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The influence of the tonometer position on canine intraocular pressure measurements using the Tonovet® rebound tonometer

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Abstract

The objective of this study was to assess the variability of readings made using the Tonovet® rebound tonometer for measurement of intraocular pressure (IOP) in the peripheral cornea and in angulated positions on the canine corneal surface. Forty-six client-owned dogs admitted for ophthalmic evaluation at the Queen's Veterinary School Hospital, University of Cambridge were included in the study. IOP readings were taken at a variety of locations and using the tonometer at a number of different angles to the cornea: 1) Perpendicularly at center of the cornea (CC); 2) At the center of the cornea but with the tonometer positioned at four angles, and 3) At four different points on the peripheral cornea. All values were compared with the values recorded at the recommended CC position. IOP values were significantly underestimated in seven positions, with median and interquartile range from 12.1 ± 4 mmHg (nasal on periphery) to 15 ± 5 mmHg (laterally angled at center), varying between 0 mmHg to 2.9 mmHg from the CC value. While dorsally angled in the central cornea were not significantly different from those at CC ($p = 0.09$). Median values were lower for measurements in peripheral positions when compared to angled central positions. These results demonstrate that angling the tonometer or measuring in peripheral regions can result in small but statistically significant underestimation of IOP values.

Keywords: Dog, Intraocular pressure, Peripheral tonometry, Rebound tonometry.

Introduction

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

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

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

The Tonovet® tonometer (Tiolat Ltd., Helsinki, Finland) uses a small magnetized probe, which is directed toward the corneal surface by an electric coil. After impacting the cornea, the deceleration of rebound is measured by the induced voltage in the sensitive coil (Kontiola, 1997; Kontiola, *et al*., 2001). The manufacturer recommends that the tonometer probe is kept in a horizontal position during measurement of IOP, preventing gravitational forces affecting the speed and deceleration of the probe. However, this is not always strictly followed by every veterinary ophthalmologist. The effect of probe position on the corneal surface has been investigated in human patients (González-Méijome *et al*., 2006; Queirós *et al*., 2007; Yamashita *et al*, 2011; Muttuvelu *et al*., 2012; Beasley *et al*., 2013) and statistically significant differences in IOP were found when IOP was measured at the periphery of the cornea and compared to the central cornea. These investigations also have shown that IOP may be significantly underestimated when measured with the probe in an angled positions (Muttuvelu *et al*., 2012; Beasley *et al*., 2013). Only one similar study was carried in dogs (Von Spiessen *et al*., 2013) using the

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probe in a number of angled positions as well as in peripheral corneal locations. When tonometry was performed using angled positions but at the center of the cornea IOP data was underestimated by up to 6.5mmHg, whereas off-center probe positioning provided a significant overestimation of 0.9 mmHg. The aim of this study was to expand this type of investigation in dogs, as anecdotal evidences did not suggest dramatic effects on IOP values when different probe positions are used. The current study evaluated potential differences of IOP values for the canine eye using a Tonovet® tonometer with the probe positioned in the periphery and in angulated positions, were compared with IOP values obtained using the recommended perpendicular position in the center of the cornea. We also aimed to evaluate the repeatability of the findings already published in the literature.

This assessment is essential since in veterinary medicine tonometry is typically performed in un-sedated dogs whose globes are in constant motion, resulting in a potential misalignment of the tonometer probe in relation to the central position of the cornea. In addition, the presence of central corneal abnormalities may sometimes impair accurate tonometry measurements at this location (Von Spiessen *et al*., 2015). For all these reasons, it is important to know if IOP values taken in a position not perpendicular to the corneal surface or at the periphery of the cornea are significantly different from those taken perpendicularly in the center of the cornea.

Materials and Methods

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

The IOP measurements were performed with a calibrated rebound tonometer Tonovet® positioned on the center of the cornea and in eight different positions, as shown in Figure 1, at a distance of 5 to 10 mm from the cornea.

