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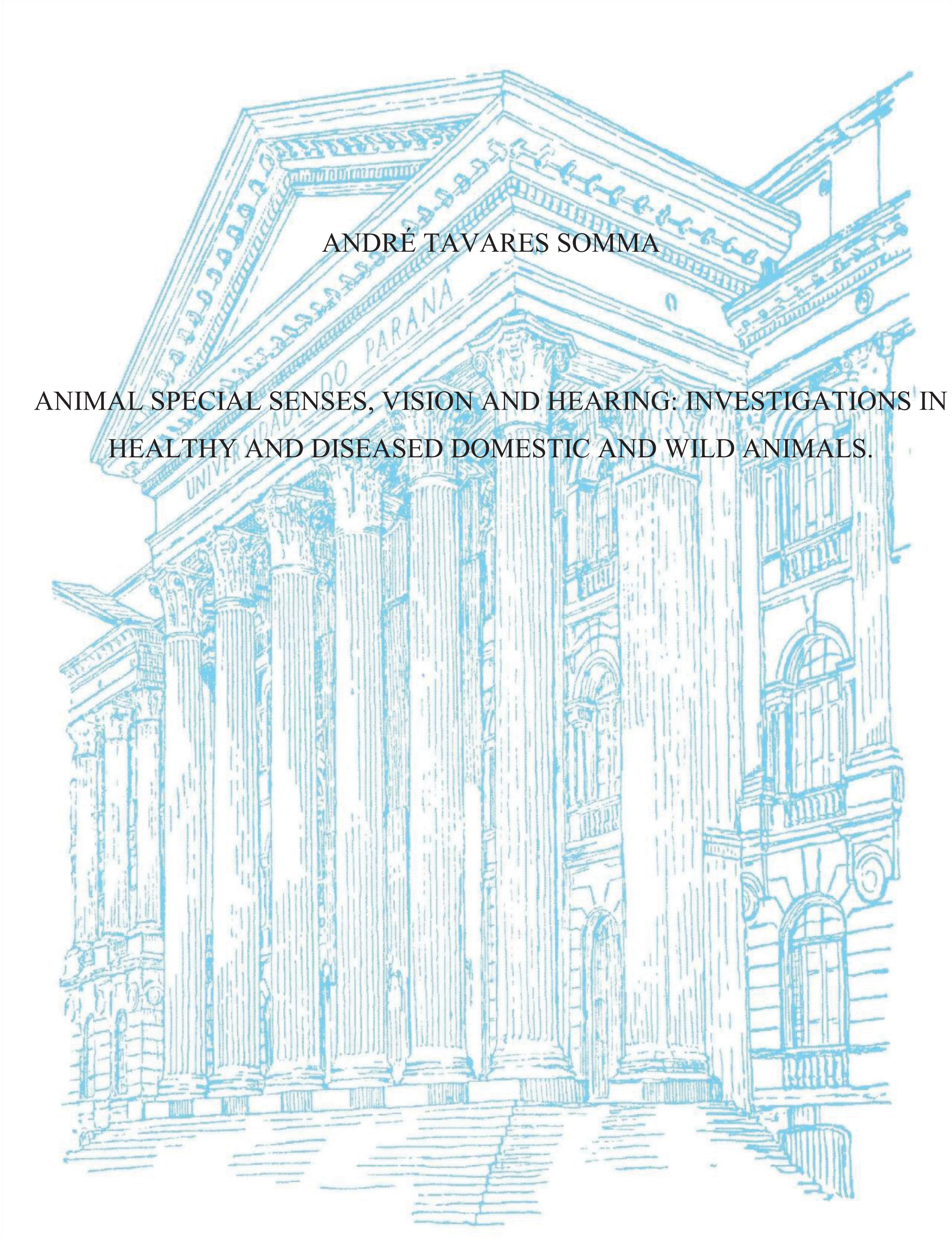

ANDRÉ TAVARES SOMMA

ANIMAL SPECIAL SENSES, VISION AND HEARING: INVESTIGATIONS IN HEALTHY AND DISEASED DOMESTIC AND WILD ANIMALS.

CURITIBA

2020

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ANIMAL SPECIAL SENSES, VISION AND HEARING: INVESTIGATIONS IN HEALTHY AND DISEASED DOMESTIC AND WILD ANIMALS.

Tese apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, do Setor de Ciências Agrárias, da Universidade Federal do Paraná, como requisito parcial para obtenção de título de Doutor em Ciências Veterinárias.

Orientador: Professor Ph.D. Fabiano Montiani-Ferreira

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ANDRE TAVARES SOMMA** intitulada: **ANIMAL SPECIAL SENSES, VISION AND HEARING: INVESTIGATIONS IN HEALTHY AND DISEASED DOMESTIC AND WILD ANIMALS**, sob orientação do Prof. Dr. FABIANO MONTIANI FERREIRA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 27 de Março de 2020.

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"There's real poetry in the real world. Science is the poetry of reality"

— Richard Dawkins

RESUMO

A presente dissertação compreende três capítulos, cada qual, composto por um artigo científico distinto. Tais capítulos englobam temas relevantes para a medicina de cães e gatos, bem como para a Medicina Zoológica, em ambos os casos, com ênfase na Oftalmologia Veterinária. Todos os estudos foram conduzidos pelo autor e colaboradores do Laboratório de Oftalmologia Comparada (LABOCO) da Universidade Federal do Paraná (UFPR). O primeiro capítulo é composto por um artigo científico denominado "Brainstem auditory evoked potentials in Whippets recorded with surface electrodes" e compreende resultados de testes de potencial evocado auditivo do tronco encefálico em cães. Este capítulo fornece informações eletrofisiológicas, contribuindo para o conhecimento de parâmetros auditivos normais em cães da raça Whippet. O estabelecimento de parâmetros fisiológicos pode auxiliar médicos veterinários no diagnóstico de doenças do aparato auditivo. O segundo capítulo, descreve a ocorrência de uma série de 13 casos de Philophtalmiase em Gaivotas (*Larus dominicanus*), em uma faixa do litoral do estado de Santa Catarina. São abordados aspectos epidemiológicos, clínicos, histopatológicos relacionados à doença, bem como aspectos morfológicos e moleculares do parasito. O terceiro e último capítulo consiste em um levantamento global com Médicos Veterinários Oftalmologistas sobre aspectos circundantes ao tema de perda visual irreversível em pequenos animais. São apresentados o perfil demográfico dos participantes da pesquisa, bem como a proporção e as respectivas causas mais frequentes de pacientes com perda visual total nas rotinas dos participantes. Ainda são abordados protocolos de manejo de animais cegos, com foco na sua contribuição para a manutenção da qualidade de vida, dos mesmos.

Palavras- chave: Oftalmologia. Audição. Whippet. Parasitos oculares. Qualidade de vida.

ABSTRACT

The present thesis encompasses three different chapters. Each one of these chapters comprises a distinct scientific manuscript, bringing relevant information about the ophthalmology of wild and domestic animals. All studies were conducted by the author and collaborators from Laboratory of Comparative Ophthalmology (LABOCO), Federal University of Paraná (UFPR). The first chapter are entitled: “Brainstem auditory evoked potentials in Whippets recorded with surface electrodes” and includes normal parameters for tests of brainstem auditory evoked potential (BAER) in dogs. This chapter provides electrophysiological information, contributing to the knowledge of auditory parameters in Whippet dogs. The establishment of physiological parameters can assist veterinarians in the diagnosis of diseases of the hearing system. The second chapter describes an occurrence of a series of cases of Philophthalmiasis in kelp gulls (*L.dominicanys)*, the south Brazilian coast, most precisely in the state of Santa Catarina. Epidemiological, clinical and histopathological aspects related to the disease are presented, as well and morphological and molecular aspects of the parasite. The third and last chapter consists of a global survey with Veterinary Ophthalmologists on aspects concerning the topic of irreversible vision loss. The demographic profile of the research participants is presented, as well the most frequent causes of irreversible blindness in pets. Management protocols also are addressed, hopefully contributing to the maintenance of quality of life.

Keywords: Ophthalmology. Hearing. Whippet. Eye Fluke. Blindness. Quality of Life.

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SUMMARY

1 GENERAL INTRODUCTION

Translational medicine can benefit enormously from studies in the Veterinary field. The establishment of electrophysiological parameters in animals can serve for both help veterinarians' clinicians during daily activities and contribute during the development of diagnostic tools for both humans and animals.

Moreover, information regarding the biological aspects of zoonotic agents can directly improve epidemiologic knowledge, and consequently allowing prophylactic strategies.

Furthermore, detailed pathologic aspects of eye disorders that affect animals can be used in order to better comprehend disease mechanisms and establish treatment protocols for humans as well. Considering the similarities between the eye and the visual apparatus of animals and humans, translational studies can provide useful data, that might increase visual health and consequently promoting high levels of Quality of life.

Therefore, in an attempt to contribute to scientific literature, with special focus in ophthalmology on both medical and veterinary areas, we present here a thesis; comprised of three distinct chapters.

The first chapter reports electrophysiologic normative data of auditory parameters in the Whippet dog breed, using an electro-diagnostic tool that measures the neuro-activation the brainstem auditory pathways, the Brain Auditory Evoked Response (BAER).

The second chapter reports clinical and histologic aspects of a series of *P. lachrymosus* cases in kelp gulls (*Larus dominicanus*), involving different localities across the Brazilian southern coast, specifically in the Santa Catarina State. The trematodes species were identified based on clinical examination combined morphological comparisons with the available species description as well as molecular analysis.

The last chapter comprises a study that investigate the opinion on irreversible blindness cases from veterinary ophthalmologists on an international scale in terms of management, quality of life (QoL) and the interaction between the pet and owner, focused to collate successful protocols for dissemination.

2. CHAPTER 1: Brainstem auditory evoked potentials in Whippets recorded with surface electrodes.

2. 1 ABSTRACT

The brainstem auditory evoked response (BAER) is an electro-diagnostic used to access neuro-activation in the cochlea, cochlear nerve, and brainstem auditory pathways. The aim of this study was to evaluate the brainstem auditory evoked potentials in a total 10 Whippet dogs, (5 males and 5 females) using surface electrodes. All dogs were anesthetized for the investigation. An air-conducted ABR Test, using clicks with stimulation speed 27.5 and rarefied polarity was used. In each test, the absolute latencies of waves I, III and V, and the I-III, III-V and I-V intervals for each side were measured at 90dBHL. The stimulus intensities were progressively decreased from 20dBHL in 20dBHL to 50dBHL. From 50dBHL the intensity was decreased by 10dBHL or 5dBHL (depending if the V wave could still be visualized). BAER values obtained in this investigation shows that the brainstem auditory evoked potentials using surface electrodes is a viable method to record the transmission of auditory information in Whippets and contributes to the obtaining BAER normative data, in healthy Whippets.

Keywords: Dogs. Brainstem Auditory Evoked Potentials. Hearing. Sighthounds. Veterinary Medicine.

2.2 INTRODUCTION

In adult humans, the sensation of hearing can be simply evaluated by clinical audiometry, in which the patient voluntary indicates that a certain frequency of sound has been perceived. On the other hand, Brain Auditory Evoked Response (BAER) in infants or young children becomes the gold standard method for hearing evaluation (Joint committee on infant hearing, 2007). Similarly, in Veterinary patients, despite the option of using clinical techniques to access auditory capacity (e.g. the observation of animal's reaction to simply clapping hands), a more complex type of behavioral response cannot be expected from most animals. Therefore, the auditory evoked responses had been largely used to access auditory function in mammals, including small (Shipley et al., 1980; Marshal et al. 1981; Harrison, 1982; Kay et al., 1984; Myers et al., 1985; Sims, 1986; Strain et al., 1989; Steiss et al., 1994; Kawasaki, 1995; Wilson, 1995; Eger, 1997; Strain et al., Wolschrijn et al., 1997; 1998; Poncelet et al., 2002; Mieskes et al., 2006;Weeb et al., 2006; Weeb, 2007; ter Haar et al.,2008; Cvejic et al., 2009; Palumbo et al., 2014; Plonek et al., 2017) large (Marshall et al., 1981; Strain, 1989; Harland et al., 2006; Melvin, 2018) exotic (Allen, 1978; Ridgway et al., 1981; Kraus ET AL., 1985; Popelar et al., 1994; Harada, 1997; Harada, 1999; Weeb et al. 2006; Pacini et al., 2011; Lima et al.,2012) and laboratory (Church, 1987; Burkard et al., 1989; Burkard et al., 1990; Gross-Sampson, 1991; van

Looij; 2004) animals. The BAER is an objective test, which records electrical potentials obtained from a specified stimulus in a form of a sound, delivered via earphones (Webb 2009). BAER can accurately measure the activation of neurons in the cochlea, cochlear nerve, and in brainstem auditory pathways. BAER normative values in dogs are extensively present in the literature (Bruce, 2019; Armasu et al., 2015; Palumbo et al., 2014; II Pallumbo, 2014; Poncelet et al., 2000; II Poncelet et al., 2000; Munro et al., 1997; Shiu et al., 1997; Haagen et al., 1989; Knowles et al., 1988; Bodenhamer, 1985; Kay et al., 1984; Sims, 1984), but according to authors knowledge, none of them in the Whippet dog breed.

Considering several intrinsic breed-differences, which could affect BAER response in dogs, some authors propose to obtain specific normative BAER data for each breed of dog (Wilson, 2005; Bruce, 2019). Therefore, the purpose of this investigation is to obtain BAER normative data in anesthetized, healthy Whippets, using surface electrodes.

2.1 MATERIALS AND METHODS

Animals

Ten 5-year-old privately-owned Whippet dogs (5 males and 5 females), with a mean weight of 12.87kg and with a mean age of 4.75 years, were included in the study. An informed consent was obtained prior to the start of the investigation. The investigation was conducted in accordance with the Federal University of Parana's Animal Use Committee. Each dog was clinically evaluated, including neurological and otoscopic examinations with the objective to exclude animals with other systemic, neurological or hearing disorders.

BAER tests were performed at the Veterinary Teaching Hospital of the Federal University of Parana (HV-UFPR), in the city of Curitiba, Paraná State, Brazil.

Anesthesia

Propofol (5mg/kg; Propovan 1%, Cristalia Produtos Quımicos e Farmacêuticos Ltda, Itapira-SP, Brazil), administered in two minutes, was used for anesthetic induction. After endotracheal intubation, the dogs received oxygen with at an inspired fraction of 100% by a rebreathing anesthetic circuit and anesthesia was maintained with a continuous rate infusion of propofol (0.3mg/kg/min).

During entire anesthetic procedure, esophageal temperature was monitored to guarantee a body temperature between 37.2 and 38^{o} C, using a warming mattress. Furthermore, heart and respiratory rates, electrocardiography, noninvasive blood pressure, pulse oximetry, end-tidal CO_2 and isoflurane concentration were continuously evaluated by a multi-parameter monitor (Life Window LW9xVet, Digicare Animal Health, Boynton Beach— Florida, USA).

BAER Test Protocol

Twenty ears from 10 dogs were tested. Electrical signals were captured using surface electrodes placed as follows: The active monopolar electrode was placed at the vertex (upper surface of the head). The reference electrode was placed just rostral to the base of the ear. The ground electrode was placed on the skin over the intersection of the frontal bone and two nasal bones in the rostral aspect of the neurocranium, between both eyes (Fig.1).

