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## ***Ex vivo* and *in vivo* study of Kowa HA-2 applanation tonometer in the measurement of intraocular pressure in dogs**

### **Estudo *ex vivo* e *in vivo* do uso do tonômetro de aplanção Kowa HA-2 na medição da pressão intraocular em cães**

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#### **Abstract**

The objective of this study was to evaluate the use of the Kowa HA-2 applanation tonometer in measuring intraocular pressure (IOP) in dogs. Twenty eyes were used in an *ex vivo* study in which the calibration curve for manometry vs. tonometry was determined by artificially raising the IOP in 5 mmHg increments up to 60 mmHg (10-60 mmHg). Both eyes of 10 anesthetized dogs were studied *in vivo* to compare manometry vs. tonometry. In the ambulatory study, 168 healthy eyes, 74 eyes with glaucoma and 60 eyes with uveitis were evaluated by tonometry, which was performed with topical anesthesia and 1% fluorescein eye drops for the formation of fluorescein semicircles. The *ex vivo* study showed an excellent correlation coefficient ( $r^2 = 0.993$ ) between the aneroid manometer and the Kowa HA-2 tonometer. In the *in vivo* study, there was no significant difference ( $P > 0.05$ ) between the IOP values by manometry and tonometry, showing the excellent accuracy of the Kowa HA-2 tonometer. In the ambulatory study using the Kowa HA-2 tonometer, the IOP values (mean $\pm$ SD, in mmHg) were  $15.1 \pm 1.8$  (12.0 – 20.0) for the healthy eyes,  $25.2 \pm 4.0$  (20.0 – 38.0) for glaucomatous eyes and  $10.1 \pm 2.3$  (5.0 – 13.7) for eyes with uveitis. There was a strong correlation between the IOP values obtained by direct ocular manometry and those from the Kowa HA-2 tonometer. In the ambulatory study, the IOP values measured by the tonometer were compatible for healthy eyes and for eyes with glaucoma or uveitis. We conclude that Kowa HA-2 applanation tonometer is accurate and practical for IOP measurement in dogs.

**Key words:** Applanation tonometer. Dogs. Glaucoma. Kowa HA-2 tonometer. Ocular manometry. Uveitis.

#### **Resumo**

O objetivo deste estudo foi avaliar o uso do tonômetro de aplanção Kowa HA-2 na medição da pressão intraocular (PIO) em cães. Vinte olhos foram utilizados no estudo *ex vivo* onde a curva de calibração para a manometria versus tonometria foi determinada aumentando artificialmente a PIO em 5 mmHg até 60 mmHg (10 – 60 mmHg). Ambos os olhos de 10 cães anestesiados foram estudados no estudo *in*

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*vivo* para comparação da manometria versus tonometria. No estudo ambulatorial, 168 olhos saudáveis, 74 olhos com glaucoma e 60 olhos com uveíte foram avaliados pela tonometria, que foi realizada com anestesia tópica e colírio de fluoresceína 1% para a formação de semicírculos de fluoresceína. O estudo *ex vivo* mostrou um excelente coeficiente de correlação ( $r^2 = 0,993$ ) entre o manômetro aneroide e o tonômetro Kowa HA-2. No estudo *in vivo*, não houve diferença significativa ( $P > 0,05$ ) entre os valores de PIO da manometria e os da tonometria demonstrando uma excelente acurácia. No estudo ambulatorial, utilizando o tonômetro Kowa HA-2, os valores de PIO (média±desvio padrão, em mmHg) foram para os olhos sadios  $15,1 \pm 1,8$  (12,0 – 20,0), para os olhos com glaucoma  $25,2 \pm 4,0$  (20,0 – 38,0), e para os olhos com uveíte,  $10,1 \pm 2,3$  (5,0 – 13,7). Houve uma forte correlação dos valores de PIO obtidos pela manometria ocular direta com os obtidos com o tonômetro Kowa HA-2. No estudo ambulatorial, os valores mensurados pelo tonômetro foram compatíveis para olhos saudáveis e com glaucoma e uveíte. Nós concluímos que o tonômetro de aplanção Kowa HA-2 é acurado e prático para a mensuração da PIO em cães.