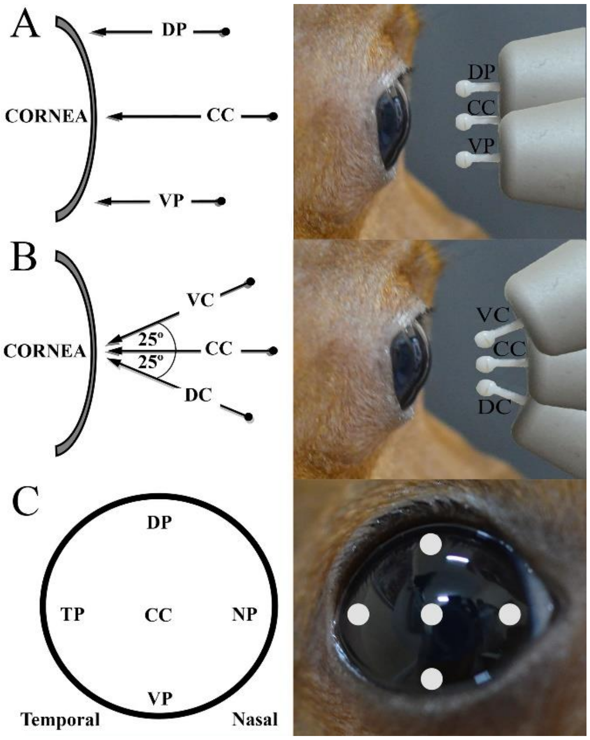

Fig. 1. The positions performed using rebound tonometer Tonovet® onto the dogs' corneal surface. A: perpendicular view of peripheral positions, showing relocation from central cornea (CC) as dorsal peripheral (DP) and ventral peripheral (VP). B: perpendicular view of angled positions, ventrally to the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

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

Measurements of IOP were performed first in the central cornea (CC). Next, the tonometer probe was displaced at 20 to 25 degrees from the CC, the angled positions being lateral to the center (LC), medial to the center (MC), dorsal to the center (DC) and ventral to the center (VC). The peripheral positions were temporal (TP), nasal (NP) dorsal (DP) and ventral (VP), and were obtained by displacing the tonometer 3 mm from the limbus, as shown in Figure 1. Measurements were made in the same order in each eye: CC, then the angled positions VC, DC, LC and MC; followed by VP, DP, NP and TP.

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

Results

The age of the dogs examined ranged from 6 months to 15 years, with a mean of 6.1 years and standard deviation of ± 3.55. The animals comprised 24 female dogs and 22 males, with a total of 79 eyes. Table 1 details additional information concerning all dogs investigated. Twenty-one different breeds were represented. Mixed-breed dogs and West White Highland Terrier appeared most frequently, from which 21 globes were evaluated. Other common breeds were English cocker spaniel, Greyhound, Golden Retriever, Labrador Retriever and Jack Russell terrier.

Table 1. Summary of 46 dogs evaluated with information about age and sex and descriptive statistics (mean and standard deviation) of intraocular pressure measurements obtained with a rebound tonometer (Tonovet®). IOP = intraocular pressure. CC = central cornea.

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

Table 2 shows median and interquartile ranges for IOP values in each position and 95% limits of agreement. Additionally, Spearman correlation coefficient (ρ) and *p*-values obtained by Friedman`s test of the IOP values for each position (MC, LC, DC, VC, DP, VP, TP, NP) compared with the IOP obtained at CC using the rebound tonometer Tonovet®.

Statistical analysis performed by Friedman`s test with Conover post-hoc analysis and Holm-Bonferroni adjustment ($p < 0.01$) showed significant underestimation of IOP in seven positions (MC, LC, VC, DP, VP, TP, NP) when compared to CC. The only position with no statistically difference when compared to CC was DC ($p = 0.09$).

Peripheral positions had lower median values than angled positions. The highest Spearman correlation coefficient (ρ) values was LC (ρ = 0.49, $p < 0.01$) and the lowest was the peripheral position NP (ρ = 0.09, $p = 0.5$).

Correlation between eyes of the same dog were statistically significant (ρ = 0.24, $p = < 0.01$).