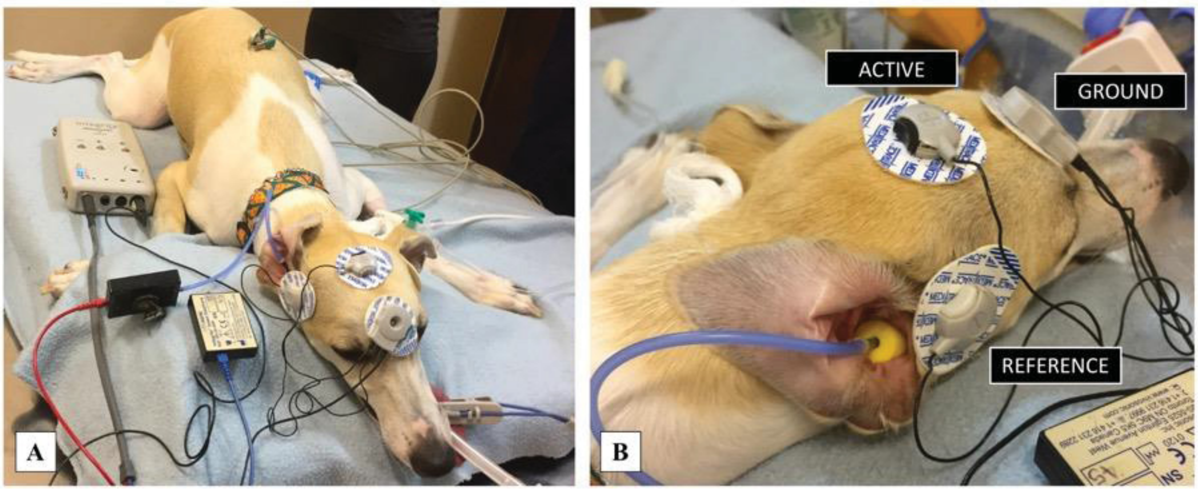

Figure 1. A) A representative Whippet dog positioned in external recumbency during BAEP evaluation. B) Detail of the position where surface electrodes and earplug were placed during BAER evaluation. The active monopolar electrode was placed at the vertex; the reference electrode was placed at a point just rostral to the base of the ear. The ground electrode was placed on the skin, between both eyes).

All examinations were performed using an auditory diagnostic system (Vivosonic Integrity V500®, Toronto, Ontario, Canada). Testing was done on both ears, separately. Before fixing the electrodes, the ear was cleaned with abrasive paste (Nuprep®, Aurora, Colorado, USA). After the electrodes were fixed, the impedance level of the electrodes before the test was required up to 1kOhm. Surface electrodes were used and insertion phone with "ear tips ER-3A" (regular, 13mm, yellow) positioned manually over the external auditory meatus of the dog (Fig. 1B). Test ABR air-conducted, clicks with stimulation speed 27.5, with rarefied polarity. In each test, the absolute latencies of waves I, III and V, and the I-III, III-V and I-V intervals for each side were measured at 90 decibels hearing level (dBHL). The intensity was decreased from 20dBHL in 20dBHL to 50dBHL. From 50dBHL the intensity was decreased by 10dBHL down to 5dBHL (Fig. 2). We have considered the last intensity in which it was possible to visualize the V wave as the Minimum Response Level.

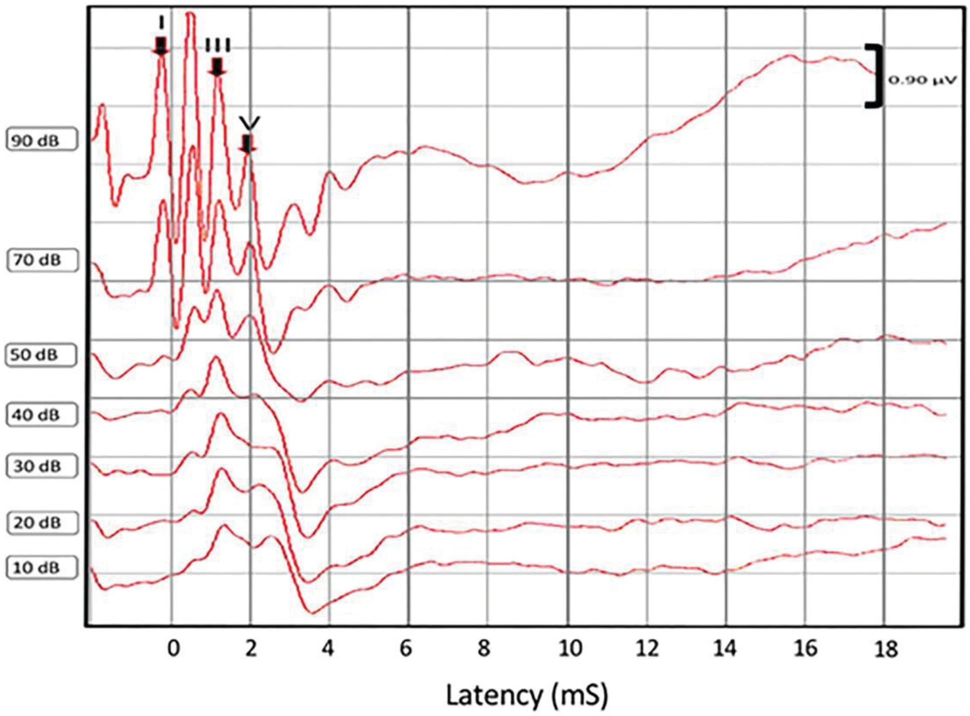

Figure 2. Typical morphology of BAER tracings, recorded after stimulation of the right ear of a PRA blind Whippet with sound pressure level intensities between 90 and 10 dBHL.

Statistical Analyses

Descriptive statistical analyses were performed. Mann-Whitney Test was used for data comparison between, right and left ears as well as data from males and females. JMP (SAS Institute, Inc., Cary, NC, USA) software was used and P-values < 0.05 were deemed significant.

2.2 RESULTS

BAER tracings with characteristic I, III and V waves and I-III, III-V and I-V inter-peaks were recognized and characterized to as normal in all records. Results did not reveal significant differences between males and females for waves I (*P* =0.354), III (*P* = 0.521), and IV (*P* = 0.232) and intervals I-III (*P* =0.411), III-V (*P* = 0.123) and I-V (*P* = 0.543). Results comparing left and right ear in males for waves I (*P* = 0.3842), III (*P* = 0.2731), and V (*P* = 0.5962) and intervals I-III (*P* = 0.3810), III-V (*P* = 0.2102) and I-IV (*P* = 0.314), and females for waves I (*P* =0.6779), III (*P* = 0.5723), and V (*P* = 0.2008) and intervals I-III (*P* =0.5821), III-V (*P* = 0.6421) and I-V (*P* =0.4528) also did not demonstrate significant differences. All values of Median and interquartile range are shown in Table 1. Median and IQR of all waves and intervals, according to sex and ear, are shown in Table 2.

Table 1. Median and interquartile range of waves (I, III and V) and intervals (I-III, III-V and I-V) of absolute latencies in milliseconds (ms) of BAER data in healthy Whippet dogs (n=20 ears).

Wave/Inter-peak	I	III	V	I-III	III-V	I-V
Median	0.955	2.560	4.150	1.620	0.920	2.530
IQR	0.045	0.050	0.930	0.060	0.070	0.095

Table 2. Median, interquartile of absolute latencies range of waves (I, III and V) and intervals (I-III, III-V and I-V) and P values according to sex and ear, in milliseconds (ms) of BAER data in healthy Whippet dogs (n=20 ears).

	Males				*P*	Females				*P*
	Left ear		Right ear			Left ear		Right ear		
Wave / Interpeak	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
I	0.94	0.03	0.94	0.013	0.3842	1	0.022	1	0.045	0.6779
III	2.56	0.04	2.56	0.05	0.2731	2.64	0.33	2.64	0.135	0.5723
V	4.04	0.88	4.17	0.85	0.5962	4.54	10.6	4.16	0.95	0.2008
I-III	1.620	0.060	1.590	0.035	0.3810	1.640	0.307	1.640	0.090	0.5821
III-V	0.940	0.052	0.910	0.040	0.2102	0.880	0.120	0.880	0.120	0.6421
I-V	2.580	0.042	2.520	0.35	0.314	2.630	0.270	2.520	0.210	0.4528

2.3 DISCUSSION

Studies using BAER in animals are common (Shipley et al., 1980; Marshal et al. 1981; Harrison, 1982; Kay et al., 1984; Myers et al., 1985; Sims, 1986; Strain et al., 1989; Steiss et al., 1994; Kawasaki, 1995; Wilson, 1995; Eger, 1997; Strain et al., 1998; Poncelet et al., 2002; Mieskes et al., 2006;Weeb et al., 2006; Weeb, 2007; ter Haar et al.,2008; Cvejic et al., 2009; Palumbo et al., 2014; Plonek et al., 2017), but comparatively, despite important aspects such as the growing perception of pet owners about the importance of specialized veterinary care (Albers, 2008), the evidences of high incidence of otologic diseases in the small animal practice (Cole et al., 1998; Murphy, 2001; Oliveira et al., 2006; Saridomichelakis et al., 2007; McGuinness et al., 2013; Lu et al., 2019) and the fact that the majority of pet owners believe that the care of their animals should take precedence over monetary aspects (Coe et al., 2007) BAER still remains somewhat not commonly used in clinical routine. Factors that might partially explain this, includes the high monetary investment to order BAER devices, and cases of unilateral deafness, that may easily be unnoticed by owners (Schemera et al., 2011).

BAER normative data were investigated in several breeds of dogs (Holliday et al., 1992; Munro et al., 1997; Palumbo et al., 2014; Strain et al., 1992; Strain 1996; Strain, 2004). However, this study appears to be the first BAER investigation in Whippets.

Congenital forms of deafness are known to occur in over 100 dog breeds (Strain, 2004; Strain, 2011), and Whippets are included in this list (Strain et al., 1992; Strain, 2004; Strain, 2011, Strain, 2014). Since BAER is recognized as an essential diagnostic tool in such cases (Holliday et al., 1992; Strain et al., 1992; Comito et al., 2012; Scheifele, 2012; Plonek et al., 2016) plus the importance of establishing auditory thresholds in the different dog breeds (Munro et al., 1997; Poma et al., 2008; Scheifele, 2012; Palumbo, 2014; Stanciu, 2015), considering the variability of canine BAER responses presented in the literature (Bruce, 2019), the data showed in this study may contribute to improve the knowledge of both, BAER reference values in normal hearing Whippets and BAER alterations found in deaf Whippets.

Normative values investigations, requires uniform data acquisition procedures. Important aspects that might influence BAER results, including sex (Jeger, 1980) age (Jeger, 1980; Harrison, 1982; Shimada et al., 1998; Harada, 1999), temperature (Kaga et al., 1979; Rossi, 1984) and head size (Pook, 1990; Meij et al., 1992; Munro et al., 1997) were considered prior this investigation started. All animals (50% males and 50% females) used were adults, with the same age and maintained with controlled body temperature and in a controlled environment. Moreover, BAER tests were conducted with all dogs under general anesthesia. Although sedation or anesthesia are usually not mandatory to perform the test in dogs (Wilson, 2005; Weeb, 2009), plus the numerous evidences of the influence of different anesthetics drugs on BAER results (Stockard, 1977; Cohen, 1982; Myers et al., 1985; Harada, 1997; Murrel et al., 2004; Murrel et al., 2005), considering that muscular activity artifacts have been proved to substantially affects BAER results (Hall, 1992; Hood, 1998; Wilson, 2005), with the aim to make a more uniform procedure and to achieve optimal recording, all dogs were anesthetized during data acquisition. The efficacy of use of Propofol during BAER tests, was previously attested both in humans (Savoia et al., 1988; Chassard et al., 1989; Thornton et al., 1989; Purdie, 1993; Reich et al., 1996; Iselin-Chaves et al., 2000; Kumar et al., 2000; Akin et al. 2005; Abulebda et al., 2019) and animals (Shi et al., 2014), including dogs (Ter Haar et al., 2002; Ter Haar et al., 2008), justifying our option for this particular drug during data acquisition.

Different types of electrodes can be used in order to perform a BAER test. While studies with humans (Kumar et al., 2000; Pokryszko-Dragan et al., 2015; Legatt, 2018) tends to use surface electrodes in veterinary medicine, needle electrodes are preferred (Holliday et al., 1992; Poncelet et al., 2002; Ter Haar et al., 2002; Wilson et al., 2005; Weeb, 2009; Wilson et al., 2011; Schemera et al., 2011; Palumbo et al., 2014; Plonek et al., 2016; Melvin, 2018;) since some authors believe that surface electrodes generate lower responses, compared with needle electrodes (Jost et al., 1994). However, since surface electrodes have already been proved to be very user-friendly and a less invasive method for animals (Musteata et al., 2013; Selvaraj et al., 2017; Selvaraj et al., 2018), the authors made the option for using this method to carry this investigation.

The types of auditory stimulus most used during BAER investigations are click (Eger, 1997, Palumbo et al., 2014), tone-burst (Rauschecker, 1993; Uzuka et al., 1998; Poncelet et al., 2002; Ter Haar et al., 2008; Dagna et al., 2014) and bone-conducted (Strain et al., 1993; Wolschrijn et al., 1997) Since rarefaction clicks were the most prevalent method used in studies in animals (Strain et al., 1992; Eger, 1997, Poncelet et al., 2006; Palumbo et al., 2014) and normative hearing parameters investigations (Kay et al., 1984; Sims, 1984; Holliday, 1985; Myers et al., 1985; Burkard et al., 1990; Thivierge, 1990; Jiang et al., 1991; Kawasaki, 1994; Uzuka et al., 1998; Esteves et al., 2009; Musteata et al., 2013; Stanciú et al., 2015) the authors made the option to use click-delivered stimulus in Whippets.

The method used in this study permitted a well-defined observation of all clinically relevant BAER waves (Arnold, 2007; Esteves et al., 2009; Bruce, 2019) I, III and V, and intervals I-III, III-V and I-V (Selvaraj et al., 2018), following what was previously proposed in BAER studies with several dog breeds including Argentinean Mastiffs (Stanciú et al., 2015), Dalmatians (Palumbo et al, 2014), Boxers (Palumbo el tal., 2014) and cats (Musteata et al., 2013).

BAER data obtained in this study did not demonstrated statistical differences between males and females. Sex have been investigated as an important individual aspect to influence the BAER response (Hall, 1992; Wilson, 2005; Bruce, 2019). Shorter latencies (Picton et al., 1981) and larger amplitudes in females (Kjaer, 1979; Michalewski et al., 1980; Picton et al., 1981) found in humans could be due shorter basilar membranes found in females and larger heads found in males (McFadden, 1998). In dogs several studies (Marshall, 1985; Pook et al., 1990; Meij et al., 1992; Munro, 1997a; Munro et al., 1997b; Shiu et al., 1997) have reported no significant differences between males and females, and this study is no exception. Literature showed no consensus about differences in BAER values attributed to differences in head size both in humans (Hall, 1992; Hood, 1998) and dogs (Pook et al., 1990; Meij et al., 1992), highlighting the importance to obtain normative data that are dog breed-specific (Wilson., 2005).

In this investigation, no significant difference was found when comparing right vs left ear, similarly to what was previously reported in other studies in dogs (Myers et al., 1985; Knowles et al., 1988; Schemera et al., 2011; Palumbo et al., 2014; Stanciu et al., 2015; Stanciu et al., 2016; Plonek et al., 2017), cats (Musteata et al., 2013) and humans (Flabiano et al., 203; Esteves et al., 2009).

BAER results presented in this study are similar to what was previously reported in dogs (Palumbo et al., 2014; Stanciu et al., 2015; Selvaraj et al., 2018).

