PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DOUTORADO EM FISIOPATOLOGIA E SAÚDE ANIMAL

CLÁUDIA LIZANDRA RICCI

AVALIAÇÃO MORFOLÓGICA DA GLÂNDULA DE MEIBÔMIO E CONJUNTIVA PALPEBRAIS E COMPARAÇÃO ENTRE TONÔMETROS PORTÁTEIS NA MENSURAÇÃO DA PRESSÃO INTRAOCULAR EM GATOS

Presidente Prudente - SP
2021

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Defesa apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Doutora – Área de concentração: Fisiopatologia e Saúde Animal.

Orientador:
Profa. Dra. Silvia Maria C. Franco Andrade

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Presidente Prudente, 27 de outubro de 2021.

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Obrigado por existirem na minha vida. Amo vocês.

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“Quem não pode fazer grande coisa,
faça ao menos o que estiver na medida de suas forças.
Certamente não ficará sem recompensa.”
(Santo Antônio de Pádua)

RESUMO

Avaliação morfológica da glândula de meibômio e conjuntiva palpebrais e comparação entre tonômetros portáteis na mensuração da pressão intraocular em gatos domésticos

Os objetivos destes estudos foram: 1^0) descrever morfologicamente as glândulas meibomianas e células caliciformes da conjuntiva palpebral; e 2^0) comparar o desempenho de quatro tonômetros de três diferentes metodologias (rebote, aplanação e aplanação com metodologia de Goldmann), bem como a tolerância ao uso por gatos domésticos, além de estabelecer valores de referência de pressão intraocular (PIO) com os tonômetros Tono-Pen Avia Vet e Tonovet Plus em animais sem alterações oculares. No 1^0 estudo: 60 amostras das conjuntivas palpebrais e da região de fórnice palpebral por microscopia eletrônica de varredura (MEV) e histopatológico oriundas de 10 pálpebras de 5 gatos (3 machos e 2 fêmeas; 2-120 meses; 0,7- 4,3 kg); e 2) a PIO de 108 olhos sadios de 55 gatos (28 machos e 27 fêmeas) sem sinais de doenças oculares mediante avaliações *ex vivo*, *in vivo* e clínica. A histopatologia palpebral identificou glândulas meibomianas, formadas for células acinares e a presença de aberturas individuais na margem palpebral; na conjuntiva do fórnice foram observadas células caliciformes entremeadas por um epitélio estratificado acompanhado de tecido conjuntivo vascularizado. A análise por MEV demonstrou um epitélio conjuntival com superfície hexagonal homogênea, com inúmeros pontos de extrusão mucosa e a presença de microvilosidades nas porções apicais com pequenas vesículas secretoras. A forma ultraestrutural das células caliciformes e glândulas meibomianas da conjuntiva palpebral de gatos descritas pela primeira vez servirá de parâmetro para avaliação de alterações morfológicas nestas estruturas, bem como parâmetro de comparação às demais espécies animais. No 2^0 estudo: A tonometria *ex vivo* determinou valores do r^2 para o Tonovet (0,923), Tonovet Plus (0,925), Tono-Pen Avia Vet (0,877) e Kowa HA-2 (0,901). Os valores da PIO em mmHg na avaliação *in vivo* foram: manômetro (16,1±2,7), Tonovet (21,1±3,6), Tonovet Plus (19,7±7,2), Tono-Pen Avia Vet (17,6±7,9) e Kowa HA-2 (16,8±2,0). As três metodologias de avaliação da PIO apresentaram altos coeficientes de correlação precisas, sendo o Kowa HA-2 o tonômetro mais acurado, e o Tonovet o mais tolerado pelos gatos. Na rotina clínica valores da PIO mais altos foram aferidos com o Tonovet e os mais baixos com o Kowa HA-2, o que reforça a necessidade de uma tabela de valores de referência de PIO para gatos para cada um desses tonômetros. Os valores de referência estabelecidos pela avaliação clínica do Tonovet Plus e Tono-Pen Avia Vet foram 16,9±1,2 e 16,2±1,1 mmHg, respectivamente.

Palavras-chave: células caliciformes, glândulas meibomianas, manometria ocular, microscopia eletrônica, tonometria.

ABSTRACT

Morphological evaluation of the palpebral meibomian and conjunctival glands and comparison between portable tonometers to measure intraocular pressure in domestic cats

The objectives of these studies were: 1st) to morphologically describe the meibomian glands and goblet cells of the palpebral conjunctiva; and 2nd) to compare the performance of four tonometers of three different methodologies (rebound, applanation and applanation with Goldmann's methodology), as well as the tolerance to use by domestic cats, in addition to establishing reference values of intraocular pressure (IOP) with the tonometers Tono-Pen Avia Vet and Tonovet Plus in animals without ocular changes. In the 1st study: 60 samples of the palpebral conjunctiva and palpebral fornix region by scanning electron microscopy (SEM) and histopathology from 10 eyelids of 5 cats (3 males and 2 females; 2-120 months; 0.7-4, 3 kg); and in the 2nd the IOP of 108 healthy eyes from 55 cats (28 males and 27 females) without signs of ocular disease by *ex vivo*, *in vivo* and clinical evaluations. Eyelid histopathology identified meibomian glands, formed by acinar cells and the presence of individual openings in the eyelid margin; in the fornix conjunctiva goblet cells were observed interspersed with a stratified epithelium accompanied by vascularized connective tissue. SEM analysis demonstrated a conjunctival epithelium with a homogeneous hexagonal surface, with numerous points of mucosal extrusion and the presence of microvilli in the apical portions with small secretory vesicles. The ultrastructural shape of the goblet cells and meibomian glands of the palpebral conjunctiva of cats described for the first time will serve as a parameter for the evaluation of morphological alterations in these structures, as well as a parameter for comparison with other animal species. In the 2nd study: *Ex vivo* tonometry determined r^2 values for Tonovet (0.923), Tonovet Plus (0.925), Tono-Pen Avia Vet (0.877) and Kowa HA-2 (0.901). The IOP values in mmHg in the *in vivo* evaluation were: manometer (16.1±2.7), Tonovet (21.1±3.6), Tonovet Plus (19.7±7.2), Tono-Pen Avia Vet (17.6±7.9) and Kowa HA-2 (16.8±2.0). The three IOP assessment methodologies showed high accurate correlation coefficients, with Kowa HA-2 being the most accurate tonometer, and Tonovet being the most tolerated by cats. In clinical routine, the highest IOP values were measured with the Tonovet and the lowest with the Kowa HA-2, which reinforces the need for a table of IOP reference values for cats for each of these tonometers. The reference values established by the clinical evaluation of Tonovet Plus and Tono-Pen Avia Vet were 16.9±1.2 and 16.2±1.1 mmHg, respectively.

Keywords: electron microscopy, goblet cells, meibomian glands, ocular manometry, tonometry.

LISTA DE SIGLAS

ARVO – Association for Research in Vision and Ophthalmology
b.i.d. – *bis in die* (duas vezes ao dia)
CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CCPA – Clínica Cirúrgica de Pequenos Animais
CMPA – Clínica Médica de Pequenos Animais
EV – endovenosa
FLPC – filme lacrimal pré corneano
HE – Hematoxilina-Eosina
HV – Hospital Veterinário
mmHg – milímetros de mercúrio
NIH – National Institute Health
OD – olho direito
OS – olho esquerdo
PAS – Ácido periódico Schiff
PIO – pressão intraocular
q.i.d. – *quarter in die* (quatro vezes ao dia)
r^2 – coeficiente de correlação
SEM – microscopia eletrônica de varredura
TRFL – tempo de ruptura do filme lacrimal
UnB – Universidade de Brasília
vs. – *versus*
µm – micra

SUMÁRIO

ARTIGO 1 (Brief Communication – *Veterinary Ophthalmology*)

Morphological and ultrastructural study of the palpebral conjunctiva of healthy domestic cats

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ABSTRACT

Objective To describe the morphology of the meibomian glands and goblet cells of the palpebral conjunctiva of healthy domestic cats without ocular alterations.

Study animals Three males and two females (10 eyelids), 2-120 months, 0.7-4.3 kg, without ocular or eyelid changes and who died from causes not related to the study.

Procedures Forty samples were collected from the upper and lower palpebral conjunctivae and 20 from the palpebral fornix region in the nasal corner. The samples were processed for scanning electron microscopy (SEM) (30 samples), and for light microscopy (30 samples).

Results In the SEM analysis of the conjunctival epithelium of the palpebral fornix, numerous points of mucous extrusion arranged between the cell junctions and the presence of microvilli in the apical portions with small secretory vesicles were visualized. A homogeneous surface was highlighted, formed by the arrangement of the cell contours in the form of hexagons. The histopathology of the fornix conjunctiva showed goblet cells interspersed with a stratified epithelium accompanied by well-vascularized connective tissue. In the samples stained with Hematoxylin-Eosin, the meibomian glands, formed by acinar cells and with the presence of individual openings of the ducts in the eyelid margin, were easily visualized in the eyelid margins.

Conclusions This study describes the ultrastructural form of the goblet cells, and the morphology by histopathology of the meibomian glands, of the palpebral conjunctiva of healthy cats without ophthalmic alterations. This description can serve as a parameter of normality and help in the detection of morphological alterations in these structures, as well as a parameter for comparison with other animal species.

Keywords: goblet cells, meibomian glands, ocular surface, scanning electron microscopy, tarsal glands.

1.1 INTRODUCTION

Meibomian glands are large sebaceous glands located in the upper and lower tarsal plates. They have the functions of synthesizing and secreting lipids and proteins on the ocular surface through the eyelid margins, close to the mucocutaneous junctions.[1] Glandular lipid is stored in acinar cells and is secreted through the duct in a holocrine process, where it spreads over the tear film to promote stability and prevent its evaporation.[1,2,3]

Goblet cells, considered specialized mucus-producing cells, contribute to the homeostasis of the ocular surface, since mucin is essential for the stability of the precorneal tear film by favoring the adhesion of tear fluid to the ocular surface.[4,5]

Regardless of sex and age, domestic cats have a higher density of these cells compared to other species, with the highest concentration being present in the palpebral areas and on the anterior surface of the third eyelid, while a lower density is found in the bulbar areas of the lower eyelid and in the marginal areas of both eyelids.[6]

The objective of the present study was to analyze, in an ultrastructural way, the goblet cells, and the morphology by histopathology of the meibomian glands, of the palpebral conjunctiva of healthy cats without ophthalmic alterations and to provide information on the characterization of these structures for feline ophthalmology.

1.2 MATERIALS AND METHODS

The experiment was approved by the Ethics Committee on the Use of Animals of UNOESTE (Universidade do Oeste Paulista) under protocol n. 5725 and conducted in accordance with the ARVO (*Association for Research in Vision and Ophthalmology – Statement for the use of animals in ophthalmic and visual research*) animal experimentation standards.

The collections were carried out at the Veterinary Hospital (HV) and the processing carried out at the Laboratory of Anatomy and Cytopathology of Campus I of UNOESTE, Presidente Prudente, SP, Brazil, and in the Laboratory of Microscopy and Microanalysis of the Institute of Biology of the University of Brasília (UnB), Brasília, DF, Brazil.

1.2.1 Samples

Forty samples were collected from the upper and lower palpebral conjunctiva and; twenty samples from the palpebral fornix regions of the lower nasal corner of five cats (of breeds 2 short-haired, 1 long-haired, 1 Persian and 1 Siamese), 3 males and 2 females, ages between 2 and 120 months, weight 3.3±1.5 (0.7-4.3) kg, with no signs of eye disease. Samples were collected up to 24 hours after death from causes not related to this study and that were authorized for necropsy examination at the Pathological Anatomy Service of the HV.

Fifty percent of the samples were processed for histopathological study and analyzed by light microscopy and fifty percent of the other samples were prepared according to scanning electron microscopy (SEM) protocols. The samples for evaluation of the meibomian glands were obtained through a “V” incision (0.3 cm) of the skin of the upper and lower eyelids with a scalpel handle n.3 and blade 15, and the conjunctival fragment was removed with curved conjunctiva scissors. Then, a sample (0.5 cm) of the palpebral conjunctiva was collected in the palpebral fornix region of each nasal corner with conjunctival tweezers and scissors.

1.2.2 Histopathological

For histopathological analysis, samples were fixed in 10% buffered formaldehyde solution for 24 hours, then sectioned and routinely processed for histopathological examination, embedded in paraffin, cut 5µm thick and stained with hematoxylin/eosin (HE). The HE stained images were captured by a Leica photomicroscope (Leica Microsistems, Switzerland) at 40x magnification and measurements of the meibomian glands were performed using ImageJ software, from the National Institute of Health (NIH) of the United States, freely available on the internet (http:/rsbweb.nih.gov/ij/) on the major and minor axis. To count goblet cells, additional sections were stained with periodic acid-Schiff (PAS) and five high-power fields of the conjunctiva were analyzed.

1.2.3 SEM

The samples were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2, for 24h at room temperature (22 to 250 Celsius). After washing in 0.1M sodium cacodylate buffer pH 7.2, the samples were later fixed in 2% osmium tetroxide,

covered with aluminum foil, for 1 hour and then washed twice in distilled water. In sequence, the samples were dehydrated in an ascending series of acetone (30, 50, 70, 90 and 100%) and then critical point drying (Critical Point Drying - CPD 030, Balzers) in liquid CO_2 was performed. After drying, the samples were fixed on metallic stubs with double-sided carbon tape. Then, they were metallized with a layer of gold at 100-200A, using a high vacuum metallizer (Leica EM SCD500). Soon after, the samples were visualized with a Jeol JSM-7000F Field Scanning Electron Microscope (Jeol Ltd.).

1.2.4 Statistical Analysis

A descriptive analysis of the goblet cell count of the palpebral conjunctiva and the measurements of the meibomian glands of the upper and lower eyelids was performed.

