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JULIANA KRAVETZ DE OLIVEIRA

OPHTHALMIC HEALTH IN WORKING DOGS AND CLINICAL INVESTIGATIONS IN CANINE OPHTHALMOLOGY, CONCERNING REBOUND TONOMETRY AND KERATOCONJUNCTIVITIS SICCA

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Ao meu дедушка João

(*in memoriam)*

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“I don’t want to believe, I want to know”

Carl Sagan

RESUMO

A estrutura dessa dissertação é composta em três capítulos sobre diferentes investigações na oftalmologia de cães. Os capítulos são artigos científicos distintos compostos por estudos sobre tonometria de rebote, ceratoconjuntivite seca e saúde oftálmica de cães de trabalho. O primeiro capítulo descreve a variabilidade das leituras realizadas pelo tonômetro de rebote Tonovet® em diferentes posições na superfície corneana do cão. Esse estudo foi realizado em colaboração com a Universidade de Cambridge, Reino Unido. O tonômetro de rebote calcula a pressão intraocular baseada em parâmetros de movimento e é utilizado na posição perpendicular em relação ao centro da córnea visando reduzir erros. O objetivo desse capítulo foi avaliar se os valores pressão intraocular mensurados pelo tonômetro de rebote variam em quatro posições periféricas e quatro anguladas no centro da córnea comparadas com a perpendicular no centro da córnea. Os achados demonstraram que sete das oito posições desalinhadas resultaram em valores subestimados significantes da pressão intraocular, sendo o tonômetro menos robusto nas posições periféricas do que nas posições anguladas no centro da córnea. Os capítulos subsequentes foram realizados no Laboratório de Oftalmologia Comparada, Universidade Federal do Paraná (UFPR). O segundo capítulo é um estudo da eficácia do oclacitinib, um inibidor da janus kinase, no tratamento de ceratoconjuntivite seca em cães. Um medicamento da mesma classe é eficaz no controle dos sinais clínicos da síndrome do olho seco em pessoas, sendo possível ter uma ação semelhante em cães. A eficácia do oclacitinib 0,1% foi comparada com o tacrolimus 0,01%, droga de escolha no tratamento de ceratoconjuntivite seca. Os cães estudados foram separados em três grupos de tratamentos e acompanhados durante um período de 45 dias, nos quais foram realizados o teste lacrimal de Schirmer e classificação dos sinais clínicos, como secreção e hiperemia ocular. Os achados demonstraram que o oclacinitib não forneceu resultados satisfatórios, sendo considerado ineficaz. Em contrapartida, tacrolimus na concentração de 0,01% foi suficiente no tratamento e pode ser utilizado como dose inicial em casos leves e moderados de ceratoconjuntivite seca em cães. O terceiro capítulo é uma pesquisa da saúde oftálmica em cães de trabalho, englobando doenças e erros refrativos e comparando com o desempenho de trabalho. Os cães estudados foram separados em cães policiais e cães-guias. Cinquenta e quatro por cento dos cães apresentaram alterações oftálmicas, sendo 60% delas consideradas doenças hereditárias. A catarata incipiente foi a doença mais prevalente. Cães-guias apresentaram uma tendência para a emetropia e não tiveram doenças no eixo visual, enquanto que, cães policiais tenderam à miopia e 17% apresentaram doenças no eixo visual. Entretanto essas doenças não parecem prejudicar o desempenho no trabalho e os animais não demonstraram sinais de deficiência visual. Ademais, devido a possibilidade de algumas doenças progredirem, reavaliações são recomendáveis e é necessário um controle na reprodução devido à alta prevalência de doenças consideradas hereditárias. Essas investigações fornecem uma contribuição relevante para o conhecimento na oftalmologia de cães.

PALAVRAS-CHAVE: tonometria de rebote, tonometria periférica, ceratoconjuntivite seca, oclacitinib, tacrolimus, cães policiais, cães-guias, erros refrativos

ABSTRACT

The structure of this dissertation is composed by three chapters concerning different investigations in canine ophthalmology. The chapters are distinct scientific papers composed by studies in rebound tonometry, keratoconjunctivitis sicca and ophthalmic health in working dogs. The first chapter describes the variability of readings made using the Tonovet® rebound tonometer in different positions on canine corneal surface. This study were carried out in collaboration with the University of Cambridge, United Kingdom. Rebound tonometer calculates intraocular pressure based on parameters of movement and it is used in a perpendicular position in the center cornea aiming to reduce errors. The purpose of this chapter was to evaluate if the intraocular pressure values measured by rebound tonometer varies in four peripheral and four angulated positions on center cornea compared to the perpendicular position on center cornea. The findings demonstrate that seven out of the eight misaligned positions resulted in significant underestimated values of intraocular pressure, being the tonometer less robust in peripheral positions than angulated positions on center cornea. The subsequent chapters were performed in the Laboratory of Comparative Ophthalmology, Federal University of Paraná (UFPR). The second chapter is an efficacy study of oclacitinib, a janus kinase inhibitor, on treatment of keratoconjunctivitis sicca in dogs. A same class drug is efficient to control clinical signs of dry eye syndrome in humans, being a possibility to have a similar action on dogs. Oclacitinib 0.1% efficacy were compared with tacrolimus 0.01%, drug of choice to treat keratoconjunctivitis sicca. The studied dogs were separate in three groups of treatment and accompanied during a period of 45 days, in which was performed Schirmer tear test and scoring of clinical signs, such as ocular discharge and hyperemia. The findings demonstrate that oclacitinib did not provide satisfactory results, being considerated ineffective. On the other hand, tacrolimus in 0.01% concentration were sufficient on treatment and could be used as starter dose in mild and moderate cases of keratoconjunctivitis sicca in dogs. The third chapter is a survey of ophthalmic health in working dogs, encompassing diseases and refractive errors and comparing them with work performance. The dogs studied were separate in police and guide dogs. 54% of them had ophthalmic disorders, being 60% of them considered inherited diseases. Incipient cataracts were the most prevalent disease. Guide dogs showed a tendency to emmetropia and did not have diseases on visual-axis, whereas police dogs tended to myopia and 17% of them had diseases on visual-axis. However, these disorders do not seem to lower work performance and the animals did not show any signals of visual impairment. Furthermore, due to possibility of progress of some diseases, follow-up is recommended and breeding control is necessary due high prevalence of considered inherited disorders. These investigations gives a relevant contribution to the knowledge on canine ophthalmology.

KEYWORDS: rebound tonometry, peripheral tonometry, keratoconjuctivitis sicca, oclacitinib, tacrolimus, police dogs, guide dogs, refractive errors

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LIST OF ABBREVIATIONS AND ACRONYMS

20°BIB	- 20° Batalhão de Infantaria Blindada
ANOVA	- Analysis of variance
APD	- Attack and protection dogs
ARVO	- The Association for Research in Vision and Ophthalmology
BOPE	- Batalhão de Operações Especiais do Paraná
C	- Celsius
CC	- Center of the cornea
DC	- Dorsal to the center
DENARC	- Departamento Estadual de Prevenção e Repressão ao Narcotráfico
DP	- Dorsal peripheral
et al	- Et alia
F	- Force
GD	- Guide dogs
GMC	- Guarda Municipal de Curitiba
Inc.	- Incorporated
IOP	- Intraocular pressure
JAK	- Janus kinase
KCS	- Keratoconjunctivitis sicca
LC	- Lateral to the center
LSD	- Least significant difference
Ltd.	- Limited
MC	- Medial to the center
mm	- Milimeter
mmHg	- Milimeter of mercury
mm/min	- Milimeter per minute
N/A	- No ophthalmic abnormalities
NP	- Nasal peripheral
O	- Oclatinib group
OD	- Other dogs

OT	- Oclacitinib + tacrolimus group
p	- p-value
R^2	- Determination coefficient
SAS	- Statistical Analysis System
SD	- Sniffer dogs
STT	- Schirmer tear test
T	- Tacrolimus group
TD	- Tracking dogs
TP	- Temporal peripheral
UFPR	- Universidade Federal do Paraná
UK	- United Kingdom
USA	- United States of America
VC	- Ventral to the center
VP	- Ventral peripheral

LIST OF SYMBOLS

® - registered trademark

% - percentage

° - degrees

± - plus-minus

ρ – rho (Spearman correlation coefficient)

< - less than

> - higher than

= - equal

+ - plus

- - minus

≥ - higher or equal than

SUMMARY

1. GENERAL INTRODUCTION

Canine ophthalmology is an important branch of comparative ophthalmology, once dogs shares similar ophthalmic diseases with human beings, being largely used as animal model for study, being spontaneous for some naturally occurring disorders, and due to the importance of them for human society, being pets or working animals. The present dissertation is divided into three chapters regarding separated investigations in this specie.

The first chapter is an investigation concerning rebound tonometry variability on intraocular pressure values measured on misaligned positions on cornea surface. Tonometry is important to measure intraocular pressure, being essential to the diagnosis of glaucoma and uveitis, guiding the clinician to treat correctly and fastly. The significance is due that; these diseases can result into blindness. Rebound tonometer Tonovet® use is increasing in canine ophthalmology due do its tolerability and accuracy. This tonometer measures intraocular pressure based on parameters of movement, and it is recommended to be used in a perpendicular position on center cornea to avoid reading errors. However, in some cases that this position is not available for the measurements, other position such as cornea periphery will be assessed, which can gives over or underestimated intraocular pressure values. Taking this into account, the purpose of this chapter study were to find if different positions like periphery or angulated in the center of cornea can give a significant different value from that of perpendicular on cornea center, giving a value information about rebound tonometry in dogs.

The second chapter is an efficacy study of oclacitinib, compared to tacrolimus, on treatment and control of ocular signs of keratoconjunctivitis sicca in dogs. Keratoconjunctivitis sicca is a condition of insufficient tear production, commonly due to immune-mediate destruction of lacrimal and nictitating glands. Tears give ocular surface nutrition, defense and lubrication and its reduction can injury cornea, resulting in inflammation, loss of transparency and predisposing to corneal ulcers, which, in severe cases, can result into blindness, by pigmentary keratitis or eye perforation. Treatment is commonly life threatening and the drugs of choice are immunomodulators, such as tacrolimus. In human patients, tofacitinib, a janus kinase inhibitor, is also effective on treatment of dry eye syndrome, disease similar to keratoconjunctivitis sicca. Oclacitinib, is the first janus kinase inhibitor authorized for use in dogs, being effective on canine atopic dermatitis, controlling pruritus and inflammation. Considering that, the aim of this chapter study was to evaluate if oclacitinib could be a new treatment option for keratoconjunctivitis sicca in dogs, by assessing its efficacy on the control of clinical signs and comparing with tacrolimus.

The third chapter is an assessment of ophthalmic health, regarding ocular disorders and refractive state of police and guide dogs. Working dogs are important to the society in which they are included, developing many activities that helps people admirably. Police dogs serves to detect narcotic and explosives, to search and rescue people, to give protection and other specific functions. Whereas guide dogs assists blind people, giving them safe mobility with confidence. These working dogs are costly to select, train and maintain, and there is an interest to keep them developing their functions for the longest period with health. Ophthalmic abnormalities are responsible for a percentage of withdrawn from service and even death or euthanasia of these dogs, and the presence of ophthalmic defocus can worsen performance. Due that, they need to be assisted by ophthalmic evaluation to detect abnormalities and refractive errors, treat them when necessary and follow-up from time to time. Maintenance of ocular health of working dogs is crucial to maintain them working, so the importance to find such abnormalities can guarantee quality of life and good performance at their jobs. Due that, the purpose of this chapter study was to investigate the presence of ophthalmic diseases and to stablish a tendency of refractive state of these animals and comparing with their performance.

These distinct investigations can contribute significantly to canine ophthalmology, being useful for the clinical routine, concerning working and pet dogs, to serves as reference to further investigations in this area and to contribute to provide new data to translational medicine.

2. INFLUENCE OF MEASURING POSITION ON CANINE INTRAOCULAR PRESSURE MEASUREMENTS USING THE TONOVET® REBOUND TONOMETER

2.1 ABSTRACT

Objective

To assess the variability of readings made using the Tonovet® rebound tonometer for measurement of intraocular pressure (IOP) in the peripheral cornea and in angulated positions on the canine corneal surface.

Animals studied

Forty-six client-owned dogs (seventy-nine eyes) admitted for ophthalmic evaluation at the Queen's Veterinary School Hospital, University of Cambridge were included in the study.

Procedures

IOP readings were taken at a variety of locations and using the tonometer at a number of different angles to the cornea: 1) Perpendicularly at center of the cornea (CC); 2) At the center of the cornea but with the tonometer positioned at four angles, and 3) At four different points on the peripheral cornea. All values were compared with the values recorded at the recommended CC position.

Results

IOP values were significantly underestimated in seven positions, with median and interquartile range from 13 ± 4 mmHg (dorsal and nasal on periphery) to 15 ± 3.8 mmHg (laterally angled at center), varying between 0 mmHg to 2 mmHg from the CC value. While dorsally angled in the central cornea were not significantly different from those at CC (p = 0.24). Median values were lower for measurements in peripheral positions when compared to angled central positions.

Conclusion

These results demonstrate that angling the tonometer or measuring in peripheral regions can result in significant underestimation of IOP values. Nevertheless while these values were statistically significantly, from a clinical perspective they did not give readings that were substantially different from that in CC.

KEY WORDS: dog, intraocular pressure, rebound tonometry, peripheral tonometry

2.2 INTRODUCTION

Tonometry is an important method for measurement of intraocular pressure (IOP) during a complete ophthalmic evaluation in dogs. It is important for the diagnosis and control of glaucoma as well as uveitis. Glaucoma is a pathological increase in IOP that causes damage to the optic nerve and retina resulting in blindness. Accurate IOP measurement is crucial to determine the therapeutic approach, since IOP abnormalities can cause significant morbidity (Plummer et al., 2013; Renwick and Petersen-Jones, 2009).

The rebound tonometer is a portable tonometer with a small probe, its use is increasing in veterinary medicine because it is well tolerated by most animals (Kontiola et al., 2001; Prashar et al., 2007), and does not require topical anesthesia. Additionally, it is the most accurate hand-held tonometer (Tofflemire et al., 2017) and can provide accurate readings even in inexperienced hands (Sahin et al., 2008; Abraham et al., 2008).

The technique of rebound tonometry was first reported in laboratory animals in the early 2000´s (Danias et al., 2003; Kontiola et al., 2001) and its use became widespread in medical (Dosunmu, et al., 2014; Muttuvelu et al., 2012; Abraham et al., 2008) and veterinary (Zhang et al., 2014; Slack et al., 2012; Selleri et al., 2012; Thompson-Hom and Gerding, 2012; Nagata et al., 2011, Reuter et al., 2010; Rusanen et al., 2010) ophthalmology in subsequent years.