Despite the limited number of animals evaluated in the present study, data presented here attest to the presence of all clinically relevant waves in all subjects evaluated. In addition, the morphological appearance of the waves was similar among all individuals tested, and the latency values were similar to other studies (Palumbo et al., 2014; Stanciu et al., 2015; Selvaraj et al., 2018) and did not differ when comparing results of right and left ears.

BAER data obtained here contributes with normal data for future auditory electrophysiology investigations in dogs. Additionally, it shows that brainstem auditory evoked potentials obtained with surface electrodes, is a repeatable method to record the central transmission of auditory information.

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3. CHAPTER 2: ***Philophthalmus lachrymosus*** **infection in free-living kelp gulls (*Larus dominicanus*) in the South of Brazil – A clinical, histologic, morphometric and molecular study.**

3.1 ABSTRACT

Thirteen kelp gulls (*Larus dominicanus*) that were admitted for rehabilitation at the Marine Animals Stabilization Unit of the University of the Itajaí Valley (UNIVALI) in the city of Penha, Santa Catarina State, between July of 2017 and September of 2018 were diagnosed with ocular an infection caused by *Philophthalmus lachrymosus*. Ophthalmic examination, histological analysis, parasite morphological, morphometric and molecular identification were used for the definitive diagnosis. The trematodes were found in the animals´ conjunctiva, especially in the lower conjunctival fornix. Eleven birds (84.61%) presented signs of systemic disease. Emaciation was detected in 8 (61.5%) birds, 5 (38.46%) presented dehydration and 3 (23.07%) showed depressed consciousness. In 11 (84.61%) birds, parasites were found on both eyes. In 1 (7.69%) parasites were just found in the left eye and in 1 (7.69%) parasites were just found in the right eye. Conjunctival hyperemia was the most prevalent ocular sign, observed in 22 eyes (84.61%). Keratitis was noted in two eyes (7.69%), corneal ulcer was observed in 2 eyes (7.69%) and corneal abscess and chemosis were detected in one eye (3.84%). The histopathologic findings were likely due to the trematode ability to attach to the ocular surface, especially in the conjunctival tissue. This study provides epidemiological and clinical details of *P. lachrymosus* infection in kelp gulls. Further research is still needed to elucidate the possible intermediary hosts.
Key Words: Eye, Veterinary Ophthalmology, Parasitology, DNA sequences, Trematode.

3.2 INTRODUCTION

The trematode *Philophthalmus* sp. is recognized for naturally infect eyes of several birds, including Galliformes,[1-3] Anseriformes,[4,5] Tinamiformes,[6] Ratites,[7-11] Passeriformes,[12] Pelecaniformes[13] and Charadriiformes.[14-16] There also are reports of Philophthalmiasis in humans,[17-23] capybaras,[24] and sea lions.[25]

The trematode *Philophthalmus* sp. have an aquatic linked life cycle: Embryonated eggs pass into the water from the eyes; Miracidia (a free-swimming ciliated larval stage) hatch almost immediately in water then invade the intermediate host (typically a snail); After the trematode reproduces asexually the cercariae are released into the water by the intermediate host; The parasite encysts on solid substrates (becoming metacercariae); The definitive host becomes infected by ingestion or directly though ocular surface contact.[26]

Nearly 50 *Philophthalmus* species have been described,[12,27] using both morphological[3,4,6,8,16,28,29] and molecular methods.[3,7,16,29,30] *P. lachrymosus* is known to occur in gulls [14,16,30] including kelp gulls (*Larus dominicanus*).[14]

Clinical cases of Philophthalmiasis in birds, typically manifests clinically through ocular surface disease signs, typically conjunctivitis [6,7,10,12,16,19,20,23,31,32] and keratitis [16,31,32] and blepharitis.[6,32] Histological analysis of infected tissues commonly reveals inflammatory infiltrates and the presence of adult form of the parasite attached to the conjunctiva.[24]

This study reports clinical and histologic aspects of a series of *P. lachrymosus* cases in kelp gulls (*L. dominicanus*), involving different localities across the Brazilian southern coast, specifically in the Santa Catarina State. The trematodes species were identified based on clinical examination combined morphological comparisons with the available species description as well as molecular analysis.

3.3 MATERIALS AND METHODS

Animals

All procedures using live birds were conducted in accordance with ARVO's Statement for the Use of Animals in Ophthalmic and Vision Research and with Federal University of Parana's Animal Use Committee. A total of 13 animals, admitted for rehabilitation at the Marine Animals Stabilization Unit of the University of the Itajaí Valley (UNIVALI) in the city of Penha, Santa Catarina State between July of 2017 and September of 2018 were examined. All birds were previously free ranging and were collected by the UNIVALI staff due to debilitating health conditions, particularly demonstrating signs of ocular disease.

Study area

The main area analyzed was an extension of approximately 67 km from the coast of the state of Santa Catarina, between the cities of Barra Velha and Bombinhas (Fig1). All medical care procedures and data acquisition were performed at the Marine Animals Stabilization Unit of the University of the Itajaí Valley (UNIVALI) as part of the Beach Monitoring Program – Santos Basin (PMP-BS) in the city of Penha, Santa Catarina State, located on the southern Brazilian coast. Detailed information about birds collecting sites, can be seen in Table 1.

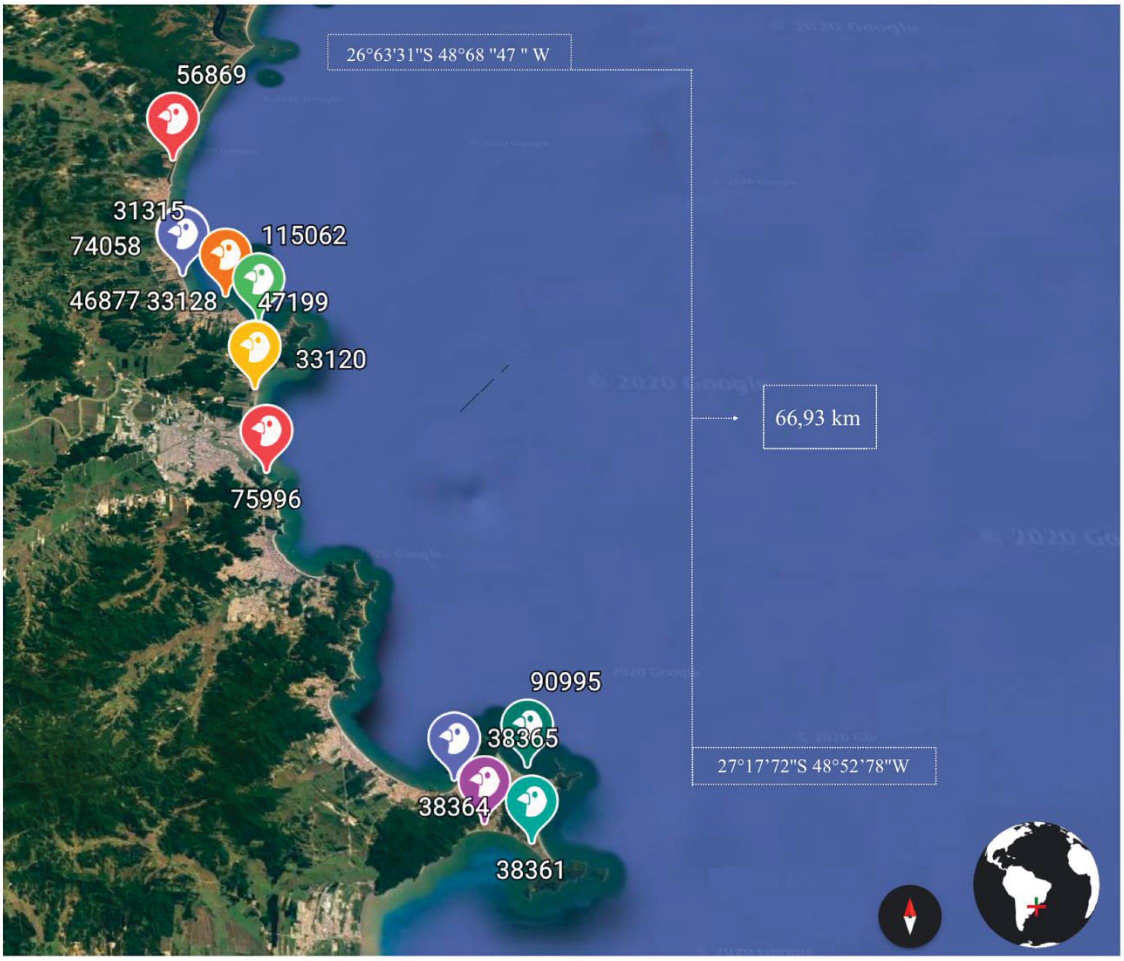

Figure 1. Image obtained with Google Earth (https://earth.google.com) showing the range coast between the cities of Barra Velha (north) and Bombinhas (south) in which 13 kelp gulls (*L. dominicanus*) were collected and diagnosed with ocular infections caused by *P. lachrymosus*. Bird balloons (Cities) and numbers (Bird identification code).

Table 1. Collection sites of 13 kelp gulls (*L. dominicanus*) infected with *P. lachrymosus* in the coast of Santa Catarina state, in the Brazilian southern coast.

City	Beach	Geographic location	Total of birds collected
Barra Velha	Bacia da Vovó	26°45'48"S 48°38'48"W	1
	Peninsula	26°36'19"S48°40'25"W	1
Navegantes	Navegantes	26°51'09"S 48°38'01" W	2
Piçarras	Piçarras	26°44'30"S 48°40'07"W	3
Bombinhas	Canto Grande	27°12'02"S 48°29'58"W	2
	Zimbros	27°10'43"S 48°32'04"W	1
Porto Belo	Baxio	27°09'11"S 48°32'36"W	1
Itajaí	Cabeçudas	26°55'35"S 48°38'00"W	1
Penha	Armação	26°47'21"S 48°37'04"W	1

Clinical exam

All birds were submitted to the clinical evaluation protocol established by Veterinarians from the Marine Animals Stabilization Unit, including feather and skin health evaluation, cardiac and pulmonary auscultation and cloacal temperature measurement.

Ophthalmic exam

All birds included in the study were manually restrained. A total of 26 eyes, from 13 kelp gulls were examined. The anterior segment structures were evaluated using a slit lamp biomicroscope (PSL One, Keeler, Malvern, PA, USA) and fluorescein stain (Drogavet; Curitiba, PR, Brazil) was performed to investigate potential corneal lesions. The same investigator conducted the ophthalmic examination in all birds. Intraocular pressure and fundus examination were not performed.

Sampling of eye trematodes.

Individual trematode parasites were visually identified in the conjunctival sac, removed using a toothless Colibri ophthalmic forceps, and preserved both in formalin 10 % and 96% ethanol.

Parasite morphological study.

Samples of ethanol fixed adult parasites obtained from the eyes of the birds were stained with alum acetocarmine, dehydrated in ethanol series, diaphanized in beechwood creosote and mounted on permanent slides with Canada balsam.

Morphological and morphometric studies were performed under an Olympus BH2 optical microscope (Olympus, Tokyo, Japan). Measurements were obtained using a micrometer eyepiece. Photographs were taken with a Leica ICC50 HD digital camera coupled to the microscope Leica DM500 and analyzed in the Leica Application software suite (LAZ EZ), version 2.0 (Leica Microsystems, Wetzlar, Germany). Measurements are expressed in micrometers and were represented by the mean and standard deviation, with amplitude in parentheses (Table 2). The relationships between some morphological traits were calculated according to Ching (1964). The taxonomic identification of the parasite was performed with the aid of taxonomic keys (Jones et al., 2005) and descriptive works by different authors (Freitas, 1955; Travassos et al., 1969; Yamaguti, 1971; Nasir and Díaz, 1972). Samples of the parasites studied were deposited in the Collection of Trematodes of the Universidade, Federal de Minas Gerais (UFMG-TRE).

Parasite molecular analyses

Specimens of ethanol fixed parasites were used for the molecular study. DNA was extracted using the Wizard® Genomic DNA Purification kit. The concentration of the extracted DNA was estimated using a microvolume spectrophotometer (NanoDrop® Lite-1000). DNA amplifications were performed using the polymerase chain reaction (PCR) in a total volume of 25 μL. In each reaction we used Platinum HotStard Master Mix 2×, 10 μM of each primer and ~50 ng of DNA. The molecular markers used was the mitochondrial cytochrome c oxidase subunit 1 gene (cox-1). The primer and PCR conditions used were those previously published: The primers JB3 and COI-R- Trema were used to amplify a fragment of ~ 800 bp the cox-1.ref PCR products were initially subjected to agarose gel electrophoresis and then purified with polyethylene glycol 8000 (20%) (Promega, Madison, WI), following the protocol described by Paithankar and Prasad (1991), with slight modifications. Sequencing in both directions was performed using capillary electrophoresis in the ABI3730 sequencer, with the POP7 polymer and the BigDye v3.1 sequencing kit (Applied Biosystems, Inc., Foster City, CA). Consensus sequences were assembled and edited using the program ChromasPro version 2.0.1 (Technelysium Pty Ltd, Tewantin, Australia). Generated contigs and sequences available from

GenBank were aligned using ClustalW implemented in MEGA7, ref. The new molecular sequences here obtained were deposited in GenBank.

Gross evaluation and histologic analysis

Six eyes from 3 birds (33120, 90995 and 115062) that died from undefined causes were collected and analyzed. After death, globes were enucleated and immersed in 10 % formalin for 14 days, followed by transfer to 70 % ethanol for storage. After fixation, transverse lengths (nasotemporal and dorsoventral), corneal diameters (nasoventral and dorsotemporal), and the axial length were measured using a steel caliper. The eyes were sectioned in the sagittal (at the optic axis) and coronal planes for gross anatomic evaluation, and two sagittal hemisections were collected and embedded in paraffin. Histologic sections of 4 μm each, were then stained with hematoxylin and eosin and *PAS* and examined microscopically using the microscope Leica DM500 with the Leica Application software suite (LAZ EZ), version 2.0 (Leica Microsystems, Wetzlar, Germany). These animals were not submitted to a full necropsy. Only the eyes were sent to the Federal University of Paraná´s Comparative Ophthalmology Lab (LABOCO UFPR).

3.4 RESULTS

Animals

Twelve birds were classified as adults and 1 was classified as juvenile according to the plumage color[33,34] Exact ages could not be determined since all animals were free ranging birds and gender could not be determined due to lack of sexual dimorphism of the species. Nevertheless, adults were considered to have at least 4-year-old, since full adult plumage is acquired in the fourth year.[35]

Clinical exam

Eleven birds (84.61%) presented signs of systemic disease. Emaciation was the most common finding during clinical evaluation, which was detected in 8 birds (61.5%). Five birds (38.46%) presented dehydration in the moment of the admission. Three cases (23.07%) of depressed consciousness. Diarrhea was present in two individuals. Cloacal prolapse and pododermatitis were found in one individual each. The left hindlimb was missing in one individual examined. Clinical exam findings can be seen in details in Table 2. 3 birds (23.07%) (33120, 90995 and 115062) died before any clinical diagnostic of systemic disease was concluded.

Ophthalmic exam

Parasites were found on both eyes in 11 birds (84.61%). In one (7.69%) bird (ID 56869) parasites were just found in the left eye (OS), and in 1 (7.69%) bird (ID 75996), parasites were just found in the right eye (OD). A total of 206 parasites were found and removed. One hundred and nine (52.91%) parasites were found in the OD and 97 (47.08%) were collected from OS. All trematodes collected in this investigation were found in the ventral conjunctival fornix (Fig.2A). Conjunctival hyperemia (Fig 2.B) was the most prevalent ocular sing, observed in 22 eyes (84.61%). Hyperemia apparently was more severe in eyes heavily infected by the parasites. Keratitis was noted in two eyes (7.69%). Corneal ulcer (Fig2D) was observed in 2 eyes (7.69%) corneal edema (Fig.2C) corneal abscess and chemosis were detected in one eye (3.84%) respectively.