1.3 RESULTS

1.3.1 Histopathological

In the HE stained samples, the meibomian glands were easily visualized in the eyelid margins. The large, rounded glands formed by acinar cells and with the presence of individual openings of the ducts in the eyelid margin were captured at 40x magnification (Fig. 1A), and measurements of the minor and major axis of three glands for each eyelid were performed. through the ImageJ software (Fig. 1B). Of the five animals evaluated, four were adults (48-120 months) and the largest glandular measurement measured in these animals was 8462 µm in its major axis and 1096 µm in its minor axis. One of the animals was younger, (animal 5) 2 months old, the highest measurement obtained was 3221 µm and the smallest measured axis was 991 µm. The smallest and largest measurements of both eyelids (lower/upper; right/left) of each animal are described in Table 1.

In the analysis of the palpebral conjunctiva collected from the fornix region stained in PAS, well-stained goblet cells were observed, interspersed by non-keratinized stratified squamous epithelial tissue supported by vascularized loose connective tissue (Figs 1C, 1D). The count resulted in (mean ± standard deviation) 49 ± 16 (26-94) goblet cells per field (0.5mm²).

1.3.2 SEM

In the observation of the SEM micrographs of the palpebral conjunctival epithelium, numerous points of mucosal extrusion arranged between the cell junctions were noted (Fig. 2A, 2B). It was also noted the presence of microvilli in the apical portions with small secretory vesicles (Fig. 2C, 2D) and a homogeneous surface, formed by the arrangement of the contours of the cells fair put in the form of hexagons (Fig. 2E, 2F).

1.4 DISCUSSION

This is the first study that ultrastructurally describes the goblet cells, and the morphology by histopathology of the meibomian glands, of the palpebral conjunctiva of healthy cats without ophthalmic alterations.

There are few reports in the literature regarding the anatomical and histological description of the meibomian gland, in humans[2] and in laboratory animals.[3] The meibomian gland is a sebaceous gland with a tubuloacinar structure and holocrine function, located in the upper and lower tarsal plates. Similar to what was described in humans, in domestic cats, it was observed that each gland is composed of numerous rounded lipid-secreting acini arranged vertically in simple rows, a long central duct and a terminal excretory duct that opens at the posterior margin of the eyelid.[1-3,7] Unlike other sebaceous glands, meibomian glands do not have direct contact with hair follicles and are lined by stratified epithelium similar to the skin epidermis, except for the absence of a granular layer.[1,2]

The morphological description of goblet cells by electron microscopy has been reported in humans,[8] dogs[9] and in animals for experimental tests.[4,10,11] Similar to other species, a high concentration of goblet cells was observed in the lower nasal fornix region, as described in cats,[6] dogs,[9] rats[10] and in humans.[12]

In the histopathological analysis of the samples stained in PAS, the goblet cells were observed in a row in the form of a barrier against external aggressions, an essential factor for the maintenance of the epithelial homeostasis of the mucosa of the ocular surface.[5] Mucin, secreted by conjunctival goblet cells, is important for the ocular surface as it not only promotes tear film integrity to maintain a moist and properly refractory film, but also serves

as an antimicrobial and anti-inflammatory substance. Therefore, the loss of goblet cells can result in ocular surface diseases.[5,6]

Araújo *et al* 2019, in the observation of canine conjunctival tissue by SEM, described a homogeneous surface formed by fair put hexagonal cells and the presence of microvilli in the apical portion of the epithelial cells, interspersed with several points of mucus extrusion, released in small fusiform vesicles by the free edges of goblet cells,[9] was also observed in this study.

The ultrastructural shape of the goblet cells and the morphology of the meibomian glands of healthy cats is similar to those already described in humans,[2,8] laboratory animals[3,4,10,11] and in dogs.[9] Furthermore, the results presented may guide the description of alterations or comparison regarding the morphology of these structures comparative between species.

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CONFLICT OF INTERESTS

The authors declare that there are no potential conflicts of interest regarding the research, authorship and/or publication of this article.

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Table 1 Measurements (μm) of the meibomian glands of the upper and lower eyelids of both eyes of 5 cats. Measurements (major axis *vs.* minor axis) obtained using the ImageJ software (NIH).

	Right lower eyelid		Left lower eyelid		Right upper eyelid		Left upper eyelid	
Animal	Major axis	Minor axis	Major axis	Minor axis	Major axis	Minor axis	Major axis	Minor axis
1	7768	1875	7463	1873	7968	1896	7463	1979
2	7497	2165	8462	1821	6973	1750	5576	1748
3	7281	1320	6279	1096	7655	1320	6681	1452
4	3027	1472	3770	1532	4386	1142	3792	1398
5	1609	1056	2956	1496	3221	1037	1537	991
Average	5436.4	1577.6	5786.0	1563.6	6040.6	1429.0	5009.8	1513.6
Standard deviation	2895.7	442.5	2360.6	310.7	2114.1	377.2	2380.9	374.8
Minimum	1609.0	1056.0	2956.0	1096.0	3221.0	1037.0	1537.0	991.0
Maximum	7768.0	2165.0	8462.0	1873.0	7968.0	1896.0	7463.0	1979.0

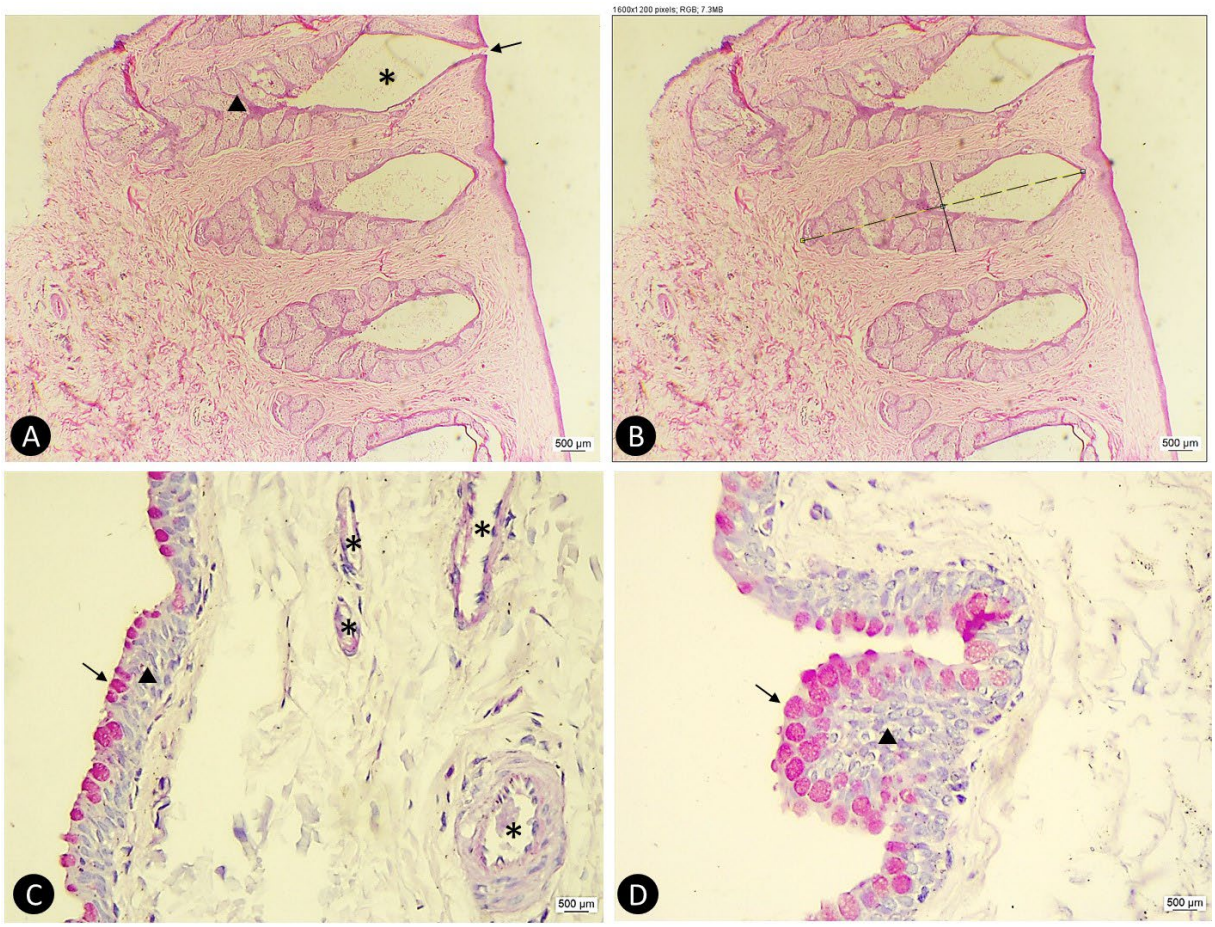

Figure 1. Histopathological. **A,B** Section of the feline right lower palpebral conjunctiva (HE, 40x). The Meibomian gland is composed of numerous rounded acini arranged vertically in single rows (arrowhead), a long central duct (asterisk) and a terminal excretory duct (arrow) that opens at the margin. **B** Lines indicate the measurement of a gland in its shortest axis (solid line) and longest axis (dotted line) using ImageJ software (NIH). **C, D** Section of the palpebral conjunctiva of a domestic feline in the nasal fornix region (PAS, 400x). **C** Presence of goblet cells (arrow) interspersed with stratified epithelium (arrowhead). Also noteworthy are the blood vessels (asterisk) wrapped in loose connective tissue. **D** Detail of goblet cells (arrow) interspersed in stratified epithelium (arrowhead) (PAS, 400x).

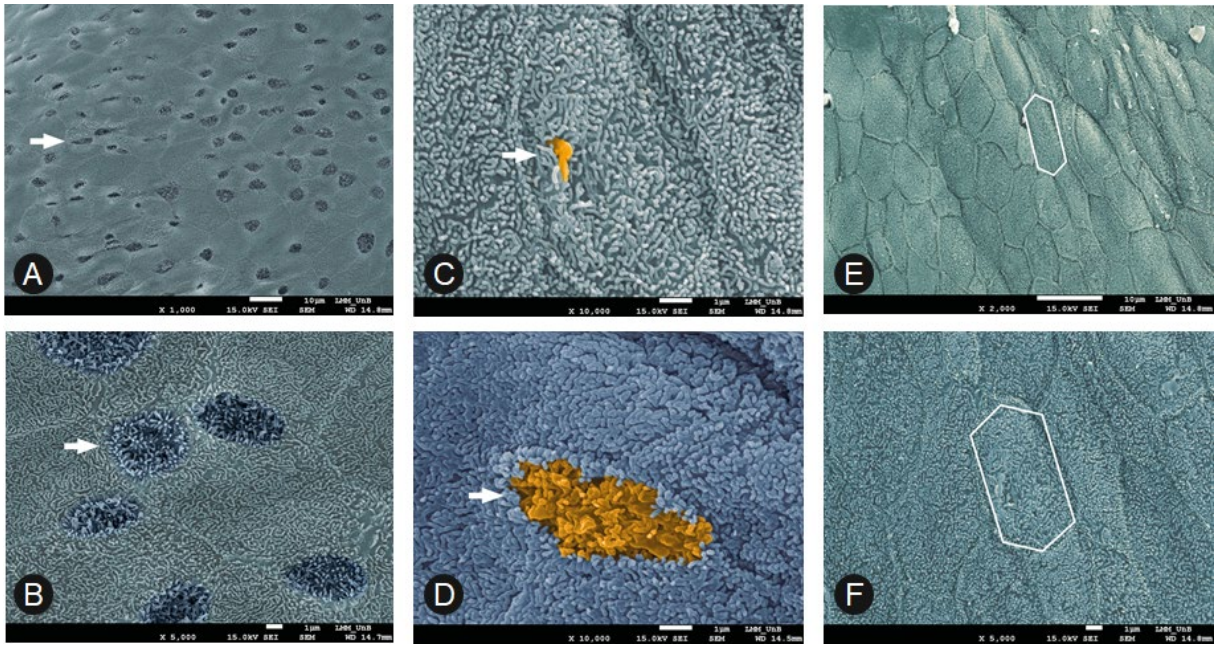

Figure 2. SEM micrographs of the feline palpebral conjunctival epithelium. **A, B** Arrows highlight mucosal extrusion points arranged between cell junctions, sample from the palpebral fornix, a portion with a high concentration of goblet cells. **C, D** At lower magnification, apical microvilli and small secretory vesicles can be seen (arrow). **E, F** The homogeneity and hexagonal contours of the epithelial cells (hexagons) stand out.

ARTIGO 2 (Original Article – *Journal of Feline Medicine and Surgery)*

Comparison between Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 portable tonometers in measuring intraocular pressure in healthy cats

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ABSTRACT

Objective To compare the use of four tonometers with different methodologies for measuring intraocular pressure (IOP), Tonovet and Tonovet Plus (rebound), Tono-Pen Avia Vet (applanation) and Kowa HA-2 (Goldmann's methodology applanation) in cats.

Methods Fifty-five healthy cats (108 eyes) were evaluated in three studies: ten eyes of five cats *ex vivo* to correlate actual IOP values by manometry *versus* tonometry and to calculate the correlation coefficient (r^2); ten eyes of five sedated cats *in vivo* compared real IOP (manometry) with tonometry; and 80 eyes of 45 unsedated cats compared to IOP measured only by tonometers in an outpatient clinical study.

Results The r^2 values observed in the *ex vivo* study were: Tonovet (0.923), Tonovet Plus (0.925), Tono-Pen Avia Vet (0.877) and Kowa HA-2 (0.901). The IOP values in mmHg in the *in vivo* study were: manometer (16.1±2.7), Tonovet (21.1±3.6), Tonovet Plus (19.7±7.2), Tono-Pen Avia Vet (17.6±7.9) and Kowa HA-2 (16.8±2.0); in the outpatient clinical study were: Tonovet (19.7±6.6), Tonovet Plus (17.1±5.4), Tono-Pen Avia Vet (16.3±4.3) and Kowa HA-2 (14.5±2.2).

Conclusions and relevance All tonometers showed a strong correlation between IOP values and manometry. In the outpatient clinical routine, the highest IOP values were measured with the Tonovet and the lowest with the Kowa HA-2, which reinforces the need for a table of IOP reference values for cats for each of these tonometers.