Figure 2 shows the dispersion graphs with the determination coefficient (R^2) for each position in comparison with CC, the which the values were 0.05 (DC), 0.11 (VC), 0.24 (MC) and 0.27 (LC) for angled positions and 0.02 (NP), 0.03 (TP), 0.03 (DP) and 0.03 (VP) for peripheral positions.

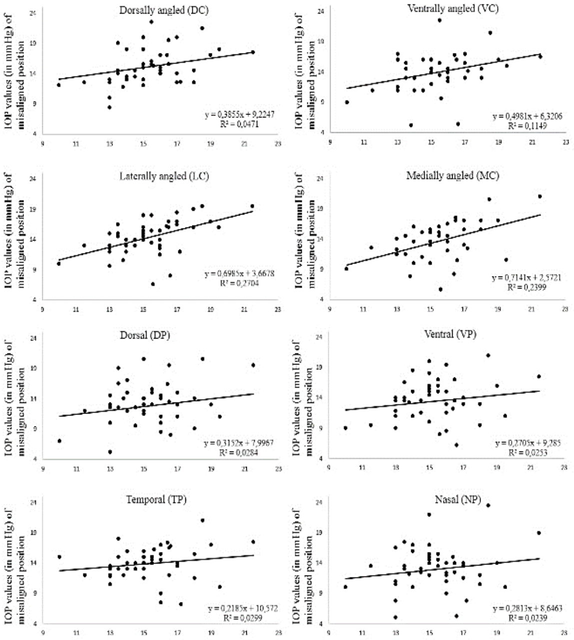

Fig. 2. Dispersion graphs for each position of measurement of IOP with the rebound tonometer Tonovet® compared with the CC. For every position there is a positive correlation. The solid line represents the CC position and dashed line represents the position indicated in the legend.

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

Probe position	Median ± interquartile range	*p values*	Limit of agreement of 95% in mmHg	Spearman correlation coefficient (ρ)
Central cornea (CC)	15 ± 3	-	-	-
Angled ventrally to the center (VC)	14 ± 4.5	< 0.01	[-5.4; 8.4]	0.39
Angled dorsally to the center (DC)	15 ± 5.3	0.09	[-8.1; 8.75]	0.31
Angled laterally to the center (LC)	15 ± 5.3	< 0.01	[-4.8; 7.0]	0.49
Angled medially to the center (MC)	14 ± 4	< 0.01	[-4.5; 8]	0.48
Dorsal position (DP)	13 ± 3.75	< 0.01	[-6.5; 11.8]	0.27
Ventral position (VP)	14 ± 3	< 0.01	[-5.9; 11.6]	0.1
Temporal position (TP)	13.6 ± 5	< 0.01	[-7.1; 11.1]	0.28
Nasal position (NP)	12.1 ± 4	< 0.01	[-6.5; 11.8]	0.09

A weak positive correlation is observed between off-center/angled readings and central corneal readings. The R^2 values for these regression lines are close to zero for most locations. Only the medially and laterally angled values are R^2 values for these slopes marginally > 0.2, which for a clinical measurement is hardly indicative of a strong association.

Figure 3 shows the Bland-Altman plots for each position, with a 95% interval of agreement, the limits values for angled positions (in mmHg) were approximately ± 6.9 (VC), ± 8.4 (DC), ± 5.9 (LC) and ± 6.2 (MC). The peripheral positions had larger limits with 95% of agreement, which were (in mmHg) ± 9.2 (DP), ± 7.1 (VP), ± 9.1 (TP) and ± 8.73 (NP). Figure 4 shows box-plot graphs of the IOPs values in mmHg, obtained by the comparison of misaligned positions with CC, including outlier's values.

Discussion

The misplacement of the rebound tonometer probe during measurements could lead to both underestimated and overestimated IOP values, being imprecise and inaccurate and could markedly differ from values obtained on CC in an unpredictable manner. Three positions off-angle (VC, LC and MC) and four positions off-axis (DP, NP, TP and VP) showed significant statistical differences from CC. The only position in which IOP measurements showed no significant difference from CC was DC.