Treatment protocol

Support therapy were performed according systemic conditions of each bird. Treatment protocol of infected birds with conjunctivitis consisted only in the mechanical removal of the eye flukes. Birds with corneal lesions received further antibiotic eye drops or ointment instillation according to the severity of each case.

Table 2. Identification number, city of origin, and clinical aspects of 13 Kelp Gulls (*L. dominicanus*) infected by P. l*achrymosus* in the coast of Santa Catarina, Brazil, between July of 2017 and September of 2018.

Case ID	Date Admission	Age	City	Clinical exam	Ophthalmic exam	Number of parasites	
						OD	OS
46877	07/18/2017	Adult	Piçarras	Emaciated	Conjunctival hyperemia OU	13	19
56869	08/20/2017	Adult	Barra Velha	Emaciated	Conjunctival hyperemia OS	0	5
47199	08/25/2017	Adult	Navegantes	Dehydrated	Conjunctival hyperemia OU	11	7
31315	10/15/2017	Adult	Penha	-	Conjunctival hyperemia OU	2	6
75996	12/31/2017	Adult	Itajaí	Emaciated	Conjunctival hyperemia OD	12	0
				Left hindlimb missing	Keratitis OU		
38364	04/01/2018	Adult	Porto Belo	-	Conjunctival hyperemia	7	9

					Chemosis OS		
38361	03/26/2018	Adult	Bombinhas	Depressed consciousness	Conjunctival hyperemia OU	5	4
				Dehydrated			
				Emaciated			
33120+	03/29/2018	Young	Navegantes	Depressed consciousness	Conjunctival hyperemia OU	5	11
				Dehydrated	Keratitis OS		
				Emaciated	Corneal ulcer OD		
					Corneal abscess OS		
33128	03/31/2018	Adult	Piçarras	-	Conjunctival hyperemia OU	13	3
					Corneal abscess		
74058	04/06/2018	Adult	Piçarras	Emaciated	Conjunctival hyperemia OU	16	8
				Diarrhea			
38365	04/17/2018	Adult	Bombinhas	Dehydrated	Conjunctival hyperemia OU	12	6
				Emaciated			
90995+	09/15/2018	Adult	Bombinhas	Depressed consciousness	Conjunctival hyperemia OU	2	4
				Diarrhea	Keratitis OS		
				Cloacal prolapse			
115062+	09/18/2018	Adult	Penha	Dehydrated	Conjunctival hyperemia OU	11	15
				Pododermatitis			

+ Birds who died during the period of the study.

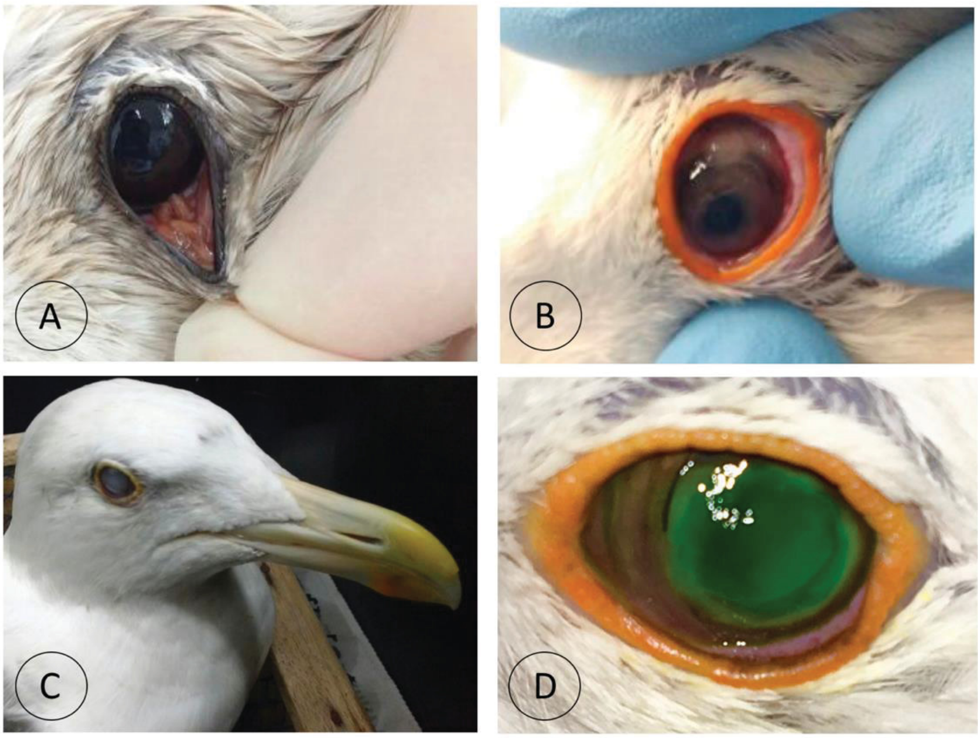

Figure 2. A: OS Ventral conjunctival fornix of a kelp gull (*L. dominicanus)* infected with specimens of *P. lachrymosus*. B: Left eye of a kelp gull (*L. dominicanus)* with conjunctival hyperemia and chemosis in the dorsal bulbar conjunctiva, near the limbus. C: Kelp gull (*L. dominicanus)* with corneal edema in the OD. D: OS corneal ulcer stained after the instillation of fluorescein dye.

Histologic evaluation

Hemi section of the globes revealed no intraocular abnormalities. No infectious agents were observed in any of the samples. Histopathology of the eye and adnexa confirmed conjunctivitis. The microscopic lesions varied according to each individual animal and the stage of the disease in which the globe was enucleated. In general, microscopic lesions consisted of varying degrees of inflammation in the conjunctival tissue, with a discrete diffuse mononuclear inflammatory cell's infiltration in the stroma (Fig. 3A). Occasionally conjunctival papillary projections (Fig. 3B) were observed. In the center of these papillae, delicate vessels were commonly observed. Caliciform cells typical of the conjunctival fornix were observed around these sites. Histology of the globes with corneal ulcers showed absence of epithelium (Fig. 3A) in the affected areas, stromal inflammatory cell infiltration and secondary stromal fibrosis.

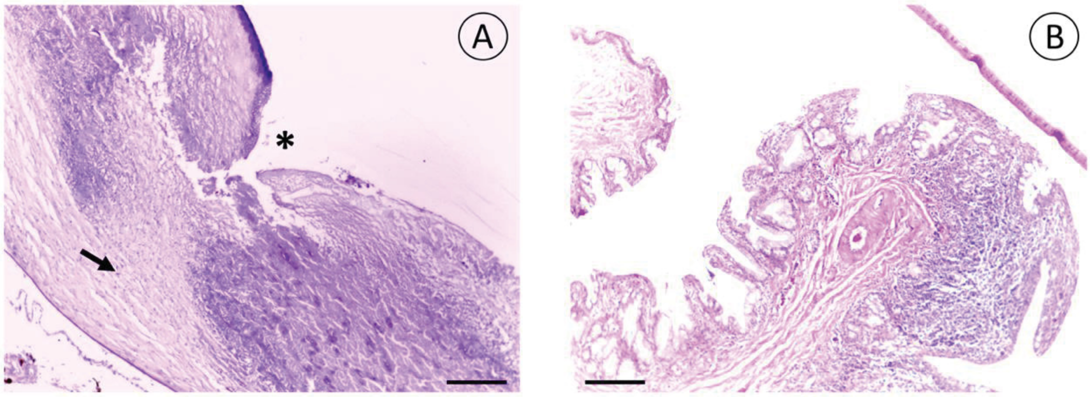

Figure 3. Microscopic findings in eyes of kelp gulls (*L. dominicanus)* infected with *P. lachrymosus.* A: Corneal ulcer evidenced by the absence of epithelium (black asterisk), and diffuse mononuclear inflammatory cells infiltration in the stroma (black arrow). B: conjunctival papillary projections.

Morphological identification

The *P. lachrymosus* specimens (Fig. 4) have an elongated body, that is an average of 4282 µm (3386-45134) long by 1549 µm (1274-1893) wide. The oral sucker subterminal is, on average, 310 (248-397) long by 407 (340-475) wide. The prepharynx is very small or absent. The muscular pharynx is located posterior to the oral sucker, and is on average 415 (355-468) long by 306 (248-425) wide. The ventral sucker is located on the anterior third of body and is an average of 664 (581-716) long by 694 (659-730) wide. The ratio of the transverse diameter of the oral sucker to that of the ventral sucker is 1:2.2 (1.6-2.6). The ratio of the transverse diameter of the oral sucker to that of the pharynx is 1:1.3 (1.2-1.6). The shape of the ovary is oval, and is situated medially in a pretesticular and post-uterine position. The ovary measures 237 (211-258) long by 266 (245-300) wide. The cirrus pouch is elongated, averaging 948 (610-1361) by 173 (106-227), in most specimens post-acetabular. The testes are oval and are situated in tandem located at the position post ovarian, intercecal, in the posterior part of body. The anterior testis measures, on average, 417 (206-624) long by 537 (241- 794) wide. Posterior testis measures, on average, 451 (255-610) long by 515 (291-744) wide. The vitellaria are bilateral, extracecal, follicular, and extended 53% (32-70%) of the distance from the anterior testis to the ventral sucker. Mature eggs (Fig. 2b) are non-operculated, with a formed miracidia bearing eyespots. The eggs were an average size of 75 (63-84) long by 34 (30-41). The excretory pore is terminal.

The morphometric data related to adult parasites obtained in the present study were compared with other neotropical records of *P. lachrymosus* and the results are presented in the Table 3, followed by the mean in brackets (in micrometers) when available. The morphological characteristics of the parasite reported here made possible the identification of the eye fluke *P. lachrymosus,* Braun, 1902.

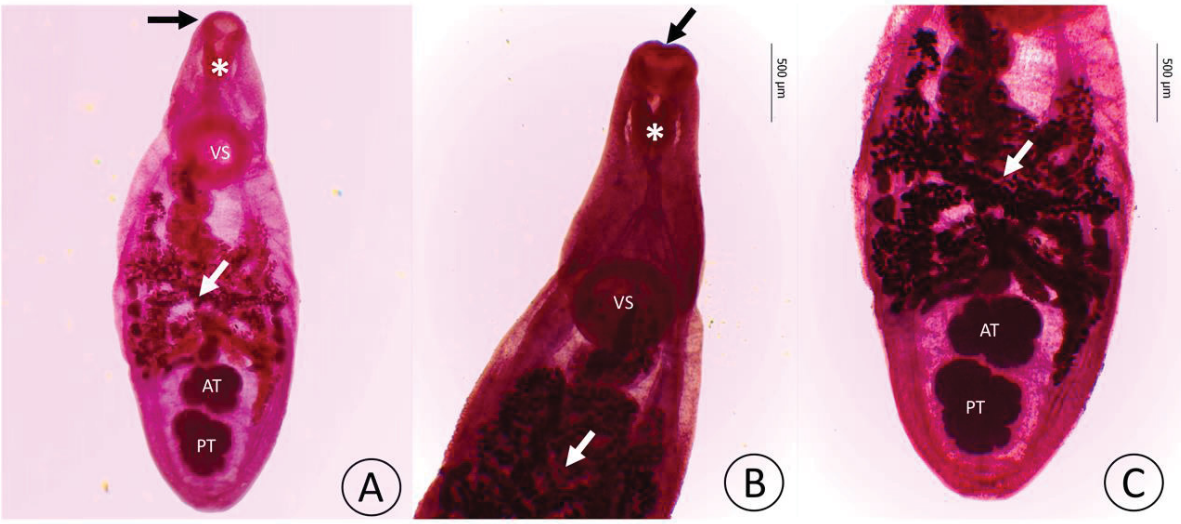

Figure 4. A: Adult specimen of *P. lachrymosus* found in ventral conjunctival fornix from kelp gulls (*L. dominicanus).* B: Detail of anterior portion. C: Posterior portion. Oral sucker (black arrow), pharynx (asterisk), ventral sucker (VS), uteri containing eggs (white arrow), anterior testis (AT), posterior testis (PT).

Parasite molecular study.

Partial sequences of cox-1 (777 bp) was obtained for *P. lachrymosus* recovered from kelp gulls. Analyses of these sequences showed 100% similarity between adult parasites and sequences available in Gen- Bank for *P. lachrymosus* (Fig. 5).

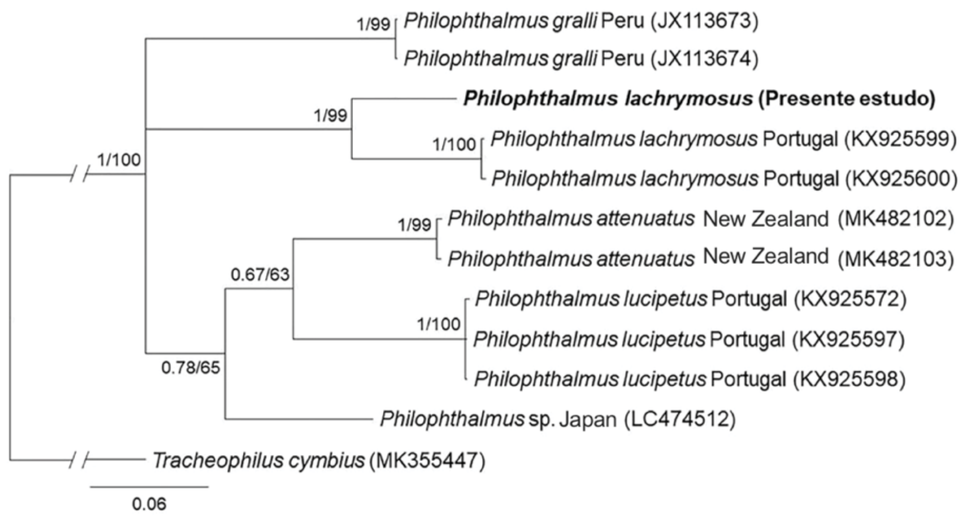

Figure 5: Phylogenetic relationships between *Philophthalmus lachrymosus* (in bold) analyzed in this study, and other reports, including comparison to other species of the genus *Philophthalmus* sp., as inferred from sequences cox-1 analyzed by Bayesian inference (BI) and maximum likelihood (ML) methods. Nodal support is indicated as ML/ BI; values < 0.90 (BI) and < 50 (ML) are indicated by a dash.

Table 3. The morphometric data of parasite specimens collected from 13 kelp gulls (*L. dominicanus*): between July of 2017 and September of 2018, in the Coast of Santa Catarina State, Brazil, and compared with previous values reported for *Philophthalmus lachrymosus* from brown-hooded gull (*Chroicocephalus maculipennis*), great egret (*Ardea alba*), willet (*Tringa semipalmata*) and humans.