Keywords: applanation tonometry, Goldmann tonometry, intraocular pressure, ocular manometry, rebound tonometry.

2.1 INTRODUCTION

Measuring intraocular pressure (IOP) plays an important role in detecting eye changes that can lead to vision loss, such as when measuring high IOP values, as in glaucoma, and low values, as in uveitis. Feline glaucoma is less common compared to canine glaucoma, and in most cases it is secondary;[1] uveitis is one of the most frequent and significant eye disorders seen in domestic cats.[2]

The most accurate way of measuring IOP is performed invasively by ocular manometry that measures the real IOP in mmHg, used only experimentally.[3,4] Tonometry, measuring IOP using devices called tonometers, is the method used in routine ophthalmic examination.[5,6,7] In cats, the most used methods are applanation with the use of the Tono-Pen tonometer, and the rebound methodology, with the Tonovet[2] and Tonovet Plus. Less commonly used, the Goldman applanation method is considered the standard in humans. In cats there are some studies to validate its use with the Perkins and Kowa HA-2 tonometers.[8,9]

Rebound tonometry is based on the measurement/recovery principle, where a lightweight, magnetized probe makes momentary contact with the cornea. A software analyzes the deceleration and the time of contact of the probe, on the impact with the cornea. The probe used is disposable to prevent contamination and measurements are performed without need topical anesthesia. Applanation tonometry is based on the force required to flatten a given area of a sphere, which is equal to the pressure inside that sphere.[4] Goldmann applanation tonometry uses a 3.06 mm diameter that measures corneal indentation and IOP with the formation of fluorescein semicircles that adjust during the exam.[8]

There is no comparative study with the use of the Goldmann tonometer, with the applanation methodology and the rebound method in cats. Thus, the aim of this study is to analyze and compare the use of Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 tonometers in cats.

2.2 MATERIALS AND METHODS

The study was carried out for a period of 24 months in the ophthalmology sector of the small animal medical clinic (CMPA), small animal surgical clinic (CCPA) and cattery of the

Veterinary Hospital (HV) of the Universidade do Oeste Paulista (UNOESTE) in Presidente Prudente, Sao Paulo, Brazil.

2.2.1 Animals

Fifty-five healthy cats (108 eyes of 28 males and 27 females) of breeds (one Maine Coon, five Siamese, six Persian, three domestic long-haired and 40 domestic short-haired) with no signs of eye disease at the time of measurement were used. In all evaluations, the animals were submitted to the Schirmer Tear Test (Ophthalmos, São Paulo, Brazil) and slit lamp biomicroscopy (SL-15, Kowa, Japan). To standardize the IOP measurement site, the devices were positioned in the central corneal region and at a 90º angle. Measurements were always performed by the same examiner between 1 pm and 5 pm. Three IOP readings were taken and the average calculated, starting with the left eye and then the right eye, with each tonometer in the following order: 1st) Tonovet – 'd' calibration, 2nd) Tonovet Plus – 'cat' calibration, 3rd) Tono-Pen Avia Vet and 4th) Kowa HA-2.

2.2.2 *Ex vivo* study

The *ex vivo* study was performed to compare actual IOP values by direct ocular manometry and IOP values measured by tonometers. The methodology was based on previously published studies.[2,10,11] This study was performed up to 24 hours after death in ten healthy eyes of five cats, 48 to 120 months of age, weight 3.5±1.1 (2-5) kg, three males and two females, at the CMPA of the HV of UNOESTE, who died from causes without ophthalmic repercussions unrelated to this study. The animals were positioned in sternal recumbency, the eyelids were parted with a Barraquer blepharostat and the anterior chamber cannulated with a 23G scalpel (Lamedid, São Paulo, Brazil) 2 mm posterior to the superior temporal limbus at the 10 o'clock position in the right eye (OD) and medial superior at the 2 o'clock position in the left eye (OS) (Figure 1). Cyanoacrylate (Superbonder, Loctite, São Paulo, Brazil) was applied around the needle to prevent leakage of aqueous humor. The needle was connected to a polyethylene tube, which was connected to a three-way stopcock (Labor Import, São Paulo, Brazil), to allow connection to another polyethylene tube and a reservoir of 0.9% saline solution. (Equiplex, Goiás, Brazil). Attached to this system, an aneroid manometer (BIC, São Paulo, Brazil) that was zeroed in relation to the center of the eye (Figure 1). The IOP was

artificially raised by opening the three-way stopcock to infuse saline at 5 in 5mmHg up to 60mmHg (10-60mmHg). Three readings were taken at each IOP level with the tonometers and the average was calculated.

2.2.3 *In vivo* study

An *in vivo* study in anesthetized cats was performed to compare the real IOP obtained by direct ocular manometry and the IOP measured with tonometers. Ten eyes from 5 cats, aged between 24 and 132 months, weight 4.5±0.9 (3.8-6.2) kg were used; 2 males and 3 females, from the Unoeste HV cattery. In the surgical center of the HV, under the supervision of an anesthesiologist, the anesthetic protocol was as follows: pre-anesthetic medication with acepromazine (Acepran 0.2%, Vetnil, São Paulo, Brazil) at a dose of 0.05mg/kg IV followed by of induction with propofol (Propovan, Cristália, São Paulo, Brazil) at a dose of 5 mg/kg IV, followed by endotracheal intubation and anesthetic maintenance with isoflurane (Isoflurane, BioChimico, Rio de Janeiro, Brazil) diluted in 100% O_2 in semi closed circuit. The animals were maintained on artificial ventilation with a 674 Takaoka Ventilator (KTK, São Paulo, Brazil), whose ventilation parameters were adjusted to maintain the end-tidal carbon dioxide concentration ($EtCO_2$) between 35-45 mmHg. To centralize the eyeball, the neuromuscular blocker atracurium besylate 10mg/mL (Atracurio, Cristália, São Paulo, Brazil) was used at a dose of 0.1mg/kg IV. The animals were positioned in sternal recumbency, as recommended in the literature, and tonometry was started with the Tonovet, followed by the Tonovet Plus. Promptly, the corneas of both eyes were topically anesthetized with 1 drop of eye drops based on 1% tetracaine hydrochloride + 0.1% phenylephrine hydrochloride (Anesthetic, Allergan, São Paulo, Brazil) to measure IOP with Tono-Pen Avia Vet and then instilled the fluorescein eye drops (Fluoresceína, Allergan, São Paulo, Brazil) in the corneas of both eyes to visualize the semicircles in the measurement with Kowa HA-2. After tonometry, ocular manometry was performed to detect IOP and then the scalp was removed to immediately instill a drop of cyanoacrylate glue with the aid of a 1 mL syringe and a 25x0.7 mm needle (BD, Paraná, Brazil) at the corneal puncture site to seal the perforation. Subsequently to the IOP readings, the effects of atracurium besylate were reversed with the use of neostigmine methylsulfate (Normastig, União Química, São Paulo, Brazil) at 0.5 mg/mL at a dose of 0.01 to 0,04 mg/kg associated with atropine sulfate at 0.25 mg/ml at a dose of 0.044 mg/kg (Pasmodex, Isofarma, Ceará, Brazil). After the completion of the study,

the animals were treated with the instillation of a drop of anti-inflammatory eye drops based on diclofenac sodium 0.1% (Still, Allergan, São Paulo, Brazil) BID and a drop of antibiotic eye drops based on tobramycin 0.3% (Tobracin, Latinofarma, São Paulo, Brazil) QID in both eyes for seven days and assessed with daily ophthalmic examination using Schirmer's Tear Test and Slit Lamp biomicroscopy.

2.2.4 Outpatient Clinical Study

To evaluate the use of tonometers in clinical routine, IOP was measured in the eyes of cats treated at the outpatient clinic in the ophthalmology sector of the CMPA of the HV of Unoeste. A total of 45 cats (88 eyes) were evaluated, of which 2 animals had only one eye as a result of post-traumatic enucleation of the contralateral eye, age between 1.5 and 180 months, weight 3.7±1.6 (0,4-7) kg, 23 males and 22 females. The cats were positioned in a station, the neck kept without pressure and the eyelids slightly apart in order to avoid changes in the IOP measurement, and there was a two-minute interval between the use of each tonometer (Figure 2).

2.2.5 Statistical analysis

In the *ex vivo* study regression lines were constructed for measured IOP values from manometry vs. tonometry, the correlation coefficient (r^2) and the linear regression equation were calculated, the Bland-Altman agreement analysis was also performed to compare two quantitative methods of measuring IOP and a series of agreements was defined as a mean bias of ± 2 standard deviations. In *in vivo* and outpatient clinical studies, the means and standard deviations of the IOP values measured with the tonometers were calculated and statistically compared by analysis of variance (ANOVA). A significance level of 5% ($p<0.05$) was adopted. The software used for the statistical analysis was the R Core Team (2020) program.

2.3 RESULTS

In the *ex vivo* study there was a strong correlation between the IOP values of manometry and all tonometers and the observed values of r^2 in decreasing order were: Tonovet Plus (0.925),

Tonovet (0.923), Kowa HA-2 (0.901) and Tono- Pen Avia Vet (0.877). The linear regression equations of each tonometer are described in Figure 3. In the analysis of agreement observed in the Bland Altman graphs (Figure 4), the Tonovet showed a slight tendency to underestimate the manometry in the reading of 15 mmHg and overestimated readings between 25 and 40 mmHg, the Tonovet Plus also showed reduced accuracy in the values measured between 25 and 40 mmHg and underestimated the IOP by 50 mmHg, the Tono-Pen Avia Vet showed less agreement in the lower pressures, with the underestimation of the readings between 10 – 15 mmHg and overestimated readings above 35 mmHg and the Kowa HA-2 tonometer overestimated IOP values at pressures above 40 mmHg.

In the *in vivo* study, the IOP values measured in manometry and in the different tonometers are described in Figure 6. There was a statistically significant difference ($p<0.05$) compared to the manometry of the Tonovet, Tonovet Plus and Tono Pen Avia Vet tonometers, and not there was a statistically significant difference ($p>0.05$) between manometry and the Kowa HA-2 tonometer (Figure 5).

In the outpatient clinical study, there was a statistically significant difference ($p<0.05$) between the Tonovet and the other tonometers and there was no statistical difference between the Tonovet Plus and the Tono-Pen Avia Vet. The Tonovet was the device that presented the greatest variation in values of IOP in relation to the others and the Kowa HA-2 was the tonometer that presented the smallest variation (Figure 6).

2.4 DISCUSSION

This is the first study in cats that compared applanation tonometry using the Goldmann methodology (Kowa HA-2) with the two methodologies frequently used by veterinary ophthalmologists, rebound (Tonovet and Tonovet Plus) and applanation (Tono-Pen Avia Vet).[2] Although applanation tonometry using the Goldmann methodology is little used in veterinary medicine, some studies have shown an excellent correlation with manometry, with tonometers, Perkins[8,12] and Kowa HA-2[9] in cats. The literature is vast on the use of applanation tonometers with Tono-Pen Vet[2,14-18] and Tono-Pen XL[11,12,19-21] and with the tonometer with the rebound methodology Tonovet[2,11,14,16,17,22] in cats, but only one study reports the applanation methodology with Tono-Pen Avia Vet[23] and two with the rebound Tonovet Plus.[14,24]

In the *ex vivo* study, there was a strong correlation (r^2>0.9) with the manometry of the tonometers in decreasing order: Tonovet Plus (0.925), Tonovet (0.923) and Kowa HA-2 (0.901), values close to those found in a similar study recently published in dogs[10] with the same devices in descending order: Kowa HA-2 (0.989), Tonovet Plus (0.984) and Tonovet (0.981). The Tono-Pen Avia Vet also demonstrated a good correlation in the present study (0.877), and similar values in the dog study (0.847). Rusanen *et al* (2010)[17] compared the Tonovet tonometers with the Tono-Pen Vet in cats and concluded that both correlated well with direct manometry. McLellan *et al* (2013)[11] concluded in a comparative study of Tonovet with Tono-Pen XL in cats, that Tonovet was significantly more accurate than Tono-Pen XL, which agrees with our results.

In the Bland Altman agreement analysis, the two rebound tonometers evaluated in this study (Tonovet and Tonovet Plus) overestimated the readings between 25 and 40 mmHg, but correlated well with direct manometry, with the best agreement in the range between 45 and 60 mmHg, which disagrees with a validation study carried out with Tonovet in cats, which demonstrated an overestimation of IOP at higher pressures, as well as an increase in variance, particularly between 40 and 60 mmHg.[11] However, in another comparative study with the Tonovet in cats, a good correlation with direct manometry was observed, with the best agreement in the range between 25 and 50 mmHg despite the tonometer underestimating IOPs below 25 mmHg and showing reduced accuracy only at pressures above 50 mmHg.[17]

An experimental study carried out with the Tono-Pen XL in cats observed that this tonometer underestimated the IOP in the IOP values between 10 and 50 mmHg. cats.[21] In another study carried out with the same tonometer, an underestimation of 3 to 5 mmHg was observed in all readings in cats.[11] In this study, the Tono-Pen Avia Vet underestimated the lower readings and overestimated the IOP values at higher pressures, especially between 35 and 40 mmHg. Better agreement was found for readings between 20 and 30 mmHg in this study in cats, which contradicts research by Martinez & Plummer, that the Tono-Pen Vet was consistent with manometry of only 10 mmHg in dogs and cats.[14]

Goldmann applanation tonometry with the Kowa HA-2 also correlated well with direct manometry in our study and showed the best agreement in readings between 10 and 40 mmHg. In addition, the Kowa HA-2 was also the device that came closest to ocular manometry in the *in vivo* evaluation. Existing works with Goldmann tonometry in cats with the Perkins tonometer[8,12,25] and the Kowa HA-2[9] also had similar results.