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

This study was designed to assess whether misalignment of rebound tonometer can result in increased or decreased values of IOP. To find such results, rebound tonometry was repeated in each position on the corneal surface. No disturbance or damage to the cornea was observed. However, it is well known that some applanation tonometers produce the tonographic effect, which is a phenomenon in which IOP values decline with repeated tonometry (Stocker, 1958; Moses, 1961; Krakau and Wilke, 1971; Gaton *et al*., 2010; Zimmermann *et al*., 2017). In a study of repeated rebound tonometry in children, the tonographic effect did not occur (Dosunmu *et al*., 2014).

However, in mice, repeated rebound tonometry resulted in a significant reduction of IOP readings, a reduction of 2 mmHg after 10 measurements (Morris *et al*., 2006). To our knowledge, there is no information about IOP values changes in dogs due to repeated measurements with rebound tonometry. Thus, we cannot discard the possibility that the tonographic effect occurred in this study, and this would have particularly affected measurements made in the peripheral positions since these were evaluated last in every case and these values did consistently underestimate IOP. Further studies are needed to investigate the tonographic effect with rebound tonometry in canine corneas.

Correlation of IOP values in angulated misaligned positions with CC were positive and statistically significant, however with a low strength, which can be due to the small variability of IOP values, which only CC values between 15 and 25 mmHg were accepted for this study. Thus, if uveitic and glaucomatous eyes were analyzed, stronger correlation will probably be seen.

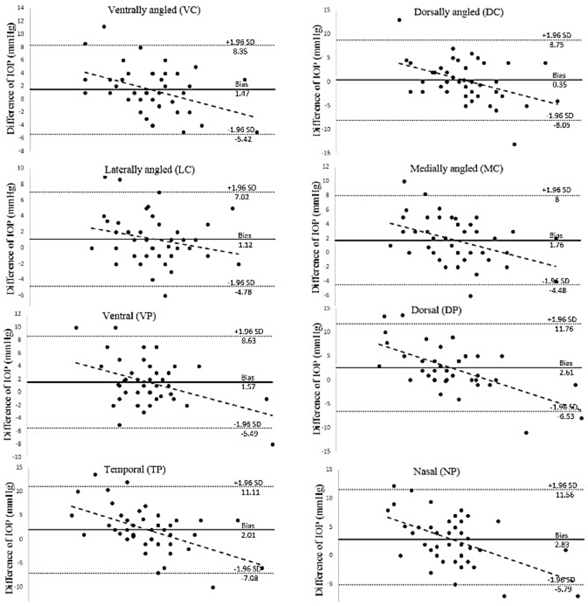

Fig. 3. Bland-Altman plots showing the lack of agreement of IOP values measured by rebound tonometer (Tonovet®) in different positions compared with CC. Vertical axis shows the difference in IOP reading versus the average CC and corresponding misaligned position value on horizontal axis. The dashed lines shows the limits of agreement of 95%. The full line is representative of the means difference and the dotted line is the linear regression with the equation and R², determination coefficient values.

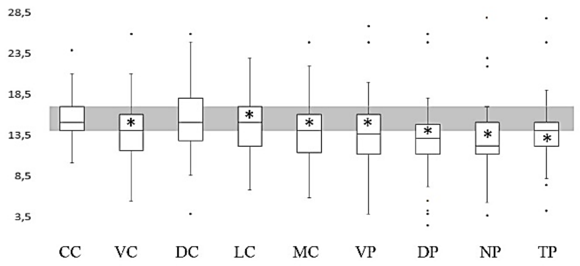

Fig. 4. Box plot graph of intraocular pressure values measured by rebound tonometer (Tonovet®) in different positions on corneal surface. * Shows the positions with statistically significant differences compared to CC.

For comparison, the dispersion graphs also show the positive correlation, but due to the small variability values, determination coefficients were also low. Nevertheless, peripheral positions had no statistically significant correlation, showing that in these positions, IOP values will not co-vary similar with CC, given results may indicate that peripheral readings undergo more variables than angulated positions. Additionally, the Bland-Altman graphs show both underestimation and overestimation of values found in misaligned positions compared to CC.