The Tonovet® tonometer (Tiolat Ltd., Helsinki, Finland) uses a small magnetized probe, which is directed toward the corneal surface by an electric coil. After impacting the cornea, the deceleration of rebound is measured by the induced voltage in the sensitive coil (Kontiola, et al., 2001; Kontiola, 1997). The manufacturer recommends that the tonometer probe is kept in a horizontal position during measurement of IOP, preventing gravitational forces affecting the speed and deceleration of the probe.

The effect of probe position on the corneal surface has been investigated in human patients (Beasley et al., 2013; Muttuvelu et al., 2012; Yamashita et al, 2011; Queirós et al., 2007; González-Méijome et al., 2006) and statistically significant differences in IOP were demonstrated when measured at the periphery of the cornea when compared to the central cornea. These investigations also demonstrated that when IOP may be significantly underestimated when measured with the probe in an angled positions (Beasley et al., 2013; Muttuvelu et al., 2012). A similar study was carried in dogs (Von Spiessen et al., 2013) using the probe in a number of angled positions as well as in peripheral corneal locations. When tonometry was performed using angled positions but at the center of the cornea IOP data was underestimated by up to 6.5mmHg, whereas off-center probe positioning provided a significant overestimation of 0.9 mmHg.

The aim of this study was to repeat the published study to expand the data, as our anecdotal evidence did not suggest such dramatic effects on IOP values with varied probe positions. The current study evaluated potential differences of IOP values for the canine eye using a Tonovet® tonometer with the probe positioned in the periphery and in angulated positions, were compared with IOP values obtained using the recommended perpendicular position in the center of the cornea. We also aimed to evaluate repeatability compared to the previous study.

This assessment is essential since veterinary medicine tonometry is typically performed in un-sedated dogs whose globes are in constant motion, resulting in a potential misalignment of the tonometer probe in relation to the central position of the cornea. In addition, the presence of central corneal abnormalities may sometimes impair accurate tonometry measurements at this location (Von Spiessen et al, 2015). Moreover, systemic and topical anesthetics drugs can increase or decrease IOP values (Kanda et al., 2015; Artigas et al., 2012; Rauser et al., 2012; Almubrad and Ogbuehi, 2007; Baudouin and Gaustad, 1994), and neither general nor topical anesthesia were used in this study reflecting the reality of canine clinical practice. For these reasons, it is important to know if IOP values taken at the periphery or not perpendicular to the corneal surface are significantly different from those taken perpendicularly in the corneal center.

2.3 MATERIAL AND METHODS

Forty-six client-owned dogs (79 eyes) undergoing ophthalmic evaluation at the Queen's Veterinary School Hospital, University of Cambridge were included in the study. The age of the dogs ranged from 6 months to 15 years, with a mean of 6.1 years and standard deviation of ± 3.55. The animals comprised 24 female dogs and 22 males, with a total of 79 eyes. Twenty-one different breeds were represented. Mixed-breed dogs and West White Highland Terrier appeared most frequently, from which 21 globes were evaluated. Other common breeds were English Cocker Spaniel, Greyhound, Golden Retriever, Labrador Retriever and Jack Russell Terrier.

All included animals were quiet and easily restrained in a sitting position, allowing the evaluation of IOP with minimal physical restraint. The study followed ARVO`s Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the Ethics Committee of the Department of Veterinary Medicine, University of Cambridge. A consent form was signed by each owner. The welfare of the animals was not compromised by the repeated measurements required in this study since rebound tonometry requires momentary contact with the cornea and does not cause irritation or damage. A routine ophthalmic examination was performed in each case, including: examination of cornea, anterior chamber, iris, and lens performed by direct and indirect ophthalmoscopy (Keeler practitioner direct ophthalmoscope and Vantage indirect ophthalmoscope, Windsor UK) and slit-lamp biomicroscopy (Kowa®, Kowa Company®, Japan) to exclude animals with corneal disease.

The IOP measurements were performed with a calibrated rebound tonometer Tonovet® positioned on the center of the cornea and in eight different positions, as shown in Figure 1, at a distance of 5 to 10 mm from the cornea. The rebound tonometer has a manufacturer's calibration of 'd' for use in dogs and cats. The "d" calibration setting was used in throughout this study. The tonometer makes six IOP readings for each evaluation. The result given by the instrument´s internal processor is a mean of these values, discarding the lower and the higher result. The values recorded in this study were those with a steady display in the instrument window (ie those with a small variation between readings). Additionally, the tonometer shows an "error" signal when it measures discrepant values and it discards these automatically. A single veterinary ophthalmologist performed all the measurements, as well as all the ophthalmic evaluations.

Measurements of IOP were performed first in the central cornea (CC). Next, the tonometer probe was displaced at 20 to 25 degrees from the CC, the angled positions being lateral to the center (LC), medial to the center (MC), dorsal to the center (DC) and ventral to the center (VC). The peripheral positions were temporal (TP), nasal (NP) dorsal

(DP) and ventral (VP), and were obtained by displacing the tonometer 3 mm from the limbus, as shown in Figure 1. Measurements were made in the same order in each eye: CC, then the angled positions VC, DC, LC and MC ; followed by VP, DP, NP and TP.

2.3.1 Statistical analysis

All IOP data collected from a single eye were analyzed individually. Median and interquartile range were calculated for variables for each position. Mean and standard deviation were calculated for age of the animals. A box-plot graph was created using Excel® (Microsoft Corporation, Washington, USA). Ninety-five percent limits of agreement were calculated according to Bland and Altman (1986) (mean difference ± 1.96 standard deviation of the differences) for each position in comparison with central cornea. A Shapiro-Wilk normality test showed that the data did not follow a Gaussian distribution. A Friedman`s test with Conover and Holm-Bonferroni post-hoc tests was used. p <0.01 were considered significant. Spearman correlation coefficients (ρ) and determination coefficient (R^2) were calculated and simple linear regressions were applied in order to summarize and analyze possible relations between IOP values performed at CC and each different corneal position or probe angle. The software used for statistical analyses was StatView® 5.0 (SAS Institute Inc., Cary, North Carolina, USA).

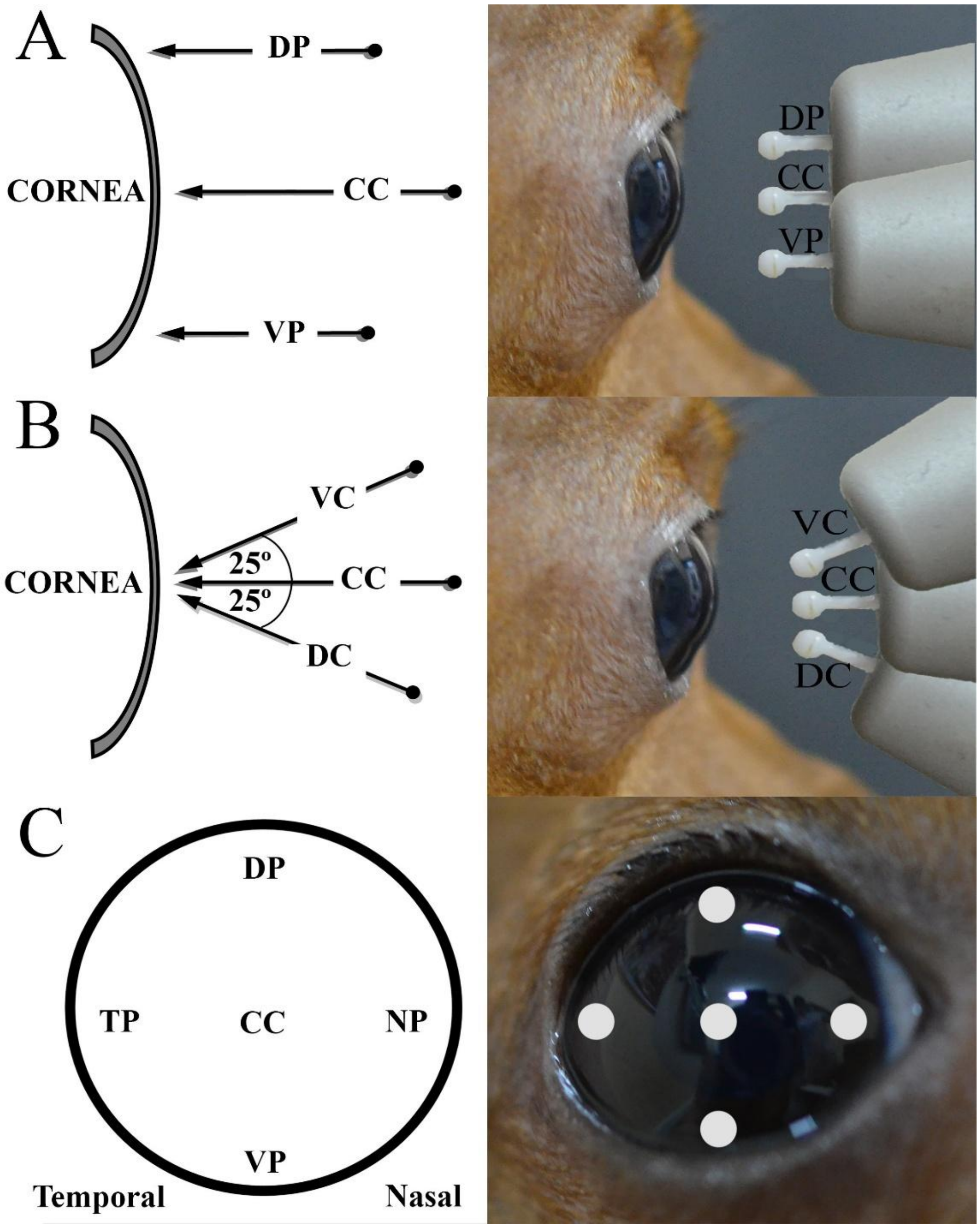

Fig. 1. The positions performed using rebound tonometer Tonovet® onto the dogs' corneal surface. A: perpendicular view of peripheral positions, showing relocation from central cornea (CC) as dorsal peripheral (DP) and ventral peripheral (VP). B: perpendicular view of angled positions, ventrally to the center (VC) and dorsally to the center (DC). C: frontal view of the peripheral positions showing relocation from central cornea (CC) as dorsal (DP), ventral (VP), nasal (NP), and temporal (TP)

2.4 RESULTS

The dogs examined had a median and interquartile range of 15 ±3 mmHg of IOP on CC. Table 1 details additional information concerning all dogs investigated. Table 2 shows median and interquartile ranges for IOP values in each position and 95% limits of agreement. Additionally, Spearman correlation coefficient (ρ) and *p*-values obtained by Friedman`s test of the IOP values for each position (MC, LC, DC, VC, DP, VP, TP, NP) compared with the IOP obtained at CC using the rebound tonometer Tonovet®.

Table 1. Summary of 46 dogs evaluated with information about age and sex and descriptive statistics (mean and standard deviation).

	Age (years)		
	Minimum and maximum	Mean ± standard deviation	Number
Sex	0.5 – 15	6.1 ± 3.55	46
Males	0.5 – 13	6.1 ± 3.73	22
Females	0.6 – 15	6.1 ± 3.45	24

Statistical analysis performed by Friedman`s test with Conover post-hoc analysis and Holm-Bonferroni adjustment (p <0.01) showed significant underestimation of IOP in seven positions (MC, LC, VC, DP, VP, TP, NP) when compared to CC. The only position with no statistically difference when compared to CC was DC (p = 0.248). Peripheral positions had lower median values than angled positions. The highest Spearman correlation (ρ) values were LC (ρ = 0.5069, p = 0) and MC (ρ = 0.3975, p < 0.01). The lowest were the peripheral positions DP (ρ = 0.1479, p = 0.19) and NP (ρ = 0.0571, p = 0.62). Dispersion graphs for each position compared to CC are present on Figure 2 with their respective determination coefficient. Correlation between eyes of the same dog were statistically significant (ρ = 0.2169, p = <0.01).

Table 2. Statistics (median and interquartile range) relating to intraocular pressure measurements obtained with a rebound tonometer (Tonovet®) in different positions on the cornea compared with central cornea (CC). P values generated by Friedman`s test with Conover post-hoc analysis and adjusted by Holm-Bonferroni method in comparison with CC. Spearman correlation coefficient (ρ) and 95% limits of agreement.

Probe position	Median ± interquartile range	*p values*	Limit of agreement of 95% in mmHg	Spearman correlation coefficient (ρ)
CC	15±3	-	-	-
VC	14±4	< 0.01	[-5.64; 8.26]	0.3296
DC	15±5.05	0.24	[-7.61; 8.11]	0.3344
LC	15±3.8	< 0.01	[-4.65; 6.29]	0.5069
MC	14±4	<0.01	[-4.86; 7.97]	0.3975
DP	13±4	<0.01	[-6.68; 11.14]	0.1479
VP	14±4	<0.01	[-6.32; 9.44]	0.1894
TP	14±5	<0.01	[-6.61; 10.19]	0.1759
NP	13±4	<0.01	[-6.24; 10.82]	0.0572