Barraquer's blepharostat was used in *ex vivo* and *in vivo* assessments as it does not cause acute changes in IOP in cats.[13] All assessments were performed in the afternoon, between 1 pm and 5 pm to avoid circadian variations[19] always in the center corneal region with the tonometers positioned at a 90º angle in order to circumvent possible differences between the measurements.[26] During the procedure, the animals were gently positioned sitting or standing, the neck kept free and the eyelids slightly apart with the help of the fingers of the contralateral hand of the examiner so as not to impair the measured IOPs.[18,22] Studies have reported that the use interval between the tonometers is 1 min,[7] 2 min[20] and 10 min.[27] In this study, a 2-minute interval was used. All eyes were assessed contiguously, as there are no significant differences in the IOP comparison between the right and left sides.[11,17]

Knowing the differences between the tonometers helps the clinician with the interpretation of the IOP values obtained by different devices.[28] In the clinical evaluation, the Tonovet measured significantly higher IOP values ($p>0.05$) with average results of 3 to 6 mmHg above the values recorded by the other tonometers, similar to what was found by Russanen *et al* (2010) who observed average IOPs of 2 to 3 mmHg above those measured with the Tono-Pen Vet applanation tonometer.[17] Similar to our finding, in the comparative study of the same four tonometers in dogs[10], a statistical difference was also observed, however with the Tonovet Plus, with readings between 3 and 5 mmHg above the others, and which also corroborated with other similar work comparing Tonovet, Tonovet Plus and Tono-Pen Avia Vet in dogs with normal eyes, where the values of Tonovet Plus also were significantly higher than the pressures obtained with the Tonovet, which were significantly higher than the Tono-Pen Avia Vet.[28] In a recent study by Kerdchuchuen *et al* (2021) comparing the use of Tonovet Plus in brachycephalic and non-brachycephalic cats the IOP values obtained from non-brachycephalic cats (18.77±0.49) are close to the values obtained in the present study, which was 17.1±5.4.[24]

Another study that compared two rebound tonometers (Tonovet and Tonovet Plus) and two applanation tonometers (Tono-Pen Avia Vet and Tono-Pen Vet) in dogs concluded that devices with rebound methodology were significantly more accurate than applanation devices, although all tonometers underestimated the IOP with increasing pressure.[29]

Rebound tonometry is well tolerated by the animals[17] and was also well tolerated in the present study. Its ease of use and degree of precision in inexperienced hands makes it a useful instrument for professionals with little ophthalmic experience.[30] Despite this, it was observed

in our study that the innovations of the recently launched Tonovet Plus do not favor its use in cats, who were startled by the beep emitted at each measurement and by the signal lights around the probe, it is recommended that these functions be deactivated in the clinical routine, when necessary.

In the outpatient clinical study, the IOP values observed between the tonometers were variable among themselves. The IOP values were from Tonovet 19.7±6.6 (9.0-39.0), Tonovet Plus 17.1±5.4 (8.0-32.7), Tono Pen Avia Vet 16 .3±4.3 (9.0-26.7), and from Kowa HA-2 14.5±2.2 (10.0-23.6). Higher IOP values with Tonovet, and lower and less variable with Kowa HA-2, are close to those reported in the cat studies described with Tonovet by Rusanen *et al* (2010)[17], and by Ricci *et al* (2017)[9]. These findings reinforce the need for a differentiated IOP table for cats.

In the outpatient clinical study, the rebound tonometers, Tonovet and Tonovet Plus, were the easiest and fastest to perform IOP measurements, but some cats were uncomfortable with the light of the Tonovet Plus.

One of the limitations of our study, which is common in research with cats, is a smaller sample size of the research compared to the number with tonometry studies with dogs, as in the studies by Passarelli *et al* (2021) (n=56),[10] and von Spiessen *et al* (2013) (n=80).[31] However, the number of eyes used in the present study *ex vivo* (n=10) and *in vivo* (n=10) for the correlation of manometry *versus* tonometry are greater than the reported by Rusanen *et al* (2010) (n=6),[17] McLellan *et al* (2013) who used 1 normal cat (n=2) and 2 cats with glaucoma (n=4),[11] and Martinez *et al* (2019) (n=6).[14]

2.5 CONCLUSIONS AND RELEVANCE

All tonometers were accurate in measuring IOP in healthy cats with normal eyes. The Tonovet Plus, Tonovet and Kowa HA-2 showed a strong correlation in relation to manometry observed in the *ex vivo* study, and the Tono-Pen Avia Vet showed a good correlation. The Kowa HA-2 tonometer was the tonometer that in the *in vivo* study showed the highest IOP approached manometry, in addition to a smaller variation. In the clinical outpatient routine, the highest IOP values were measured with the Tonovet and the lowest with the Kowa HA-2, which reinforces the need for a table of IOP reference values for each of these tonometers in

cats. Therefore, the current research may contribute with more information about these devices in this species.

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CONFLICT OF INTERESTS

The authors declare that there are no potential conflicts of interest regarding the research, authorship and/or publication of this article.

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ETHICAL APPROVAL AND CONSENT STATEMENTS

The experiment was approved by the Ethics Committee on the Use of Animals of UNOESTE under protocol n. 4979, conducted in accordance with the ARVO (Association for Research in Vision and Ophthalmology – Statement for the use of animals in ophthalmic and visual research) animal testing standards and the International Guiding Principles for Biomedical Research Involving Animals (1985) of the Council for International Organizations of Medical Sciences. The inclusion of animals in the study was voluntary and the tutors signed the Free and Informed Consent Form.

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Table 1 Characteristics observed by the authors in this study using the Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 tonometers in the clinical evaluation of cats without ocular alterations.

Caracteristics	Tonovet	Tonovet Plus	Tono-Pen Avia Vet	Kowa HA-2
Accuracy	+++	+++	++	+++
Acceptance by cats	+++	++	++	++
Ease of use	+++	+++	++	+
Disposable probe	Yes	Yes	Yes	No
Topical anesthesia	No	No	Yes	Yes

+: low, ++: moderate, +++: high.

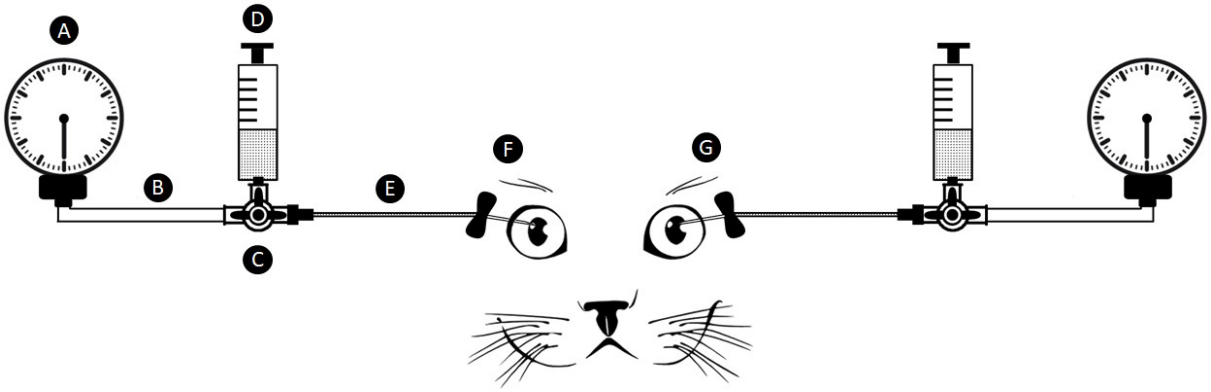

Figure 1 Schematic diagram *ex vivo* study. Aneroid manometer **(A)** positioned at the same height in relation to the center of the eye, connected to a polyethylene tube **(B)** connected to a three-way stopcock **(C)** with a saline solution reservoir **(D)** and another polyethylene tube **(E)**. Cannulated anterior chamber with a 23G scalp 2 mm posterior to the temporal limbus at 10 o'clock in the right eye **(F)** and 2 o'clock in the left eye **(G)**.

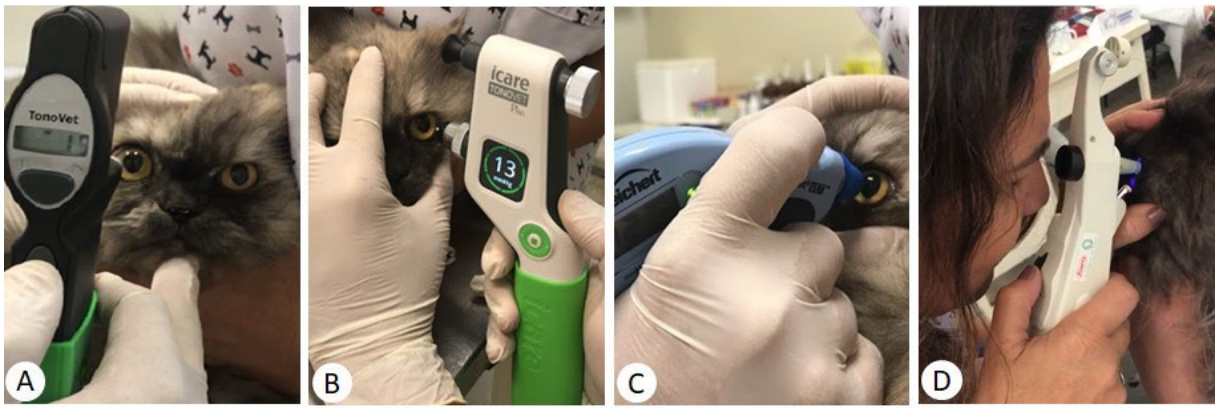

Figure 2 Clinical assessment. IOP reading with **A** Tonovet, **B** Tonovet Plus, **C** Tonopen Avia Vet, and **D** Kowa HA-2.

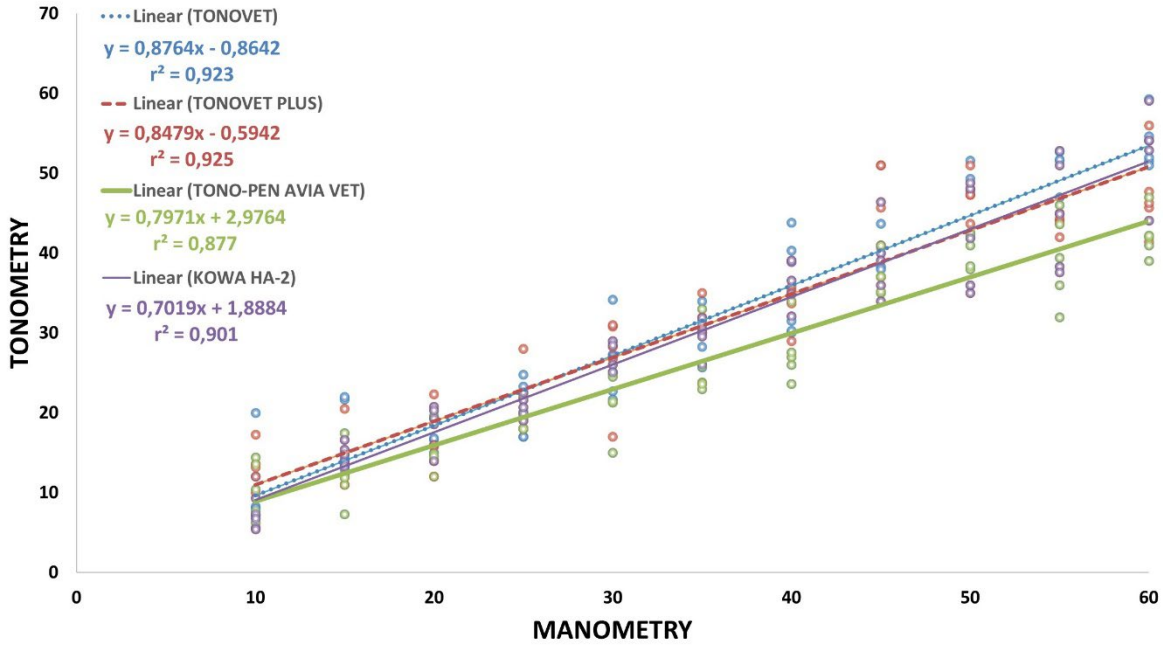

Figure 3 Regression analysis between manometry (aneroid manometer) and tonometry (Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2) in ten eyes of five cats in the *ex vivo* study.

