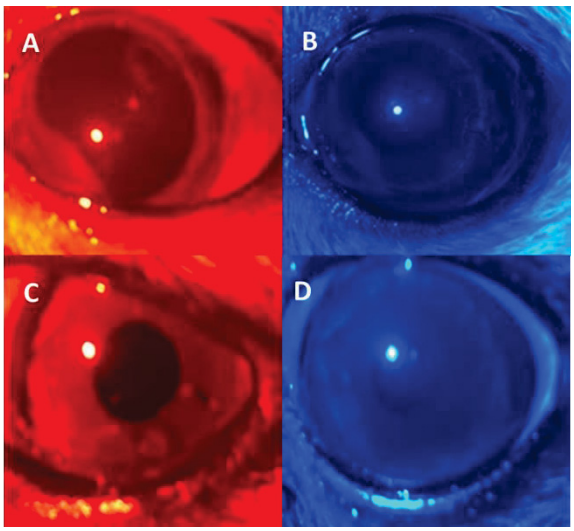

Figure 1- Chromatic pupillary light reflex test. A – Red light stimuli in affected animal (number 01). Note the large pupillary diameter. B- Blue light in affected dog (number 01). Note the pupil response. C – Red light stimuli in unaffected animal (number 07). Note the more intense pupil response compared to figure A. D – Blue light stimuli in unaffected dog (number 07). Note the intense pupil response.

3.4.2 Clinical evaluation of humans

A total of 18 patients were evaluated by the medical group of the Retina e Vítreo Clinic. Six people represented each group (group 01 – retinal degeneration; group 02 – optic neuropathies and group 03 – control). There were five women (83.33%) and one man (16.77%) in group 01, four women (66.66%) and two men (33.34%) in group 02, and three women (50%) and three men (50%) in group 03 (Table 4). The mean age of each group was 414 ± 247.56, 720 ± 139.26, and 360 ± 183.56 months respectively. All patients of group 01 had a non-recordable ERG under scotopic conditions.

Table 4 – Distribution of patient groups according to sex, age, and disease.

Group	Patient ID	Sex	Age (Months)	Ocular disease
01	Number 01	Female	660	RP OU
01	Number 02	Female	300	RP OU
01	Number 03	Female	624	RP OU
01	Number 04	Male	72	RP OU
01	Number 05	Female	156	RP OU
01	Number 06	Female	672	RP OU
02	Number 07	Female	684	NAION OD
02	Number 08	Female	876	AAION OD
02	Number 09	Female	456	LHON OU
02	Number 10	Male	804	NAION OD
02	Number 11	Male	828	NAION OD
02	Number 12	Female	672	NAION OU
03	Number 13	Female	516	None
03	Number 14	Female	480	None
03	Number 15	Male	204	None
03	Number 16	Male	48	None
03	Number 17	Male	564	None
03	Number 18	Female	348	None

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*)*; OU (*Oculus uterque* or both eyes); RP (Retinitis pigmentosa); NAION (Non-arteritic Anterior Ischemic Optic Neuropathy); AAION (Arteritic Anterior Ischemic Optic Neuropathy).

The ratio B/R OD of the groups was significantly higher in group 02 compared to group 01 and 03. The pupillary diameter under red and blue light was significantly higher in group 01 to compared with other groups ($p<0.05$) (Tables 5,6, and 7). All patients had pupil response under blue and red light.

Table 5 – Pupillary diameter of patients in group 01 under blue and red light.

	Pupillary diameter (mm)						
Stimuli	Patient 01	Patient 02	Patient 03	Patient 04	Patient 05	Patient 06	Mean
Red OD	5	4.5	4	3	5	4	4.25 ± 0.69
Red OS	4.5	4.5	4	3	5	4	4.17 ± 0.62
Blue OD	3.5	3	3	1.5	3	3	2.83 ± 0.62
Blue OS	3.5	3.5	3	1.5	2	3	2.75 ± 0.75
B/R OS	0.78	0.78	0.75	0.50	0.40	0.75	0.66 ± 0.15
B/R OD	0.70	0.67	0.75	0.50	0.60	0.75	0.66 ± 0.09

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*).* B/R (ratio between the pupillary diameter under the blue light by the puppillary diameter under the red light).