**Palavras-chave:** Cães. Glaucoma. Kowa HA-2. Manometria ocular. Tonômetro de aplanção. Uveíte.

## Introduction

Glaucoma and uveitis are two of the most frequent causes of blindness in dogs. Intraocular pressure (IOP) measurement is an important tool for decision-making in both the screening and management of these ophthalmopathies (MILLER, 2013; MAGGS, 2013). The glaucoma is characterized by increased IOP with resultant retinal and optic disk dysfunction and the main clinical signs are dilated pupil, bulbar conjunctival venous congestion, corneal edema, blepharospasm, buphthalmos and partial or complete loss of vision (MILLER, 2013). The uveitis is an inflammation of the iris and ciliary body that often leads to the reduction of IOP and the main clinical signs are red eye, miosis, photophobia, blepharospasm, pain, epiphora, hypopyon, hyphema, episcleral vascular injection and corneal edema (MASSA et al., 2002).

In veterinary medicine, the most popular method of IOP measurement is the use of direct and portable tonometers that use applanation tonometry. Applanation tonometry is based on the Imbert-Fick Law, which states that the pressure is equal to the force per unit area of applanation for a spherical container that is assumed to be infinitely thin, dry and perfectly elastic in form. The most popular applanation tonometer used in veterinary medicine is the Tono-Pen (XL, VET, AVIA, AVIA VET) (MAGGS, 2013).

Recently, a number of published veterinary studies with dogs, cats, horses and cattle have described the use of another method and applanation tonometer, the Goldmann method with a Perkins tonometer, which uses a prism adapted to a light source to form fluorescein semicircles. These studies show an excellent accuracy with this tonometer, which has the advantage of being cheaper than the Tono-Pen (ANDRADE et al., 2009, 2011, 2012, 2013). Another handheld applanation tonometer that uses Goldmann methodology is the Kowa HA-2 tonometer. The differences between the Perkins and Kowa HA-2 tonometers are: scale (Perkins, 0 – 50 mmHg; Kowa HA-2, 0 – 60 mmHg), battery requirement (Perkins, 4 AA 1.5 V batteries; Kowa HA-2, 2 AA 1.5 V batteries), weight (Perkins, 250 g; Kowa HA-2, 240 g) and manufacturer (Perkins, Clement Clarck (Great Britain); Kowa HA-2, Kowa (Japan)) (SCHOTTENSTEIN, 1996).

There are no veterinary studies utilizing the Kowa HA-2 handheld applanation tonometer in dogs. The purpose of this study was to evaluate the use of the Kowa HA-2 tonometer in dogs, with the objectives being to calibrate the tonometer through an *ex vivo* study, to verify its accuracy through an *in vivo* study and to validate its use in an ambulatory study.

## Materials and Methods

This experiment was approved by the Ethical Committee in Research of UNOESTE (Protocol no. 954). All care and handling of dogs and cats adhered to the Association for Research in Vision and Ophthalmology (ARVO, 2013) statement for the use of animals in ophthalmic and vision research. All measurements with the tonometer were obtained by the same examiner (SFA).

### Animals

For the *ex vivo* study, animals were obtained immediately after sacrifice from several cases authorized for necropsy in the Veterinary Teaching Hospital of Unoeste. Twenty healthy eyes from 10 dogs, sex (6 males and 4 females, age  $4.2 \pm 2.9$  (2.0 – 10.0) years, weight  $14.4 \pm 7.4$  (6.9 – 25.1) kg, breed (4 mixed breed, 2 Poodle, 2 Lhasa Apso, 1 Rottweiler and 1 German Shepherd) were evaluated.

For the *in vivo* study, 20 healthy eyes as determined by ophthalmic examination, pupillary light reflex, slit-lamp biomicroscopy (Kowa, Japan), direct ophthalmoscopy (Pocket Jr Ophthalmoscope 12850, Welch Allyn, USA), Schirmer tear test (Ophthalmos, Brazil) and fluorescein test (Ophthalmos, Brazil), from 10 anesthetized dogs from the UNOESTE kennel were used (5 males and 5 females, age  $3.3 \pm 2.6$  (1.0 – 6.0) years, weight  $11.4 \pm 3.5$  (7.9 – 17.0) kg, all mixed breed).