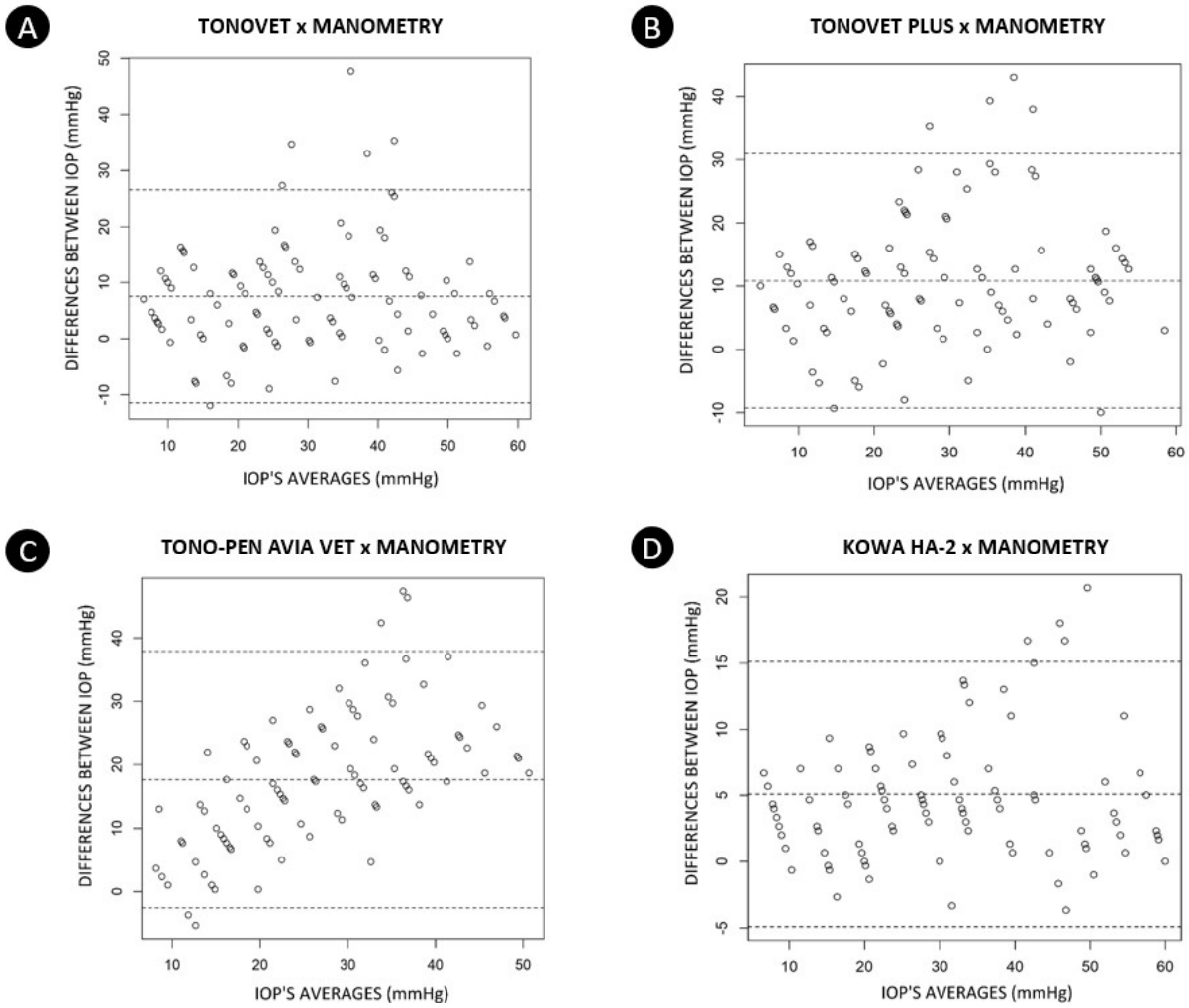

Figure 4 Bland-Altman analysis of means and differences between IOP readings in mmHg obtained between the **A** Tonovet, **B** Tonovet Plus, **C** Tono-Pen Avia Vet and **D** Kowa HA-2 tonometers and manometry (aneroid manometer) and in ten eyes.

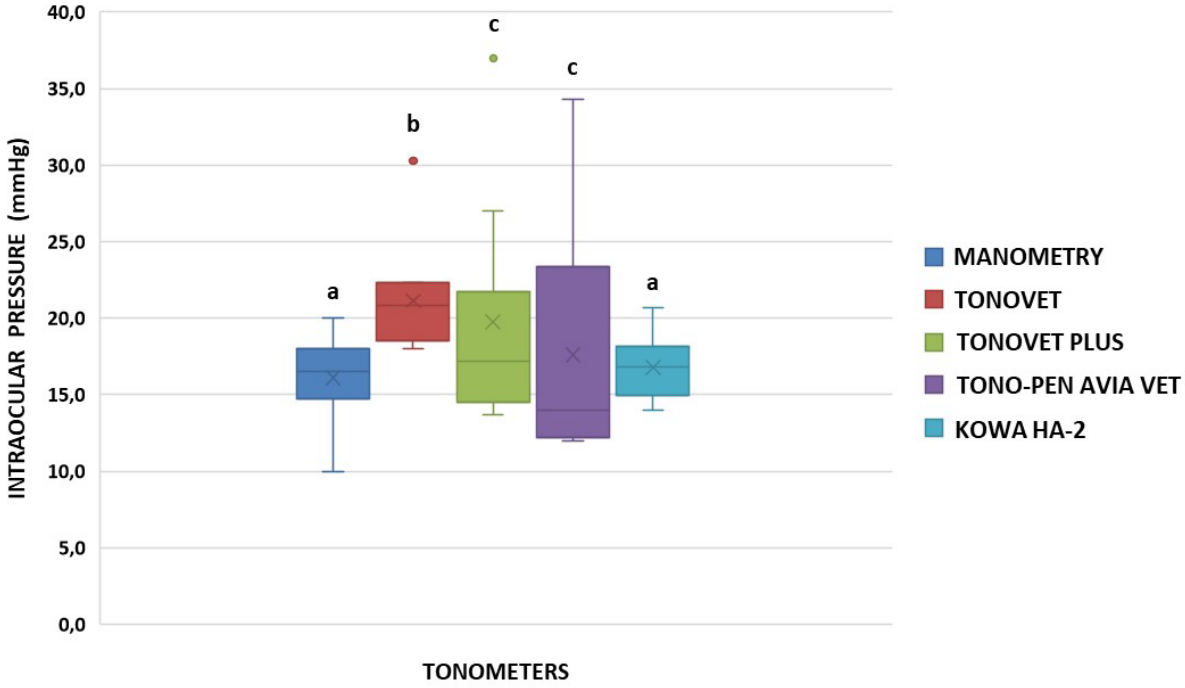

Figure 5 Boxplot of intraocular pressure (mmHg) readings between manometry (aneroid manometer) and tonometry (Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2) in 10 normal eyes of five cats in the *in vivo* study. Different letters indicate significant differences ($p < 0.05$). ･:outlier.

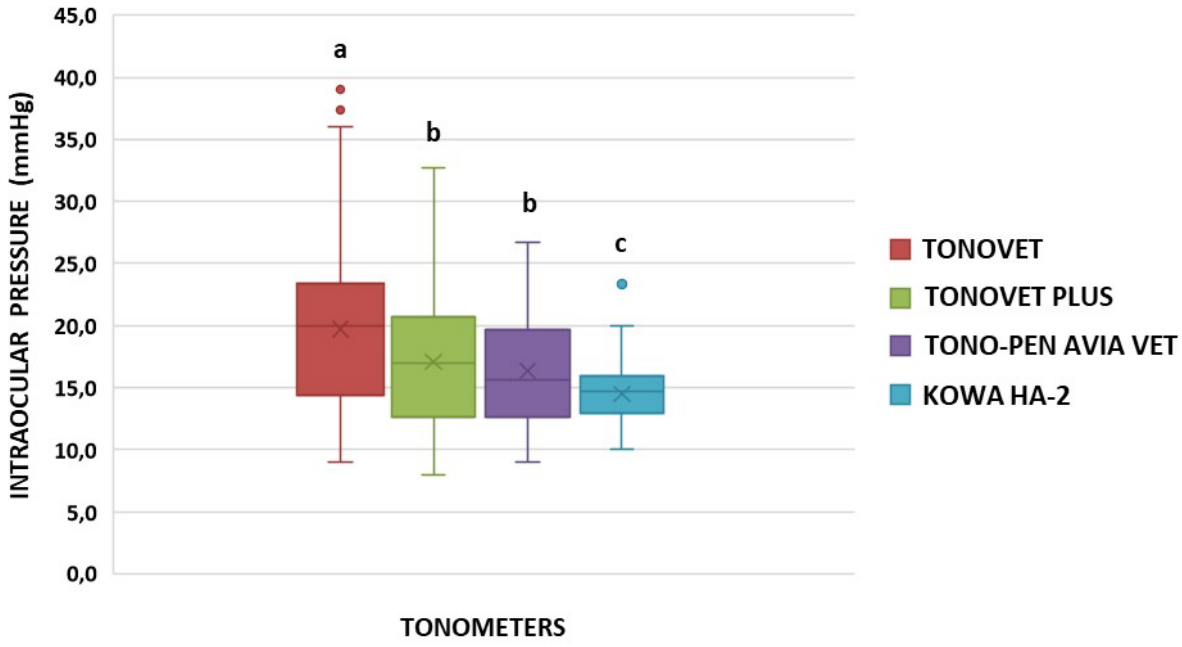

Figure 6 Boxplot of intraocular pressure (mmHg) readings from tonometers (Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2) in 88 normal eyes of 45 healthy cats, evaluated in the outpatient clinical study. Different letters indicate significant differences ($p<0.05$). ·:outlier.

ANEXOS

ANEXO A-

Clinical evaluation of IOP measurement in mmHg with the Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 tonometers in 88 healthy of 45 healthy cats from March 2019 to February 2020 in the ophthalmology department of the CMPA of the UNOESTE HV, Presidente Prudente, SP, Brazil.

Animal		**Mean±Standard Deviation / (Minimum – Maximum)**							
Sex /	**Breed**	**TONOVET**		**TONOVET PLUS**		**TONO-PEN AVIA VET**		**KOWA HA-2**	
Age (months)		**OD**	**OS**	**OD**	**OS**	**OD**	**OS**	**OD**	**OS**
1. M/180	PERSIAN	19±1 (18-20)	26.3±0.6 (26-27)	18.7±0.6 (18-19)	21.3±4.2 (18-26)	19.7±0.6 (19-20)	12.7±2.5 (10-15)	18.7±1.2 (18-20)	13±1 (12-14)
2. M/12	SIAMESE	21.7±0.6 (21-22)	16.3±0.6 (16-17)	19.3±1.5 (18-21)	15.7±0.6 (15-16)	23.7±2.5 (21-26)	15.3±1.2 (14-16)	11.7±1.2 (11-13)	15±1 (14-16)
3. F/7.92	DPC	11.3±3.2 (9-15)	12.3±1.5 (11-14)	12.7±2.1 (11-15)	16±1 (15-17)	10.7±1.2 (10-12)	11.7±0.6 (11-12)	9.3±0.6 (9-10)	12.7±1.2 (12-14)
4. F/24	DPC	41.3±3.5 (38-45)	36±1.7 (35-38)	32.7±1.2 (32-34)	29.7±2.9 (28-33)	23±1 (22-24)	26.3±3.1 (23-29)	15±0 (15-15)	15.3±0.6 (15-16)
5. M/30	SIAMESE	9±1 (8-10)	7.3±0.6 (7-8)	10.3±0.6 (10-11)	8.7±1.5 (7-10)	10.7±1.5 (9-12)	10.7±1.5 (9-12)	10±0 (10-10)	8±0 (8-8)
6. M/48	DPC	11.7±2.5 (9-14)	13±1 (12-14)	10.7±1.2 (10-12)	11.3±0.6 (11-12)	10±0 (10-10)	11.7±1.2 (11-13)	11.7±0.6 (11-12)	11±0 (11-11)
7. M/72	DPC	35.3±3.8	27.7±1.5	25.7±2.1	23±1	20.7±3.5	22.7±2.1	13.3±1.5	14.3±2.1

		(31-38)	(26-29)	(24-28)	(22-24)	(17-24)	(21-25)	(12-15)	(12-16)
8. F/24	SIAMESE	37.3±1.5 (36-39)	26.3±1.5 (25-28)	23.3±2.9 (20-25)	18.7±0.6 (18-19)	20±1 (19-21)	14.7±2.3 (12-16)	10.7±0.6 (10-11)	10.3±0.6 (10-11)
9. F/132	DPC	30.7±2.3 (28-32)	26±1 (25-27)	30±1.7 (28-31)	26.3±2.1 (24-28)	21.7±0.6 (21-22)	16.3±1.5 (15-18)	11±1 (10-12)	14.7±2.1 (13-17)
10. M/36	PERSIAN	15.7±1.2 (15-17)	17±2.6 (14-19)	16±2 (14-18)	14±0 (14-14)	13.7±1.5 (12-15)	15±4.4 (12-20)	15.3±0.6 (15-16)	14±1.7 (12-15)
11. F/24	DPC	20±1 (19-21)	25.3±2.1 (23-27)	17.7±0.6 (17-18)	18±2 (16-20)	21.3±2.1 (19-23)	26.7±3.2 (23-29)	13±1 (12-14)	14.7±0.6 (14-15)
12. F/3	DPC	10.7±1.5 (9-12)	12.3±1.2 (11-13)	10±2.6 (8-13)	8.3±0.6 (8-9)	7.7±0.6 (7-8)	11±2 (9-13)	3.3±0.6 (3-4)	5.7±0.6 (3-4)
13. M/60	DPL	23.3±1.5 (22-25)	22.7±2.1 (21-25)	24±1 (23-25)	16.7±1.5 (15-18)	16.3±3.5 (13-20)	24.3±2.3 (23-27)	9.7±0.6 (9-10)	9.7±0.6 (9-10)
14. F/84	SIAMESE	14.7±2.5 (12-17)	13.3±1.2 (12-14)	12.7±1.2 (12-14)	12±1 (11-13)	11±1.7 (10-13)	11.3±1.2 (10-12)	14.7±1.5 (13-16)	10±2.6 (8-13)
15. M/60	DPC	28±2 (26-30)	22.7±0.6 (22-23)	25±2 (23-27)	18.3±1.5 (17-20)	19±2.6 (16-21)	18±3.5 (16-22)	23.3±1.2 (22-24)	16±0 (16-16)
16. M/60	DPC	12.7±11 (11-14)	21.3±2.9 (18-23)	17±2 (15-19)	20.3±4.6 (15-23)	19.3±0.6 (19-20)	19.7±1.5 (18-21)	12±0 (11-13)	13.3±2.5 (11-16)
17. F/3	DPC	12±0 (12-12)	13.3±1.5 (12-15)	11±3.6 (7-14)	9.7±0.6 (9-10)	14.3±2.1 (14-16)	12.7±1.5 (11-14)	10.3±1.5 (9-12)	15.5±0 (15-15)
18. M/24	DPC	22±3 (19-25)	25±3 (22-28)	16.3±0.6 (16-17)	21.3±1.2 (20-22)	23.7±0.6 (23-24)	22.7±2.1 (21-25)	14.3±2.1 (12-16)	11.3±0.6 (11-12)