Table 6 – Pupillary diameter of patients in group 02 under blue and red light.

	Pupillary diameter (mm)						
Stimuli	Patient 07	Patient 08	Patient 09	Patient 10	Patient 11	Patient 12	Mean
Red OD	3.5	3	3	3.5	3	3	3.25 ± 0.24
Red OS	3.5	3	3	3.5	3	3.5	3.50 ± 0.25
Blue OD	3	2	3	3	2.5	2	2.50 ± 0.45
Blue OS	2	2	3	2	1.5	2	2.00 ± 0.45
B/R OS	0.57	0.67	1.00	0.57	0.50	0.57	0.57 ± 0.17
B/R OD	0.86	0.67	1.00	0.86	0.83	0.67	0.76 ± 0.12

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*).* B/R (ratio between the pupillary diameter under the blue light by the puppillary diameter under the red light).

Table 7 – Pupillary diameter of patients in group 03 under blue and red light.

	Pupillary diameter (mm)						
Stimuli	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	Mean
Red OD	2.5	3	3	3.5	3.5	3	3.08 ± 0.34
Red OS	2.5	3	3	3.5	3.5	3	3.08 ± 0.34
Blue OD	1	1	1.5	1.5	1.5	1.5	1.33 ± 0.24
Blue OS	1	1.5	1.5	1.5	1.5	1.5	1.42 ± 0.19
B/R OS	0.4	0.5	0.5	0.43	0.43	0.50	0.46 ± 0.04
B/R OD	0.4	0.33	0.5	0.43	0.43	0.50	0.43 ± 0.06

OD (*Oculus dexter* or right eye*);* OS (*Oculus sinister* or left eye*).* B/R (ratio between the pupillary diameter under the blue light by the puppillary diameter under the red light).

3.5. DISCUSSION

This study showed that retinal dystrophies and optic neuropathies affect the intensity of pupillary constriction under chromatic pupillography as demonstrated previous works[18-24]. Other authors have demonstrated that these diseases can also affect the latency of pupillary constriction and re-dilation after the end of the light stimulus[20,23]. These parameters could not be evaluated in the present study.

Affected dogs showed no response under red light, a smaller blue light response and a bigger ratio B/R compared to the control group. Assessment of pupillometry under red light appears to be an objective criterion in dogs with early onset retinal dystrophies. Humans with retinal dystrophies showed a smaller response under blue and red light compared to patients of control group similar previous study[20]. PRA in the German Spitz is an early onset disease[18], the progression of retinal dystrophies is faster in these dogs than RP in humans, causing more severe degeneration of the photoreceptors in a short time and important visual impairment in very young

dogs, recently was identified a frameshift mutation in GUCY2D of German Spitz like Leber's Congenital Amaurosis[27,28], retinal dystrophy that causes early onset visual impairment in humans[29], which explains the lack of response under red light in very young dogs in the present study. The lower pupillary constriction under blue light in group 01 may have been influenced by the degeneration of cones and rods and the light stimuli might not have been sufficient to individualize intrinsically photosensitive photoreceptors and ganglion cells. The rods are sensitive to low-brightness blue light and the ipRGCs to high-brightness blue light, the equipment used in our study did not allow this feature[30].

Puppies and children performed better when stimulated with blue light compared to older patients, probably due to a lesser degree of photoreceptor degeneration and preservation of ganglion cells in retinal degenerations[19]. Previous studies showed a delay in pupil contraction under blue light and in the re-dilatation after the end of the light stimulus in people with RP[20]. Herbst et al. (2012)[31] showed that one of the main factors that interfere with pupillary latency and re-dilation is age, with parameters being directly proportional. Furthermore, under normal conditions, the basal pupillary diameter of children is larger than that of the elderly[32], nonetheless, these changes cannot be observed due to the study method.

