Intraocular pressure measurements in cattle, sheep and goats with two different tonometers

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ABSTRACT

The aim of this study was to investigate normal intraocular pressure (IOP) values of cattle, sheep and goats with two different tonometers (TonoVet® (TV) and Tono-Pen AVIA® (TPA)) and to determine correction functions for the two devices. Twenty healthy cattle, sheep and goats each underwent slit-lamp biomicroscopy. IOP readings from both eyes were taken with the two different tonometers and statistically analyzed. For calibration purposes, the IOP was preset from 5 to 60 mmHg using 5 mmHg increments in 10 bovine, 8 ovine and 6 caprine freshly enucleated eyes. For each interval, readings were taken with both tonometers and compared to the manometrically controlled IOP (Mann-Whitney-U-test, $P \leq 0.05$; Bland-Altman plot, regression analysis). In cattle, sheep and goats the median IOPs (min-max) obtained with the TV were 23 mmHg (12-40 mmHg), 11 mmHg (7-20 mmHg) and 23 mmHg (9-37 mmHg), respectively. Using the TPA, IOP measurements for cattle, sheep, and goats were 16 mmHg (8-27 mmHg), 10 mmHg (5-18 mmHg) and 13 mmHg (4-25 mmHg), respectively. There were statistically significant differences between the readings taken with the TV and the TPA in all species (Wilcoxon-test, $P \leq 0.05$). All measurements obtained with the TV and the TPA during the calibration procedure differed statistically significantly from the manometrically controlled IOP measurements (Mann-Whitney-U-test, $P \leq 0.05$). For both instruments, regression formulas were calculated to correct the measurements. Both tonometers can be used effectively to assess intraocular pressure in ruminants considering the specific regression formulas.

INTRODUCTION

The normal and relatively constant intraocular pressure (IOP) is maintained by the balance of aqueous humor production and outflow. This pressure is essential to maintain the shape of the eye and a stable position of the intraocular structures. (1, 2)

IOP measurement should be performed in each patient presented for ophthalmic examination, as tonometry is an essential diagnostic procedure for diagnosing and monitoring uveitis and glaucoma. The most accurate method of measuring IOP is direct tonometry using a manometer, but due to the invasiveness of this procedure, it is impractical for clinical use. In current clinical veterinary ophthalmology applanation and rebound tonometry are the most widely used techniques for measuring IOP. Both methods indirectly determine IOP by measuring corneal tension. (3)

There are multiple published studies that compare the different methods and available instruments for measuring IOP in various animal species. In veterinary ophthalmology, the Mackay-Marg applanation tonometer has been widely used in the past and has been shown to have good accuracy. It seems to be the most reliable device for measuring IOP in different animal species both with and without ocular abnormalities. The accuracy and usefulness of the commercially available Tono-PenXL®, the Perkins handheld tonometer, and the TonoVet® (TV) have been extensively studied in different animal species. (4-15)

For accurate pressure measurement, each type of tonometer has to be calibrated for the different animal species because of varieties in their ocular anatomy (e.g., corneal thickness and curvature, corneal and scleral rigidity, tear film viscosity, etc.). Consequently, calibration curves for the different tonometers are necessary. There are several studies in dogs, cats, horses, cows, sheep, rats, mice and chinchillas available. (3, 11, 15-18) Calibration studies for the TV and newly designed Tono-Pen AVIA® (TPA) in domestic ruminants are lacking.

Depending on the instrument used, the mean IOP reported for cattle, sheep and goats ranges from 23 – 29 mmHg, 13 –16 mmHg and 8 – 12 mmHg, respectively (Table 1). (5, 6, 12, 19-22)

Although the incidence of glaucoma is described to be very low, even less than 1%, in cattle, various ocular diseases (inflammatory or neoplastic conditions) can lead to alterations in aqueous humor production and outflow, resulting in an increased or decreased IOP. (23-25) Furthermore, ocular hypertension has been experimentally induced in cattle and sheep using topical application of corticosteroids. (21, 26)

To the author's knowledge, there are not currently any studies comparing applanation and rebound tonometry or calibration curves for the TV or the TPA in ruminants.

MATERIAL AND METHODS

Patient examination

Twenty healthy privately owned cattle, sheep and goats each were examined due to owner´s request as a part of a general health check. All procedures were conducted according to the guidelines of the Association for Research in Vision and Ophthalmology (ARVO).The animals were examined in their normal environment to keep the stress level as low as possible. The cows were restrained in their feed fence with the head fixed with a halter to either the right or left side to avoid any pressure on the jugular vein. All sheep were positioned on their backside as routinely done for shearing, and their heads were gently held straight ahead. Goats were kept in a standing position with their heads restrained by their horns, looking straight ahead.