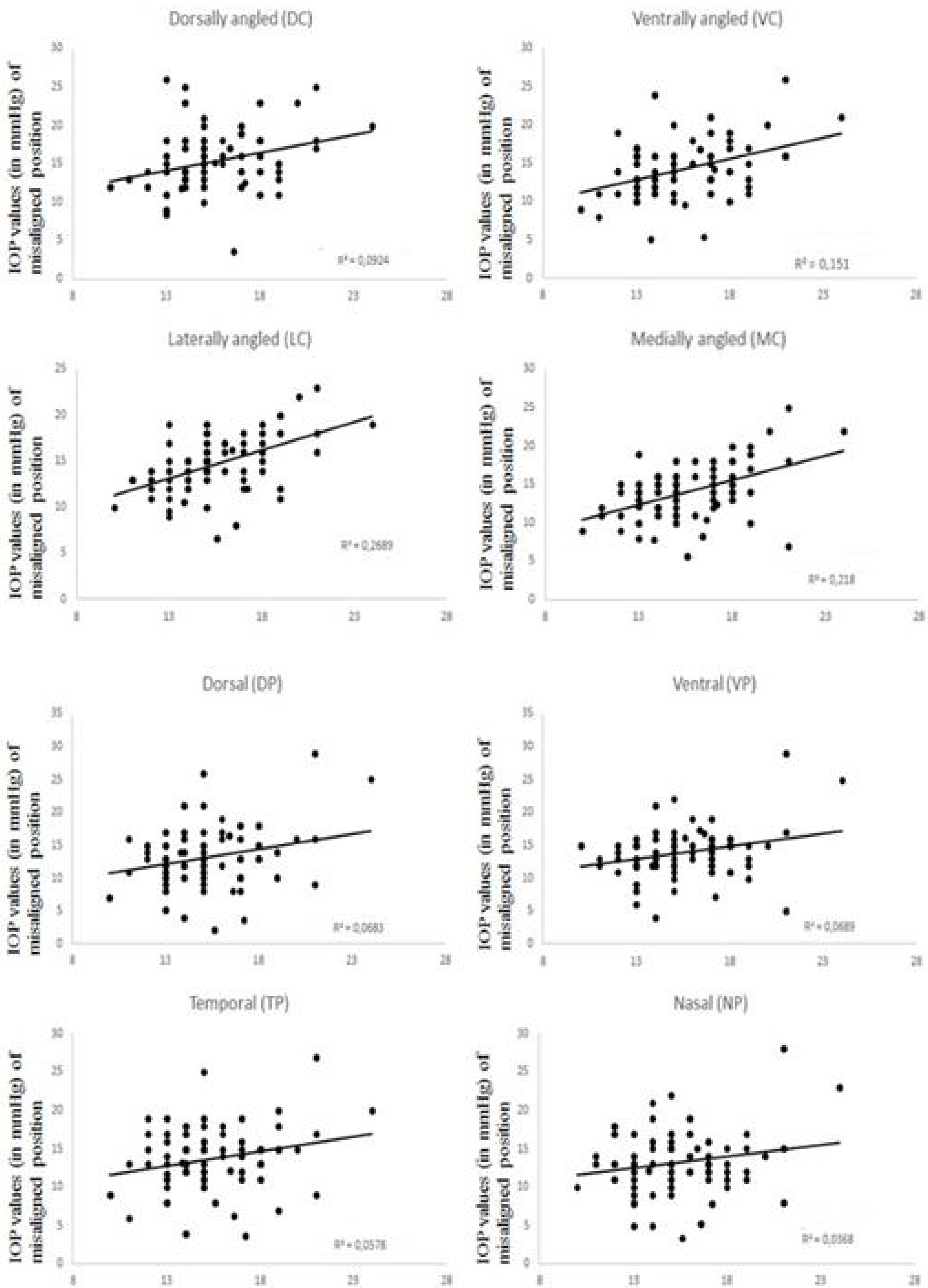

Fig. 2. Dispersion graphs for each position of measurement of IOP with the rebound tonometer Tonovet® compared with the CC. For every position there is a positive correlation. The solid line represents the CC position and dashed line represents the position indicated in the legend.

Box-plot graphs of the IOPs values in mmHg are present in Figure 3, and were obtained by the comparison of misaligned positions with CC, including outlier's values. Figure 4 shows the Bland-Altman plots for each position, with a 95% interval of agreement, the limits values for angled positions (in mmHg) were approximately ±6.95 (VC), ±7.74 (DC), ±5.47 (LC) and ±6.41(MC). The peripheral positions had larger limits with 95% of agreement, which were (in mmHg) ±8.91 (DP), ±7.88 (VP), ±8.40 (TP) and ±8.53 (NP).

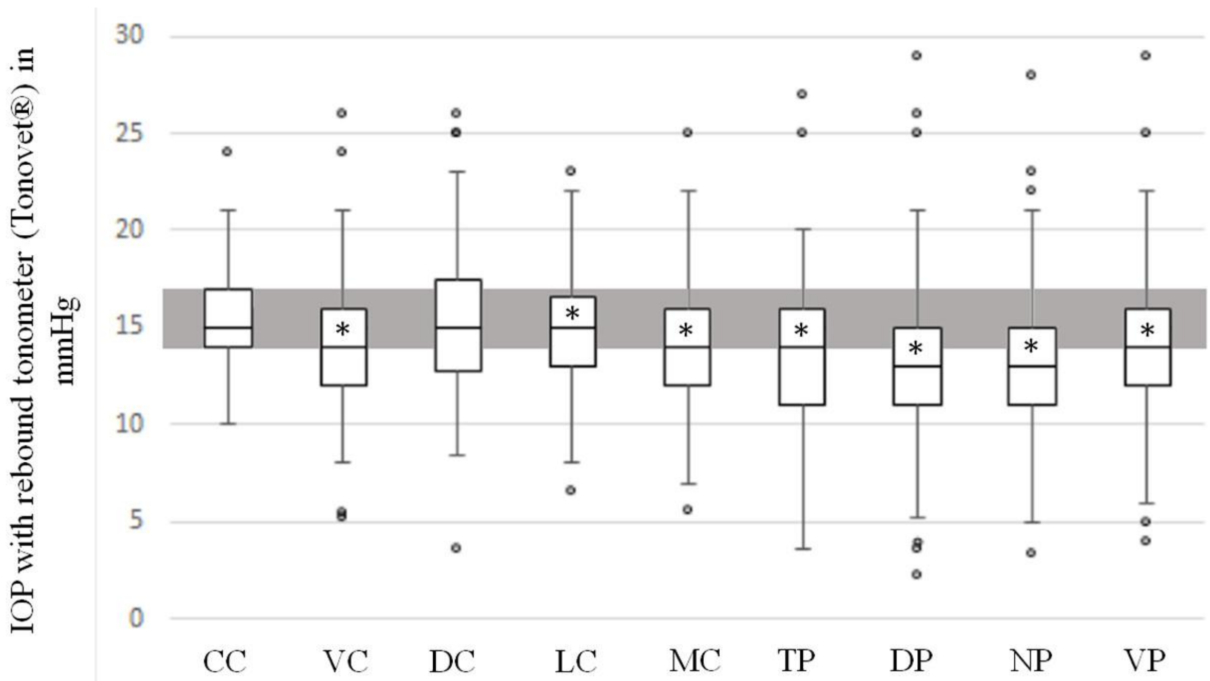

Fig. 3. Box plot graph of intraocular pressure values measured by rebound tonometer (Tonovet®) in different positions on corneal surface. * Shows the positions with statistically significant differences compared to CC.

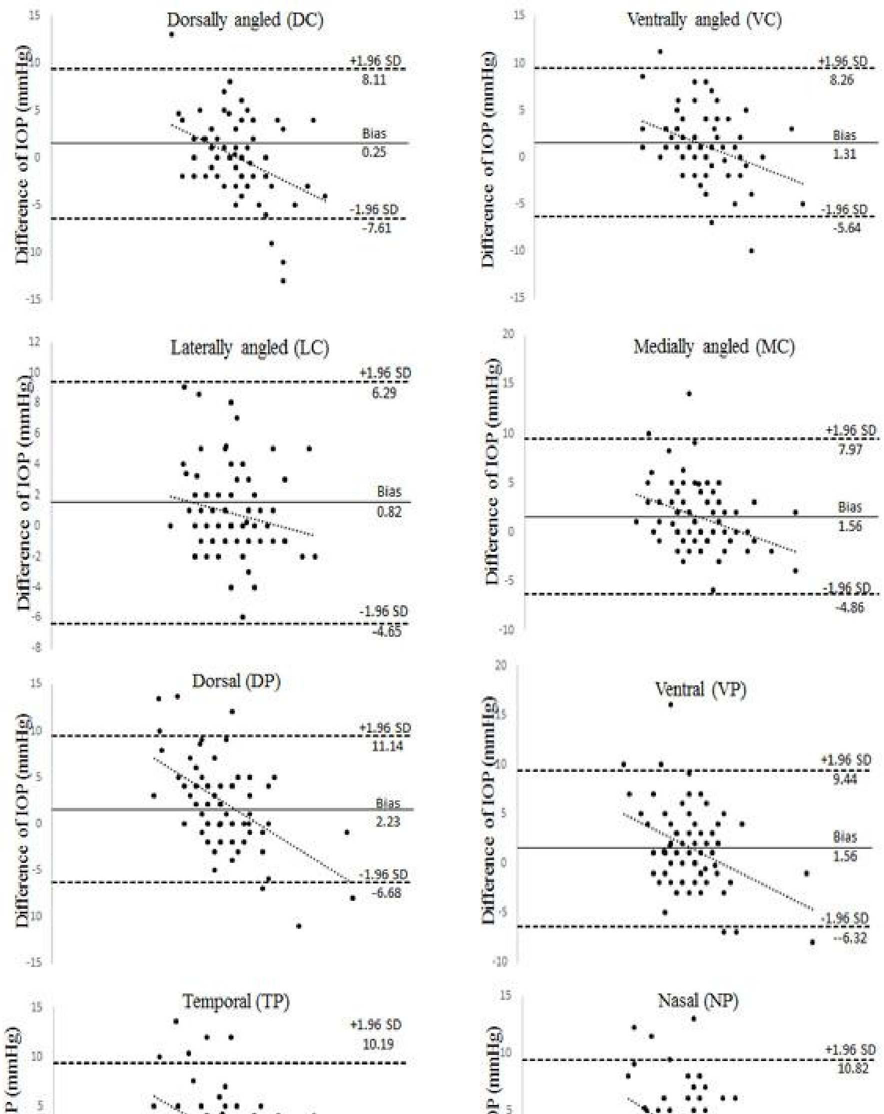

Fig. 4. Bland-Altman plots showing the lack of agreement of intraocular pressure values measured by rebound tonometer (Tonovet®) in different positions compared with Central Cornea (CC). The dashed lines shows the limits of agreement of 95%. The full line is representative of the means difference and the dotted line is the linear regression with the equation and R^2, determination coefficient values

2.5 DISCUSSION

The misplacement of the rebound tonometer probe during measurements could lead to underestimated IOP values when compared to the perpendicular position in the central cornea. Three positions off-angle (VC, LC and MC) and four positions off-axis (DP, NP, TP and VP) showed significant statistical differences from CC. The only position in which IOP measurements showed no significant difference from CC was DC. Having said that these peripheral positions and angled measurements gave statistically significant differences from a value obtained with the probe perpendicular and central, these variations were not markedly different from CC, remaining in IOP normal range, and that they did would not have lead to a misdiagnosis in a clinical patient.

The use of rebound tonometer is becoming increasingly popular in veterinary medicine due to its practicality and accuracy when compared to manometry (Tofflemire et al., 2017; Ma et al., 2016; McLellan et al., 2013; Knollinger et al., 2005), which is an invasive method that documents true IOP values. One of the limitations of this study is that true IOP values given by manometry were not available at the data collection. Nevertheless our research question was whether there is a significant difference between the standard result and that obtained when the probe is positioned angled or peri-centrally and this does not require determination of the true IOP.

This study was designed to assess whether misalignment of rebound tonometer can result in increased or decreased values of IOP. To find such results, rebound tonometry was repeated in each position on the corneal surface. No disturbance or damage to the cornea was observed. However, it is well known that some applanation tonometers produce the tonographic effect, which is a phenomenon in which IOP values decline with repeated tonometry. (Zimmermann, et al., 2017; Gaton et al, 2010; Krakau and Wilke, 1971; Moses, 1961; Stocker, 1958). In a study of repeated rebound tonometry in children, the tonographic effect did not occur (Dosunmu et al., 2014). However, in mice, repeated rebound tonometry in resulted in a significant reduction of IOP readings, a reduction of 2 mmHg after 10 measurements (Morris et al., 2006). To our knowledge, there is no information about canine corneal behavior due to repeated measurements with rebound tonometry. Thus, we cannot discard the possibility that the tonographic effect occurred in this study, and this would have particularly affected measurements made in the peripheral positions since these were evaluated last in every case and these values did consistently underestimate IOP. Further studies are needed to investigate the tonographic effect with rebound tonometry in canine corneas.

Spearman coefficients showed a positive correlation of IOP values in misaligned positions with CC, however it was ≤0.5069 (LC), due to the small range of IOP values. For comparison, the dispersion graphs also show the positive correlation, but due to the small range of values, determination coefficients were also low. Additionally, the Bland-Altman graphs show that the wider range of limits of agreement for the peripheral positions means that the rebound tonometry measurements in these positions are less reliable.

Von Spiessen and colleagues (2013) also found underestimation of IOP in dogs when the tonometer was in an angled position. In human patients, 10º degrees of angulation can result in a statistically significant underestimation of IOP readings (Beasley et al., 2013), however this effect was not seen in rats with tonometer used in an angled position of 25º degrees (Kontiola et al., 2001). Rebound tonometry calculates IOP based on parameters of movement; such as the time spent in contact with the cornea, return velocity and deceleration. The last parameter is intimately correlated to IOP (Kontiola, 1997). According to Newton`s third law (action-reaction principle) when a body exerts a force on a second body, simultaneously, the second body exerts an equal and opposite force (Newton and Motte, 2016). Force is a vector quantity and depends on mass and acceleration (F = mass * acceleration). In the case of rebound tonometry, the deceleration of the probe when contacting the corneal surface will be one of the main determining factors for the IOP result, since the mass of the probe remains constant. When the probe is applied at an angle, the force will be distributed according to vectors, and consequently, the IOP values will be lower. The equation used in mechanics can be extrapolated to give the resultant force at a determined angle and can be written as $F_{\text{angled position}} = F_{\text{central cornea}}$ * Cosine (angle), in which cosine will be always be < 1, resulting in a lower IOP. This equation is for a hypothetical environment without considering the effects of variables such as gravity, air resistance or probe slippage, nevertheless it gives an approximate IOP value for any determined angle.

Interestingly, the only position in which there was no significant difference compared with CC was DC. IOP measurements are lower at DC than CC, as would be expected in angled positions; however, in the DC position gravity facilitates the probe´s return to its original position.

There was thus some degree of agreement between the results of Von Spiessen and colleagues (2013) and the results of our investigation. In both studies, angled positions underestimated IOP. The difference was however, lower in our study. This disparity between the studies may be due to differences in the positions investigated, since Von Spiessen and colleagues (2013) assessed only two angulations (dorsally and ventrally), whereas our study investigated laterally and medially angled as well, providing more results with less interference of gravity.

The IOP results obtained in peripheral positions in this study are in disagreement with the values found by Von Spiessen et al. (2013) in which the values of IOP in these locations were overestimated. Our results show an underestimation of IOP when measurements were made in the peripheral cornea, a similar finding to results previously reported in human medicine (Muttuvellu et al., 2012; Queirós et al., 2007, González-Méijome et al., 2006). Is well known that corneal thickness has a positive correlation with IOP values measured by rebound tonometry in different species, including dogs (Chui et al., 2008; Sahin et al, 2008; Prashar et al., 2007; Rao et al., 2014; Park et al., 2013; Park et al., 2011; Poostchi et al., 2009; Harada et al., 2008; Martinez-de-la-Casa et al., 2005). The cornea is thicker peripherally than centrally (Strom et al., 2016; Gilger et al., 1991.), however in paraxial positions there was no significant difference from central corneal thickness (Strom et al., 2016). In our study, measurement was made at a distance of 3 mm from the limbus, whereas Von Spiessen et al. (2013) made measurements at approximately 1.5 mm from the limbus. This difference in distance might possibly explain the disparity of IOP values in both studies, since the cornea is thicker nearer the limbus.