.Patients with ischemic and hereditary mitochondrial neuropathies showed higher pupillary diameter values under blue light compared to the control group, nonetheless, it showed an lower response compared to group 01. Optic neuropathies such as LHON, AAION and NAION typically cause optic nerve injuries and ganglion cell death[33-35], although, intrinsically photosensitive ganglion cells appear to be more resistant to such damage[36]. The ratio B/R OD in group 02 was significantly higher than other groups. This parameter has good potential for the evaluation of optic neuropathies according to our study.

There were limitations in the present work. A digital pupillometer was not available to facilitate the recording of parameters such as latency, duration of contraction, baseline pupil diameter, speed of contraction and time for re-dilation. In addition, parameters that may influence the pupillary response such as the circadian cycle and exaggerated sympathetic stimulation or altered emotional conditions were also not considered and the sample of animal and human patients was heterogeneous, which may have prejudiced the research results. Coronavirus-19 pandemic caused difficulty in the organizing the project and many financial restrictions.

3.6 CONCLUSION

Chromatic pupillometry methods can be a simple, non-invasive, reasonable way of subjectively diagnosing retinal retinopathies and dystrophies. Pupillary responses in dogs with PRA and humans with RP are similar and lower than individuals of control groups. The ratio B/R is a potential method for evaluation in optic neuropathies in humans and retinal dystrophies in dogs. Assessment of pupillometry under red light appears to be an objective criterion in dogs with early onset retinal dystrophies. New studies of pupillometry using automated evaluation methods and a larger sample of patients should provide a more objective evaluation, allowing the evaluation of important parameters such as the speed and intensity of pupillary constriction in an easy, fast and non-invasive way when applied to humans and animals.

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5.0 ANEXOS

Pontifícia Universidade Católica do Paraná
Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação
Comissão de Ética em Pesquisa no Uso de Animais