Meanwhile box-splot graph shows that interquartile range were below CC, showing the tendency for underestimation. In short, the findings showed that rebound tonometry in all misaligned positions could give unreliable results. Additionally, the wider range of limits of agreement for the peripheral positions in Bland-Altman plots, and lowers values of Spearman correlation and determination coefficients means that the rebound tonometry measurements in these positions were less robust.

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

Interestingly, the only position in which there was no significant difference compared with CC was DC. IOP measurements are lower at DC than CC, as would be expected in angled positions; however, in the DC position gravity facilitates the probe´s return to its original position. Another possibility that DC may not be significantly different presumably due to their considerable variability. Although there was no significant difference, this position is also not recommended for measurement of IOP, due to interference of variables, especially gravity, which were not considered by the tonometer during the calculations of IOP value.

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

The IOP results obtained in peripheral positions in this study are in disagreement with the values found by Von Spiessen *et al*. (2013) in which the values of IOP in these locations were overestimated. Our results show an underestimation of IOP when measurements were made in the peripheral cornea, a similar finding to results previously reported in human medicine (González-Méijome *et al*., 2006; Queirós *et al*., 2007; Muttuvellu *et al*., 2012).

It is well known that corneal thickness has a positive correlation with IOP values measured by rebound tonometry in different species, including dogs (Martinez-de-la-Casa *et al*., 2005; Prashar *et al*., 2007; Chui *et al*., 2008; Harada *et al*., 2008; Sahin *et al*, 2008; Poostchi *et al*., 2009; Park *et al*., 2011, 2013; Rao *et al*., 2014).

The cornea is thicker peripherally than centrally (Gilger *et al*., 1991; Strom *et al*., 2016), however in paraxial positions there was no significant difference from central corneal thickness (Strom *et al*., 2016). In our study, measurement was made at a distance of 3 mm from the limbus, whereas Von Spiessen *et al*. (2013) made measurements at approximately 1.5mm from the limbus. This difference in distance might possibly explain the disparity of IOP values in both studies, since the cornea is thicker nearer the limbus.

However, the increase in thickness of the peripheral cornea does not necessarily mean an increase of hardness. The distribution of collagen fibers can change corneal elasticity, and these are more compressed in the central cornea than in the periphery in dogs (Nagayasu *et al*., 2009). Similar changes in collagen distribution have been reported in man (Boote *et al*., 2003). The underestimated IOP values found in peripheral positions in this study resembles the studies in human eyes (González-Méijome *et al*., 2006; Queirós *et al*., 2007; Muttuvelu *et al*., 2012).

The underestimation of IOP values in peripheral positions may be a due to greater elasticity of peripheral cornea, resulting in a smaller deceleration of the rebound tonometer probe and, consequently, a lower value of IOP. Conversely, Yamashita *et al*. (2011) found overestimation of IOP in peripheral positions in the human eye; however the experimenters asked the patients to move their eyes to make the measurements, and this can apply tensile forces to the cornea due to extraocular muscles movements.

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

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

Although an underestimation tendency were found, rebound tonometry in misaligned positions also resulted in overestimated values in both angled and peripheral positions. These findings can be the result of increased deceleration of probe rebound on cornea, giving higher IOP values. Potential reasons for this were variations on individual corneal biomechanical properties and the presence of tensile forces on cornea by extra ocular muscles (Yamashita *et al*., 2011; Von Spiessen *et al*., 2013).

Correlation between eyes of the same dog were statistically significant, showing that in the same dog the resulting values of both eyes were similar. Which can due to individual variations of anatomy and corneal biomechanical proprieties, shared by both eyes of the same dog, which will influence IOP readings.

Conclusion

The present study has shown that there are small, but significant, differences between TonoVet® measurements taken in different corneal positions and angles, with a tendency for underestimation. Misaligned positions can differ from CC markedly and unpredictably, giving underestimated and overestimated values.

Conflict of interest

The authors declare that there is no conflict of interests.

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