However, the increase in thickness of the peripheral cornea does not necessarily mean an increase of hardness. The distribution of collagen fibers can change corneal elasticity, and these are more compressed in the central cornea than in the periphery in dogs (Nagayasu et al., 2009). Similar changes in collagen distribution have been reported in man (Boote et al., 2003). The underestimated IOP values found in peripheral positions in this study resembles the studies in human eyes (Muttuvelu et al., 2012; Queirós et al., 2007, González-Méijome et al., 2006). The underestimation of IOP values in peripheral positions may be a due to greater elasticity of peripheral cornea, resulting in a smaller deceleration of the rebound tonometer probe and, consequently, a lower value of IOP. Conversely, Yamashita and colleagues (2011) found overestimation of IOP in peripheral positions in the human eye; however the experimenters asked the patients to move their eyes to make the measurements, and this can apply tensile forces to the cornea due to extraocular muscles movements.

The underestimated IOP values found in the current study in peripheral positions could also be a result of the angled position of the tonometer assumed due to natural corneal curvature, and besides that, slip of rebound tonometer on corneal surface could also be a possibility. The values obtained show that the rebound tonometer measurements are somewhat less robust in peripheral positions, as previously shown in a study in chickens (Prashar, et al., 2007), which also showed weaker positive correlation with CC and a wider limit of agreement in Bland-Altman analysis.

Besides central corneal thickness, there are other corneal biomechanical properties that may influence IOP values measured by tonometry (Liu and Roberts, 2005), such as hysteresis, corneal resistance factor (Chui et al., 2008; Liu and Roberts, 2005; Deol et al., 2015; Ogbuehi and Osuagwu, 2014), corneal curvature (Harada et al., 2008; Liu and Roberts, 2005; Matsumoto et al., 2000) and pre-corneal tear film (Zeng et al., 2008). These biomechanical features may have a greater influence than thickness on IOP readings in the central cornea and have a significant correlation with IOP values measured by rebound tonometry (Chui et al., 2008; Deol et al., 2015). All of these properties should be considered during IOP investigations in dogs, given that they may markedly reduce or increase the measured value of IOP. However, investigating such factors is difficult and costly given the complex equipment required, this probably explains the lack of validated studies of corneal biomechanical properties in dogs.

2.6 CONCLUSION

The present study has shown that there are small, but significant, differences between TonoVet® measurements taken in different corneal positions and angles. However, since the values all fall within the normal IOP range, these differences are not substantially different from CC and thus is appropriate to say that the device still provides clinically relevant results when positioned off-center and off-angle.

2.7 CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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3. TOPICAL OCLACITINIB 0.1% AND TACROLIMUS 0.01% COMPARATIVE EFFICACY STUDY IN CANINE KERATOCONJUNCTIVITIS SICCA

3.1 ABSTRACT

Objective

Assess the efficacy of oclacitinib 0.1% as a single drug and combined with tacrolimus 0.01% to control ophthalmic signs of keratoconjunctivitis sicca (KCS) in dogs.

Animals studied

Thirty-two dogs diagnosed (57 eyes) with idiopathic KCS were used. Inclusion criteria were Schirmer Tear Test (STT) values lower than 15mm/min and consequential clinical signs such as ocular hyperemia and discharge.

Procedures

The animals were submitted to a randomized, open-label, 5-week-study and divided in three treatment groups treated with the following ophthalmic solutions: (1) Oclacitinib 0.1%; (2) Oclacitinib 0.1% + tacrolimus 0.01% and; (3) Tacrolimus 0.01%. All eye drops were instilled twice a day on the affected eyes. In the follow-up, STT, clinical sign and drug tolerability information were assessed.

Results

Oclacitinib improved the average STT values in 2.4mm/min, but did not reach significant difference ($p < 0.05$), the same occurred with clinical scores. Tacrolimus alone and associated with oclacitinib increased mean STT values in 10.7mm/min and 11.1mm/min, respectively, and both groups achieved significant improvement on day 7 ($p < 0.05$), that persisted until the end of the study. Clinical scores also improved significantly in both groups.

Conclusions

Oclacitinib 0.1% twice a day is inefficacious to treat and control the ocular signs of KCS in dogs. Tacrolimus in a dose of 0.01% increased STT values significantly and can be used as starter dose for mild to moderate cases of KCS. The combination of both drugs were similarly efficient to tacrolimus, however a synergism did not occur, which does not justify its use in KCS.

KEY WORDS: dog, keratoconjunctivis sicca, JAK inhibitor, oclacitinib, calcineurin inhibitor, tacrolimus

3.2 INTRODUCTION

Keratoconjunctivis sicca (KCS) is a condition of insufficient tear production. It has multiple etiologies.[1-6] However, in the majority of cases, it occurs as a consequence of an immune-mediated disease, in which mononuclear inflammatory infiltration can promote lacrimal and/or nictitating gland atrophy, epithelial cell apoptosis and fibrosis by acantholysis and conjunctival degeneration, reduction of goblet cells density and hyperkeratosis.[1,7-10] This disease commonly decreases aqueous portion production to pathological levels, causing tear instability. Furthermore, tear quality also depends on preserved mucin and lipid components of the tear film.[8,11-12] The importance of the disease remains in its high prevalence and that can be deleterious for the animal´s quality of life. Additionally it can be used as a spontaneous animal model for similar disease in human beings.

Tears contributes to the ocular surface´s nutrition, as a main source of oxygen, and bring mechanical and biochemical protection by bringing lubrication and helping the eyelids´debris removal and by producing lysozyme, lactoferrin and immunoglobulins, respectively. Its deficiency can cause corneal and conjunctival inflammation and injuries. In severe cases can result in blindness by pigmentary keratitis or ulcerative keratitis leading to corneal perforation.[13] The first ocular signs perceived by the owner in aqueous deficiency are the loss of corneal brightness and transparency, ocular congestion and the presence of a mucopurulent discharge.[1] To diagnose KCS, specific ophthalmic tests can be used, such as Schirmer Tear Test (STT), break-up time of the tear film with fluorescein staining and corneal staining with lissamine green and Rose Bengal.[14] STT is the most used diagnostic test and have different types, being the type 1 the most common. STT type 1 consists in the measurement of baseline and reflex production of lacrimal gland by using a strip of Whatman filter number 41, of 5 mm of width and 35 mm of length put inside ventral conjunctival fornix. This test does not require topical anesthesia, once reflex production by the presence of the paper in the conjunctiva is necessary to validate the test. Normal STT1 values stablished for dogs are between 15 and 25 mm in 1 minute, and results below 15 mm/min accompanied to ophthalmic signs such as hyperemia, mucoid discharge accumulation and keratitis, confirm KCS.[13]

The widespread treatment of choice is based on topical calcineurin inhibitors, such as cyclosporine and tacrolimus that have lacrimostimulatory and immunomodulatory effects.[11,15-22] These drugs are capable to control clinical signs and restore corneal health. Both drugs interfere on interleukin-2 transcription and reduce lacrimal gland inflammation. The treatment aims to reestablish tear production, avoiding KCS complications. Currently, alternative and supporting treatments are being studied to achieve better overall results, to be applicable in refractory cases, reduce costs and improve treatment acceptance by the owner.[23-28]

Oclacitinib maleate is a novel drug for treatment of pruritus related to allergic skin diseases and atopic dermatitis in dogs.[29] It is a janus kinase (JAK) inhibitor, with JAK1 selectivity and well-tolerable by dogs.[30-31] JAK are protein-tyrosine kinases that play an important role in intracellular signaling, regulated by cytokines, such as interleukins, hormones and interferons. The activation of JAK results in specific gene expression due its pathway and its dysregulation can lead to autoimmune, inflammatory and neoplastic disorders development.[17] Tofacitinib, that is a JAK inhibitor, were studied in dry eye disease in human beings, showing a significant clinical response[32] so, the hypothesis that oclacitinib can have an action on KCS in dogs appeared to be applicable.

The main objectives of this study were: to evaluate the efficacy of oclacitinib, compare it to tacrolimus, to control KCS clinical signs, such as ocular inflammation and tear production assessed by STT1.

3.3 MATERIAL AND METHODS

Thirty-two client-owned dogs that were presented to the Comparative Ophthalmology Laboratory at Veterinary Teaching Hospital of Federal University of Paraná with clinical sings of quantitative KCS were submitted to a randomized, open-label efficacy study. A total of 57 eyes were submitted to the treatments. In which 19 were females and 13 males. Different breeds were present in this study, in which mixed-breed, Poodle and Lhasa Apso were over-represented, as 8, 7 and 6 individuals, respectively.

Others breeds presented were Akita, English bulldog, Cocker Spaniel, Pekingese, Pinscher, Pug, Scottish terrier and Yorkshire terrier.

The 5-week study followed the ARVO Statement for the use of Animals in Ophthalmic and Vision Research. The Institution´s Animal Care and Use Committee approved the research and the owners signed a consent term of the participation of the study.

All patients were submitted to a complete eye and adnexa examination, performed before and after mydriasis promoted by a tropicamide 1% eye drop (Mydriacil®, Novartis®, São Paulo, Brazil), which consisted in tonometry (Tonovet®, Tiolat Ltd.®, Helsinki, Finland) and slit lamp evaluation (Hawk Eye®, Optotek Medical®, Slovenia), indirect ophthalmoscopy (Welch Allyn®, New York, United States of America), Schirmer Tear Test (STT) type 1, Fluorescein staining with strips (Drogavet®, Curitiba, Brazil) and photographic records. All the procedures were made with a gentle physical restraint.

The inclusion criteria to the study were STT less than 15mm/min and no previous history of treatment for KCS. The dogs with less than 15mm/min of tear production and presenting ophthalmic signs that included ocular discharge, ocular surface inflammation, corneal vascularization and/or pigmentary keratitis, were diagnosed with KCS.

The drugs used in this study were tacrolimus and oclacitinib, manipulated aseptically into corn-oil based eye drops by Drogavet® (Curitiba, Brazil) compounding pharmacy in a sterile manner, in a controlled environment for humidity and temperature. The developed eye drops were placed in white opaque polypropylene bottles.

The 32 were sorted in three groups of treatment, which were: (O) oclacitinib maleate 0.1%, (OT), oclacitinib maleate 0.1% + tacrolimus 0.01% and; (T) tacrolimus 0.01%. Two groups had 11 dogs, O and OT, and one group had 10 dogs (T). The owners were instructed to instill one drop on each affected eye every 12 hours, to maintain as close as possible the interval between instillations, and to store the eye drops in -4° C (refrigerator). Exclusion criteria were previous use of other drugs such as ophthalmic solutions or systemic medications, failure of the owner to attend to the follow up consultation or the diagnosis of a different cause of KCS, other than immune-mediated disease, such as neurogenic, iatrogenic and infectious diseases. The follow up consultations were made in the days 0, 7, 15, 30 and 45 after the beginning of the treatment and consisted in ophthalmic evaluation plus questions about drug tolerability. Objective results were obtained by STT. Subjective evaluation consisted in ocular discharge accumulation and hyperemia, both classified using a score system: None (0); Moderate (1) and; Severe (2). Photographic records were made for before and after comparative assessment of clinical aspects such as secretion accumulation and corneal and conjunctival inflammation.

3.3.1 Statistical analysis

The collected data of each eye were evaluated separated for statistical analysis. The STT values obtained in the follow-up of the drugs were submitted to a Shapiro–Wilk normality test. STT was normally distributed and ocular discharge and hyperemia scores were not. Normally distributed data were subjected to ANOVA followed by Tukey-Kramer *post hoc* tests. Kruskal-Wallis were used for ocular discharge and hyperemia scores. All statistics were analyzed by StatView 5.0 (SAS Institute Inc, Cary, North Carolina, USA) for Windows.

3.4 RESULTS

The average value of STT of all dogs before treatment were 6.61 ± 4.94 (0 to 14 mm/min). Concerning individual eyes, left eye showed higher values than right eyes on day 45 ($p < 0.05$), being more responsive to treatments. Data of gender, age and breed are detailed of the dogs treated are on Table 1. Besides the 32 dogs treated, other 8 were withdraw from this study due to systemic drugs usage (4 dogs) and owner noncompliance (4 dogs). The 4 dogs withdrawn for systemic drug usage were due to surgical treatment of mammary tumors (1) and clinical treatment of atopic dermatitis (2) and intervertebral disc disease (1), these patients received oral anti-inflammatories and/or antibiotics. The causality of the disease development correlated to ophthalmic treatments were unlikely, because prior ophthalmic treatment, they already had these comorbidities diagnosed. Inside the groups, this study showed significant increase of STT values of OT and T groups, but no in O group. Table 2 summarizes mean and standard deviation of each group separately. The same occurred with ocular discharge and ocular hyperemia scores, being OT and T groups with significant improvement ($p < 0.05$), which did not occur with

O group. The figures 1 and 2 illustrate with graphs the results STT values obtained during follow-up.

Table 1. Summary of 31 dogs submitted to three different treatments divided into their respective groups. The information about gender and breed, as well as descriptive statistics (mean and standard deviation) for age in years. O: Oclacitinib 0.1%; OT: Oclacitinib 0.1% and Tacrolimus 0.01%; T: Tacrolimus 0.01%.

	Groups			
	O	OT	T	Total
Gender (n[%])	11	10	10	31
Female	5 [45.5]	7[70]	6[60]	18[58.1]
Male	6 [54.5]	3[30]	4[40]	13[41.9]
Breed (n[%])	11	10	10	31
Purebred	7 [63.7]	7[70]	9[90]	23 [74.2]
Cross-breed	4 [36.3]	3[30]	1[10]	8 [25.8]
Age in years (mean ± standard deviation [minimum; maximum]	11 ± 4.86 [4;17]	9.55 ± 3.05 [4;13]	9.4 ± 3.37 [5;15]	10 ± 3.82 [4;17]

Table 2. Descriptive statistics (mean and standard deviation) and ANOVA analysis of variance of Schirmer Tear Test (STT) in mm per minute of dogs submitted to three different treatments on follow-up days of the study. * Means statistical significant difference compared to day 0 ($p < 0.05$). O: Oclacitinib 0.1%; OT: Oclacitinib 0.1% and Tacrolimus 0.01%; T: Tacrolimus 0.01%.