For the ambulatory study, the animals were obtained from the kennel and from the ophthalmology ambulatory service of the Veterinary Hospital of Unoeste, from November 2011 to November 2012, and the same ophthalmic examination described in the *in vivo* study was performed. The ambulatory study subjects were divided into three groups: dogs with healthy eyes, dogs with signs of glaucoma, and dogs with signs of uveitis. In total, 168 healthy eyes from 84 dogs (34 males and 50 females, age  $4.2 \pm 2.9$  (1.0 – 12.0) years, weight  $11.0 \pm 8.8$  (1.0 – 40.0) kg), 74 eyes from 47 dogs (23 males and 24 females, age  $7.8 \pm 3.1$  (2.0 – 13.0) years, weight  $11.0 \pm 8.8$

(1.0 – 40.0) kg) were evaluated with one or more clinical signs of glaucoma (enlarged episcleral vessels, blepharospasm, partial or complete loss of vision, corneal edema, buphthalmos, fixed dilated pupil, alterations in the anterior chamber, lens dislocation, retina degeneration and cupping of the optic disc, among others). Sixty eyes from 36 dogs were evaluated (17 males and 19 females, age  $4.5 \pm 2.5$  (1.0 – 10.0) years, weight  $15.3 \pm 10.0$  (2.0 – 38.0) kg) with one or more clinical signs of uveitis (photophobia, blepharospasm, pain, epiphora, aqueous flare, keratic precipitates, hypopyon, hyphema, episcleral vascular injection, corneal edema, miosis and anterior or posterior synechiae, among others).

### Ex vivo study

For the *ex vivo* study, calibration of the Kowa HA-2 applanation tonometer with ocular direct manometry was performed based on a method previously described in dogs (ANDRADE et al., 2009, 2012), with the exception that the calibration curve for manometry vs. tonometry was determined by raising the IOP artificially, in 5 mmHg increments up to 60 mmHg (10-60 mmHg) because the scale of Kowa HA-2 tonometer is up to 60 mmHg, in open stopcock mode. Three readings were taken at each level of IOP with the Kowa HA-2 tonometer (Kowa, Tokyo, Japan). Prior to taking Kowa HA-2 tonometer IOP readings, one drop of 1% fluorescein (Allergan, Guarulhos, SP, Brazil) was instilled for the formation of fluorescein semicircles. The tonometer was calibrated according to the manufacturer's instructions.

### In vivo study

For evaluation of the accuracy of the tonometer, an *in vivo* study was performed, comparing a real IOP reading obtained by ocular manometry with the IOP reading obtained using the Kowa HA-2 tonometer in anesthetized dogs always between 08h00 and 10h00. The animals were anesthetized

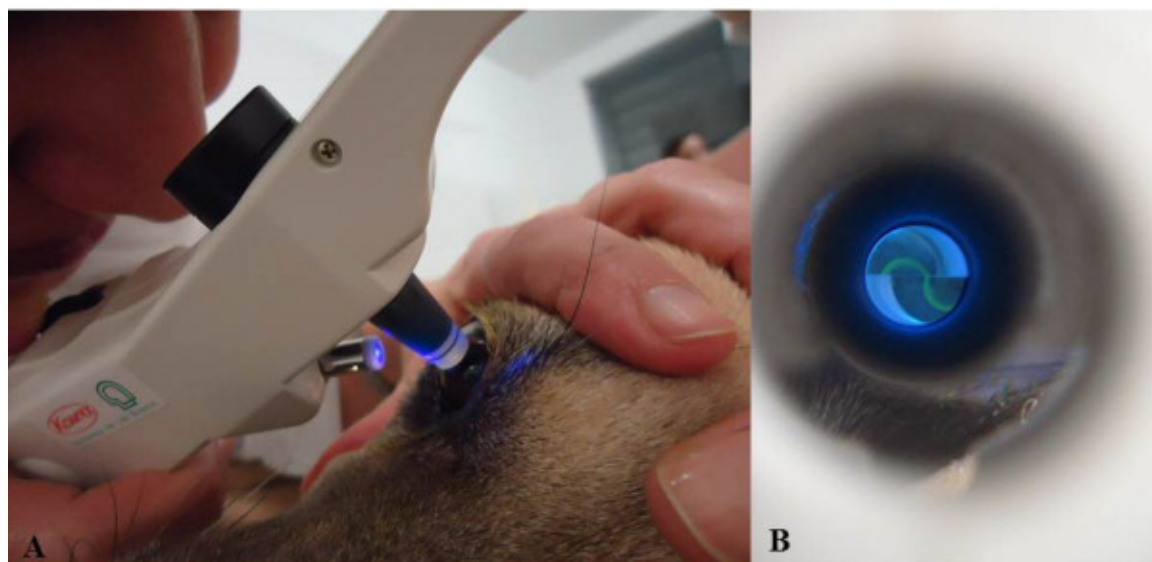
according to the following protocol: pre-anesthetic medication with 0.5 mg/kg i.v. diazepam (Valium®, Roche, São Paulo, Brazil) followed soon afterwards by 12.5 mg/kg i.v. sodium thiopental (2.5% solution of Thiopentax®, Cristalia, Itapira, SP, Brazil) and, for centralization of the eyeball, 0.05 mg/kg i.v. of a neuromuscular blocking agent with vecuronium (Norcuron®, Organon, São Paulo, SP, Brazil). The cornea was topically anesthetized with two drops of 1% tetracaine + 0.1% phenylephrine (Anestésico®, Allergan, Guarulhos, SP, Brazil). First, one drop of 1% fluorescein was instilled, and three readings were taken with the Kowa HA-2 tonometer. The mean was calculated and multiplied by 10, as in the calibration in the *ex vivo* study. To avoid transmission of infectious ocular diseases, the prism was removed and washed in a 0.9% physiologic saline solution after each use of the Kowa HA-2 tonometer. It was then submerged for 10 min in a solution of 3% hydrogen peroxide and washed again in 0.9% saline solution before being dried with sterile gauze (LINGEL; COFFEY, 1992). After the IOP reading by the tonometer, the manometry measurements were taken as described for the *ex vivo* study. After the IOP readings by the manometer were made, the needle was removed