19. M/24	DPL	23.7±3.2 (20-26)	21.3±1.2 (20-22)	20.7±2.9 (19-24)	13.3±0.6 (13-14)	12.7±2.1 (11-15)	11.3±0.6 (11-12)	18±0 (18-18)	18.3±1.5 (17-20)
20. M/7.9	DPC	27±3.5 (25-31)	22.7±2.9 (21-26)	22.3±3.2 (20-26_	21±3 (18-24)	20.3±0.6 (20-21)	19.7±1.2 (19-21)	12.3±3.2 (10-16)	10.3±0.6 (10-11)
21. M/120	DPC	-	26.3±2.1 (24-28)	-	22.3±3.1 (19-25)	-	19±1 (18-20)	-	12±1 (11-13)
22. F/72	DPC	24.3±0.6 (24-25)	22±1 (21-23)	20.7±1.5 (19-22)	21.3±2.9 (18-23)	14.7±2.2 (13-17)	13.3±1.2 (12-14)	15±1.7 (13-16)	14.3±0.6 (14-15)
23. M/3.6	DPC	-	15±1 (14-16)	-	15.3±0.6 (15-16)	-	165±1 (15-17)	-	10.3±0.6 (10-11)
24. M/1.9	DPC	13±1.7 (12-15)	14.3±1.2 (13-15)	9±1 (8-10)	9.7±2.1 (8-12)	9±1 (8-10)	23.3±3.5 (20-27)	13.3±0.6 (13-14)	13.7±0.6 (13-14)
25. M/24	DPC	18±1 (17-19)	20.3±1.2 (19-21)	17±0 (17-17)	17.3±1.2 (16-18)	17±1 (16-18)	18.3±1.5 (17-20)	15±0 (15-15)	15.3±0.6 (15-16)
26. M/3	DPC	19.3±2.5 (17-22)	15.3±1.2 (14-16)	11.3±0.6 (11-12)	15.7±0.6 (15-16)	15.3±1.5 (14-17)	15±1 (14-16)	12.3±0.6 (12-13)	14.7±1.5 (13-16)
27. F/48	DPL	23±1.7 (22-25)	21±1.7 (20-23)	15±1 (14-16)	17±1 (16-18)	14±1 (13-15)	13.3±1.5 (12-5)	12.3±0.6 (12-13)	14.3±0.6 (14-15)
28. F/48	PERSIAN	20.7±1.5 (19-22)	17.3±1.2 (16-18)	15.3±0.6 (15-16)	13.7±1.2 (13-15)	20±1 (19-21)	12±0 (12-12)	14.7±0.6 (14-15)	14.7±0.6 (14-15)
29. M/96	DPC	20.3±3.5 (17-24)	20.3±4 (18-25)	18.3±6 (12-24)	15.3±1.2 (14-16)	15.7±2.1 (14-18)	16±0 (16-16)	14.7±0.6 (14-15)	14±0 (14-14)
30. F/36	DPC	22.3±1.5	21±1.7	18.3±1.5	20±4.6	17.7±3.2	14.3±1.65	12±2	5.3±1.5 (4-

		(21-24)	(19-22)	(17-20)	(16-25)	(16-21)	(13-16)	(10-14)	7)
31. M/0.6	DPC	9.7±0.6 (9-10)	10.3±0.6 (10-11)	4.3±1.5 (3-6)	10±2.6 (8-13)	10.7±2.1 (9-13)	15±3.6 (11-18)	12±0 (12-12)	11±0 (11-11)
32. M/72	DPC	23.3±3.2 (21-27)	21.3±3.1 (18-24)	20.3±3.2 (18-24)	17.3±3.5 (14-21)	16±1 (15-17)	15±1 (14-16)	9.7±1.5 (8-11)	12.3±0.6 (12-13)
33. F/36	SIAMESE	24.7±0.6 (24-25)	15±2 (13-17)	16±1 (15-17)	14.7±0.6 (14-15)	18.7±1.2 (18-20)	14.3±0.6 (14-15)	12.7±1.2 (12-14)	8.7±1.2 (8-10)
34. F/7.9	DPC	27.7±0.6 (27-28)	22.3±0.6 (22-23)	22.7±1.5 (21-24)	23.3±1.2 (22-24)	21±1 (20-22)	24±1 (23-25)	14±2 (12-16)	16±1.7 (12-16)
35. M/156	PERSIAN	39±2.6 (36-41)	26.3±2.3 (25-29)	30.7±2.3 (28-32)	25.3±4 (23-30)	16±1 (15-17)	14.3±0.6 (14-15)	14.7±1.2 (14-16)	12±0 (12-12)
36. F/24	PERSIAN	23.7±1.5 (22-25)	18±0 (18-18)	17.3±1.2 (16-18)	19.7±1.2 (19-21)	12.3±1.2 (11-13)	18.3±2.1 (16-20)	14±0 (14-14)	16.7±0.6 (16-17)
37. F/12	DPS	16.7±1.2 (16-18)	20±1 (19-21)	16.3±1.5 (15-18)	18±1.7 (16-19)	17.7±0.6 (17-18)	16±1 (15-17)	13.7±1.2 (13-15)	14±1.7 (13-16)
38. M/72	MAINE COON	13.3±1.2 (12-14)	13.3±1.5 (12-15)	12.7±2.1 (11-15)	13±1 (14-12)	12.3±0.6 (12-13)	11.7±0.6 (11-12)	13.7±0.6 (13-14)	12±0 (12-12)
39. F/1.9	DPC	9.3±0.6 (9-10)	14.7±2.1 (13-17)	8±1 (7-9)	9.3±0.6 (8-9)	7.3±0.6 (7-8)	11.7±1.2 (11-13)	9.7±1.5 (8-11)	10±1 (9-11)
40. M/12	DPC	19±1 (18-20)	17±1.7 (15-18)	21±2 (19-23)	21±0 (21-21)	22.7±4.2 (18-26)	15±1.7 (14-17)	14.7±1.5 (13-16)	12±2 (10-14)
41. F/12	DPC	14.3±2.5 (12-17)	17±1 (16-18)	11.7±0.6 (12-12)	17.3±2.3 (16-20)	14.7 ±1.2 (14-16)	15.7±1.5 (14-17)	11.7±1.5 (10-13)	10.7±1.5 (9-12)

42. F/12	DPC	18±1.7 (17-20)	13.3±0.6 (13-14)	12±0 (12-12)	12±0 (12-12)	18.3±3.2 (16-22)	15±2.6 (13-18)	15±1 (14-16)	12±0 (12-12)
43. F/12	DPC	8.7±0.6 (8-9)	11±1.7 (9-12)	8.3±0.6 (8-9)	8.3±0.6 (8.9)	12±1 (11-13)	12.3±1.5 (11-14)	14±0 (14-14)	11±0 (11-11)
44. F/12	DPC	18±1.7 (16-19)	20±0 (20-20)	17.7±1.5 (16-19)	17.7±0.6 (17-18)	17±2 (15-19)	13.7±0.6 (13-14)	15±2.6 (13-18)	14.3±1.5 (13-16)
45. F/12	DPC	21.3±2.1 (19.23)	18±0 (18-18)	17±1.7 (15-18)	14.7±1.2 (14-16)	21±6 (15-27)	15±1 (14-16)	11.3±2.3 (10-14)	12±1 (11-13)

*Average of three readings performed with each tonometer. F: female; M: male; DPL: domestic long hair; DPC: domestic shorthair; IOP: intraocular pressure; RE: right eye; OS: left eye

ANEXO B– APROVAÇÃO DOS TRABALHOS PELO COMISSÃO DE ÉTICA EM USO DE ANIMAIS (CEUA) DA UNIVERSIDADE DO OESTE PAULISTA (UNOESTE)

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PEIC - Programa Especial de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"ESTUDO MORFOLÓGICO DA CONJUNTIVA PALPEBRAL DE GATOS SAUDÁVEIS"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **5725** e tendo como participante(s) **CLAUDIA LIZANDRA RICCI (discente), JOAO VICTOR GOULART CONSONI PASSARELI (discente), GIOVANA JOSE GARCIA ESTANHO (discente), DEBORA DA SILVA ALVES (discente), JOSÉ RAIMUNDO CORRÊA (participante externo), PAULA DINIZ GALERA (participante externo), ROSÉLIA DE LIMA SOUZA ARAÚJO (participante externo), GISELE ALBORGHETTI NAI (docente), SILVIA MARIA CALDEIRA FRANCO ANDRADE (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, 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), tendo sido APROVADO em reunião realizada em 11/09/2019.

Vigência do projeto: 08/2019 a 10/2021.

ANIMAL VIVO

Espécie/Linhagem	Nº de Animais	Peso	Idade	Sexo	Origem
FELINO	2	5 quilos	5 anos	M	ATENDIMENTO ABULATORIAL HV
FELINO]	3	5 quilos	5 anos	F	ATENDIMENTO ABULATORIAL HV]

Presidente Prudente, 13 de Setembro de 2019.

Prof. Dr. Nair Rodrigues Garcia Jr.
Coordenador Científico da CPDI

Profª Ms. Adriana Falcão de Brito
Coordenadora da CEUA - UNOESTE

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PEIC - Programa Especial de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"COMPARAÇÃO ENTRE OS TONÔMETROS PORTÁTEIS TONOVET, TONOVET PLUS, TONO-PEN AVIA VET, KOWA IIA-2 NA MENSURAÇÃO DA PRESSÃO INTRAOCULAR EM GATOS"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **4979** e tendo como participante(s) **CLAUDIA LIZANDRA RICCI (discente), LUIS FELIPE DA COSTA ZULIM (discente), FELIPE FRANCO NASCIMENTO (discente), JOAO VICTOR GOULART CONSONI PASSARELI (discente), TAINA MARIA PAULINO LEOPOLDO (discente), THAIS ANGELONI DE OLIVEIRA BARBOZA (discente), GLAUCIA PRADA KANASHIRO (docente), SILVIA MARIA CALDEIRA FRANCO ANDRADE (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, 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), tendo sido APROVADO em reunião realizada em 14/11/2018.

Vigência do projeto: 10/2018 a 10/2021.

ANIMAL VIVO

Espécie/Linhagem	Nº de Animais	Peso	Idade	Sexo	Origem
Felino, DPC	5	4 quilos	4 anos	F	Gatil Unoeste
Felino, DPC	20	4 quilos	4 anos	M	Gatil/Atendimento Ambulatorial
Felino, DPC	20	4 quilos	4 anos	F	Gatil/Atendimento Ambulatorial

Presidente Prudente, 22 de Novembro de 2018.

Prof. Dr. Jair Rodrigues Garcia Jr.
Coordenador Científico da CPDI

Profª Ms. Adriana Falcão de Brito
Coordenadora da CEUA - UNOESTE

ANEXO C- NORMAS DE PUBLICAÇÃO DA REVISTA *VETERINARY OPHTHAMOLOGY*

Mission Statement:

Veterinary Ophthalmology is a peer-reviewed. online. international journal that welcomes submission of manuscripts whose target audience includes Diplomates of the ACVO and ECVO. members of international ophthalmic societies and associations. academic researchers with interests in veterinary or comparative ophthalmology. specialists and general practitioners with an ophthalmology interest. *Veterinary Ophthalmology* strives to serve as the primary journal for all articles pertaining to veterinary and comparative ophthalmology published worldwide.

Veterinary Ophthalmology publishes original material relating to all aspects of clinical and investigational veterinary and comparative ophthalmology. The following types of material will be considered for publication:

- Original articles including clinical (prospective and retrospective clinical studies) and investigational studies. Studies involving animals must have the approval of the institution's animal care and use committee or other appropriate regulatory group and be acceptable to the Editor-in-Chief.
- Review articles (including papers which clarify. summarize and critically evaluate the current literature). These may be invited by the Editor or a member of the editorial board.
- Case Reports (limited to 2000 words. 4 figures and 18 references). In general. single case reports describing observations in an animal will not be considered unless the report makes a substantial contribution to ophthalmic knowledge (important mechanistic insights. illuminate a novel principle or technique. or describe a newly recognized disease or important variation or increased depth of understanding of a recognized disease) and is not merely additive to the existing literature. Reporting a procedure previously established but performed for the first time in a new species generally does not add sufficient new knowledge to justify publication. unless unique aspects of the procedure or management have been adjusted. Justifying innovation and unique aspects of the case is the responsibility of the authors.
    - Authors of case reports rejected by *Veterinary Ophthalmology* may be offered the option of having their manuscript. along with any related peer review comments. automatically transferred for consideration by the editorial team of *Clinical Case Reports*. Authors will not need to reformat or rewrite their manuscript at this stage. and publication decisions will be made a short time after the transfer takes place. *Clinical Case Reports* is an Open Access journal. and article publication charges apply. For more information. please visit: https://onlinelibrary.wiley.com/journal/20500904

- Viewpoint articles (papers which challenge existing concepts or present an alternative interpretation of available information) are usually invited by the Editor or a member of the editorial board.
- Brief communications: Brief research and clinical communications (limited to 1500 words. 2 figures and 12 references). Brief communications are used when the extent of the investigation or the findings do not warrant a full paper. but are still considered of value to the ophthalmic community.
- Letters to the editor will be limited to 750 words. including references. and one image / figure / table; letters may cover a variety of topics and these may include but are not restricted to: a. Briefly highlighting or commenting on an issue in a previously published paper. b. Seeking to generate discussion or awareness of a developing area.

Double-Masked Peer Review Process

Veterinary Ophthalmology uses a double-masked peer review process for all manuscripts (i.e. the reviewers and authors are masked each other's identity). Authors are required to submit BOTH masked and unmasked versions of their main document. It is crucial that authors conscientiously redact identifying information from their manuscripts for a double masked review to be successful. Responsibility for ensuring that manuscripts are adequately masked rests entirely with the authors and not with the Journal.

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Ethics approval statements that refer to your institution

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Author institution details
Author contact details
Acknowledgements
Conflicts of interest (if declared)
Ethics approval statements that refer to your institution

Author Guidelines

Veterinary Ophthalmology uses the guidelines and criteria for authorship of the International Committee of Medical Journal Editors (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). The editorial staff is not responsible for resolving disputes between authors or potential authors of manuscripts submitted or accepted for publication.