	Days of follow-up				
	0	7	15	30	45
O	7.95 ± 5.0	10.10 ± 7.9	10.42 ± 7.3	9.89 ± 6.1	10.36 ± 7.1
OT	4.89 ± 5.0	11.16 ± 6.9*	11.32 ± 7.5*	13.21 ± 6.8*	15.68 ± 7.8*
T	7 ± 4.42	12.97 ± 6.45*	15.84 ± 5.48*	17.42 ± 4.4*	17.79 ±5.47*

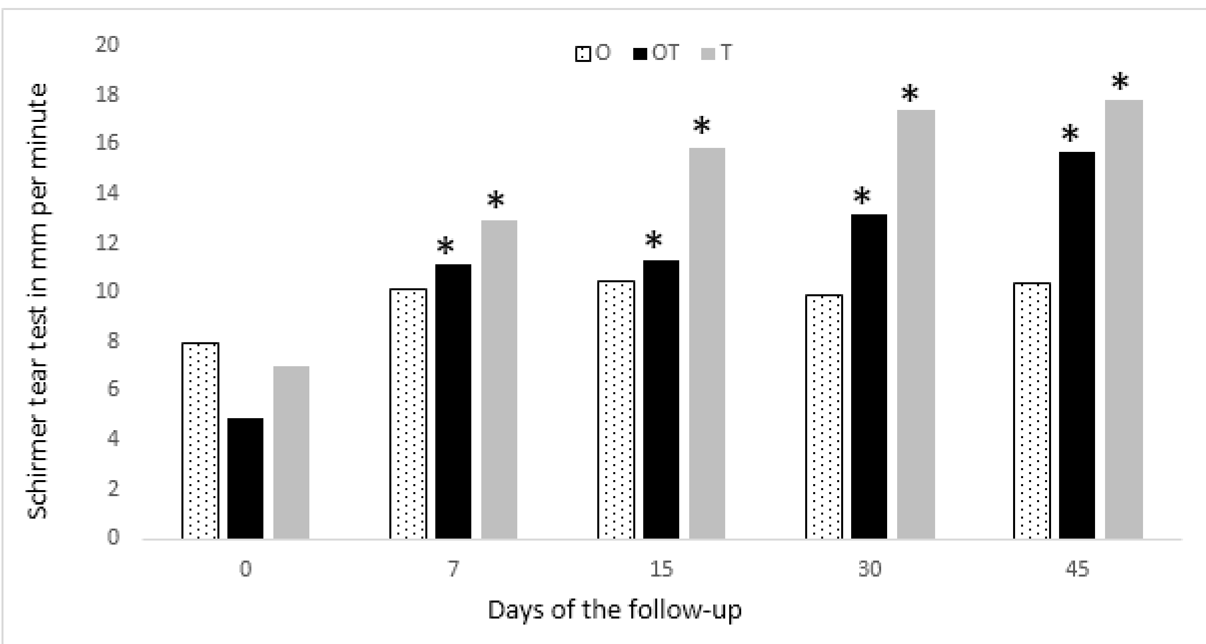

Figure 1. Graph of Schirmer tear test (STT) mean in mm per minute of three treatment groups of dogs with keratoconjunctivitis sicca on follow-up days. *statistical significant difference compared to day 0 ($p <0.05$). O: Oclacitinib 0.1%; OT: Oclacitinib 0.1% and Tacrolimus 0.01%; T: Tacrolimus 0.01%.

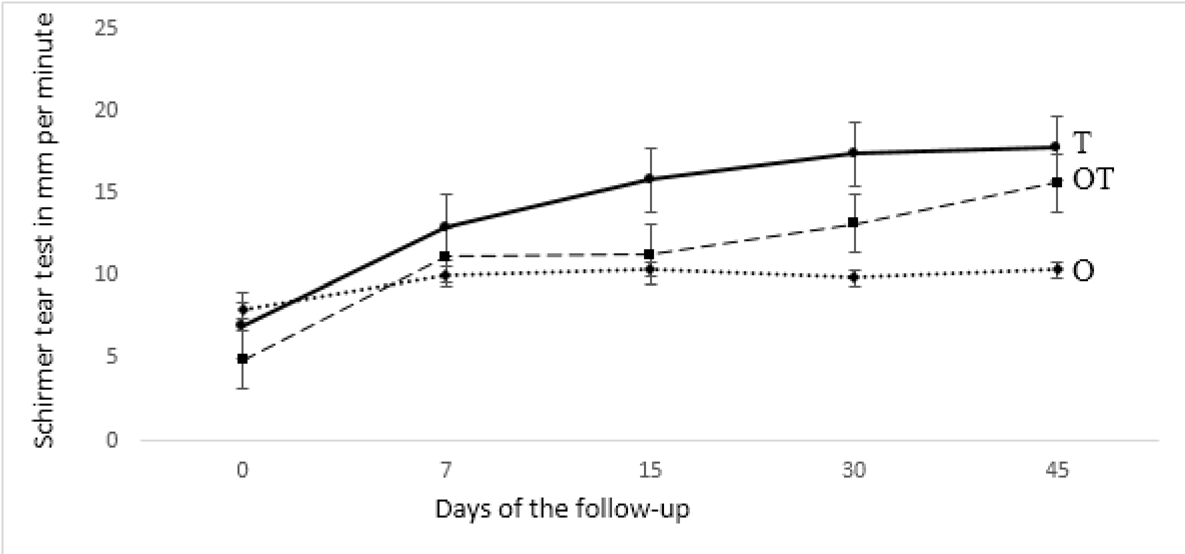

Figure 2. Linear graph of Schirmer Tear Test (STT) mean (and standard error) in mm per minute of three treatment groups of dogs with keratoconjunctivitis sicca on follow-up days. O: Oclacitinib 0.1%; OT: Oclacitinib 0.1% and Tacrolimus 0.01%; T: Tacrolimus 0.01%.

3.4.1 Oclacitinib (O group)

In O group, STT values started day 0 with mean and standard deviation of 7.95 ± 5.05, and the final day (day 45), these values increased to 10.37 ± 7.10, with a mean increase of 2.42 of STT values. However, with significance level of 5%, the p-value were > 0.05, showing no significant statistical improvement during the study. Considering ophthalmic clinical signs scores, ocular hyperemia and discharge did not had a significant reduction (p >0.05), with median of 1 unaltered from day 0 to 45. Despite low resolution of ocular signs, the acceptance of the owners and patient tolerability were high, except for one case, in which the dog, a Yorkshire terrier, became intolerant to the eye drops instillation at day 30 of this study. No other patient demonstrated this behavior. Figure 3 shows photographs of a Lhasa Apso during follow-ups submitted to treatment of O group.

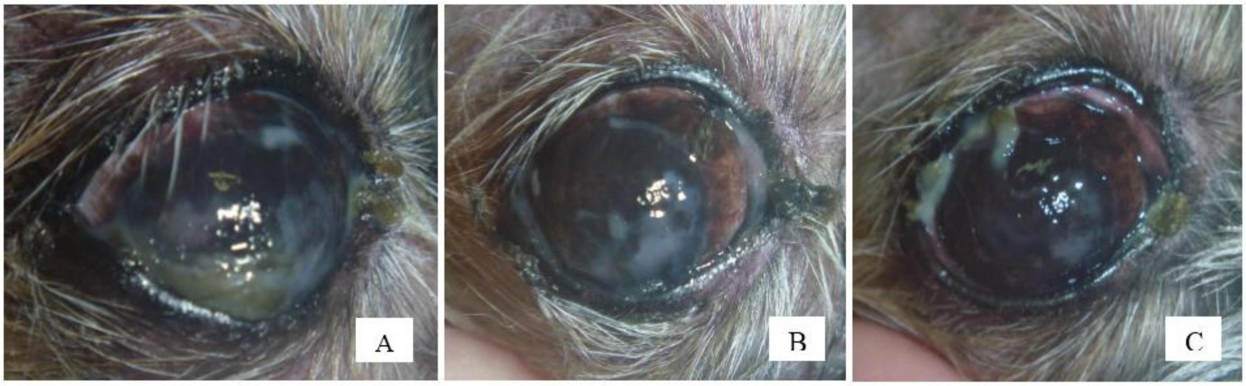

Figure 3. A ten-year old female Lhasa Apso with keratoconjunctivitis sicca on left eye during follow-up with Oclacitinib 0.1% treatment. A: Day 0, STT value of 0 mm/min. B: Day 15, STT value of 0, with low reduction of ocular discharge. C: Day 45, STT value of 0 mm/min, no clinical improvement were noted.

3.4.2 Oclacitinib + tacrolimus (OT group)

During the study, OT at day 0 started with a mean and standard deviation of STT values of 4.89 ± 5.06, and at day 7 already achieved significant improvement ($p < 0.05$) of tear production of 11.16 ± 6.9, and in the last day of study (45 days) showed a mean and standard deviation of 15.68 ± 7.86. The improvement on tear production were 10.79 on STT values comparing the first day with the last day. Statistical significance difference were obtained in all days of follow-up compared to day 0 ($p < 0.05$). Concerning ophthalmic signs scores, clinical improvement were observed following the increase of STT values, in which ocular hyperemia and discharge scores median varied from 1 to 0, with significant difference ($p < 0.05$). Owner acceptance and patient tolerability were high. No irritability, such as pruritus or pain were observed in the patients. Figure 4 shows a Pekingese treated with OT group eye-drops on follow-up days.

Figure 4. A twelve-year old male Pekingese with keratoconjunctivitis sicca on right eye during follow-up with Oclacitinib 0.1% and Tacrolimus 0.01% treatment. A: Day 0, STT values of 4 mm/min. B: Day 15, STT values of 9 mm/min, ocular improvement with reduction of ocular redness and discharge. C: Day 45, STT values of 8 mm/min, ocular appearance remained improved.

3.4.3 Tacrolimus (T group)

The T group started day 0 with STT values of 7 ± 4.43 (mean and standard deviation), at day 7 achieved significant improvement ($p < 0.05$) with values of 12.95 ± 6.45 and at the last day (45) showed results of mean of 17.79 ± 5.47. The difference between the last and first day were 10.74. Statistical significant improvement remained all days compared to day 0 ($p < 0.05$). About ocular signs, ocular hyperemia and discharge scores had a significant reduction ($p < 0.05$) from 1 to 0. There was no ocular irritability by patients and all owners showed good acceptance. Figure 5 shows a Pug treated on group T during follow-up days.

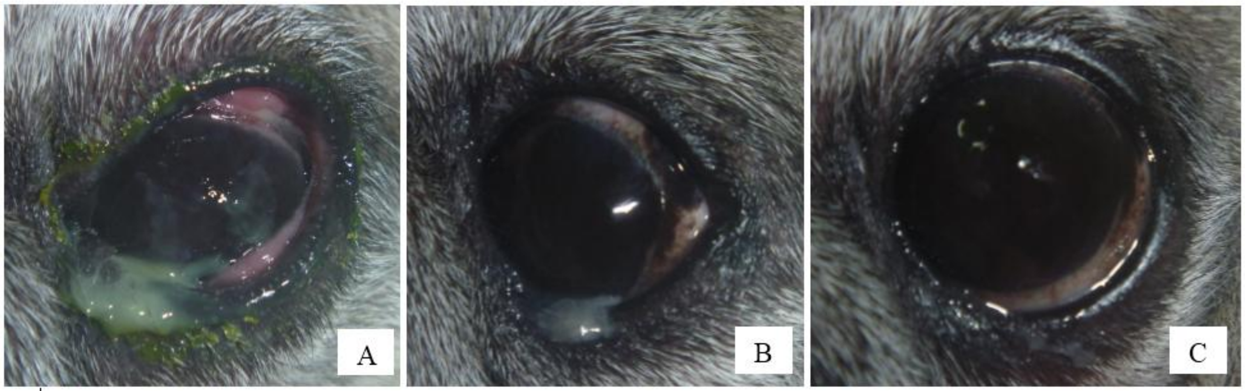

Figure 5. An eight-year-old female Pug with keracotonjunctivis sicca on right eye during follow-up with Tacrolimus 0.01% topical treatment. A: Day 0, STT values of 4 mm/min. B: Day 15, STT values of 10 mm/min, note reduction of ocular redness and ocular discharge and increase of ocular brightness. C: Day 45, STT values of 13 mm/min, ocular appearance still improved and no ocular discharge observed.

3.4.4 Comparison between groups

Comparison between groups showed the beginning of significant difference between T and O on day 15 ($p < 0.05$), which continued thorough the study, until day 45, with higher STT values on group T. The same occurred between OT and O only on day 45 ($p < 0.05$), with higher STT values on group OT. Among groups OT and T, significant difference occurred on day 15 and 30 ($p < 0.05$), with higher STT values on group T. At day 45, both groups reach similar results.

Details of STT mean differences between groups and days of follow up are summarized on table 3.

Table 3. Mean differences between treatment groups of Schirmer Tear Test (STT) in mm per minute values of dogs with keratoconjunctivitis sicca. Statistical analysis with ANOVA analysis of variance and Fisher`s post hoc least significant difference (LSD) to compare results. * Means statistical significant difference between groups (p < 0.05). O: Oclacitinib 0.1%; OT: Oclacitinib 0.1% and Tacrolimus 0.01%; T: Tacrolimus 0.01%.

	Days of follow-up				
	0	7	15	30	45
O, OT	3.05	-1.05	-0.89	-3.32	-5.32*
O, T	0.97	-2.84	-5.42*	-7.53*	-7.42*
OT, T	-2.11	-1.78	-4.52*	-4.21*	-2.12

3.5 DISCUSSION

Topical oclacitinib maleate is inefficacious on treatment and control of the signs of KCS in dogs, even it discreet increase STT values. When compared to its combination with tacrolimus or tacrolimus alone, there was a significant statistical difference (p <0.05), and with faster ophthalmic improvement achieved by tacrolimus alone, showing absence of synergism of drugs association. Nevertheless however, appropriate efficacy were seen in many dogs with a lower dose of tacrolimus (0.01%) than previously reports (0.02%).[15,18]

Oclacitinib is the first JAK inhibitor developed and liberate for use in dogs and it is considerate selective for JAK1 enzyme.[33] JAK-dependent cytokines and some growth factors utilize JAK pathway to activate DNA transcription, the result is transduction of interleukins that evokes and maintain inflammation and pruritus in allergic diseases[34-35] and also reduces T-cell proliferation.[33-35]

When used for atopic dermatitis, oclacitinib decreases pruritus in less than 24 hours,[35-37] and significant clinical improvement in one week of use.[38-39] Due to oclacitinib immediate cytokine downregulation, it was expected that resolution of clinical signs or increase of tear production occur fast, at least, less than 45 days as objective of this study. However, the action mechanisms on lacrimal gland autoimmune diseases may be different when compared with pruritus resolution in skin allergic diseases.