from the anterior chamber, and soon afterward, cyanoacrylate glue was instilled with a 25x7 mm needle (Injex, Ourinhos, SP, Brazil) at the site of the corneal puncture to prevent continued leakage of aqueous humor (VOTE; ELDER, 2000). After the procedure, the dogs were given one drop of antibiotic eye drops (chloramphenicol-Visalmin®, Bunker, São Paulo, SP) and anti-inflammatory eye drops (diclofenac sodium – Still®, Allergan), twice daily for 1 week, and were evaluated through daily basic ophthalmic exams (pupillary light reflex, slit-lamp biomicroscopy, Schirmer tear test and fluorescein test) until the induced corneal lesion healed.

#### *Ambulatory study*

For validation of the tonometer for clinical use, an ambulatory study was performed in dogs with healthy eyes and also in animals with clinical signs of glaucoma and uveitis from the kennel and the ophthalmology ambulatory service for small animals in the Veterinary Hospital of Unoeste. The procedure for taking IOP readings using the Kowa HA-2 tonometer is represented in Figure 1. The disinfection of the prism and the tonometer calibration were performed as described previously.

**Figure 1.** Ambulatory study. (A) Use of the Kowa HA-2 tonometer. (B) The formation of fluorescein semicircles, as seen through the viewing lens of the tonometer.





### Statistical Analysis

The mean IOP values measured using ocular manometry and tonometry in the *ex vivo* study were used to create a calibration curve. Linear regression analysis was performed to analyze the relationship between the *ex vivo* manometry vs. tonometry IOP measurements, and a correlation coefficient ( $r^2$ ) was calculated. The manometer and tonometer readings taken from the *in vivo* study were compared and analyzed by Student's t-tests. A significance level of  $P < 0.05$  was adopted. The Bland-Altman assessment for agreement was used to compare

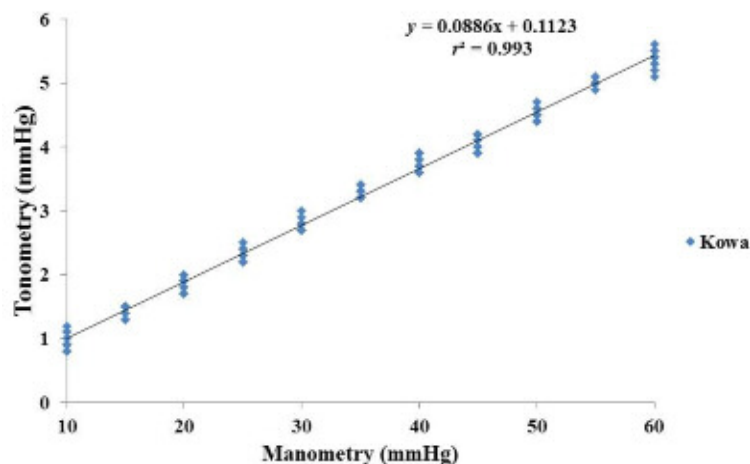
the two methods of IOP measurement. A range of agreement was defined as mean bias  $\pm 2$  standard deviations.