		Philophthalmus lachrymosus				
			Braun, 1902	**Freitas, 1955**	**Nasir & Díaz, 1972**	**Lamothe-Argumedo et al., 2003**
Host		***Larus dominicanus***	***Chroicocephalus maculipennis***	***Ardea alba***	***Tringa semipalmata***	**Human**
Location		**Present study**	**Brazil**	**Brazil**	**Venezuela**	**Mexico**
n		**14**	**3**	**5**	**1**	**1**
Body Size	L	4282 ± 502 (3386–5134)	3500–4500	4190–4620	4000	5860
	W	1549 ± 196 (1274–1893)	1200–1700	1380–1640	1500	1530
Oral Sucker (OS)	L	310 ± 40 (248–397)	355	300–310	416d	400
	W	407 ± 49 (340–475)	477	360–430		480
Pharynx (PHA)	L	415 ± 40 (355–468)	600	310–350	543d	480
	W	306 ± 45 (248–425)	399	380–460		550
Ventral Sucker (VS)	L	664 ± 45 (581–716)	611	610–690d	782d	720
	W	694 ± 26 (659 –730)	733			690
OS/VS		1:2,2 (1,6–2,6)	1:1,7*	1:2	1:1,9	1:1,8
OS/ PHA		1:1,3 (1,2–1,6)	1:1,7*	1:1–1,3*	1:1,3	1:1,2
Ovary	L	203 ± 28 (163–227)	322d	200–210	400d	160
	W	254 ± 29 (206–284)	–	180–220		200
Cirrus pouch	L	948 ± 229 (610–1361)	–	1000–1240	–	–
	W	173 ± 47 (106–227)	–	100–150	–	–
Anterior testis	L	417 ± 115 (206–624)	–	360–480	347d	190
	W	537 ± 163 (241–794)	–	460–550		210
Posterior testis	L	451 ± 100 (255–610)	–	380–480	347d	240
	W	515 ± 133 (291–744)	–	450–530		250
Vitellaria	%	53 ± 10 (32–70)	36 e 47*	45 ± 5 (37–54)*	41 e 54*	–
Eggs	L	75 ± 5 (63–84)	67	94–97	–	70–80
	W	34 ± 3 (30–41)	23	38–42	–	30

The provided measurements are based on 14 (*P. lachrymosus*). All measurements are given in µm, with the range followed by the mean and standard deviation. d = diameter, L: length; W: width.

3.5 DISCUSSION

The kelp gull (*L. dominicanus)* is broadly distributed in the Southern America. [36-38]Santa Catarina State have the largest colony recognized in the Brazilian coast,[36,37,39] Moreover, the opportunistic behavior of the kelp gull, associated to dense human areas,[40-42] makes this bird as a potential vector of pathogens.[42,43]Additionally, considering the fact that *P. lachrymosus* is reported to infect other than avian, capybaras[24] and humans [20] this zoonotic facet of this parasite, must be highlighted.

Assembling these both factors, it is easy to accept that data obtained in studies about Philophthalmiasis in synanthropic animals such as *L. dominicanus,* may help to understand the dynamic of this zoonosis, and might generate clinical data to improve ophthalmic veterinary care in this bird species.

According to the literature, *P. lachrymosus* was reported to infect eyes of 4 gulls' species: brown-hooded Gull (*L. maculipennis*),[15,27] black-headed gull (*Chroicocephalus ridibundus*),[27] and kelp gulls (*L. dominicanus.*[15] Recently the infection of *L. fuscus* was reported for the first time in Portugal.[16] However, according to the authors' knowledge, there are no studies addressing epidemiological, clinical and histological details of the infection of *P. lachrymosus* in kelp gulls.

Despite the three mentioned descriptions of infection of *P. lachrymosus in* gull species were reported on basis of Brazilian samples of the parasite, [27] the present study appears to be the first to report cases of Philophthalmiasis in kelp gulls in the Brazilian coast, since Travassos et al. (1960) who reported for the first time this specific relation parasite x host between P. *lachrymosus* and L. *dominicanus* in Brazilian southeast coast.

Emaciation was reported in cases of *Philophthalmus* sp. infection in both birds[10] and mammals.[24] However, one limitation of the present study, is that was not possible to determine whether the ocular and systemic conditions were caused solely by the trematode infection itself or if the ocular philophthalmiasis occurred secondary to the existence of a systemic disease, since many of the birds examined also presented other important clinical conditions in the moment of admission (see Table 2). In such cases, the probability of opportunistic infection by the ocular parasite should be considered. Unfortunately, necropsy data that would help elucidating this question was not available. Moreover, the development of studies focusing on the investigation of the most common comorbidities in birds infected by *Philophthalmus* sp. might help to elucidate the systemic effects of the infection.

Ocular lesions observed in this study, were most likely related to the attachment of the ventral suckers of the eye fluke, to obtain nourishment from lacrimal secretions, as previously reported.[27,44] The most frequent ocular clinical sign observed was conjunctival hyperemia (84.61%), similar to what previously described in other species.[6,7,32] Even though no quantitative method was used to describe the degree of hyperemia. The authors had the impression that there was a direct correlation between number of parasites found in each eye and degree of hyperemia. It was not possible to determine if the corneal ulcers present were primary or secondary. The authors believe it was probably due to self-inflicted traumatic abrasion, secondary to the conjunctivitis and the presence of parasite acting as a foreign body. These clinical features have been previously described in other species as well.[32] This might be an important feature, considering the proposed hypothesis that *P. lachrymosus* may have greater pathologic effects when comparing to other *Philophthalmus* sp.[16] that generally have their clinical presentation restricted to moderate conjunctival inflammation, not involving corneal damage [7]as we reported here, with the presentation of corneal edema, keratitis, corneal abscess and corneal ulcer. *P. lachrymosus* in contrast, is known to cause serious ocular lesions, including deep corneal ulcers, absence of one eye, and blindness,[16]reported respectively in gulls and capybaras.[24] Nevertheless, in the single published case of a human.[20] *P. lachrymosus* infection, serious lesions were not present. Animals high density infections, comparing to human cases of *P. lachrymosus* infection has been proposed to explain serious ocular damages in other species than in humans. [24]

Macroscopic lesions were not found in all eyes submitted to histological analyses. Despite other studies report the presence of the eye fluke in fixed eye tissues, 24 in the preset study, this might be the result of previous mechanical remove of the parasites followed by topical treatment of all infected birds, including the 3 individuals that died prior enucleation. Inflammatory infiltrates were the most prevalent microscopic finding in Philophthalmus sp. infections with few reports of corneal damage,25 following what was presented here, in kelp gulls.

Several treatment protocols for *Philopthtalmus* sp. infections were described in the literature, including the use of systemic and topical anti-parasitic drugs,[6,7,11,16]mechanical removal[10] and topical antibiotic agents.[45] In the present study, the mechanical removal was established for most cases when only conjunctivitis was present, follow what was previously described in other birds infected with eye flukes.[10] Birds with corneal lesions were treated by the use of chloramphenicol ointment (Cristália LTDA, Cotia, Brazil), a topical agent already used in *Philophthalmus* sp. infection in ostriches.[11]

Despite morphometric and morphological analyses represent valuable methods for identification of different *Philophthalmus spp.,*[3,16,24] differences related to intrinsic factors (e.g. host specificity) and extrinsic factors (e.g. sampling/storage methods) have been proposed to affect the size and morphology of anatomic structures inside one single species.[24] Therefore, despite the similarity between body measurements presented here and other descriptions of *P. lachrymosus* based in morphological assessments, in other hosts (Table 3), supplemental molecular analyses were performed to confirm that the kelp gulls trematodes represent *P. lachrymosus.*

The molecular data presented in this study from sequences of cox-1 analyzed by Bayesian inference, supports the classification of trematodes obtained from help gulls eyes, as *P. lachrymosus*, identical to what was previously described by Henenberg et al. (2018). The molecular data presented here, apparently, represent the second available for the specie, and the first in Brazil. Except by the molecular data reported for *P. lachrymosus* in gulls in the Iberian Peninsula,[16] all the two remaining reports of *P. lachrymosus* infection, both in capybaras,[24] humans[20] and gulls, did not provide molecular analyses.

The absence of intermediate hosts identification in these cases of Philophthalmiasis in kelp gulls, is an important limitation of this study, and further investigations are needed to confirm the life cycle of *P. lachrymosus* in this region on Brazilian southern coast. Species of aquatic snails are recognized as intermediate hosts of *Philophthalmus* sp.[10,16,29] The marine snail *Lampanella minima* as have been described as intermediate hosts of *P. lachrymosus.*[46] However, according to authors knowledge, there were no descriptions of this specie in the Brazilian coast.[47,48] The invasive freshwater mollusk *Melanoides tuberculata* is widely introduced in Brazil,[49,50] including the state of Santa Catarina.[51] The importance of this mollusk in the *Philophthalmus* sp. life cycle goes further than his vast distribution in the Brazilian territory. *M. tuberculata* involvement in previously reported cases of Philophthalmiasis in birds [10,12,16] and mammals [24f] and its act as a natural source of infection by *Philopthlhalmus gralli* [3] indicate the necessity of future studies focusing in the participation of this snail in kelp gull's infection.

Despite the infection of *P. lachrymosus* in kelp gulls have been known, the detailed clinical data presented here, might help bird clinicians to manage cases of Philophthalmiasis. Furthermore, since molecular identity in Philophthalmus sp. are still not completely understood, the molecular data reported here, may contribute to future taxonomic studies.

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4. CHAPTER 3: A survey of veterinary ophthalmologists to assess the advice given to owners of pets with irreversible blindness.

4.1 ABSTRACT

The primary purpose of this study was to survey how veterinary ophthalmologists manage cases of irreversible blindness and report its most common causes. Respondents completed a questionnaire sent by e-mail with the cooperation of The American College of Veterinary Ophthalmologists, The European College of Veterinary Ophthalmologists and The Latin American College of Veterinary Ophthalmologists, which was constructed containing 12 questions, with both open and closed multiple-choice response options. One hundred and eight veterinary ophthalmologists answered the questionnaire. Eighty-three percent of the respondents had been graduated more than 10 years. Glaucoma (63.56%) was the main cited cause of blindness, followed by progressive retinal atrophy (PRA) (17.80%) and retinal detachment (6.78%). The major concerns of owners refer to the impact in QoL, (39.31%) followed by depression, anxiety (20%) and environment adaptation (11.72%). General recommendations include avoidance of changes in the domestic environment (18.45%), the use of auditory stimulation (14.09%) and avoidance of dangerous areas (12.75%). Almost 31% of the professionals do not recommend sources of literature about how to deal with blind pets. Glaucoma and PRA were the most common causes of irreversible blindness in pets detected in this survey. Several recommendations frequently given to blind pet owners are presented.

Key Words: eye, survey, blind, ophthalmology, veterinarian, QoL

4.2 INTRODUCTION

The existing worldwide veterinary ophthalmology associations encourage education, training, and research in veterinary ophthalmology, provide organizational structure to facilitate efficient exchange of ideas, establish minimum standards of training and experience, and recognize individuals who have fulfilled such standards to become a certified professional practitioner. There are however no specific guidelines for the management of irreversible blindness in dogs and cats, and there is a paucity of information in the veterinary literature.

Irreversible blindness is a complex scenario in terms of the disease itself, the animal's coping mechanism and the emotional involvement of the owners. Thus, appropriate management of blind animals is always challenging, requiring several skills in addition to clinical knowledge.

The aims of the study were to: a) canvas opinion on irreversible blindness cases from veterinary ophthalmologists on an international scale in terms of management, quality of life (QoL) and the interaction between the pet and owner, and b) to collate successful protocols for dissemination.

4.3 MATERIALS AND METHODS

Sampling and data collection

An online questionnaire was developed and consisted of seven open questions and five multiple-choice questions. The questionnaire had three main objectives:

1) to establish the demographic profile of the respondents (gender, geographic location, number of years since graduation, number of years as a veterinary ophthalmologist, and affiliation to an association or college of ophthalmology) (Questions 1 – 4);

2) to establish the main causes of irreversible blindness in the respondent's routine clinic (Questions 5 - 6);

3) to compile information on the major concerns of the owners and the blindness management protocols followed by the respondent ophthalmologist (Questions 7 – 12).

The target population of the questionnaire was veterinary ophthalmologists who are members of recognized international, continental or national veterinary ophthalmology colleges or associations. The survey was disseminated via the membership list of the respective association/college via “Google Docs” (docs.google.com).

Data management and analysis

Returned online responses were manually entered into a Microsoft Excel (2010, Microsoft Corporation) spreadsheet and classified according to the questions. Chi-square tests were used to compare potential differences in the proportion of answers for each question. *P* values of less than 0.05 were regarded as statistically significant

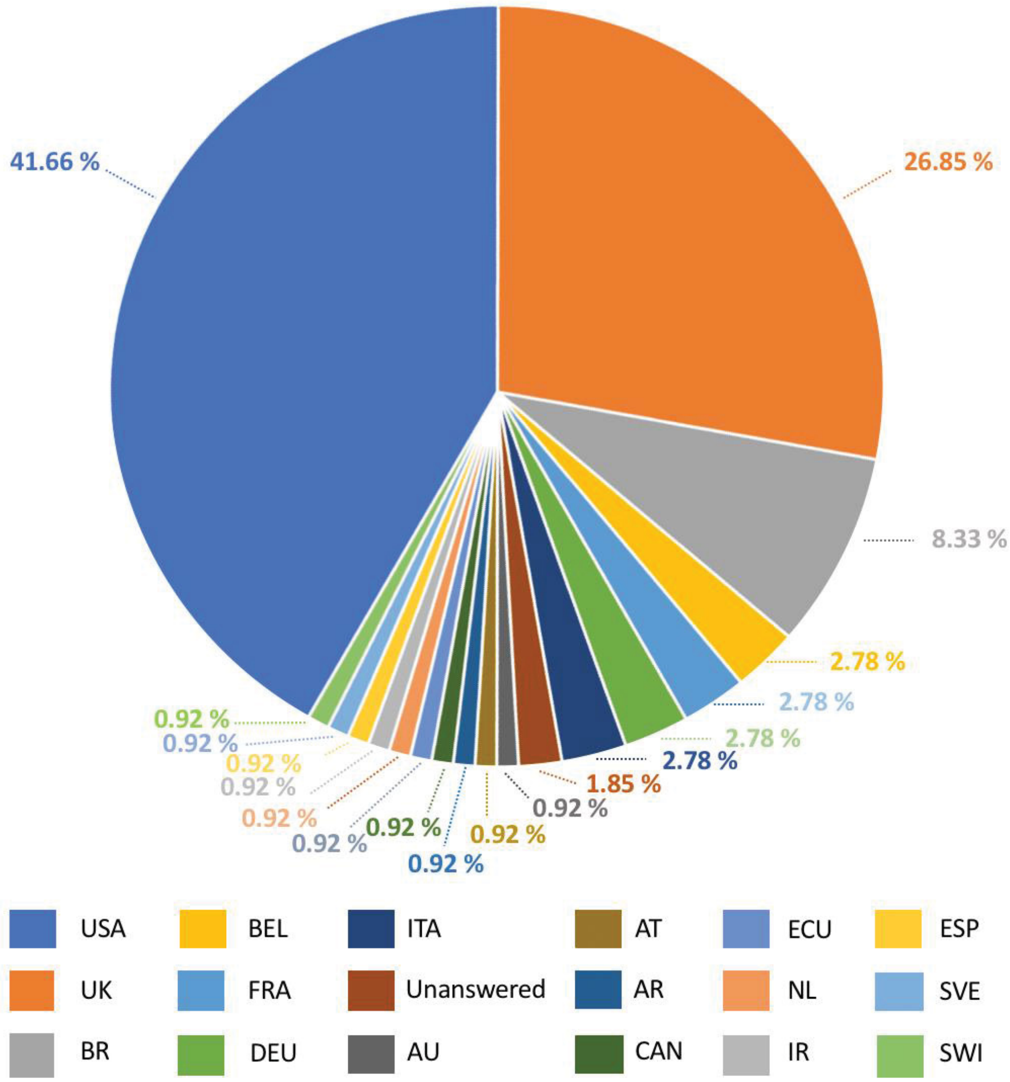
4.4 RESULTS

Data analysis 1: Demographic profile of respondents (Questions 1-4).