Topical JAK inhibitors were studied in human beings and mice,[34,40-41] however, to the best of the authors’ knowledge, there is no topical studies in dogs, neither for skin nor ophthalmic. The advantage in topical solutions is that JAK inhibitors are small molecules that easily penetrate skin and mucosa barrier and are well tolerate by human patients.[42] Topical oclacitinib and tofatinib in mice showed reduction of the skin swelling that was not seen in orally treated animals, consequence of reduction of epidermal hyperplasia, parakeratosis, dermal edema and inflammatory cells infiltration.[34-41]

Since, the inflammatory response depends on dose of JAK inhibitors,[43] in our study, we used oclacitinib in a dose of 0.1%, less than topical previous studies in mice, which 0.25% concentration already had a positive result on skin.[34] However it`s difficult to establish that the absence of clinical improvement occurred due to small concentration of oclacitinib, and other pharmacokinetics characteristics must be assessed. Drug delivery throughout conjunctival tissue is also a concern about general efficacy. Conjunctival permeability for ophthalmic compounds suggest that there is a dependence on molecular size, due to conjunctival permeability prefer lipophilic molecules.[44] Oclacitinib is a molecule with lower weight when compared to tacrolimus and cyclosporine[45] and its expected more ocular tissue absorption and consequently, efficacy. About conjunctival retention, is known that ocular surface tissues provides tacrolimus a prolonged retention of hours.[46] There is no information available about drug delivery on ophthalmic tissues for oclacitinib, so absorption and release of the drug could also influence the results.

Tofacitinib is a JAK3 inhibitor studied in dry eye disease in human patients with good efficacy, showing immunomodulatory activity, reducing cytokines and inflammatory biomarkers.[47-48] Ocular comfort and clinical signs and symptoms significantly improved with tofacitinib, being even more effective than cyclosporine,[32] however with no correlation with STT values, which not increase significantly.[32,48-49] Different from tofacitinib, Oclacitinib is predominantly JAK1 inhibitor, which could lead to different pathways of action, leading to poor efficacy seen in this study. However, the dry eye disease of human patients are different from KCS. The parameters of efficacy for dry eye disease are clinical improvement and for KCS are STT levels. JAK inhibitors improved the clinical symptoms in human patients, however not increased STT values.[32,48-49] In our study, STT values still low with oclacitinib, clinical signs improvement not occurred due to tear deficiency and severity of KCS also contribute to treatment failure.

Topical tofacitinib were also studied in experimental corneal injuries and suppressed corneal neovascularization e epitheliopathy pro-inflammatory chemokine gene and immunoreactivity of inflammatory cells.[47,49-50] This was not seen in the cases of oclacitinib use, corneal inflammation and vascularization still with same intensity during the treatment, analyzed by clinical scores and photographic register. Which can occurred by non-improvement of tear production. Further investigations are required on corneal inflammation and the use of JAK inhibitors in dogs without KCS, because the absence of tear could constantly damage cornea, avoiding its amelioration.

The improvement of STT in some cases with the use of oclacitinib only, but maintaining of clinical signs such as secretion accumulation and conjunctival hyperemia can suggest that ocular irritation is a possibility, and the increase of tear production could be a reflex response for this or by the use of the vehicle. However, ocular hyperemia alleviate in the group OT, so, tacrolimus could increase the tear production alone and alleviate clinical signs of irritation. The absence of a placebo group is a limitation for this study, once that there is a possibility of vehicle causing irritation to eye surface.

Tacrolimus is a macrolide antibiotic and topical calcineurin inhibitor that avoids transcription of pro-inflammatory cytokine genes by acting on T-cell activation cycle, having similar pharmacological profile of cyclosporine, and considered more potent, achieving same results with lower concentrations.[17,19] This is the first report of tacrolimus efficacy with the dose of 0.01%, half the dose of previously reports (0.02%).[15-18] Our study achieved significant improvement and can be used as starter dose in mild and moderate cases. This diminished dose can also be used in small dogs, with regards to potential collateral effects due to conjunctival absorption and systemic distribution, as seen in dogs receiving cyclosporine,[51] but not yet studied with tacrolimus.

Tear production is also related to neural and hormonal control[7,52] so, besides all immunosuppresive action, lacrimoestimulants effects of tacrolimus and cyclosporine may occur due in this pathway. This action is not well comprehended, however it can be due to specific binding protein cyclophilin, which is ligand for natural prolactin. Prolactin associated with neuro-hormonal signaling acts on peroxidase release on lacrimal tissue and consequently, on lymphocytes, being regulatory for tear production.[52-53] This drug action cannot be achieved by some drugs, such as corticosteroids, and based on this study, the same for oclacitinib.

Tacrolimus eye drops have a good stability if stored correctly at low temperatures inside plastic bottles.[54] Other JAK inhibitor maintained efficacy as eye drops on human patients, however, it cannot be affirmed that all JAK inhibitors will stay stabilized on eye drops and, maybe, oclacitinib diminished its concentration during the study and lost effectiveness, even if there was just five weeks.

The mucin layer of the trilaminar tear film is also important to ocular surface health maintenance, once its biochemical properties guarantee quality, providing better attachment to aqueous layer. In KCS, there is a reduction of goblet cells and hypersecretion of bad quality mucin stimulated by interleukins, giving the clinical appearance of KCS.[1,8,12,55] If oclacitinib improves mucin layer, we expected to see an amelioration on ocular surface brightness and clinical score, even without improvement of STT levels. This occurred in the groups with tacrolimus, with reduction of ocular redness and discharge, even with low STT values, similar to what occurs with cyclosporine.[22]

Others limitations of this study refers to the small number of patients assessed and open-label study. The open-label study can induce owners and evaluator to perceive false clinical improvement, however, STT serve as an objective evaluation and confirm response to the treatments, however we cannot exclude that bias have occurred.

This is the first report of JAK inhibitor use on ocular disease in dogs, studied in an immune-mediated disease with no efficacy seen. However, due to its inflammation control proved actions on cornea,[47,49,50] it should be considered in further studies of ocular inflammation.

3.6 CONCLUSION

This study showed that oclacitinib maleate 0.1%, twice a day, is inefficacious on treatment of canine KCS, even it increased STT lightly, it was not sufficient to be significant. On the other hand, tacrolimus showed efficacy and with a lower dose (0.01%) than previously reported. Synergism between drugs were not observed.

3.7 ACKNOWLEDGMENT

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3.8 CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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4. OPHTHALMIC HEALTH AND REFRACTIVE ERRORS IN WORKING DOGS

4.1 ABSTRACT

Objective

To assess ophthalmic abnormalities and refractive errors in police and guide dogs in Brazil.

Animals studied

Seventy-one dogs were used, comprising 141 eyes evaluated. Of them 10 were guide dogs, and 61 police dogs.

Procedures

Work performance were assessed by a questionnaire to each dog´s owner. All dogs underwent a complete ocular examination, and the abnormalities found were classified by disease and then in active/inactive and if they were located or not at the visual-axis. Sixty-three dogs were submitted to streak retinoscopy and its eyes classified in emmetropic or ametropic.

Results

Ophthalmic abnormalities were detected in 38 (54%) of the dogs, in which 10 were located in the visual-axis and 25 considered active diseases. Inherited disorders comprised 60% of the diseases. Incipient cataracts were the most prevalent disease. None guide dog had a disease on the visual-axis. The most common refractive error was myopia with median and interquartile range of -0.75 ± 0.75 diopters, among these, police dogs had -1.0 ± 0.5 diopters, whereas guide dogs +0.38 ± 0.71 diopters. The overall work performance were considerate good in these dogs and no signs of visual impairment were seen in any dog.

Conclusions

A considerable number of ophthalmic abnormalities and refractive errors were diagnosed in working dogs. Performance, however, seemed not be considerably affected. Police dogs had a tendency to present myopia and guide dogs for emmetropia. Frequent ophthalmic follow-ups is advisable for working dogs. Many of the ocular conditions observed are inherited. Exclusion of severely affected dogs for breeding programs is recommended.

KEY WORDS: police dogs, guide dogs, eye, cataract, myopia, emmetropia, performance.

4.2 INTRODUCTION

Working dogs were always present on human history and currently are being used for a variety of purposes, being fundamental to the society in which they are included.[1-4] In large urban centers worldwide, two subgroups of working dogs are relatively common: police dogs and service dogs.

Police dogs develop a diverse number of activities and are chosen according to their main ability, including: Superior sense of smell to find narcotics or explosives and/or ability to track people; Capacity to provide protection and help in dangerous situations.[3-5] On the other hand, service dogs may assist people possessing disabilities, which include guide dogs for the blind. Guide dogs aid visually-impaired people, functioning as their "eyes" on their daily routines with confidence and attentiveness, ensuring safe mobility on their pathways.[2,6,7]

Working dogs are costly to select, train and maintain, for this reason there is pressure to keep these dogs developing their duties with quality for the longest period possible.[3,8] Disease development, can be particularly deleterious for these animals, forcing them to be withdrawn from service, putting them in early retirement or, in some cases, leading to euthanasia.[8-10] Maintenance of a good general health is crucial to provide a good work performance with decent life quality.

Ophthalmic health and visual acuity are very important to keep a working dog on duty. Excellent visual acuity requires absence of abnormalities on every transparent ocular structure located in the visual axis, adequate refractive proprieties and appropriate neuronal processing in the retina, visual pathways and visual cortex.[11] Ocular diseases can lead to some degree of visual impairment or even blindness. Refractive errors can cause defocusing leading to poor vision and an overall negative impact on performance.[12]

In previous surveys of police dogs, ocular diseases were responsible for 1.4% of medical emergencies,[8] 2.4% of discharges from the service[13], and, surprisingly, 11% of deaths and euthanasia.[10] For guide dogs, eye diseases constitute the cause of 3.2% of withdrawn from their services.[14]

Some of the breeds commonly used to develop these activities in fact have high predisposition to the development of ophthalmic disorders that can lead to blindness,[15-20] such as corneal diseases, cataracts and progressive retinal atrophy. These abnormalities can lead to progressive visual loss and may go unnoticed by the handlers, trainers or owners until it causes important visual impairment.

The presence of refractive errors greatly jeopardize visual acuity, and some breeds can develop myopia and hyperopia spontaneously,[21-27] causing incorrect focusing of the light rays onto the retina.[12] Defocus by -2.0 diopters can reduce visual acuity[28] and by -1.5 already worsens significantly dog`s performance to find distant objects.[12]

Therefore, the purpose of this study were to evaluate the prevalence of ophthalmic abnormalities and the presence of refractive errors in a population of police and guide dogs in the south of Brazil, besides to compare eventual differences between the type of job performed by the dog, the breed and age with the data found.

4.3 MATERIAL AND METHODS

The animals used were service dogs belonging to four different military and police corporations known as 20° Batalhão de Infantaria Blindada (20° BIB), Batalhão de Operações Especiais do Paraná (BOPE), Guarda Municipal de Curitiba (GMC) and Departamento Estadual de Prevenção e Repressão ao Narcotráfico (DENARC) and to the guide dog training school Helen Keller and three guide dog owners. The study were conducted by the Comparative Ophthalmology Laboratory of Federal University of Paraná State (UFPR).

A total of 71 dogs, comprising 141 eyes were evaluated. All dogs performed complete eye examination and ocular reflexes, among these, 63 made streak retinoscopy and the refractive errors were recorded. Twelve different breeds were present in this study and were American Pit Bull Terrier, Belgian Malinois, Bloodhound, Doberman Pinscher, Dutch Shepherd, Flat Coated Retriever, German Shepherd, Golden Retriever, Labrador Retriever, Rottweiler and White Swiss Shepherd. In which Belgian Malinois were overrepresented with 30 individuals, being 42% of the total. The handlers, trainers and users responsible for dogs give permission to use them in this study and signed a consent term. Aggressive dogs were not used because it would risk examiner`s safety and to avoid non-perception of small ophthalmic abnormalities.

The study were divided in three parts, the first consists on collecting dogs information, including their breed, gender, age, function and a survey of their performance. The second part were assessment of their ophthalmic health and the third, the refractive errors.

4.3.1 Working dogs information and classification

Data were collected of the dog’s gender, age and breed. After the identification, the handler/owner of the dogs answered basically three questions: (1) Definition of the dog function (e.g. guide dog, narcotic sniffer), (2) the ability to perform their tasks, (3) and if they observed ophthalmic signs of low vision such as stumble on obstacles.

Initially, dogs were separate by occupations, including police dogs and guide dogs (GD). Subsequently, the police dogs were also divided in 4 different categories and were sniffer dogs (narcotic and explosives) (SD); tracking dogs (search and rescue dogs and criminal tracking dogs) (TD); attack and protection dogs (patrol, scout and sentry dogs) (APD); and others (OD) (agility or still under training to define a service function).

4.3.2 Ophthalmic evaluation

The ophthalmic evaluations were made in semi-dark rooms and the dogs were submitted to a complete eye and adnexa examination, made before and after mydriasis promoted by tropicamide 1% eyedrop (Mydriacyl®, Novartis®, São Paulo, Brazil), indirect ophthalmoscopy performed with a Finoff transiluminator (Welch Allyn®, New York, United States of America) and a 20 diopters indirect lens (Volk Optical Inc.®, Mentor, Ohio, United States of America). Ocular reflexes tested consisted in performing pupillary light reflex, menace response, dazzle reflex, palpebral reflex and cotton ball reflex. Tonometry (Tonovet®, Tiolat Ltd.®, Helsinki, Finland), slit lamp evaluation (Hawk Eye®, Optotek Medical®, Slovenia), Schirmer Tear Test type 1 and Fluorescein staining (Drogavet®, Curitiba, Brazil) also were performed.

The ophthalmic abnormalities found were classified according to the disease and categorized in active or inactive and whether or not the location of the lesion was on visual-axis. Those considered active were due the need of clinical or surgical treatment and, and/or that can worse vision or ophthalmic health over time. The other variable summarized were the potential to prejudice vision due to obstruction of the visual axis. Retinal diseases, despite of the location, also were considered on visual-axis.

4.3.3 Refractive errors

The refractive errors were assessed by streak retinoscopy after pharmacologic mydriasis, using a retinoscope (Welch Allyn®, New York, United States of America) and a set of skiascopic lenses (Sunwin®, China). Briefly, streak retinoscopy were made with the retinoscope placed in front of evaluator's eye with a distance of approximately 65 centimeters of the dog. The equipment emits a streak light that allows see the ocular fundus reflex, vertical movements were made to find if this reflex follow the same direction as the emitted light. Afterwards, the skiascopy lenses were positioned 2 cm from dog's eyes and began at 0.25 diopters, increasing until 10 diopters. This applies to positive and negative diopters, which are equivalent to hyperopia and myopia, respectively. When the light fulfill the ocular fundus and light movement not occurs, means that the skiascopic lens neutralized the light, corresponding to the refractive error of that eye. By means of correction due to the distance of the evaluator and the dog, a reduction in –1.5 diopters from the found value were made. All the procedures were performed by the same veterinary ophthalmologist and were made with physical restraint of the animals.

For purposes of classification suggested by veterinary literature,[23] emmetropia were considered when the streak retinoscopy values were between –0.5 and +0.5 diopters. Ametropia were considered when an eye had myopia or hyperopia, being myopia when refractive errors were below -0.5 diopters, and hyperopia when the value were higher than +0.5 diopters. Furthermore, anisometropia were considered when a difference of refractive errors between the eyes of the same dog were ≥ 1.0.