All 60 animals underwent slit-lamp biomicroscopy of the anterior segments of both eyes using a Kowa-SL 15® (Kowa, Tokyo, Japan). Measurement of the intraocular pressure of each eye was initially performed with the TonoVet® (Tiolat Oy, Helsinki, Finland) using the "d" setting.After rebound tonometry, two drops of the local anesthetic agent oxybuprocaine

hydrochloride (Novesine® 0.4%, OmniVision GmbH, Puchheim, Germany) were applied to both eyes. Thirty seconds after instillation of this medication, the Tono-Pen AVIA® (Reichert, Depew NY, USA) was used to take IOP readings from each eye. For each eye triplicate readings were taken with both devices and then averaged. Which eye was evaluated first was randomly selected. Care was taken to open the eyes without applying any pressure on the globe.

Manometric examination

Prior to the manometric examination, the manometer (D D-890, ATP Messtechnik GmbH, Ettenheim, Germany) was checked by the Bureau of Standards of the Federal States of Berlin and Brandenburg, Germany.

Ten bovine, eight ovine and six caprine freshly enucleated eyes from slaughtered animals were used for the ex vivo measurements. After enucleation, the eyes were immersed in 0.9% saline solution and stored at room temperature. All measurements were performed within 6 hours after enucleation.

For the manometric experiments the enucleated eyes were placed on a bed of modeling material on top of a plastic cup to avoid any pressure or movement during the examination. The eyes were cannulated through the sclera into the vitreous cavity with a 23-gauge needle. The needle was connected to the manometer and to a NaCl solution reservoir via a three-way stopcock. An open system was used for all measurements. The IOP was sequentially increased from 5 to 60 mmHg in increments of 5 mmHg by adjusting the height of the saline reservoir. Minimal changes in the manometrically controlled IOP (± 0.1 mmHg) were tolerated, but higher differences were corrected immediately. There was no leakage of fluid observed around the needles during all measurements.

The rebound tonometer was always used first, the applanation tonometer second. To keep the cornea moist throughout the whole examination, 4 - 5 drops of saline were applied to its surface

before each measurement. For each interval six consecutive measurements with both devices were taken. In each measurement series the lowest and the highest value were excluded from statistical analysis. All values were compared to the manometrically controlled IOP.

Statistical analysis

The statistical software R (version 3.1.0) was used for statistical analysis. Data were tested for a normal distribution with the Shapiro-Wilk test.

The Wilcoxon test was performed to detect differences between the measurements of the TV and the TPA for the right and left eyes of living animals and for comparison with the manometric results at each IOP level. For evaluation of differences between the right and left eye measurements, the Mann-Whitney-U test was used. Linear regression analysis was performed, and a regression formula was calculated to correct the results obtained from the TV and the TPA. The level of significance of all comparisons was set at $P \leq 0.05$.

RESULTS

Clinical tonometry

A total of 120 eyes from 60 healthy domestic ruminants (20 cows, 20 sheep, and 20 goats) were examined. All cattle were Holstein-Fresian dairy cows with a median age of 4 years old (2 - 10 years). The sheep were German heaths from a hobby breeder. Eleven animals were female, and 9 were male, = with a median age of 0.5 a year old (0.5 - 12 years). The goats were all female Toggenburgers and were kept for milk production and reproduction. They had a median age of 6.5 years old (2 - 14 years). Based on the ophthalmic exam, all animals were free of ocular disease.

The obtained values were not normally distributed. The results of the intraocular pressure measurements (median, min-max, mean ± standard deviation (SD), *P*-values) are listed in Table 2.

There were statistically significant differences between the measurement results from the TV and the TPA in all three species (Table 2). Differences between the left and right eyes were statistically significant for the measurements made using the TV in cattle and goats.

Manometric examination

All results from the measurements with the TV and the TPA (with exception of one IOP level in sheep) differed significantly from the manometrically set pressure level.

In cattle, the TV underestimated the manometrically determined pressure between 5 and 25 mmHg and overestimated it between 30 and 60 mmHg. The TPA underestimated the pressure throughout the whole range of IOP levels (Fig. 1a + 2a, 3a + 4a).

In sheep and goats, both instruments underestimated the manometric pressure from 5 to 60 mmHg (Fig. 1b,c + 2b,c; 3b,c + 4b,c).

To correct the measured results, regression formulas for the two tonometers for all three species were calculated (Table 3).

DISCUSSION

An adequate interpretation of IOP values requires reference values for the particular device and the relevant species. For use in different species, tonometers need to be calibrated, as ocular anatomy varies between different animal species.