One hundred and eight veterinarians returned the questionnaire, comprising 26 women (24.07%) and 19 men (17.60%). The majority of respondents (n = 63, 58.33%) did not identify their gender.

One hundred and seven respondents (99.07%) provided their geographical location (Question 1). There were 17 locations; the USA was the top-listed country with 45 respondents (41.66%), followed by the UK, with 30 respondents (26.85%) and Brazil with eight respondents (8.33%). A complete list of geographical localization of respondents is shown in Figure 1.

The two geographic locations that had the most respondents were the USA (45) and the UK (30). There was no significant difference between the number of respondents from these two (P=0.08) locations.



Legend: USA: United States of America; UK: United Kingdom; BR: Brazil; BEL: Belgium; FRA: France; DEU: Germany; ITA: Italy; AU: Australia; AT: Austria; AR: Argentina; CAN: Canada; ECU: Ecuador; NL: Netherlands; IR: Iran; ESP: Spain; SVE: Sweden; SWI: Switzerland.

Figure 1. Geographic distribution of respondents (by country).

All respondents answered the questions about the year of graduation (Question 2) and the number of years working in ophthalmology (Question 3). Ninety respondents (83.33%) had worked in the veterinary profession for more than ten years and 64 (59.81%) had worked in ophthalmology for ten years or more.

The question about affiliation to an association or college of veterinary ophthalmology (Question 4) was answered by 103 respondents (95.37%). About twenty-eight percent (27.18 %) respondents were affiliated to more than one institution and eight (7.62%) respondents were unaffiliated. Twelve associations or colleges were cited. The majority of respondents were members of the American College of Veterinary Ophthalmologists (ACVO) (n = 48, 36.09%), followed by British Association of Veterinary Ophthalmologists (BrAVO) (n=30, 22.55%) and European College of Veterinary Ophthalmologists (n = 23, 17.29%). The number of ACVO members was statistically greater than all other colleges or associations (P < 0.0001). Data from the respondents is shown in Table 1.

Table 1. Demographic profile of respondents.

Time working as a veterinarian (years)	**Number**	**Percentage**
≥ 10	90	83.33
5 – 10	15	13.89
≤ 5	3	2.78
Time working as an ophthalmologist (years)		
≥10	64	59.81
≤ 5	22	20.56
5 – 10	21	19.63
Affiliation (ophthalmology college or association)		
American College of Veterinary Ophthalmologists (ACVO)	48	36.09
British Association of Veterinary Ophthalmologists (BrAVO)	30	22.55
European College of Veterinary Ophthalmologists (ECVO)	23	17.29
Unaffiliated	8	6.01
International Society of Veterinary Ophthalmology (ISVO)	7	5.26
Colégio Latino Americano de Oftalmólogos Veterinários (CLOVE)	4	3.01
International Equine Ophthalmology Consortium (IEOC)	4	3.01
Unanswered	3	2.25
Austrian Working Group for Veterinary Ophthalmology (AKVO)	1	0.75
American Society of Veterinary Ophthalmology (ASVO)	1	0.75
Australian Veterinary Ophthalmology Society AVOS	1	0.75
Association for Research in Vision and Ophthalmology (ARVO)	1	0.75

Italian Society of Veterinary Ophthalmology (SOVI)	1	0.75
Société Française d'Études et de Recherches en Ophtalmologie Vétérinaire (SFEROV)	1	0.75

Data analysis 2: Main causes of irreversible blindness (Questions 5-6).

The percentage of patients diagnosed with irreversible blindness was specified by 106 respondents (98.15%) (Question 5). Sixty respondents (55.55%) reported that the percentage of cases of irreversible blindness was between 0.1% and 5% of the total number of patients. A range of 6 - 10% was reported by 22 respondents (20.37%) and 11 - 20% by 14 respondents (12.96%).

All respondents described the most frequent cause of irreversible blindness among their patients (Question 6). Glaucoma was the most common cause – it was mentioned 75 times (63.56%), which was significantly greater than all other cited causes ($P < 0.0001$). Progressive retinal atrophy (PRA), cited 21 times (17.80%) and retinal detachment (RD) cited eight times (6.78%) were, the second and third most common causes, respectively. A complete list of the causes is shown in Table 2.

Table 2. Reported Commonest Cause of Irreversible Blindness.

Cause	**N**	**%**
Glaucoma	75	63.56
Progressive retinal atrophy (PRA)	21	17.80
Retinal detachment (RD)	8	6.78
Sudden Acquired Retinal Degeneration (SARD)	6	5.08
Cataract	2	1.69
Optic neuritis	2	1.69
Unanswered	2	1.69
Chronic corneal disease	1	0.85
Central blindness	1	0.85

Data analysis 3: General recommendations for adaptation to blindness and owners' major concerns (Questions 7-12).

Question 7 assessed the general recommendations to help pets to adapt to blindness. Based on the response similarities, the answers related general recommendations were grouped and classified into 18 different categories, respectively (Figure 2). One hundred and seven respondents (99.07%) answered about recommendations for adaptation to blindness. The most common recommendation was "to avoid changes in the domestic environment", with 55 citations (18.45%). This was followed by "the use of auditory stimulation" with 42 citations (14.09%) and "avoid dangerous areas", with 32 citations (12.75%). All recommendations are displayed in Figure 2.

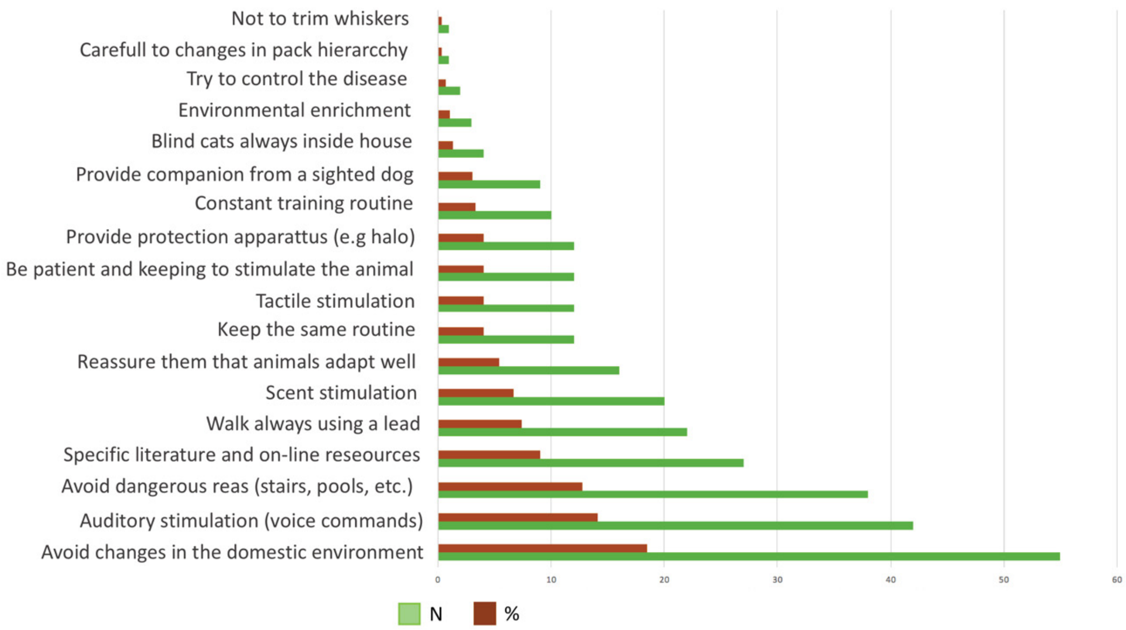


Figure 2. General recommendations in order to better adapt pets to the blindness according to respondents.

One hundred and seven respondents (99.07%) answered Question 8, regarding the respondent's opinion of the owners´ major concerns. All answers were combined into eight categories. The most common concern was the impact of blindness on the QoL, cited 57 times (39.31%). Depression and anxiety were mentioned 29 times (20%), and adaptation to being blind was cited 17 times (11.72%). The QoL factor was significant, compared to all other factors (P = 0.01). A complete list with results of the combined answers is shown in Figure 3.

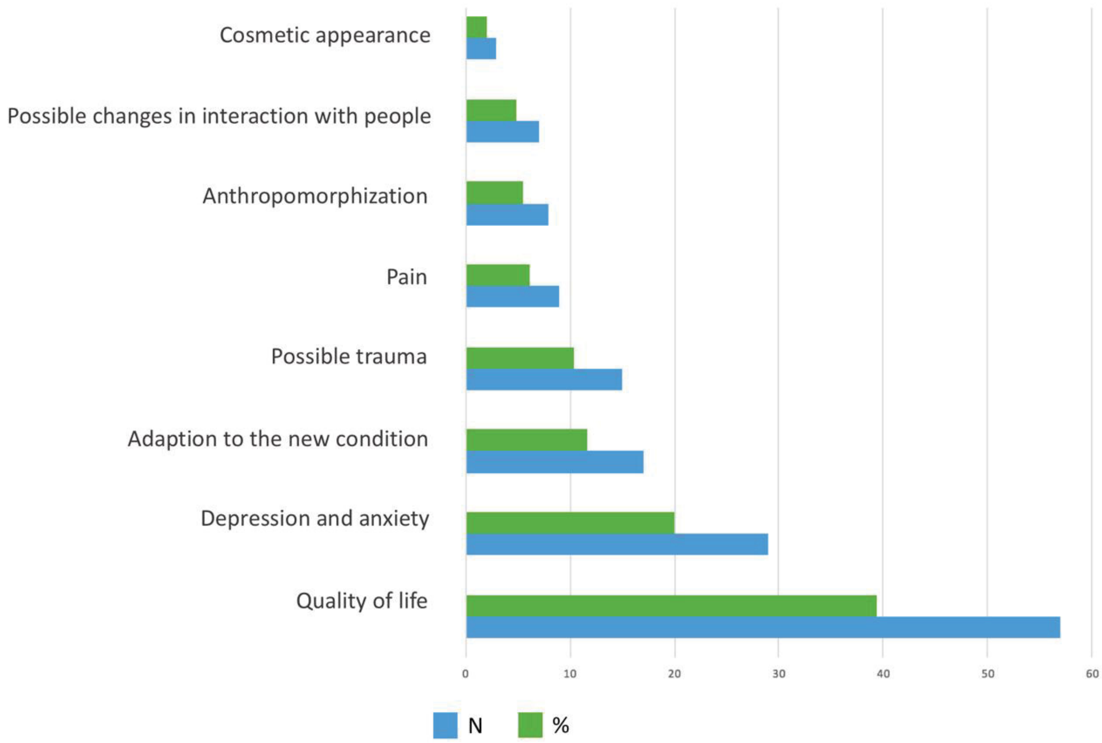


Figure 3. Major concerns of owners of blind pets, according to respondents of the survey.

In Question 9 respondents were asked a close-ended multiple-choice question, to choose, in order of importance, five recommendations offered to owners: training routine; use of protective apparatus (halo); living with another animal; avoid moving furniture; restricting access to some areas; and other. The recommendation "avoid moving furniture" was regarded as the most important (cited 48 times). The suggestion to "restricting access to some areas" was chosen as a first option only 18 times, which was less cited than the recommendation of creating a "training routine", cited 46 times. All recommendations can be seen in Figure 4A.

Question 10 assessed specific recommendations for pets with PRA. Ninety-eight respondents (90.74%) described different recommendations that were divided into 16 sub-categories. Thirty-three (22.45%) respondents indicated that they did not provide any specific recommendation for cases of PRA. The most common suggestion was to keep a source of light near the animal, cited 27 times (18.36%), followed by establishing a training routine, cited 15 times (10.20%) and nutritional supplementation, cited 12 times (8.36%). All recommendations are displayed in Figure 4B.

In Question 11, 106 respondents answered whether or not they routinely prescribe treatments without evidence of efficacy (in order to produce a placebo effect) in cases of irreversible blindness. One hundred and one (93.52%) respondents answered that they do not make this recommendation, which was significantly greater ($P < 0.0001$) than the total amount of respondents who stated that they do prescribe some sort of placebo (five respondents - 4.62%). Two respondents (1.85%) did not answer this question.

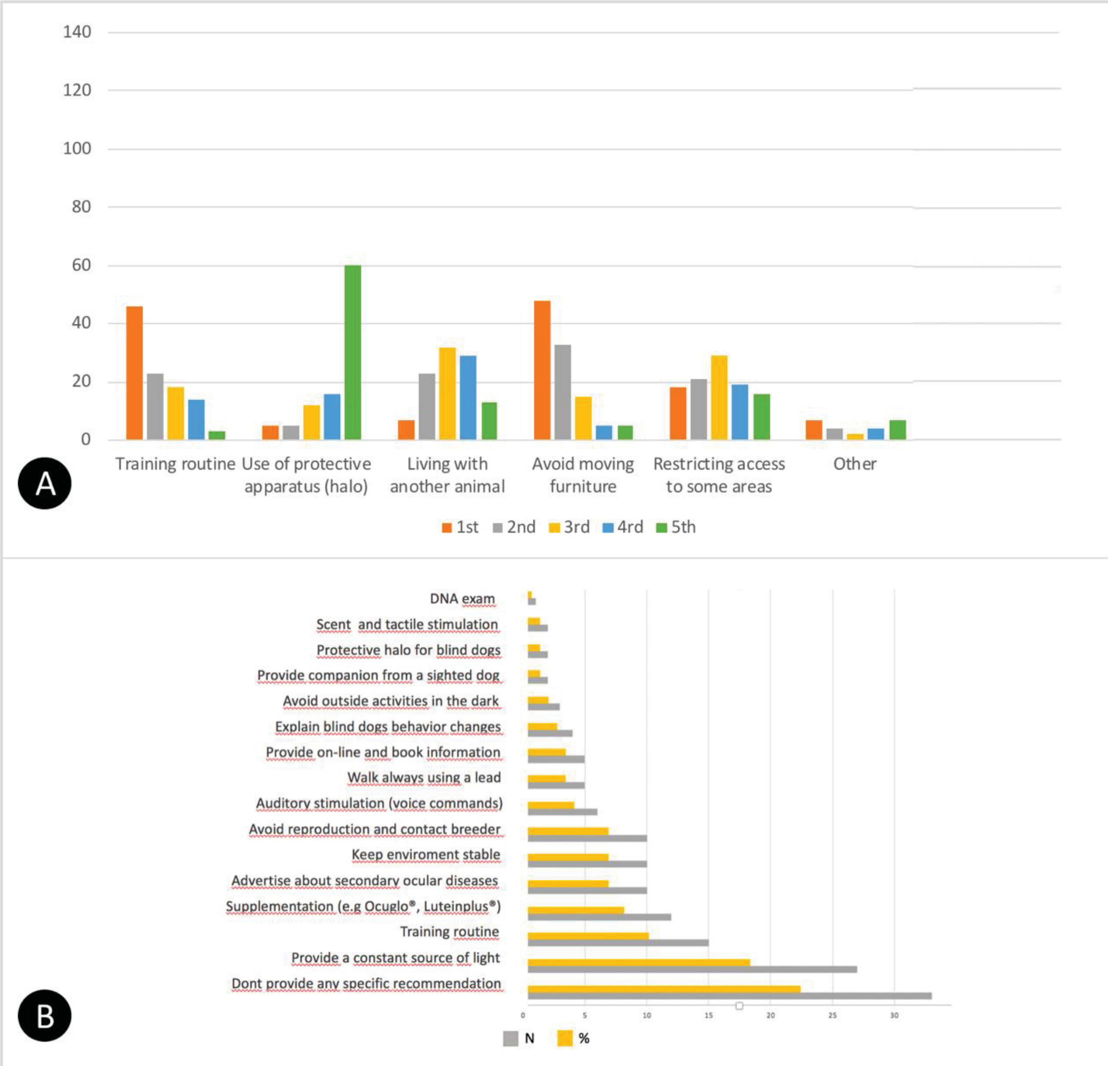


Figure 4. Recommendations in order to better adapt pets to the blindness. A) Recommendations to owners in order to help pets to adjust to blindness in order of importance. B) Specific recommendations to instruct owners of pets with PRA.