4.3.4 Statistical analysis

The refractive errors values obtained and age of the animals were submitted to Kolmogorov-Smirnov normality test to detect if the data follow a normal distribution. The test showed that the distribution was non-Gaussian, and then, Spearman correlation were made for comparison between eyes of the same dog and for age and refractive errors. Median, interquartile range and range were calculated for refractive errors and separated by dog function and breed. Chi square tests were made to find if there was relationship between ocular disease and gender. For comparison between groups, Kruskal Wallis test were made, further, adjusted by Dunn post hoc method and Benjamini-Hochberg false discovery rate. Mann-Whitney test were carried to find if the dogs with ophthalmic disorders have significantly difference on age compared to those without diseases and to compare refractive errors between guide dogs and police dogs. *P*-value were fixed in 0.05 for significant statistical differences. All statistics were analyzed by StatView 5.0 (Cary, North Carolina, United States of America) for Windows.

4.4 RESULTS

Of the 71 examined dogs, 29 were females and 42 males, with the median age and interquartile range, expressed in years, of 1 ± 2.09, in which females have 1 ± 1.52 (ranging from 4 months to 10 years) and males 2 ± 2.52 (ranging from 2 months to 9 years). The details of the groups with information of breeds and gender are seen in table 1.

4.4.1 Working dogs distribution and performance survey

The quantity of dogs belonging to the categories were 10 guide dogs and 61 police dogs, of them: 24 sniffers dogs; 10 tracking dogs; 17 attack and protection dogs and 10 in training or other functions. Altogether, handlers, trainers and owners said that all dogs perform their tasks efficiently, and they not perceive any signals of visual impairment.

4.4.2 Ophthalmic abnormalities

In all, 38 (54%) of the 71 dogs had some ophthalmic abnormality detected, comprising 47 identified abnormalities. These dogs had a median age in years and interquartile range of 4 ± 2.5, compared to 1 ± 0.17 of dogs without ocular disorders. The age difference were statistically significant ($p < 0.05$) between the affected and non-affected dogs. There was no significant difference on gender ($p > 0.05$).

The most prevalent disease were incipient cataract, affecting 15 (21%) dogs, which they had a median age and interquartile range of 5 ± 1.5. Twelve have cortical incipient cataracts occupying less than 5% of the lens diameter and off-axis of vision, and three with cataracts in the visual axis, two located nuclear and one on anterior capsule. Of the breeds affected, were Belgian Malinois (5), Labrador Retriever (3), German Shepherd (3), Bloodhound (2), Doberman Pinscher (1) and Rottweiler (1).

The second most prevalent abnormality were persistent pupillary membrane, affecting 5 dogs. One dog had the right eye removed due a trauma in early months of age. Other diseases were nuclear sclerosis (4), chorioretinal scars (3), vitreous degeneration (3), follicular conjunctivitis (2), ectropion (2), blepharitis (2), distichiasis (2), iris atrophy (2), ectropion uveae (2), corneal dystrophy (1), Peters' anomaly (1), retinal dysplasia (1), palpebral nodule (1) and discoria (1). Of these abnormalities, 28 (60%) of them were considered inherited and are cortical incipient cataracts, persistent pupillary membrane, vitreous degeneration, ectropion, distichiasis, corneal dystrophy, Peters` anomaly and retinal dysplasia.

Considering the location of the diseases, 10 dogs had some abnormality in the visual axis, of them, 4 corioretinal abnormalities, 3 nuclear sclerosis alone, one dog with pupillary persistent membrane, Peters' anomaly and cataract and two dogs with nuclear incipient cataract, one of them with nuclear sclerosis. These dogs belongs to the SD (5), TD (1) and APD (4) groups. None of GD and OD group had ophthalmic abnormality on visual-axis.

About active diseases, 25 diseases affecting 24 dogs were included, and were cataracts (15), follicular conjunctivitis (2), blepharitis (2), distichiasis (2), retinal dysplasia (1), ectropion (2) and palpebral nodule (1). These dogs belongs to the GD (3), SD (7), TD (7), APD (6) and OD (1) groups.

The number of dogs that had disease that are active or in visual-axis were 28. Combining both variables for ophthalmic abnormalities, active disease and on visual-axis location, only 5 dogs had them. Details of the breeds and groups affected are seen in table 1. Photographs of the 4 dogs with ophthalmic diseases are seen in figure 1.

Table 1. Data of working dogs divided by breeds and working categories, with details on ophthalmic abnormalities. *N/A means that there was no abnormalities seen

Breeds	**Groups**	**Total**	**Ophthalmic abnormalities**	
			Dogs affected	**Diseases**
Belgian Malinois	SD TD APD OD	18 1 7 4	8 1 2 0	Incipient cataract, corioretinal scar, vitreous degeneration, follicular conjunctivitis, pupillary persistent membrane, Peters' anomaly, enucleated, retinal dysplasia, corneal dystrophy
Labrador Retriever	GD SD OD	8 2 2	5 2 2	Pupillary persistent membrane, incipient cataract, palpebral nodule, nuclear sclerosis, vitreous degeneration
German Shepherd	SD TD APD OD	2 2 2 1	1 1 2 0	Incipient cataract, discoria, distichiasis, nuclear sclerosis,
Bloodhound	TD	7	6	Ectropion, incipient cataract, blepharitis, iris atrophy
Rottweiler	APD OD	5 1	3 1	Incipient cataract, corioretinal scar, pupillary persistent membrane
Golden Retriever	GD	1	1	Distichiasis
Flat Coated Retriever	GD	1	1	Follicular conjunctivitis
Dutch Shepherd	SD	2	1	Ectropion uveae
Doberman Pinscher	APD	2	1	Incipient cataract, iris atrophy
American Pit Bull Terrier	APD	1	0	N/A
White Swiss Shepherd	OD	2	0	N/A
	Total	**71**	**38**	

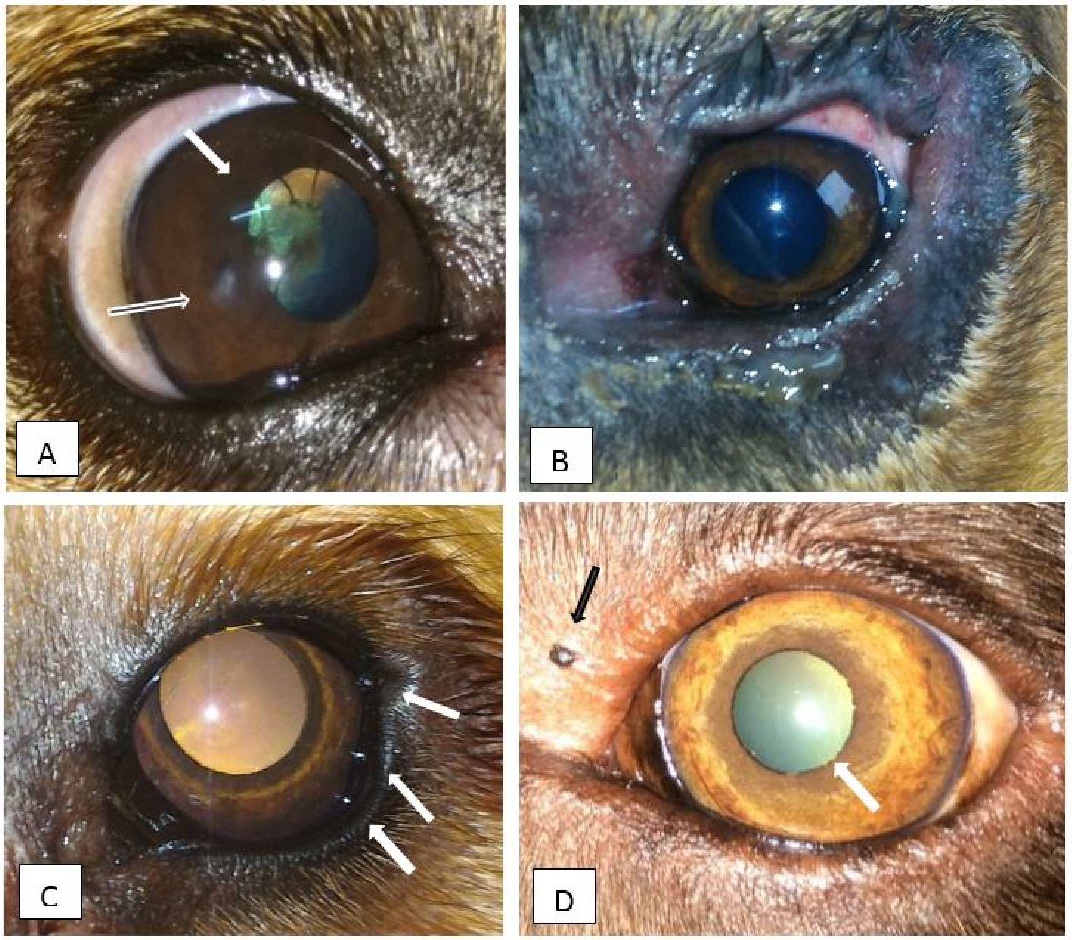

Figure 1. Photographs of four cases of ophthalmic abnormalities found in working dogs. A: Peters' anomaly with pupillary persistent membrane (white arrow) and corneal opacity (black arrow) in a four-year-old male Belgian Malinois. B: Blepharitis in a one-year-old female Bloodhound. C: Small distichiasis (white arrows) in a one-year-old female Golden Retriever. D: Pigmented palpebral nodule (black arrow) and nuclear sclerosis (white arrow) in an eight-year-old female Labrador Retriever.

4.4.3 Refractive errors

Concerning refractive errors of the 63 submitted to streak retinoscopy, 125 eyes were evaluated. Median and interquartile range of refractive errors for all eyes were -0.75 ± 0.75, meaning that 50% of the dogs had refractive errors between -1.5 and 0. A histogram graph of the distribution of refractive error is seen in figure 2.

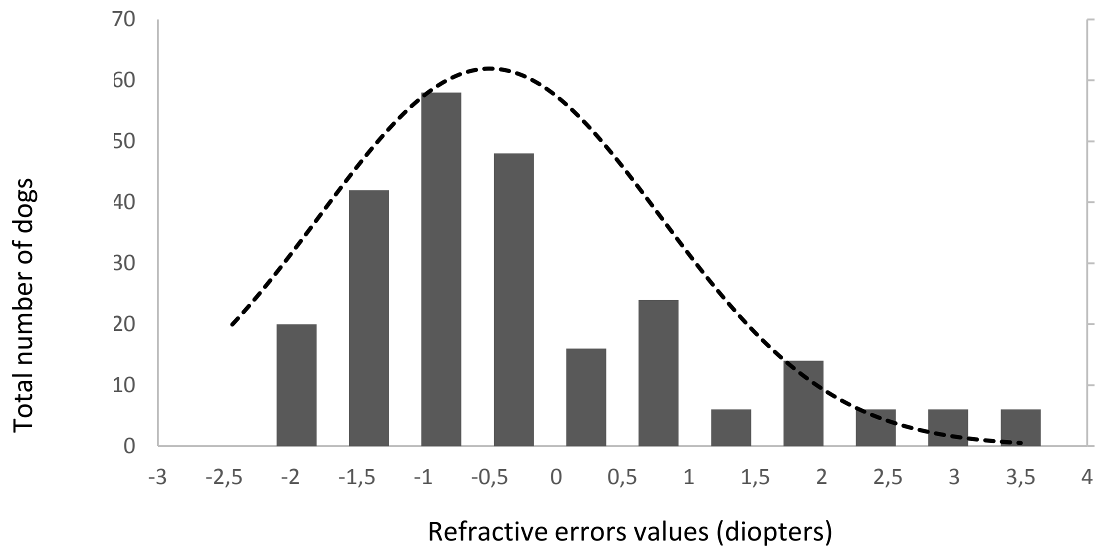

Figure 2. Histogram graph of the refractive errors distribution of 63 police and guide dogs, comprising 125 eyes. Note the right-skewed distribution of the values, emphasizing the concentration of dogs with tendency to myopia. Dashed line represent the distribution.

Police dogs had median and interquartile range of -1.0 ± 0.11, whereas guide dogs 0.38 ± 0.71, with significant difference between than ($p < 0.05$). Details of dog function and refractive errors are seen in table 2 and the distribution among the groups is seen on a box splot graph in figure 3.

Table 2. Data of median and interquartile range of refractive errors of the service dogs divided in 5 categories, with details divided on breed and eye evaluated

Group	Breeds	Refractive errors of the eye (median)			Refractive error median and interquartile range
		Right	Left	Both eyes	
Guide dogs	Labrador Retriever	+ 0.5	+ 0.13	+ 0.5	0.38 ± 0.71
	Golden Retriever	- 0.75	- 1	- 0.86	
	Flat Coated Retriever	- 2	- 2.25	- 2.13	
Sniffer dogs	Belgian Malinois	- 0.86	- 1	- 1	- 0.75 ± 0.75
	German Shepherd	- 0.5	- 0.75	- 0.75	
	Labrador Retriever	- 0.38	-0.63	- 0.38	
Tracking dogs	Bloodhound	+ 0.5	+ 0.5	+ 0.5	- 0.5 ± 1.13
	German Shepherd	- 1.38	- 1.38	- 1.38	
	Belgian Malinois	- 1.5	- 1.75	- 1.63	
Attack and protection dogs	Belgian Malinois	-0.63	- 0.75	- 0.63	- 1.25 ± 0.38
	Rottweiler	- 1.5	- 1.25	- 1.38	
	German Shepherd	- 2	- 1.13	- 1.87	
	Doberman Pinscher	- 1	- 1	- 1	
	American Pit Bull Terrier	- 1.5	- 1.5	- 1.5	
Others	Belgian Malinois	- 0.63	- 0.38	- 0.38	- 1.13 ± 0.41
	White Swiss Shepherd	- 1.25	- 1.63	- 1.5	
	Labrador Retriever	- 0.13	- 0.13	- 0.13	
	German Shepherd	- 1.25	- 1.25	- 1.25	
	Rottweiler	- 1	- 1	- 1	

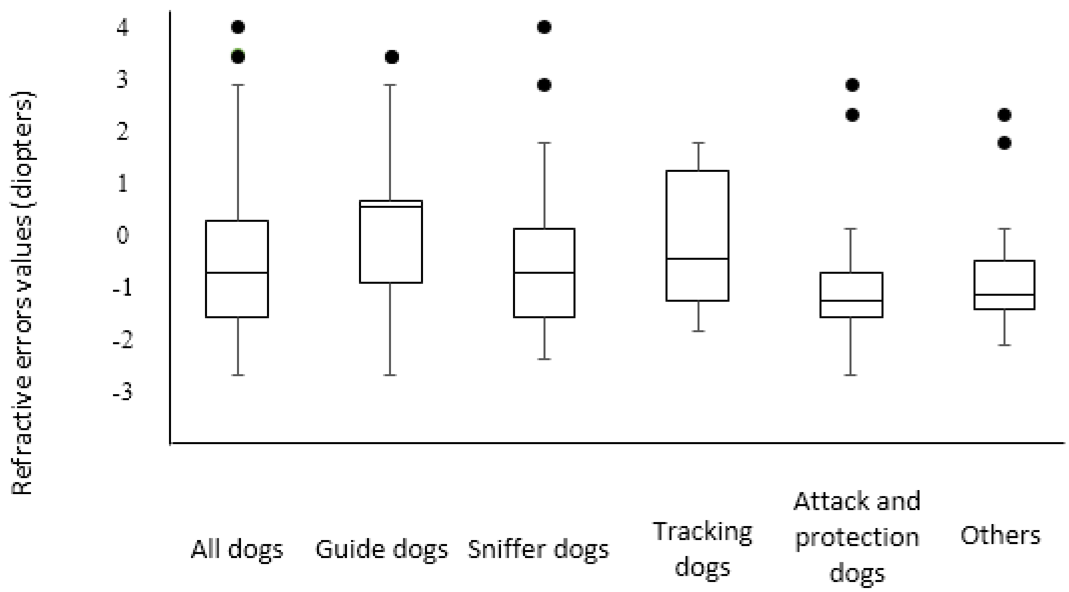

Figure 3 Box splot graph of the refractive errors of 63 police and guide dogs, comprising 125 eyes. The graph is divided in all dogs and then, in the 5 divided groups. Note that the groups: "attack and protection dogs" and "others" have narrower ranges, whereas the opposite is seen in the tracking dogs group.