Authorship should be based on the following criteria:

- Substantial contributions to the conception or design of the work; or the acquisition. analysis. or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Participation solely in the acquisition of funding or the collection of data (such as recruiting cases in multi-center drug trials) does not justify authorship and. except in the case of complex large-scale or multi-center research. the number of authors should usually not exceed six. Contributors who do not qualify as authors should be mentioned under Acknowledgements

Animals in Research

The Journal is committed to the ethical use of animals and ethical involvement of people in all studies that it publishes. The Journal also notes that requirements for ethical approval differ among countries and states or provinces and therefore we hold authors and their institutions primarily responsible for the ethical conduct of studies. We require that authors indicate during submission of their manuscript if the study required institutional or governmental approval in the jurisdiction in which it was conducted. and whether such approval was granted.

The Journal reserves the right to decline to publish manuscripts in which approval by the appropriate regulatory group is not reported. when the Journal has concerns about the welfare or treatment of animals used in the study. where there is reason to believe that animals have been subjected to unnecessary or avoidable pain or distress or when the Journal has concerns about involvement of people in the study.

The Journal is not obliged to reveal the basis for such a decision.

The Journal may request further information about care and use of animals. or involvement of people. including evidence of regulatory approval or compliance with local regulations.

Ethical approval for studies involving animals

Studies of animals including. but not restricted to. those describing induction of disease. administration of drugs or other substances. surgical interventions or clinical trials must be conducted in accordance with regulations governing the use of animals at the authors' institution. It is the responsibility of the authors to obtain approval by the appropriate regulatory group (e.g.. Institutional Laboratory Animal Care and Use Committee. Animal Ethics Committee. Hospital Board) and report this approval. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.

The design of studies involving client-owned animals with spontaneous disease (e.g.. clinical trials. prospective or retrospective studies. case reports) should be approved by the appropriate regulatory group (e.g.. hospital board) and should include a statement of informed consent by the animal's owner or the owner's representative.

It is incumbent upon the authors to ensure that care of animals meets standards required by the authors' institution.

Prior Publication. Plagiarism and self-plagiarism

Manuscripts submitted to *Veterinary Ophthalmology* may be screened electronically for plagiarism. *Veterinary Ophthalmology* is a member of CrossCheck. a service offered by CrossRef and powered by iThenticate software. iThenticate is a plagiarism screening service that verifies the originality of content submitted before publication. iThenticate checks submissions against millions of published research papers. and billions of web content.

Manuscripts are accepted for consideration on the understanding that they are being considered for publication solely in *Veterinary Ophthalmology* and that they have been neither published nor are under consideration for publication elsewhere. Manuscripts will be considered for publication with the clear understanding that their contents have not been previously published (abstracts $\leq$ 250 words presented at scientific meetings are excepted) and have not been submitted or published elsewhere while acceptance by *Veterinary Ophthalmology* is under consideration. Authors should not include entire paragraphs from their previous publications in a new submission. For example. sections related to the significance of a condition or background knowledge about the topic should be unique to each publication.

Veterinary Ophthalmology is published in online-only format. This is a proactive step towards reducing the environmental impact caused by the production and distribution of printed journal copies and will allow the journal to invest in further digital development. Published articles will continue to

be disseminated quickly through the journal's broad network of indexing in the Web of Science. MEDLINE and Scopus. Articles will also continue to be discoverable through popular search engines such as Google.

EarlyView

Veterinary Ophthalmology is part of Wiley's Early View service. All articles are published online in Early View. These articles are fully peer reviewed. edited and complete – lacking only page numbers and volume/issue – and are considered fully published from the date they first appear online. This date is shown with the article in the online table of contents. Because Early View articles are considered fully complete. changes cannot be made to an article after the online publication date. even if it has not yet appeared in an Issue. Early View articles are given a Digital Object Identifier (DOI). which allows the article to be cited and tracked before it is allocated to an issue. After issue publication. the DOI remains valid and can continue to be used to cite and access the article.

Submitting Your Manuscript

Veterinary Ophthalmology only accepts manuscripts through our submission website. To submit a manuscript. please follow the instructions below:

Getting Started

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   - Enter your institution and address information as prompted then click 'Next.'
   - Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID) and then select your area of expertise.
   - Click 'Finish' when done.
4. Log-in and select 'Author Center.'
5. After you have logged in. click the 'Submit a Manuscript' link on the Author Center screen.
6. Enter data and answer questions as prompted.
7. Click on the 'Next' button on each screen to save your work and advance to the next screen.
8. You will be prompted to upload your files:
   - Click on the 'Browse' button and locate the file on your computer.
   - Select the description of the file in the drop down next to the Browse button.
   - When you have selected all files you wish to upload. click the 'Upload' button.

9. Review your submission (in both PDF and HTML formats) before sending to the Editors. Click the 'Submit' button when you are done reviewing.

You may stop a submission at any phase and save it to submit later. After submission. you will receive a confirmation via e-mail. You can also log-on to ScholarOne Manuscripts at any time to check the status of your manuscript. The Editors will send you information via e-mail once a decision has been made. A covering letter. signed by all authors. must be included. This should state that the work has not been published and is not being considered for publication elsewhere. and that all authors meet the journal's criteria for authorship. Information on any financial or other conflict of interest which may have biased the work should be provided (even if precautions were taken and authors are satisfied that bias was avoided).

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Data Protection

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Manuscript Style

The manuscripts must be in Microsoft Word format (.doc or .docx). The manuscript (including footnotes. references. figure legends. and tables) must be double-space typed. using 12-point Times New Roman font. 1-inch margins. and left justification. Original Research papers and Review Articles should usually not be longer than 5000 words. Viewpoint articles and Case reports will not normally exceed 2000 words. and Brief communications 1500 words.

Title Page

The title page should include a descriptive title for the article. the names [first name. initials of middle name(s). surnames]. qualifications and affiliations of all authors. and the full postal address. e-mail and telephone number of the author to whom correspondence should be addressed. A suggested running title of not more than 50 characters including spaces should be included.

Abstract and Keywords

The abstract should be on a separate page and should not exceed 250 words. Where possible. the

abstract should be structured. Suggested headings for abstracts of primary research are: Objective; Animal studied. Procedure(s). Results. and Conclusions.

Key words are used by indexes and electronic search engines. and should appear after the abstract. Use the heading 'Key words'. typed in bold and followed by a colon. and then the key words separated by commas. Include up to six key words. Also enter the key words where prompted during the submission process.

Main Text

This should begin on a separate page. Sections within the main text should be appropriately sub-headed: Introduction; Materials and methods. Results. and Discussion. Abbreviations and footnotes should be avoided where possible.

References

References should be in the AMA style. For more information about AMA reference style - AMA Manual of Style

All references should be numbered consecutively in order of appearance and should be as complete as possible. In text citations should cite references in consecutive order using Arabic superscript numerals. Sample references follow:

Journal article:

1. King VM. Armstrong DM. Apps R. Trott JR. Numerical aspects of pontine. lateral reticular. and inferior olivary projections to two paravermal cortical zones of the cat cerebellum. *J Comp Neurol* 1998;390:537-551.

Book:

2. Voet D. Voet JG. *Biochemistry*. New York: John Wiley & Sons; 1990. 1223 p.

Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

Please note that work that has not been accepted for publication and personal communications should not appear in the reference list. but may be referred to in the text (e.g. M. van der Burgh. personal communication). Also. it is the authors' responsibility to obtain permission from colleagues to include their work as a personal communication.

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The figures should be referred to as 'Fig.' and numbered consecutively in the order in which they are referred to in the text. Captions to figures. giving the appropriate figure number. should be typed on a separate page at the end of the manuscript; captions should not be written on the original drawing or photograph. In the full text online edition of the journal. figure legends may be truncated in abbreviated links to the full screen version. Therefore. the first 100 characters of any legend should inform the reader of key aspects of the figure. Further guidelines regarding the submission of artwork can be found at https://authorservices.wiley.com/asset/photos/electronic_artwork_guidelines.pdf.

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The journal will consider up to 2 video files to accompany articles. For video files to be accepted. they must clearly show a dynamic condition that cannot be adequately captured in still images. The Editor and/or Associate Editors will scrutinize all video submissions very carefully to assure they meet the intent of providing unique information. Video files of routine imaging findings will not be accepted. Up to 2 video files will be considered for each paper. Video files must be submitted in Quicktime format and each file must be less than 5MB in size.

Tables

Clear tables which contain essential data are welcome. however. a complete list of all data and/or case information is discouraged. except to be considered as supplementary data available online only. Tables should be formatted with the table function in a word processor. such as MS Word. on a separate page with the legend typed above. Column headings should be brief. with units of measurement in parentheses. All abbreviations must be defined in footnotes to the table. Number tables consecutively in the order they occur in the text. with Arabic numerals.

Acknowledgements

Acknowledgements should be brief and must include reference to sources of financial and logistical support. Author(s) should clear the copyright of material they wish to reproduce from other sources. and this should be acknowledged.

Conflict of Interest

Veterinary Ophthalmology requires that all authors include a conflict of interest statement in their manuscript (in a Conflict of Interest or Acknowledgements section). Any interest or relationship.

financial or otherwise. that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership. membership of a company board of directors. membership of an advisory board or committee for a company. and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript. they can query the editorial office at wilkie.1@osu.edu. The existence of a conflict of interest does not preclude publication in this journal. **If the authors have no conflict of interest to declare. they must also state this in the manuscript and at submission.** It is the responsibility of the corresponding author to review this policy with all authors and collectively to list in the manuscript. and in the online submission system ALL pertinent commercial and other relationships.

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Peer Review

A peer review process involving at least 2 reviewers who are members of the editorial review board or other veterinarians or scientists with disciplinary expertise is used. Up to one reviewer may be selected based on authors' recommendations. **Authors may suggest potential reviewers that have expertise in the field and can provide an unbiased evaluation.** Previous or current mentors. mentees. collaborators. colleagues. or personal relations are not suitable recommendations. Authors can also oppose reviewers who would have a conflict of interest in reviewing their manuscripts.

The reviewers' comments are reviewed by an associate editor who makes a recommendation on the suitability of the report for publication to the editor-in-chief. who makes the final decision. The editors reserve the right to seek consultation on appropriateness of study design. survey instruments. and methods used for statistical analysis of data.

Manuscripts that report studies that are otherwise scientifically sound may be rejected before or after peer review because they lack breadth of appeal. impact. or are outside the interest area of the journal. Likewise. studies that are simply additive to the literature and do not provide substantial discovery. mechanistic insight. challenge dogma. or highlight novel understanding are not likely to be considered suitable for publication.

Manuscripts that are poorly prepared or written will be returned without peer review. It is the authors' responsibility to submit manuscripts that are written in a style that enhances readability (clear. direct. concise. with appropriate grammar and spelling). Authors with limited experience in scientific writing in English are encouraged to seek assistance from professional editing services.

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Further Information

If you wish to discuss prospective submissions or to clarify the guidance outlined above. please contact Dr. David A. Wilkie at the editorial office (email: wilkie.1@osu.edu).

Further details about the peer review process and arrangements for the final submission of accepted articles and proofs will be sent to authors of accepted manuscripts and are available from the editorial office.

Author Services

Veterinary Ophthalmology currently offers article tracking for authors. This is a reminder to our authors to enroll in Wiley's Author Services production tracking service

You need to register in order to add your article to the article tracking system and be able to track your article online. As well as tracking the production of your article online. as a registered author you can also:

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ANEXO D- NORMAS DE PUBLICAÇÃO DA REVISTA *JOURNAL OF FELINE MEDICINE AND SURGERY*

1. Article types

Journal of Feline Medicine and Surgery (JFMS) considers manuscripts submitted in the following formats.

Original Articles

Papers should be as concise as possible. and generally not exceed 3.000 words (excluding references). Each paper should have a self-contained Abstract (up to 300 words. structured with subheadings as detailed in Preparing your manuscript). followed by Introduction. Materials and methods. Results. Discussion. Conclusions. Acknowledgements. Conflict of Interest. Funding. Ethical Approval and Informed Consent statements. and References. Note that Original Articles on well-recognised diseases that report valuable national or regional data on disease prevalence. or other relevant data. should be submitted to the sister journal. the Journal of Feline Medicine and Surgery Open Reports (jfmsopenreports.com) as a Short Communication. The manuscript submission guidelines for JFMS Open Reports can be found here.

Review Articles

Offers of reviews and topics for consideration should be directed to the Editors. via the editorial office (jfms@icatcare.org). for initial editorial approval. Reviews should provide an update on recent advances in a particular field and the length should not generally exceed 4.000 words. They should include an abstract (up to 300 words). followed by subheadings directed by the content. as well as Conclusions. Acknowledgements. Conflict of Interest. Funding. Ethical Approval and Informed Consent statements. and References.

Short Communications

Short communications reporting relevant research or sufficiently substantial pilot studies are considered for JFMS. They should generally not exceed 1.500 words. They should include a self-contained Abstract (up to 300 words. structured with subheadings as detailed in Preparing your manuscript). followed by Introduction. Materials and methods. Results. Discussion. Conclusions. Acknowledgements. Conflict of Interest. Funding. Ethical Approval and Informed Consent statements. and References.

Short Communications on well-recognised diseases that report valuable national or regional data on disease prevalence. or other relevant data. should be submitted to the sister journal. the Journal of Feline Medicine and Surgery Open Reports (jfmsopenreports.com) – the manuscript submission guidelines for which can be found here.

Case Series

Large prospective and retrospective case series are considered for JFMS. Depending on the information contained. a Case Series may be up to 2.500 words in length. It should include a brief Abstract (up to 300 words structured with subheadings detailed in Preparing your manuscript) followed by Introduction (optional). Case series description. Discussion. Conclusions. Acknowledgements. Conflict of Interest. Funding. Ethical Approval and Informed Consent statements. and References. Small case series and individual case reports that provide novel information should be submitted to the Journal of Feline Medicine and Surgery Open Reports – the manuscript submission guidelines for which can be found here.

Letters to the Editor

Letters commenting on papers recently published in JFMS will be considered for publication in the journal. Letters should not exceed 1.000 words (including references and one table or figure). The Editors may send the letter to the authors of the original paper for comment so that both letter and reply may be published together.