### Results

#### *Ex vivo study*

The correlation coefficient ( $r^2$ ) between the manometry measurements and the Kowa HA-2 tonometer measurements was 0.993, and the corresponding linear regression equation was  $y = 0.0886x + 0.1123$  in the *ex vivo* study (Figure 2).

**Figure 2.** Intraocular pressure (IOP) measurement comparison (in mmHg) between manometry (aneroid manometer) and tonometry (Kowa HA-2 tonometer) in dogs (n = 10 eyes) in an *ex vivo* study. The solid line is the calculated regression line.  $r^2$  is the correlation coefficient.



#### *In vivo study*

In the *in vivo* study, the mean IOP reading with the manometer in dogs was  $15.6 \pm 1.6$  mmHg (range 14.0-18.0 mmHg) and with the Kowa HA-2 tonometer was  $15.5 \pm 1.0$  mmHg (range 14.3-17.2). There was no significant difference ( $P > 0.05$ ) between the IOP measurements obtained via manometry and those obtained by tonometry.

#### *Ambulatory study*

The mean IOP reading with the Kowa HA-2 tonometer in the ambulatory study in dogs with

healthy eyes was  $15.1 \pm 1.8$  mmHg (12.0 – 20.0 mmHg). The mean IOP value in dogs with clinical signs of glaucoma was  $25.2 \pm 4.0$  mmHg (20.0 – 38.0 mmHg). The prevalent breeds in this study group were Poodle (32.4%), Shitzu (13.5%), Lhasa Apso (10.8%) and mixed breed (10.8%). With regard to sex, 51.4% were female and 48.6% were male, and the ages of the animals were 2 – 4 years (18.7%), 5 – 7 years (27.3%), 8 – 10 years (32.5%) and 11 – 13 years (21.5%). These cases were classified as primary glaucoma (without an ocular or systemic disease) in 39% of the cases and secondary glaucoma (secondary to other ocular or systemic

disease) in 61% (of which, 40% involved cataracts, 30% trauma, 20% lens luxation and 20% diabetes mellitus).

The mean IOP value in dogs with clinical signs of uveitis was  $10.1 \pm 2.3$  mmHg (5.0 – 13.7 mmHg). The prevalent breeds in this study group were mixed breed (23.2%), Poodle (20.0%), Basset hound (10.0%) and Border Collie (10.0%). With regard to sex, 53.4% were female and 46.6% were male, and the ages of the animals were 1 – 3 years (46.6%), 4 – 7 years (40.0%) and 8 – 10 years (13.4%). The cause of uveitis was trauma in 50% of cases, infection in 40% (80% ehrlichiosis and 20% leptospirosis) and autoimmune disease in 10% (uveodermatologic syndrome).

## Discussion

The *ex vivo* study verified a strong correlation between the aneroid manometer and the Kowa HA-2 tonometer ( $r^2 = 0.993$ ) (Figure 2 and 3), which is similar to other studies describing use of the Perkins tonometer in dogs, which uses the same Goldmann methodology. In one such study the correlation coefficient ( $r^2$ ) was 0.982 (ANDRADE et al., 2009), while in another, the  $r^2$  value was 0.981 (ANDRADE et al., 2012). The tonometer was calibrated according to the linear regression equation ( $y = 0.0886x + 0.1123$ ), where  $y$  = IOP reading by the tonometer and  $x$  = IOP real reading by the manometer in mmHg. Therefore,  $x = y - 0.1123/0.0886$ . So, for example, an IOP reading of 1.2 from the tonometer would be equal to  $x = 1.2 - 0.1123/0.0886 = 12.2$  mmHg in the equation. Therefore, in clinical practice, the Kowa HA-2 scale readings are converted to estimates of IOP in mmHg by multiplying the reading by 10, which is done routinely in humans (SCHOTTENSTEIN, 1996) and was also verified in other studies with the Perkins tonometer in dogs, cats, equine and cattle (ANDRADE et al., 2009, 2011, 2012, 2013).