Respondents were asked if they usually indicate some source of information (book, paper or website) for further instruction for the owners (Question 12). Thirty-seven respondents (31.35%) replied that they did not usually recommend any source of additional information. The book "Living with Blind Dogs" [1] was cited by 34 of the respondents (28.81%), followed by several websites cited 9 times (7.62%) and the book "Blind Devotion" [2] cited by six respondents (5.08%). All sources of information mentioned are shown in Table 3.

Table 3. Recommended reading sources for owners.

Source of information		N	%
Books			
	Living with Blind Dogs	34	28.81
	Blind Devotion	6	5.08
	Siehst du es (You see…)	2	1.69
	Caring for a blind cat	2	1.69
Websites			
	Diverse Websites	9	7.62
	www.blindtails.com	5	4.23
	www.google.com	3	2.54
	www.blinddogs.com	3	2.54
	www.youtube.com	1	0.84
	www.jordycanid.com	1	0.84
	www.pepedog.com	1	0.84
	Purina Web Page	1	0.84
	blinddogtrainer.com	1	0.84
Others			
	Yes, but source not specified	6	5.08
	Contact with other owners of blind pets	2	1.69
	blinddogs@yahoo.com	1	0.84
	Website: Facebook Profile - Living with SARDs	1	0.84
	Information leaflet for blind pet owners	1	0.84
	How to manage a blind pet	1	0.84

4.5 DISCUSSION

The use of questionnaires is a common practice to assess how ocular problems can affect the QoL in people.[3-7] In the veterinary field, questionnaires have been proven to be a useful tool to establish how physical disease can affect QoL in animals. Examples include emergency disorders,[4] pain,[8-10] dermatology,[11] cardiac,[12] neoplastic,[13 14] neurology,[15] and otologic [6] diseases. Questionnaires have also been helpful to evaluate non-physical conditions[17] including temperament and behavior.[18 19] Only sporadic studies using clinical vignette questionnaires to evaluate ophthalmic conditions in animals are available[20 21]. Furthermore, rarely eye disorders were mentioned in published articles constructed through veterinarians' survey results. In a survey with 3982 veterinary clinicians, the eyes or ocular structures were not mentioned in the classification of body systems concerning diseases versus clinical signs.[22] Therefore, to our knowledge, this is the first study with an international basis to survey veterinarian ophthalmologists for their professional attitudes and approaches about irreversible blindness condition in pets.

We believe that the results in this study most likely reflect the opinion of veterinary ophthalmologists around the world, since responses were received from a representative sample of veterinary ophthalmologists, from various countries and continents and types of workplaces.

Demographic characteristics of questionnaire survey respondents are often included in the literature.[22-28] The majority of women respondents was shown in other studies regarding the attitudes of veterinarians.[22 25 26 29] In this study was impossible to conclude whether more women received the questionnaire, were more likely to participate, or if there are more women working in the field of veterinary ophthalmology.

Possible explanations for the fact that most respondents were from the USA and the UK are the high number of veterinary ophthalmologists working in these two places, the fact that the specialty colleges in these two locations (ACVO and BrAVO) are well established, and were more efficient in sharing the survey with the membership. The predominance of respondents with more than 10 years working as a veterinarian is in accordance with another study from Europe.[30] The predominance of respondents with at least ten years of practice in the ophthalmology area may reflect post-graduate learning.

According to our knowledge, there are no published studies comparing the proportion of members of colleges or professional associations of veterinary ophthalmology. Thus, it is not possible to assure that the greater participation of ACVO with 48 members (36.09 %), BrAVO with 30 members (22.55%) and ECVO with 23 members (17.29%), results from better dissemination of the survey link, better scientific engagement and/or a higher level of membership.

In this study, glaucoma was the main cause of irreversible blindness, followed by PRA and retinal detachment. The results were expected because the existing literature demonstrates that these are indeed the most common eye diseases leading to irreversible blindness found in small animal ophthalmology practice [31 32 33 34 35 36 37] Other diseases or conditions also were cited in this question (Table 2). Some of these diseases are interesting in the medical sense, but not very common in the ophthalmology practice, such as central blindness. Other diseases are not typically classified as "irreversible", but sometimes it might just be the case, such as pigmentary keratitis (a chronic corneal disease) which is difficult to control, particularly in brachycephalic breeds. Lastly, some of the ocular diseases cited are not truly irreversible, such as cataracts.   Since glaucoma is an important cause of impairment on QoL in humans [38 39] and an important cause of chronic pain in animals [40 41] and assuming that chronic pain can reduce QoL in animals, it seems reasonable that veterinary ophthalmologists should always emphasize the importance of establishing an appropriate pain control management in such cases, including the discussion of enucleation.

Although the literature suggests that blind dogs and cats may have an excellent capacity for adaptation, the owners usually do not appreciate this, since this item appears preceded only by QoL and depression, as the tree major concerns for owners of blind pets.. The ophthalmologists should prioritize advice regarding these concerns.

In addition, behavioral particularities regarding how each individual reacts to blindness, need to be considered far beyond than only differences between species, such cats and dogs (e.g. special attention to avoid falls from heights for blind cats). Other factors that may be decisive for how a certain animal will react to the privation of sight, including the primary activity for which ta certain dog breed was adapted. For example, possibly a sighthound may naturally have greater difficulty than a scent hound. Furthermore, future studies should focus on how species or breed-specific characteristics might affect QoL once the animal gets irreversible blind. The most common patient in the small animal practice (*Canis familiaris*) is the animal species with the greatest morphological variability, [42] which was shown to affect sensory modalities such as olfaction and vision (particularly the distribution of ganglion cells). [42 43]

The two most common recommendations from the ophthalmologist were "to avoid changes in the domestic environment", and the "use of auditory stimulation". These actually are common recommendations in textbooks on the subject. [1 2 44] Anecdotal evidence indicates that the owners can themselves recognize the need for these two recommendations as they commonly report the difficulty in negotiating in an unfamiliar environment or when moving furniture in the home. Auditory cues include new verbal commands (e.g. immediately prior to bumping), devices attached to sight co-inhabitants' animals (e.g. bell on the collar) or specific locations (e.g. furniture, inside toys, motion sensor alarm near to steps). These strategies help the animal to feel more confident navigating the house, keeping it safe and avoiding potentially dangerous areas, which was the third most frequent general recommendation (12.75%). Auditory warning systems can be augmented by gates and barriers and by establishing a training program. It was unexpected that many respondents (22.45%) do not provide any specific recommendation for PRA cases, given that PRA is a major cause of irreversible blindness in animals,[45 46] and particularly in dogs[47 48]. The clinical signs of PRA, including nyctalopia and slow progression, are widely known but only 18.36% of respondents recommend the use of a light source near the animal.[49-51] Twelve respondents (8.36%) prescribe nutritional supplementation for animals with PRA, which in the opinion of the authors, may be considered a type of placebo given the lack of evidence for affecting the progression of the disease.[52] The majority of respondents (93.52%), however, do not prescribe treatment protocols without scientific evidence. Considering both findings in this study, the authors conclude that some veterinarian surgeons adopt this approach. It is worth mentioning that PRA is a group of conditions with similar clinical features, not a single disease. Several forms of PRA were not yet characterized. Regarding progression, there are two main clinical forms of PRA recognized in dogs, an early onset, which is typically diagnosed in puppies, and a late onset form that is detected in adult dogs. None of the respondents spontaneously mentioned whether or not the stage or the type of disease regarding progression would influence their approach to giving owners advices.

Despite the availability of multiple information sources, 31.35% of respondents do not recommend any extra source of information for owners. It is impossible to know whether this is because most of the sources cited were not written by veterinary ophthalmologists, or because the verbal information provided at the time of diagnosis is considered sufficient. Perhaps this is partly because there are few publications or because key textbooks do not address the issue in any detail, unlike clinical and surgical subjects.

Limitations of this study included the form of data acquisition. It is important to recognize that all data was collected by convenience sampling with lack of randomization and the results can be influenced by that. Convenience sampling was chosen because it is a quick and easy method to collect preliminary information about a subject. This is particularly true when considering the little information available about this specific subject in the scientific literature. Another aspect that could be further explored in the future is how the level of academic qualification might have affected the answers (such as possessing an MSc or PhD degree). Since ophthalmic treatments may vary substantially for the same ocular conditions, particularly between general practitioners and specialized professionals, mainly for conditions for which guidelines nor high-quality evidence exists [53] there is enough reason to believe that recommendations given for owner's blind pets also may vary. Nevertheless, belonging to a recognized international, continental or a national professional veterinary ophthalmology association or college is considered an academic qualification and the minimum attribute to be considered as a specialist in many countries. In this survey, only eight (7.62%) out of 108 respondents were unaffiliated.

A relatively small number of the existing veterinary ophthalmologists in the world were represented in this survey. However, the authors believe that the resulting data reflects important management actions, from a globally distributed sample of qualified professionals, and since there was no similar studies about this topic, the results presented here may help clinicians to draw ideas and advices to better support cases of irreversible blindness. Equally important is the fact that the respondents were very experienced in the field, since the great majority of the respondents (almost 60%) had worked in ophthalmology for ten years or more.

When considering the veracity of the answers regarding cases of irreversible blindness in question five, we believe that some of these responses were indeed very precise, based on medical records, while others were probably based on an estimation of the caseload. This may be another limitation of the present study. Nevertheless, the result presented here may serve at a starting point for future investigations concerning irreversible blindness among Veterinary Ophthalmologists.

Irreversible blindness is a serious condition, which affects not only the affected individual but all members of the household.[7 54] There is however limited information for owners, and an apparent gap in the veterinary literature.[1 2] By identifying the common approaches taken by veterinary ophthalmologists when looking after blind patients, we can rank and prioritize methods to benefit veterinary surgeons. For this reason, that the data compiled in the present study might both contribute to the literature and better equips veterinary surgeons to improve the QoL of their blind patients.

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5 APÊNDICE

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ORIGINAL ARTICLE

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Reference values for selected ophthalmic diagnostic tests in two species of microchiroptera bats (*Artibeus lituratus* and *Anoura caudifer*)

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Abstract

Objective: To establish reference values for ophthalmic tests in two bat species.

Bats: Fourteen bats including seven great fruit-eating bats (*Artibeus lituratus)* and seven tailed tailless bats (*Anoura caudifer*).

Procedures: Normal values for following ophthalmic tests were investigated as follows: (a) aqueous tear production using the standardized endodontic paper point tear test (EPPTT), (b) rebound tonometry, and (c) horizontal palpebral fissure length.

Results: Aqueous tear production was 2.53 ± 1.65 mm/min for *A lituratus* and 1.89 ± 0.62 for *A caudifer*. Intraocular pressure measured in the upright position was 11.0 ± 3.28 mm Hg for *A lituratus* and 7.28 ± 2.70 for *A caudifer*. Horizontal palpebral fissure length was 5.04 ± 0.45 mm for *A lituratus* and 3.92 ± 0.51 for *A caudifer*.

Conclusions: The data obtained in the present study may serve as a reference for ophthalmic parameters and help practitioners in the diagnosis and management of eye diseases in bats, as well for future investigations about microchiroptera bats.

KEYWORDS

chiropteran, eye, IOP, lacrimal production, ophthalmic tests, palpebral

1 | INTRODUCTION

Bats have received considerable attention among the scientific community being the only mammals capable of true flight. One remarkable aspect of bat flight is their unique set of sensory systems that enable and contribute to complex flight dynamics, including echolocation, vision, olfaction, thermoperception, taste, and even touch.[1,2] Interest in echolocation often overshadows studies on vision and other sensory systems in bats; however, our understanding of bat vision is increasing, not only as an adjunct sensory modality but also as a necessary component for successful echolocation. Although bats are not regarded to have particularly high visual acuity, their visual system does contribute significantly to gathering spatial information needed for successful flight.[3] Vision in bats has been shown to be a prominent sensory modality for short-range orientation during flight,[4] for foraging for passive prey[5] and hunting,[6] and for color discrimination during intraspecific communication.[7,8] Vision has even been shown to be used preferentially over echolocation when sensory cues between the two modalities are conflicting.[4] Normal physiologic parameters of bat eyes have shown that their vision is dominated by rod photoreceptors rather than cone photoreceptors.[9] Nevertheless, unlike many nocturnal mammalian species, many bats have been shown to hold the potential for dichromatic vision with intact genes for short- and long-wavelength opsins. However, some loss of function of these genes in an evolutionary basis has been noted[1,10] possibly due to a sensory trade-off with increasing olfactory and echolocation capabilities.[11] Despite a growing body of literature on bat vision, very little is known about many of the normal parameters for ocular anatomy and clinical tests in this unique and diverse order of mammals. Two reports are available for review of such data, and both studies were conducted using Chiropteran bats from the suborder megachiroptera: *Pteropus vampyrus*, *Pteropus pumilus*, and *Pteropus hypomelanus*.[12,13] No investigation to date has been performed to examine normal parameters for ophthalmic tests in bats of the microchiropteran suborder.

When studying vision in bats, it is important to note that the two major groups of genera *Chiroptera* (megachiropteran and microchiropteran) have significant ecological and physiological differences between the visual systems.[14] Megachiropteran bats have a highly developed visual system[9,12] and an advanced retinotectal pathway.[15] On the other hand, microchiropteran seems to depend primarily on the use of echolocation mechanisms for their biological activities, although studies have shown that even echolocating bats can perform important activities, such as hunting, without the use of echolocation.[16] This may suggest an undervalued participation of other sensory modalities such as vision. Therefore, the objective of this study was to establish normal reference values for ophthalmic diagnostic tests in these two microchiropteran species, which may contribute to future investigations help guide clinical management of ocular disease in animals that may depend on vision more than previously thought. Here, we evaluated the ocular morphology and biometrics in two common microchiropteran bat species of the family Phyllostomidae (*A lituratus* and *A caudifer)* found throughout Brazil. The large fruit-eating bat *A lituratus* is one of the most commonly studied bat species in Brazil.[17,18] This species is considered one of the largest Brazilian bats weighing between 44 and 87 g[19] and reaching up to 70 mm of forearm length.[20] It is found throughout the entire Brazilian territory and forages primarily on fruits by use of echolocation and vision.[21] The tailed tailless bat *Anoura caudifer* of the subfamily Glossophaginae is a smaller bat with a forearm length ranging from 34 to 38 mm[22] that forages primarily on nectar and pollen.[23] This species is more widely distributed in South America, also occurring in other countries such as Colombia, Bolivia, Venezuela, Guyana's, Ecuador, Peru, and Argentina.[21]

2 | MATERIALS AND METHODS

All the procedures using live bats were conducted in accordance with ARVO's Statement for the Use of Animals for Ophthalmic and Animal and Vision Research and with the Federal University of Paraná's Animal Use Committee.