Manuscripts should be clearly labelled ‘Original Article’. ‘Review Article’. ‘Short Communication’. ‘Case Series’ or ‘Letter to the Editor’.

Table 1. Overview of the requirements for manuscript submission to JFMS

Article type	Abstract	Main text word limit	References	Figures/Tables
Original Articles	300 words	3.000 words*	As necessary	As necessary
Review Articles	300 words	4.000 words*	As necessary	As necessary
Short Communications	300 words	1.500 words*	As necessary	As necessary
Case Series	300 words	2.500 words*	As necessary	As necessary
Letters to the Editor	None	1.000 words**	As necessary	Either one table or figure

*Excludes abstract. references. tables and legends

**Includes references. tables and legends

Journal of Feline Medicine and Surgery Resident Best Paper Award

The award recognises quality and excellence for early career authors who publish in JFMS. Authors who are in a recognised veterinary residency programme (eg. ABVS. EBVS and ANZCVS residency) at the time of submission of their paper will automatically be eligible for consideration for the award. subject to its acceptance for publication. Accepted papers will be considered for the award in the year in which they are published. Details about this award for interested residents and resident supervisors can be found here.

Back to top

2. Editorial policies

2.1 Peer review policy

JFMS operates a single-blinded peer review process in which the reviewer's name is withheld from the author. The reviewer may at their own discretion opt to reveal their name to the author in their review but our standard policy is for the reviewer's identity to remain concealed. Each manuscript is reviewed by at least two referees. All manuscripts are reviewed as rapidly as possible. and an editorial decision is generally reached within 6–8 weeks of submission. Generally. JFMS does not accept more than two revisions to a paper.

2.2 Authorship

Papers should only be submitted for consideration once consent is given by all contributing authors. Corresponding authors should carefully check that all those whose work contributed to the paper are acknowledged as contributing authors.

The list of authors should include all those who can legitimately claim authorship. This is all those who:

1. Made a substantial contribution to the concept and design. acquisition of data or analysis and interpretation of data.

2. Drafted the article or revised it critically for important intellectual content.

3. Approved the version to be published.

4. Have participated sufficiently in the work to take public responsibiity for appropriate portions of the content.

Authors should meet the conditions of all of the points above. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

When a large. multicentre group has conducted the work. the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship.

Acquisition of funding. collection of data. or general supervision of the research group alone does not constitute authorship. All contributors who do not meet the criteria for authorship should be listed in the Acknowledgements section (see 2.3 below). Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship guidelines for more information on authorship.

Please note that manuscripts must be submitted with declaration statements in the following order: Acknowledgements (where relevant). Conflict of Interest. Funding. Ethical Approval and Informed Consent. Manuscripts may be returned if these statements are not included.

2.3 Acknowledgements

All contributors who do not meet the criteria for authorship should be listed in an Acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help (see also section 2.2).

2.3.1 Third party submissions

Where an individual who is not listed as an author submits a manuscript on behalf of the author(s). a statement must be included in the Acknowledgements section of the manuscript and in the accompanying cover letter. The statements must:

• Disclose this type of editorial assistance – including the individual’s name. company and level of input

- Identify any entities that paid for this assistance
- Confirm that the listed authors have authorized the submission of their manuscript via third party and approved any statements or declarations. e.g. conflicting interests. funding. etc.

Where appropriate. SAGE reserves the right to deny consideration to manuscripts submitted by a third party rather than by the authors themselves.

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It is not necessary to disclose the use of language polishing services.

Any acknowledgements should appear first at the end of your article prior to your Conflict of Interest statement.

2.3.2 Prior presentation at conferences

Details of any prior presentation of study findings/results at conferences or meetings should be included in an 'Author note' section after the 'Acknowledgements' and before the 'Conflict of Interest' statement.

2.4 Declaration of conflicting interests

It is the policy of JFMS to require a declaration of conflicting interests from all authors enabling a statement to be carried within the paginated pages of all published articles.

Please ensure that a 'Conflict of Interest' statement is included at the end of your manuscript. after any acknowledgements and before the 'Funding' statement. If no conflict exists. please state that 'The authors declared no potential conflicts of interest with respect to the research. authorship. and/or publication of this article'.

For guidance on conflict of interest statements. please see the ICMJE recommendations here.

2.5 Funding

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2.6 Clinical and Research Ethics. and Informed Consent

Prior to undertaking studies and prior to submitting a manuscript to JFMS. authors should read these guidelines to ensure requirements have been adequately met.

Circumstances relating to the use of animals in clinical and experimental studies must meet international standards as set out in:

- The International Guiding Principles for Biomedical Research Involving Animals (1985) from the Council for International Organizations of Medical Sciences. available at https://cioms.ch/shop/product/international-guiding-principles-for-biomedical-research-involving-animals-2/ (or from the Executive Secretary CIOMS. % WHO. Via Appia. CH-1211 Geneva 27. Switzerland)

- The Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals from the International Association of Veterinary Editors. available at http://www.veteditors.org/consensus-author-guidelines-on-animal-ethics-and-welfare-for-editors/

In addition to the above. for manuscripts submitted to JFMS. the Editors would not normally support publication of:

- Any experimental studies directly resulting in euthanasia of the cats.

- Studies using non-experimental (eg. client-owned) cats that may cause the cat a level of pain. suffering. distress or harm higher than that induced by inserting a hypodermic needle. and/or where the procedure is not part of ‘Recognised Veterinary Practice’. Recognised Veterinary Practice would include investigations. procedures and therapies that are part of normal clinical practice and that would be of direct benefit for the individual cat (or potentially to the group to which it immediately belongs). Where investigations. procedures or therapies are unproven. or where there is deliberate exposure of cats to procedures or interventions that might be deleterious to their health without direct clinical benefit to them. it

is highly likely that experimental cats should be used with appropriate attention to their health and welfare. with the requisite ethical approval (see below). If authors are in any doubt. they are encouraged to contact the Editors prior to manuscript submission.

The Editors would also expect that for all manuscripts submitted:

- Where appropriate. analgesia. sedation and/or anaesthesia must have been used and the authors should have adequately discussed the use of analgesia for the welfare of the cats involved.

- Any drugs or therapeutic agents used must have been obtained legally and ethically. following all relevant locally applicable regulations.

- Research involving experimental animals must always have received prior approval from an appropriate ethics committee with oversight of the facility in which the studies were conducted. and this may also apply to some studies involving client-owned animals (see 2.6.1 Ethical approval).

The Editors reserve the right to reject manuscripts on ethical or welfare grounds when. in their opinion. studies involve unnecessary pain. distress. suffering. harm. or potential harm to animals; and where the above guidelines have not been followed.

2.6.1 Ethical Approval

All material published in JFMS must adhere to high ethical standards concerning animal welfare and meet with the above guidelines. Irrespective of the nature of the work (eg. prospective. retrospective or experimental studies. case series or review). JFMS requires all authors to make one of the following four ethical approval declarations (using the exact wording) in an ‘Ethical approval’ section at the end of their manuscript. stating:

a) The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards (‘best practice’) of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in JFMS. Although not required. where ethical approval was still obtained. it is stated in the manuscript

b) The work described in this manuscript involved the use of non-experimental (owned or unowned) animals and procedures that differed from established internationally recognised high standards (‘best practice’) of veterinary clinical care for the individual patient. The study therefore had prior ethical approval from an established (or ad hoc) committee as stated in the manuscript.

- This statement might. for example. apply to randomised and/or controlled trials (including where established interventions are being compared with each other). as well as studies where novel medications. techniques. devices or interventions established as safe but not currently part of ‘Recognised Veterinary Practice’ (see 2.6 Clinical and research ethics. and informed consent) are used.

- Authors must state in the Materials and methods the nature of the institutional. national or international ethical review body used. and. if available. the ethical approval number.

- If an existing ethical review body was not available. authors should state why in the Materials and methods. and should describe the nature of an ad hoc committee that was used (which must have included at least some individuals independent of the institute[s]/clinic[s] involved in the work).

c) The work described in this manuscript involved the use of experimental animals and the study therefore had prior ethical approval from an established (or ad hoc) committee as stated in the manuscript.

- Authors must state in the Materials and methods the nature of the institutional. national or international ethical review body used. and. if available. the ethical approval number.

- If an existing ethical review body was not available. authors should state why in the Materials and methods. and should describe the nature of an ad hoc committee that was used (which must have included at least some individuals independent of the institute[s]/clinic[s] involved in the work).

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JFMS requires all authors to make one of the following two informed consent declarations (using the exact wording below) in an 'Informed consent' section at the end of their manuscript. stating:

a) Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals. including cadavers) for all procedure(s) undertaken (prospective or retrospective studies).

b) This work did not involve the use of animals (including cadavers) and therefore informed consent was not required.

• Authors may select this option if. for example. the manuscript is solely a clinical review or clinical guidelines using previously published data. or reports on questionnaire or in vitro findings. and does not involve the publication of any novel animal-specific data.

In addition to informed consent for use of animals within a study. informed consent for publication is required where any animal or person may be identifiable as a result of the publication (eg. a recognisable photograph. description or unique identifiable features. etc). Authors are therefore required to also state within the 'Informed consent' section either:

a) For any animals or people individually identifiable within this publication. informed consent (verbal or written) for their use in the publication was obtained from the people involved.

b) No animals or people are identifiable within this publication. and therefore additional informed consent for publication was not required.

2.7 Reporting guidelines

Authors and researchers are encouraged to consult the relevant EQUATOR Network reporting guidelines for different studies. including. for example. the Consolidated Standards of Reporting Trials (CONSORT) for randomized controlled trials. Other resources can be found at NLM's Research Reporting Guidelines and Initiatives

2.8 Data

JFMS requests that any primary data used by authors in their research article is published as Supplementary material. or that detailed information is provided in the article on how the data can be obtained. This information should include links to third-party data repositories or detailed contact information for third-party data sources. Data available only on an author-maintained website will need to be loaded onto either the journal's platform or a third-party platform to ensure continuing accessibility. Examples of data types include (but are not limited to) statistical data files. replication code. text files. audio files. images. videos. appendices. and additional charts and graphs necessary to understand the original research. The Editors may consider limited embargoes on proprietary data. The Editors can also grant exceptions for data that cannot legally or ethically be released. All data submitted should comply with Institutional or Ethical Review Board requirements and applicable government regulations. For further information. please contact Jennie Atkinson (Jennie.atkinson@sagepub.co.uk). Publishing Editor at SAGE Publications.

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3. Publishing policies

3.1 Publication ethics

SAGE is committed to upholding the integrity of the academic record. We encourage authors to refer to the Committee on Publication Ethics' International Standards for Authors and view the Publication Ethics page on the SAGE Author Gateway.

3.1.1 Plagiarism

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4. Preparing your manuscript for submission

This section explains how to format. style and reference your paper for JFMS. The title. keywords and abstract are key to ensuring readers find your article online through online search engines such as Google. Please refer to the information and guidance on how best to

title your article. write your Abstract and select your keywords by visiting the SAGE Journal Author Gateway for guidelines on How to Help Readers Find Your Article Online.

4.1 Formatting

The preferred format for your manuscript is Word. LaTeX files are also accepted. Word and LaTeX templates are available on the Manuscript Submission Guidelines page of our Author Gateway.

The text should be double-spaced throughout and with a minimum of 3 cm for left and right hand margins and 5 cm at head and foot. Text should be standard 10 or 12 point. All lines should be numbered on manuscripts using continuous line numbering. Figures. tables and Supplementary material should all be cited in the text in numerical order.

Title page

The title should be concise (20 words maximum) with no abbreviations

Abstract

The second page of the manuscript must contain only the abstract. which should be of no more than 300 words and must be clearly written and comprehensible to readers before they come to read the paper.

For Original Articles and Short Communications. the Abstract should be structured with the following four subheadings: ‘Objectives’. ‘Methods’. ‘Results’. and ‘Conclusions and relevance’. For Case Series. the abstract should be structured with the following two subheadings: ‘Case series summary’. and ‘Relevance and novel information’. For Reviews. the abstract can either have no subheadings or subheadings of the author’s choice.

Abbreviations should be avoided and reference citations are not permitted.

Any manuscripts submitted without a structured abstract will be returned to the author prior to peer review. thus delaying the evaluation process of the manuscript.

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For guidance on the preparation of illustrations. pictures and graphs in electronic format. please visit SAGE's Manuscript Submission Guidelines. Figures supplied in colour will appear in colour online and in print.Tables should be provided in an editable format (eg.

drawn in Microsoft Word or Microsoft Excel). The minimum image quality required is 300dpi at 1000 x 1000 pixels.

4.3 Style Guide

JFMS has its own style guide: JFMS Style Guide 2020

4.4 Abbreviations. symbols and drug names

Each scientific abbreviation must be explained at its first occurrence in the paper; for example:

• complement fixation test (CFT).

Do not use propriety symbols (eg. ® or ™) or ltd. etc. in medications or company names.

Medications should be referred to by their recommended International Nonproprietary Name (rINN). A list of these generic names is coordinated by the World Health Organization at http://www.who.int/medicines/services/inn. Where appropriate. the proprietary name and the manufacturer should be given in parentheses when first mentioned; for example:

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4.5 Supplementary material

This journal is able to host additional materials online (eg. datasets. podcasts. videos. images. etc) alongside the full-text of the article. These will still be subjected to peer review. For more information please refer to our guidelines on submitting supplementary files.

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You will be asked to provide contact details and academic affiliations for all co-authors via the submission system and identify who is to be the corresponding author. These details must match what appears on your manuscript. At this stage please ensure you have included all the required statements and declarations and uploaded any additional supplementary files (including reporting guidelines where relevant). Manuscripts must be submitted with

declaration statements in the following order: Acknowledgements (where relevant). Conflict of Interest. Funding. Ethical Approval and Informed Consent.

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As an example of how to supply this information please see the example below:

Joe Bloggs. Department of Veterinary Science. University Hospital. Town. Zip code. USA

Email: JoeBloggs@email.com

Twitter: @drjoebloggs

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6. On acceptance and publication

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6.2 Access to your published article

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7. Further Information

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