There was no significant difference between the IOP values obtained with the Kowa HA-2 tonometer

( $15.5 \pm 1.0$  mmHg) and the IOP values obtained with a manometer ( $15.6 \pm 1.6$  mmHg) in the *in vivo* study. In other words, the IOP reading by the tonometer was very close to real IOP measurements made using the manometer. These results were very similar to those obtained with the Perkins tonometer in that species ( $15.3 \pm 2.9$  mmHg) and those obtained with the manometer ( $15.0 \pm 1.7$  mmHg) (ANDRADE et al., 2009).

In the ambulatory study of healthy eyes, the IOP results with the Kowa HA-2 tonometer ( $15.1 \pm 1.8$  mmHg) were very similar to those obtained with the Perkins tonometer ( $15.3 \pm 2.1$  mmHg) in a comparative study with the Tono-Pen XL tonometer ( $17.5 \pm 3.7$  mmHg), which justifies the need for the normal range of IOP values for each tonometer (ANDRADE et al., 2012). In another study with the Tono-Pen XL in dogs, the IOP values were  $19.2 \pm 5.9$  mmHg (GELATT; MACKAY, 1998). Based on the results of the present study, we suggest that the Kowa HA-2 tonometer, like the Perkins tonometer, have a differentiated table of IOP values compared with the Tono-Pen tonometer.

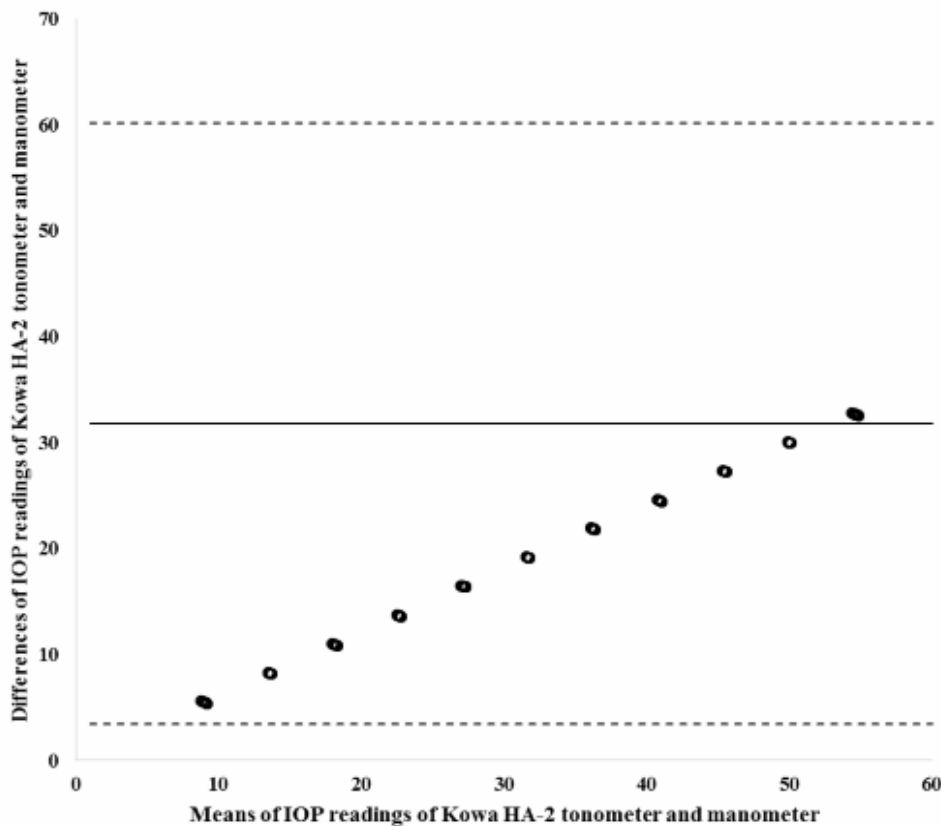
This is the first study of the Goldmann methodology with a portable tonometer in dogs with glaucoma and uveitis. The mean IOP value in dogs with glaucoma was  $25.2 \pm 4.0$  (20.0 – 38.0), which is greater than the mean measured value in healthy eyes  $15.5 \pm 1.0$  (12.0– 20.0), and the mean IOP value in dogs with uveitis was  $10.1 \pm 2.3$  (5.0 – 13.7), which is less than the mean measured value in healthy eyes. The IOP values above the normal range are compatible with glaucoma, while IOP values below this range are compatible with uveitis, which is the trend that was observed in this study (TOWNSED, 2008). It is well-known in human medicine that corneal thickness affects the accuracy of IOP measurement by Goldmann applanation tonometry, although this methodology is still the gold standard for measuring IOP in humans. People with thicker corneas will get an artificially high reading and people with thinner corneas will get an artificially low reading (SCHOTTENSTEIN, 1996). Corneal