PARECER CONSUBSTANCIADO DA CEUA

TÍTULO DA PESQUISA	**CARACTERIZAÇÃO CLÍNICA DA ESPOROTRICOSE OFTÁLMICA; IDENTIFICAÇÃO DO FUNGO NA CONJUNTIVA EM GATOS DOENTES**		
Nº DO PARECER / VERSÃO	02060 – versão II		
PESQUISADOR RESPONSÁVEL	Marconi Rodrigues de Farias		
ESPECIE DO ANIMAL	*Felis catus*	**Nº DE ANIMAIS**	60
NOME COMUM DO ANIMAL	Gatos	**Nº SISBIO** (animais de vida livre)	*Não se aplica*
SEXO / IDADE / PESO	Variados	**ATIVIDADES** (animais de vida livre)	*Não se aplica*
ORIGEM DO ANIMAL	CVE	**GP TAXONÔMICOS** (animais de vida livre)	*Não se aplica*
DATA DE INICIO DA PESQUISA	Maio/2021	**LOCAL (IS)** (animais de vida livre)	*Não se aplica*
DATA DE TÉRMINO DA PESQUISA	Outubro/2022	**Nº SISGEN**	*Não se aplica*
APRESENTAÇÃO DO PROJETO	Coleta de dados O experimento será conduzido em conjunto com a Pontifícia Universidade Católica do Paraná (PUCPR). Os animais serão selecionados pelo serviço de atendimento da Unidade Hospitalar para Animais de Companhia (UHAC-PUCPR), os proprietários receberão um termo de consentimento livre e esclarecido para o uso dos animais no experimento, os dados referentes ao número da ficha, espécie, raça, idade, sexo, peso, características das lesões, exames realizados e tratamento serão coletados e tabulados em planilha do software Excel. Os dados clínicos e anamnese serão colocados em duas fichas padronizadas, uma dermatológica e outra oftálmica. O experimento estará enquadrado nas diretrizes do Comitê de Ética Animal (CONCEA-UFPR) e da Comissão de Ética no Uso de Animais (CEUA-PUCPR). Desenho experimental Serão feitos quatro grupos de gatos, um com lesões cutâneas fixas, mas sem lesões oftálmicas (Grupo 1), outro com lesões cutâneo disseminadas e conjuntivite granulomatosa (Grupo 2) e outro com quadro disseminado sem conjuntivite granulomatosa (Grupo 3) e por fim outro com quadro exclusivamente extra-cutâneo respiratório ou nasal (Grupo 4), cada grupo terá 20 animais. Só serão considerados para os grupos gatos com diagnóstico definitivo de esporotricose atendidos pelo UHAC- PUCPR por meio do histórico, exame clínico, visualização das leveduras pelo exame citopatológico ou histopatológico ou identificação do fungo por meio de cultura fúngica ou por técnica biomolecular. Todos os gatos serão avaliados, será feito a avaliação oftálmica por meio de retroiluminação, reflexo pupilar a luz, ofuscamento, reação à ameaça, tonometria, fluoresceína e lissamina verde.		
OBJETIVO DA PESQUISA	A principal proposta desse estudo é testar a hipótese de os quadros de blefaroconjuntivite em gatos são realmente causados pela esporotricose e se gatos sem lesão oftálmica podem ter o fungo em sua conjuntiva.		
RISCOS E ATITUDES MITIGATÓRIAS	As possíveis avaliações podem causar um nível de estresse nos gatos, tal fato será observado com a apresentação de formas de contenção mais adequadas a espécie. Trata-se de uma zoonose, o uso de EPI's durante a consulta será obrigatório, assim como o uso de máscaras e antissepsia devido ao ambiente de pandemia. Serão respeitados o número máximo de pessoas durante o atendimento, que será limitado em 3 pessoas. Todos os integrantes serão treinados para tal pesquisa.		
CONSIDERAÇÕES SOBRE A PESQUISA	Não há.		
CONSIDERAÇÕES SOBRE OS TERMOS DE APRESENTAÇÃO OBRIGATÓRIA	Todos os termos de apresentação obrigatória foram submetidos e estão de acordo.		

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 013/2021, referente ao projeto de pesquisa **"Avaliação comparativa do reflexo pupilar cromatográfico em doenças degenerativas da retina em cães",** sob a responsabilidade de **Fabiano Montiani Ferreira –** que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 04/05/2021.

Finalidade	Pesquisa
Vigência da autorização	Maio/2021 até Junho/2022
Espécie/Linhagem	*Canis lupus familiaris* (canino)
Número de animais	20
Peso/Idade	Variável
Sexo	Variável
Origem	Hospital Veterinário da UFPR, Curitiba, Paraná, Brasil

*A autorização para início da pesquisa se torna válida a partir da data de emissão deste certificado.

CERTIFICATE

We certify that the protocol number 013/2021, regarding the research project **"Comparative evaluation of the chromatic pupillary light reflex in dogs with retinal degenerative diseases."** under **Fabiano Montiani Ferreira –** which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE FEDERAL UNIVERSITY OF PARANA, BRAZIL, with degree 1 of invasiveness, on May 4th, 2021.

Purpose	Research
Validity	May/2021 until June/2022
Specie/Line	*Canis lupus familiaris* (canine)
Number of animals	20
Weight/Age	Various
Sex	Various
Origin	Veterinary Hospital of UFPR, Curitiba, Paraná, Brazil.

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 04 de maio de 2021

Maity Zopollatto

Coordenadora pro-tempore
CEUA/AG/UFPR

UFPR - HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ -

CEP/HC/UFPR

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ACHADOS CLÍNICOS E GENÉTICOS DE DOENÇAS OFTALMOLÓGICAS HEREDITÁRIAS

Pesquisador: Mario Teruo Sato

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 5

CAAE: 24561313.3.0000.0096

Instituição Proponente: Hospital de Clínicas da Universidade Federal do Paraná

Patrocinador Principal: Financiamento Próprio
Hospital de Clínicas da Universidade Federal do Paraná
Universidade Federal do Paraná - Setor de Ciências da Saúde/ SCS

DADOS DA NOTIFICAÇÃO

Tipo de Notificação: Envio de Relatório Parcial

Detalhe:

Justificativa: Envio de relatório parcial para notificar sobre o andamento da pesquisa.