A total of 14 bats were used in the current study, including seven adult *A lituratus* (four females and three males) and seven adult *A caudifer* (five females and two males). All the *A lituratus* bats were from captivity, living at the Capão da Imbuia's Natural History Museum, located in Curitiba, Paraná State, Brazil. The approximate age of all *A lituratus* individuals was 3 years, according to the biological and medical records of the museum. The *A caudifer* bats were all free-living animals captured by the Paraná State´s Agricultural Defense Agency during a routine capture procedure. The precise ages were not known. For this reason, only individuals with a head length at least 50 mm[22] compatible with adult size were used in this study. All animals were manually and carefully restrained for sex determination and measurements of body weight, which was obtained by use of a precision scale (Pesola®, Baar, Switzerland) with a 1-kg scale. Procedures were split between the investigators. However, to avoid discrepancies related to different observers, the same person always performed the same ocular test. Ophthalmic measurements were performed in the following order: aqueous tear production, tonometry, and palpebral measurements.

Aqueous tear production was measured using the endodontic paper point tear test (EPPTT) method.[24-28] The method was developed for animals with very small eyes and consists of using standardized sterile endodontic paper points (Roeko Color®, size 30) rather than the larger Schirmer tear test strips traditionally used for measurements of lacrimal production. Each bat was manually restrained, and the point of the paper was inserted into the bottom of the conjunctival fornix, remaining there for 1 minute (Figure 1A). After this time, the paper

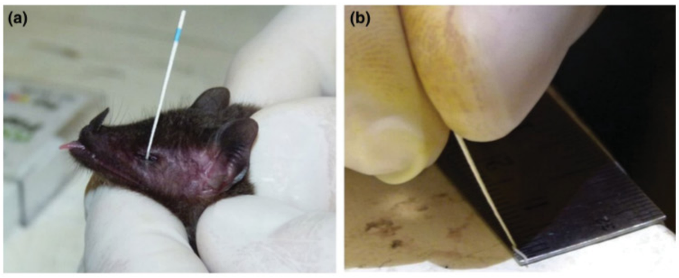

FIGURE 1 A, Measurement of tear production using a standardized endodontic absorbent paper point in the left eye of, *A caudifer*. B, The reading method using a graduated rule to measure the absorption of the aqueous portion of the tear film on the paper point

point was removed and read using a graded ruler with values expressed in millimeters per minute (mm/min) (Figure 1B).

Intraocular pressure (IOP) was measured in 28 eyes from 14 animals, using a rebound tonometer (TonoVet®, Kruuse), with the P setting for undefined species. The IOP (mm Hg) was measured with the animal in an upright position (feet toward the floor and nose parallel with the ground) (Figure 2). Six measurements were taken and averaged by the tonometer's internal software.

Palpebral fissure length (PFL) of both eyes was measured in all 14 manually restrained animals using a stainless-steel caliper with an LCD display and an accuracy of 0.02 mm (Neiko Tools®) (Figure 3). Values were obtained by measuring the distance in millimeters (mm) between the lateral canthus to the medial canthus of the eyelids.

To verify the normality of distribution, the Shapiro-Wilk test was used. Unpaired *t* tests were used for data comparison between males and females. Paired *t* tests were used for data comparison between right and left eyes. Values of $P < 0.05$ were considered significant.

3 | RESULTS

The male-to-female ratio in the group of *A lituratus* was 42.86% male and 57.14% female (three males and four females). In this species, females weighed significantly more than males (59 ± 5.9 g vs 44 ± 10.82 g, respectively; $P = 0.007$). The male-to-female ratio in the group of *A caudifer* was 71.43% male and 28.57% female (five males and two females). The difference in weight between the two sexes was significant (females 14.6 ± 0.8 g vs males 16 ± 1.1 g; $P = 0.025$). Interspecifically, a significant weight difference was found between the two species: *A lituratus* 52.7 ± 10.9 g and *A caudifer* 15 ± 1.1 g. ($P = 0.001$). All the weight values are provided in Table 1.

The mean values of the production of the aqueous fraction of the tear film for *A lituratus* were 2.38 ± 1.7 mm/min for males and 2.65 ± 1.7 mm/min for females. There were no significant differences between males and females ($P = 0.778$) and no significant differences between left and right eyes ($P = 0.197$). The mean values for the production of the aqueous fraction of the tear film for *A caudifer* were 2.1 ± 0.6 mm/min for males and 1.8 ± 0.6 for females. No significant difference was observed between males and females ($P = 0.401$) or between left and right eyes ($P = 0.543$). No significant difference in aqueous tear levels was found between the two species *A lituratus* (median 2.0 mm and range 4.6 mm) and *A caudifer* (median 2.0 mm and range 2.5 mm) ($P = 0.185$) (Figure 4).

The mean ± SD IOP value for *A lituratus* was 12.1 ± 3.3 mm Hg for males and 10.1 ± 3.1 mm Hg for females. No significant difference was observed between males and females ($P = 0.265$) and between left and right eyes ($P = 0.987$). The mean ± SD IOP value for *A caudifer* was 6.7 ± 2.7 mm Hg for males and 7.5 ± 2.7 mm Hg form females. No significant difference was observed between males and females ($P = 0.657$) and between left and right eyes ($P = 0.776$). However, IOP was significantly different between *A lituratus* (11 ± 3.2 mm Hg) and *A caudifer* (7.3 ± 2.7 mm Hg) ($P = 0.003$) (Figure 5).

The mean values of PFL for *A lituratus* were 4.86 ± 0.5 mm for males and 5.17 ± 0.1 mm for females. No significant difference was observed between males and females ($P = 0.214$) and between left and right eyes ($P = 0.587$). The mean values of PFL for *A caudifer* were 4.06 ± 0.5 mm for males and 3.86 ± 0.5 for females. No significant difference was observed between males and females ($P = 0.552$) and between left and right eyes ($P = 0.449$). However, PFL was significantly different between *A lituratus* (2.5 ± 1.6 mm) and *A caudifer* (1.9 ± 0.6 mm) ($P = 0.001$) (Figure 6).

All values from the performed tests from both species are summarized in Table 2.

FIGURE 2 Intraocular pressure evaluation in an *A caudifer* using a rebound tonometer (TonoVet®, Kruuse, Langeskov, Denmark)

FIGURE 3 Palpebral fissure length measurement using a stainless-steel caliper in the right eye of an *A caudifer*

TABLE 1 Mean values of body weight in grams (g) with standard deviation (std.) of male and female *A lituratus* and *A caudifer*

	Female + male	Female			Male			
Species	**Mean ± SD (g)**	**Mean ± SD (g)**	**Median (g)**	**Range (g)**	**Mean ± SD (g)**	**Median (g)**	**Range (g)**	***P***
Artibeus lituratus	52.7 ± 10.9	59 ± 5.9	60.5	15	44.33 ± 10.8	40	23	0.007
Anoura caudifer	15 ± 1.1	14.6 ± 0.8	15	2	16 ± 1.1	16	2	0.025

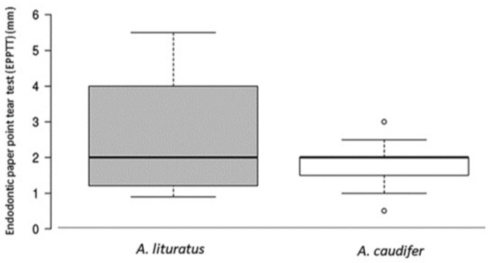

FIGURE 4 Boxplots for EPPTT values obtained from two populations of *A caudifer* (white box) and *A lituratus* (gray box)

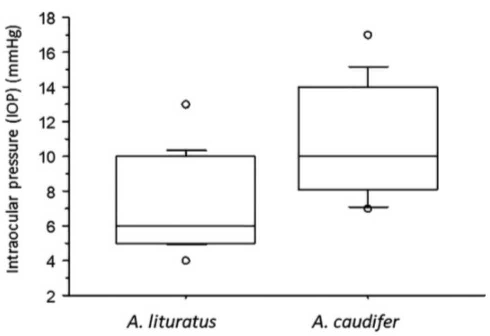

FIGURE 5 Boxplots for IOP values obtained from two populations of *A caudifer* and *A lituratus*

4 | DISCUSSION

Tear production is an important diagnostic test for many species and is commonly included in investigations of wild animals' ophthalmology.[12,13,24-29] To the authors' knowledge, there is only one study with information regarding this parameter in bats, in which the tear production of megachiropteran species was evaluated using the phenol red thread test (PRTT).[12] The measurement of the aqueous fraction of the tears using the standardized endodontic paper point test (EPPTT) is particularly useful to evaluate lacrimal production in small animals, or animals that have relatively small eyes, since the thickness of the paper point is compatible with the length of the palpebral fissure in such cases.[24] Reference values using this method have been reported for several other

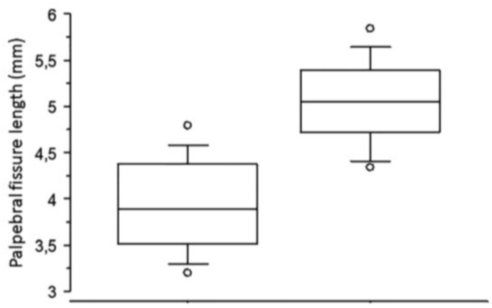

FIGURE 6 Boxplots for PFL values obtained from two populations of *A caudifer* and *A lituratus*

species of wild and exotic animals.[24-27] The results obtained for aqueous tear production in *A lituratus* and *A caudifer* are considerably lower compared to the values from the previously mentioned studies. The low rate of tear production found in the two species of microchiropteran examined in this study may be attributed to the width of the paper relative to the size of the eye. The small size of the conjunctival sac in megachiropterans makes placement of a phenol red thread nearly very difficult (Blackwood et al[12]) and even more so in microchiropteran species. Alternatively, tear production might be affected by biological behavior. Being nocturnal, normal activity hours for these two species are at dusk/dark, and all measurements were performed in daytime. Similarly, among avian species, nocturnal raptor families have lower tear production when compared to other raptor families.[29] Thus, it would be interesting to examine the circadian properties of these measurements and define how they differ according to activity hours, especially for bats. Additionally, it would be interesting to compare our measurements to those of other bat species that hunt during daytime, such as the *Hipposideros rube*.[30] Nevertheless, the reference values presented here are still relevant as most veterinary patients including nocturnal animals are examined during daytime, but the time of examination must be taken into consideration when evaluating tear production in nocturnal bats.

To date, intraocular pressure has only been reported in megachiropteran bats.[12,13] The mean values obtained for *A lituratus* are similar to several megachiropteran species: *P vampyrus* (14.1 ± 1.31 mm Hg), *P hypomelanus* (11.6 ± 1.38 mm Hg),

TABLE 2 Mean values obtained for EPPTT (endodontic paper point tear test), IOP (intraocular pressure), and PFL (palpebral fissure length) for two bat species (*A lituratus* and *A caudifer*)

	A literatus				*A caudifer*			
	Total	**Male**	**Female**	***P***	**Total**	**Male**	**Female**	***P***
PPTT (mm/min)								
Mean ± SD	2.53 ± 1.65	2.38 ± 1.70	2.65 ± 1.72	0.778	1.89 ± 0.62	2.12 ± 0.62	1.80 ± 0.63	0.401
Median (range)	2.0 (4.6)	1.6 (4.1)	2.0 (4.5)		2.0 (2.5)	2.0 (1.5)	2.0 (2.0)	
IOP (mm Hg)								
Mean ± SD	11.0 ± 3.28	12.16 ± 3.31	10.12 ± 3.18	0.265	7.28 ± 2.70	6.75 ± 2.75	7.50 ± 2.79	0.657
Median (range)	10.0 (10.0)	12.0 (8.0)	9.0 (8.0)		6.0 (9.0)	6.5 (6.0)	6.0 (8.0)	
PFL (mm)								
Mean ± SD	5.04 ± 0.45	4.86 ± 0.57	5.17 ± 0.32	0.214	3.92 ± 0.51	4.06 ± 0.58	3.86 ± 0.50	0.552
Median (range)	5.0 (1.51)	4.7 (1.51)	5.2 (0.89)		3.89 (1.6)	3.96 (1.2)	3.89 (1.3)	

and *P giganteus* (12.4 ± 2.9 mm Hg).[11,12] However, the mean value was slightly lower compared to that established for *P pumilus* (18.2 ± 1.08 mm Hg).[13] The mean values obtained for *A caudifer* were markedly lower than all previously studied megachiropteran species suggesting that microchiropteran bats may have lower IOP values. Additionally, it is important to note that in the present study IOP was only evaluated in animals in the upright position, contrary to how megachiropteran bats have been measured (in inverted position with the head toward the floor). The upright position for IOP measurements was chosen because it was technically simpler for the researchers to restrain the bats this way. Higher intraocular pressure readings may have been obtained if bats in the present study were measured upside down, and therefore, the authors encourage future studies to measure bats in both positions to provide a better understanding of the dynamics of intraocular pressure in bats.[12] Furthermore, the IOP for *A caudifer* was found to be significantly lower than the IOP for *A lituratus*, showing variation even within microchiropterans, encouraging future investigations into the relevance of variation in IOP between microchiropteran species, and megachiropteran species, taking into consideration other aspects that may play a role in IOP (eg, natural body and head position during normal activity both in day and night).

PFL in the species studied here was considerably lower than that obtained in three species of megachiropteran bats.[12,31] This difference is likely because of the notable differences in size and ecology between megachiropteran and microchiropteran bats. For example, megachiropteran bats from the family *Pteropodida* (*P vampyrus*, *P pumilus,* and *P hypomelanus*) have relatively large eyes[32] and also do not possess an echolocation system[21] in contrast to microchiropteran bats. The fact that the PFL values obtained for *A lituratus* and *A caudifer* are significantly different perhaps is justified by the simple fact that *A caudifer* are significantly smaller than *A lituratus*. On the other hand, the authors encourage that efforts should be targeted in the search for other possible explanations, such as ecological behaviors or biological aspects, as these features normally are present in morphometric studies in bats.[22,31]

To our knowledge, this is the first study focusing on the clinical ocular values of microchiropteran bats. Other than aqueous tear production (which is not significantly different between species more phylogenetically distant: dog and cat),[33] we have shown significant interspecific variation in all ocular parameters studied. Similarly, our results show differences between ocular parameters of microchiropteran bats and previous reports of ocular parameters of megachiropteran bats. This is interesting considering the large degree of variability in ecological and physiologic parameters among and between both megachiropteran and microchiropteran bats.

The data obtained in the present study may serve as a reference for ophthalmic reference parameters in the diagnosis and management of disease, as well as for future investigations. Future studies should evaluate the implications of the differences in ocular parameters for visual ecology of megachiropteran and microchiropteran bats. One limitation of the present study was the small sample size, which had the potential to be limiting in enabling identification of other significant interspecific differences.

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6 ANEXOS

6.1 APROVAÇÃO NO COMITÊ DE ÉTICA DO SETOR DE CIÊNCIAS AGRÁRIAS DA UFPR.

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CERTIFICADO

Certificamos que o protocolo número 067/2014, referente ao projeto "Caracterização Clínica e Eletrorretinográfica da Atrofia Progressiva de Retina em Cães da Raça Whippet", sob a responsabilidade de André Tavares Somma, na forma em que foi apresentado (grau B de invasividade), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias da Universidade Federal do Paraná - Brasil, em reunião realizada dia 12 de dezembro de 2014.

CERTIFICATE

We certify that the protocol number 067/2014, regarding the project "Clinical and electroretinographic characterization the Retina Atrophy Progressive in Whippet dogs", under André Tavares Somma supervision, in the terms it was presented (was classified as grade B of invasiveness), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on December 12, 2014.

Curitiba, 13 de Dezembro de 2014.

Ricardo Guilherme D'Otaviano de Castro Vilani
Presidente CEUA-SCA

Ananda Portella Félix
Vice-Presidente CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

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