thickness was not evaluated in this study, and more research in this regard should be conducted in the future. Another important aspect observed in this study was the prevalence of the breed, age and sex of the dogs affected with glaucoma and uveitis.

The prevalence of most affected breeds with glaucoma of this study, 32.4% (Poodle), 5% (Shitzu), 4% (Lhasa Apso and mixed-breed dog)

and 2% (Basset Hound, Cocker and Pinscher), differs from a study in North America (GELATT; MACKAY, 2004) that the breeds more commonly affected by glaucoma was Cocker Spaniel (5.52%), Basset Hound (5.44%), Chow Chow (4.7%), Shar-Pei (4.4%), Boston Terrier (2.88%) and Wire Fox Terrier (2.28%), probably due to the greater popularity of these breeds in each country.

**Figure 3.** Bland-Altman plot comparing the intraocular pressure (IOP) readings in mmHg of Kowa HA-2 tonometer and manometer in dogs (n = 10 eyes) in an *ex vivo* study.



Regarding age, the largest prevalence detected in this study was in the interval from 5 to 10 years (27.3% from 5 to 7 years and 32.5% from 8 to 10 years) which is similar to the findings of the study in North America mentioned above (GELATT; MACKAY, 2004). Regarding sex, the percentage of dogs with glaucoma that were female was a little greater (51.4%) than the percentage of males (48.6%). Female dogs in general present a greater

predisposition to primary glaucoma (STROM et al., 2011), which has been described more commonly in females of breeds such as Cocker Spaniel, Basset Hound, Cairn Terrier, Chow Chow, English Cocker Spaniel, Samoyed, and Siberian Husky and in males of the Australian Cattle Dog and Saint Bernard breeds (GELATT; MACKAY, 2004).

Regarding etiology, 39% of the cases was primary glaucoma and 61% secondary glaucoma



(40% involved cataracts, 30% trauma, 20% lens luxation and 20% diabetes mellitus). In primary glaucoma, genetic causes are important factors, as a pre-disposition to lens dislocation and primary closed angle glaucoma is prevalent in some breeds, and secondary glaucoma can be a complication of another eye disease such as diabetes mellitus, uveitis, displacement of the lens, or trauma to the eye (GELATT; MACKAY, 2004). The prevalent cause of uveitis was trauma in 50% of cases, followed by infection in 40% (80% ehrlichiosis and 20% leptospirosis) and autoimmune disease in 10% (uveodermatologic syndrome). The causes of uveitis usually include inflammatory processes and autoimmune, infectious and traumatic diseases, among others (MASSA et al., 2002; TOWNSED, 2008).

In our study, we observed that the ability to use the Kowa HA-2 tonometer as well as the Perkins tonometer was acquired in a few days while maintaining the appropriate quality of the exam, and the use of the Kowa HA-2 tonometer is therefore feasible and has a more accessible cost, which is approximately three to five times less than that of the Tono-Pen and Tonovet tonometers. In addition, this tonometer can be used in any position, is extremely resistant, is easy to calibrate and disinfect and has a lighter weight than the Perkins tonometer.

The results observed in *ex vivo* and *in vivo* studies demonstrate an excellent accuracy in IOP readings by the Kowa HA-2 tonometer due the strong correlation between the IOP values obtained by direct ocular manometry and this tonometer. In the ambulatory study, the IOP values measured by the tonometer were compatible for healthy eyes and eyes with glaucoma or uveitis. Thus, we conclude that the Kowa HA-2 applanation tonometer is accurate and practical for IOP measurement in dogs.

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