Data do Envio: 25/07/2019

Situação da Notificação: Parecer Consubstanciado Emitido

DADOS DO PARECER

Número do Parecer: 3.509.144

Apresentação da Notificação:

ACHADOS CLÍNICOS E GENÉTICOS DE DOENÇAS OFTALMOLÓGICAS HEREDITÁRIAS

Objetivo da Notificação:

Relatório parcial

Avaliação dos Riscos e Benefícios:

Não há riscos ou benefícios relacionados ao relatório parcial

Comentários e Considerações sobre a Notificação:

Data de início do projeto em 13/01/2014.

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

UFPR - HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ -

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Envio de Relatório Parcial	RELATORIO_PARCIAL_CEP_CHC_2019.pdf	25/07/2019 10:05:31	LETICIA MIDORI KONDO IWAMOTO	Postado

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 15 de Agosto de 2019

Assinado por:
maria cristina sartor
(Coordenador(a))

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ORIGINAL REPORT

WILEY

Ocular lesions in cats diagnosed with systemic sporotrichosis

Henrique M. Freitas[1] | Renata C. B. da Rocha[2] | Marconi R. de Farias[2] | Bret A. Moore[3] | Fabiano Montiani-Ferreira[1]

[1]Veterinary Medicine Department, Comparative Ophthalmology Laboratory (LABOCO, Federal University of Paraná (UFPR), Curitiba, Brazil

[2]School of Life Sciences, Pontifical Catholic University of Paraná (PUCPR), Curitiba, Brazil

[3]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida (UF), Gainesville, Florida, USA

Correspondence
Fabiano Montiani-Ferreira, Veterinary Medicine Department, Federal University of Paraná. Rua dos Funcionários, 1540, Curitiba, PR, Brazil.
Email: montiani@ufpr.br

Abstract

Objectives: To describe the most common ocular lesions and demonstrate the frequency of ophthalmic involvement in a group of cats with systemic sporotrichosis.

Animals Studied: Two hundred seventy-four cats diagnosed with systemic sporotrichosis. The inclusion criteria included previous positive cytopathological examination, histopathological examination, or fungal culture.

Procedures: In a prospective case–control study, 274 cats diagnosed with systemic sporotrichosis underwent ophthalmic evaluation and received treatment for systemic sporotrichosis. Of these animals, 63 had ocular abnormalities which were recorded, and conjunctivitis was scored from 0 to 5. Diagnostic techniques utilized included fungal culture, as well as cytopathological (10 eyes; 10 cats), and histopathological examination of the palpebral conjunctiva and eyes (2 eyes).

Results: Cytopathological and histopathological examination of the conjunctiva, as well as fungal culture, proved to be important tests for the detection of *Sporothrix sp.* Five cats without the evidence of ophthalmic abnormalities also had a positive fungal culture. The identified ocular lesions in animals with systemic sporotrichosis included increased serous discharge (79 eyes; 53 cats), blepharoconjunctivitis (33 eyes; 25 cats), conjunctivitis (39 eyes, 20 cats), blepharitis (9 eyes; 8 cats), uveitis (5 eyes; 3 cats), and Florida keratopathy-like lesions (2 eyes; 1 cat).

Conclusion: Sporotrichosis should be considered a differential diagnosis for conjunctivitis and blepharoconjunctivitis, especially in endemic areas. Fungal culture and cytopathology of ocular discharge and histopathological examinations of the conjunctiva are important for the diagnosis of ophthalmic sporotrichosis, although not all cats underwent laboratory testing in this study. Ocular discharge could be a source of contagion transmission.

KEYWORDS

blepharitis, conjunctival swabs, conjunctivitis, feline blepharoconjunctivitis, fungal culture, sporotrichosis