For right eye, median and interquartile range were -0.75 ± 0.94, and for the left eye were -1 ± 0.75. Correlation coefficient between the eyes of the same dog were R = 0.94 and $p < 0.05$, being statistically significant. Furthermore, correlation between refractive error and age were R = 0.10 and $p > 0.5$, being not significant.

Of the 125 eyes, only eight had were no refractive errors (0) and only two dogs had both eye without any refractive error. Details of refractive errors and the dog breed independent on their function are seen in table 3.

Table 3. Data of median, interquartile range and range of refractive errors of working dogs, separate by breed.

Breed	Number of dogs	Refractive errors	
		Median and interquartile range	Range
Belgian Malinois	24	- 1 ± 0.69	-2.25; +3.5
Labrador Retriever	12	+0.5 ± 0.72	-2.5; +2.5
German Shepherd	7	- 1.25 ± 0.34	-2.5; 0
Bloodhound	7	+ 0.5 ± 0.94	-1.25; +1.5
Rottweiler	6	- 1.13 ± 0.38	-2.25; -0.5
Doberman Pinscher	2	- 1 ± 0.25	-1.25; -0.75
Swiss White Shepherd	2	-1.5 ± 0.34	-2.0; -0.75
Flat Coated Retriever	1	- 2.13 ± 0.06	-2.25; -2.0
Golden Retriever	1	- 0.86 ± 0.06	-1.0; -0.75
American Pit Bull Terrier	1	-1.5 ± 0	-1.5; -1.5

Comparison between groups showed no statistical significant differences of the groups SD, APD, TD and OD ($p > 0.05$). GD were significant different from all groups ($p < 0.05$) with exception of TD ($p = 0.52$).

Of the 63 dogs, only 12 (19%) were considered emmetropes, in which five of them were Labrador Retrievers. Fifty (81%) dogs were ametropes, being 40 (64%) myopics and 11 (17%) hyperopics. Only three dogs (0.05%) were anisometropic, and the difference between the eyes were 1.0 for them, there were two Belgian Malinois and one Labrador Retriever.

4.5 DISCUSSION

Ophthalmic abnormalities and ametropia were present in the most part of the working dogs studied. Older dogs appears to have more ocular diseases than younger ones and considerable inherited disorders were more common. Incipient cortical cataract and pupillary persistent membrane were the most prevalent abnormality detected. However only few of them had disturbances that can effectively impair vision locating off visual-axis. Refractive errors distribution of the working dogs showed that they tends to be myopic, and beyond that, GD and TD tends to be emmetropes. Although alterations were seen in the majority of the dogs, working performance were good and no signals of visual impairment were seen by their handlers, trainers and owners.

Due to meticulous ophthalmic evaluation performed, tiny abnormalities were detected, such as the incipient cataract found, which comprised subjectively less than 5% of the lens volume. These alterations increased the number of dogs with ocular disease, however due to small size of the cataracts, will be a prejudice to vision depending on position. Because of that, the classification of ophthalmic abnormalities based on visual-axis position were made, and the results are more reliable to the probability to impair vision.

Some of the ocular diseases diagnosed in this study are presumably to be inherited.[17,29-30] Since the dogs evaluated were purebred, there is a possibility that they had a genetic heritance for this abnormalities.

Considering the location of ophthalmic diseases, only 10 of them were in the visual axis, with diseases that can reduce vision significantly, all of these dogs were police dogs. Dogs that are used for detection, such ones of the groups SD and TD, relies their perception more on olfaction than vision[5], so the ocular abnormalities and refractive errors found probably will not prejudice their performances. Interestingly, one sniffer dog were enucleated of the one eye, and, nevertheless, performs her duty with excellence.

On the other hand, APD besides to have good sense of smell, also needs good vision to intercept potential criminals, however they`re the group with more frequency of ophthalmic abnormalities on visual-axis. Worsen of dog`s visual perception were already hypothesized as a factor that can increase aggressiveness[31], which can be a concern to control the dog by the trainer and handler. Moreover, even there was no complains about dogs` vision in this study, some degree of vision worsening must be occurred.

Interestingly that GD had no ophthalmic abnormalities on visual-axis and have a tendency to emmetropia, the same were seen in past studies.[23,27] Guide dogs are firstly selected by their ability to interpret the environment by senses, specially vision, and conduct a blind person throughout safe pathways. During the first months of age, potential guide dogs are analyzed concerning their behavior, quality of interaction and training, and the time to train a dog with good visual acuity is faster than the one with poor visual acuity.[23] Due to this selection based on performance, the presence of low refractive errors could be the phenotype directly related to their ability to guide, being possible to be heritable.[23,27] Past studies showed that same breeds had significantly difference of refractive errors between guide dogs and non-working dogs, being guide dogs with higher concentration of emmetropes than non-working dogs, strengthening the heritability hypothesis.[23]

The ophthalmic diseases were more present in older dogs, showing that the nature of some diseases have a late onset, and may be progressive with age, specially nuclear sclerosis and age-relate cataracts.[32-34] Knowing this, older dogs need a special attention because they`re more frequently withdrawn from service than younger ones due ocular disorders.[13]

Cataracts were seen in 15 (21%), that is similar to previous large surveys of ophthalmic abnormalities in dogs that varies from 14.7% to 31.4%.[35-36] However in investigations concerning specific breeds and large dogs, our prevalence were at least three times higher.[17,19,32-33] Purebred dogs can have breed-relate cataracts and, probably this was the main reason for the high prevalence found. This type of cataracts has been increasing significantly the incidence throughout time.[33] The absence of a foundation or a group that register inherited eye diseases in purebred dogs in Brazil can be one of the factors influencing such findings, because, with the lack of control of affected dogs, many of them are used for breeding, perpetuating these diseases.

Previous criteria used to stablish a cut-off for senile cataracts in large breeds are the onset after six years,[36] and is known that prevalence increases with age and unusually became mature,[34] so it`s expected that older dogs develop them. Median of the dogs' age with cataracts were five years, higher than non-affected dogs, showing that some dogs must have senile cataracts.

Labrador Retriever is a breed with predisposition to the development of diseases that can cause blindness, such as cataracts, and retinal progressive atrophy,[17-19,33,37] the latter being the most common ocular diseases that leads to early retirement of guide dogs.[9] Retinal progressive atrophy is autosomal recessive in this breed and gene mutation is more prevalent in pet dogs than guide dogs.[15] In our sample, none of the Labrador Retrievers, had this disease detectable during the examination, and can be due to the rigorous selection of these animals. However, three of them had incipient cortical cataracts, compatible with posterior polar cataract, inherited in this breed.[17,19] Nevertheless, this type of cataract presentation is not proved to be stationary and it may progress further, so future ophthalmic evaluations are necessary.

Ocular fundus abnormalities in police dogs, such as chorioretinitis scars found, were similar to previously identified in police German Shepherds dogs with no determinate cause.[38] Although chorioretinal lesions can reduce the perception of image by retina, good visual performance of the animals affected remain unaltered, same as found by Balick and colleagues.[38] Determinant factor to the development of these lesions still unclear, however have been speculate that intense physical activities or stressing situations that increase blood pressure, can cause microvascular hemorrhages and/or retinal detachment and scarring of retina.[38,39] Other underlying causes such as infectious and inherited chorioretinal diseases should also be considered as possibility, however a complete health investigation was not present at the time.

The majority of dogs presented ametropias, with a tendency to low myopia, similar to some studies of pet dogs and Brazilian police dogs.[31,40] These findings disagree with others reports from Brazil,[25,41-42] in which they found a tendency of hyperopia in a limited survey of 10 animals and in a population of Brazilian Mastiff. Otherwise, in larger studies, emmetropia were found in the majority of dogs.[21-24]

Myopia were general tendency of this study and can be due to breed predisposition and the type of work, being Rottweiler, German Shepherd and Belgian Malinois breeds with this tendency,[27,31] seen specially in SD, APD and OD groups.

Working dogs can identify moving objects in a distance of 810 to 900 meters and stationary objects at 585 meters.[43] However, when they have mild and severe myopia, at least worse than -2.0, it can reduces visual acuity,[28] and by consequence, impact negatively on their performance, diminishing their perception to less than 140 meters.[12] However no subjective signal of poor visual acuity or low performance were seen by the trainers, handlers and owners, and it can be due to low degrees of myopia found in the majority of dogs. Those who present severe degrees of ametropia could relies their perception more on other senses to perform their jobs, as seen in sniffer dogs.[5]

Correlation between age and refractive errors were not seen, probably due to the low number of old dogs, which develops nuclear sclerosis and can changes the refractive state predisposing to myopia.[23,27,44] Moreover, the variety of breeds or even their working condition can also influenced this absence of correlation o age and refractive state.

Interestingly, the groups with emmetropic tendency were GD and TD, and the breeds Labrador Retrievers and Bloodhounds, which are in greater number in their respective groups.

Labrador Retrievers has a naturally occurring myopia due to lengthening of vitreous chamber.[23,26] However, this breed had the higher number of emmetropic eyes of this study, similar to previous surveys[24,26] and it could be to the major number of the dogs belonging to GD group. In a German Shepherd population of guide dogs, the majority of them were emmetropic,[27] showing that even if the breed has predisposition to myopia, this specialized population have better visual acuity.

This is the first report of refractive errors in Bloodhounds, showing that they have a median refractive state tending for emmetropia, even if was a few numbers of dogs evaluated. Some individuals had hyperopia, the same occurred in previous findings in Brazilian Mastiffs,[25] which are similar breeds. We recommend further investigations of this breed, with higher number of dogs, including both working and non-working dogs to find if exists a predisposition, and the possibility to be a naturally occurring animal model for hyperopia.

Concerning anisometropia, the prevalence were lower than previous reports,[23,25,27] in which German Shepherd the most affected, however, in our study, anisometropia occurred in two Belgian Malinois and one Labrador Retriever. The two breeds more prevalent in this study and this can be a factor that influenced these findings.

Subjective evaluation of performance with questions made to the handlers, trainers and guide dog users, and none of them perceived difficult of their dogs to perform the activities or signals of visual impairment. However a more objective evaluation were not performed, such as maze on escotopic and photopic vision, being a limitation for this analysis. The number of dogs evaluated were also a limitation for this study, specially concerning about some breeds that had low number of individuals, which made it difficult to find a correlation of some breeds and refractive errors and ophthalmic abnormalities.

Moreover, assessment of non-working dogs could serve as a comparison for these clinical findings, and could elucidate if the alterations found would have the same prevalence.

Notwithstanding, in summary, Brazilian working dogs have a good performance on their duties, despite the ophthalmic abnormalities and refractive errors found in this study. Guide dogs have a tendency to emmetropia and absence of ophthalmic diseases on visual-axis, while Police dogs have a tendency to myopia and few dogs present ocular diseases on visual-axis, which may be the result of their selection for the type of work. Since guide dogs relies more on vision and police dogs, on other senses, especially sniffer dogs.

4.6 CONCLUSION

Considerable ophthalmic abnormalities and refractive errors were seen in the working dog population studied, and although some of the diseases and ametropias found, it will not effectively cause a significant vision loss. Is reasonable to admit that they need special attention to their ophthalmic health. Police dogs have a tendency for myopia and guide dogs for emmetropia. Ophthalmic evaluations must be performed in working dogs to maintain eye health and visual acuity. In addition, some of these ocular diseases can progress and the follow-up is essential. Many of these findings are genetically inheritable, thus, removal of the dogs affected and their parents from breeding are recommended.

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4.8 CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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6. ANNEX

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

CERTIFICADO

Certificamos que o protocolo número 105/2016, referente ao projeto **"Estudo da eficácia do oclacitinib oftálmico no controle da ceratoconjuntivite seca em cães e gatos"**, sob a responsabilidade de **Fabiano Montiani Ferreira** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 19/10/2016.

Vigência do projeto	Outubro/2016 até Abril/2017
Espécie/Linhagem	*Canis familiaris* (cão) e *Felis catus* (gato)
Número de animais	50 (40 cães e 10 gatos)
Peso/Idade	2 a 30 kg / 1 a 15 anos e 2 a 8 kg / 1 a 15 anos
Sexo	Ambos
Origem	Hospital Veterinário da Universidade Federal do Paraná (HV/UFPR)

CERTIFICATE

We certify that the protocol number 105/2016, regarding the project **"Efficacy study of ophthalmic oclacitinib to control keratoconjunctivitis sicca in dogs and cats"**, under **Fabiano Montiani Ferreira** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 19/10/2016.

Duration of the project	October/2016 until April/2017
Specie/Line	*Canis familiaris* (dog) and *Felis catus* (cat)
Number of animals	50 (40 dogs and 10 cats)
Wheight/Age	2 to 30 kg / 1 to 15 years and 2 to 8 kg / 1 to 15 years
Sex	Both
Origin	Veterinary Hospital of the Federal University of Paraná

Curitiba, 19 de outubro de 2016.

Simone Tostes

Simone Tostes de Oliveira Stedile

Coordenadora CEUA-SCA