Our IOP readings in cattle made using the TV were almost consistent with the values from a previous study by Kotani et al. (19) obtained using the Mackay-Marg tonometer and were

slightly lower than those reported by Gum et al. (12) using the Mackay-Marg tonometer and the Tono-Pen XL®. The values measured with the TPA in our study were quite similar to those reported by Andrade et al. (5) using the Perkins handheld tonometer.

Gerometta et al. (21) found a mean IOP (±SD) of 10.6 ± 1.4 mmHg in sheep measured with the Perkins tonometer. Our measurements taken with the TPA were quite similar. Our measurements with the TV revealed slightly higher results, which were comparable to those from Ribeiro et al. (22) using the Tono-Pen XL®.

There is only one study from Broadwater et al. (6) that established reference values for IOP measurements in goats. They measured IOP using the Tono-Pen XL® and the TV. Compared to our study, their results were quite similar to the values we obtained with the TPA. We found significantly higher values for the IOP in goats measured with the TV. This may be due to breed differences, different fixation methods or diurnal variation. All the cattle and goats in our study were females, but gender is not reported to have a significant influence on IOP in several other species. (6, 27, 28)

In contrast to previous studies, where no differences between the left and right eyes were detected (6, 12, 14, 20, 22), we found statistically significant differences between the left and right eyes in cattle and goats, but only for the measurements made with the TV. The eye measured first was always randomly selected, so the order of measuring cannot account for this phenomenon. As this side difference was only detectable in cattle and goats and was only observed with the use of the TV, an examiner-related cause seems to be unlikely. The reason for these statistically significant differences remains unknown. However, the side difference is only up to 2 mmHg and may not be of any clinical importance. In a study with a larger number of animals, this difference may no longer be significant.

The TV offers three settings for evaluation of the intraocular pressure in different species with respect to various globe sizes and anatomic variations (“h” = horse, “d” = dog and “p” = other

species). In the clinical part of the study, at first the “h” setting was used in cows (n = 10), as it was assumed that the bovine globe was most similar to the equine globe. In only 2/10 animals, readings without error were obtained. Therefore, the “d” setting was tried, and evaluable readings were obtained in 10/10 animals. Similar issues were discussed by Tofflemire et al., who stated that it is unclear which setting is the most accurate for use in cattle. (20) The manometric results of our study show that the “d” setting can be used for tonometry in cattle, sheep and goats.

Although glaucoma is rarely seen in ruminants, tonometry is still an important part of the ophthalmic examination. Both instruments (the TV and the TPA) used in this study are handheld devices that can be easily transported and used in mobile food animal practice. Measurements made with the TV appeared to be more difficult to obtain in cows due to the difficulty of restraining their heads in an appropriate position. The TV needs to be held in a perpendicular position to the cornea with the tip parallel to the ground, whereas the TPA can be used independently of the head position.

Comparing the two tonometers, we found statistically significant differences in all three species. We always measured with the rebound tonometer first because the measurements can be taken without the use of a topical anesthetic agent. Various authors have reported a tonographic effect of a rebound tonometer to be unlikely. They assume that the order of tonometer application does not affect the IOP results when using a rebound tonometer before an applanation tonometer. (11, 29)

A study by Miller et al. (10) showed that the Tono-Pen® significantly underestimated the pressure in normal healthy cat eyes in vivo when compared to the Mackay-Marg tonometer. Interestingly, with regard to the order of instrument application, different values were obtained with the TonoPen® when used after the Mackay-Marg tonometer. Furthermore, they found that both instruments tended to underestimate the pressure in open and closed in vitro systems in

cat eyes compared to direct manometry. No significant differences between two different applanation tonometers were found by Miller et al. (9) and Gum et al. (12) for in vivo measurements made with either the Mackay-Marg or the TonoPen XL® in horses and cows, respectively.

Compared with manometry in freshly enucleated eyes, all values from both tonometers (except one made using the TPA in sheep eyes) differed significantly from the manometrically set IOP. We excluded any tonographic effect, at least for the manometric study, by immediately adjusting the saline reservoir if pressure changes exceeded 0.1 mmHg.

In general, there were different results regarding the over- or underestimation of rebound tonometers. The underestimation of IOP from 5 – 25 mmHg and an overestimation from 30 – 60 mmHg in cattle eyes using the TV in our study is consistent with a previous study in cats from Rusanen et al. (30) In sheep and goat eyes, we found an overall underestimation with the TV. The study by Knollinger et al. (31) found a good agreement for the TV in enucleated dog eyes throughout the whole pressure range (5– 80 mmHg), whereas in enucleated horse eyes the TV significantly underestimated the IOP for pressures >70 mmHg. An equine study from Guese et al. found that the TV tends to slightly overestimate IOP in the clinically relevant pressure range from 10 – 60 mmHg. For pressure values >70 mmHg, the device underestimated the true IOP. (18)

The overall underestimation found when using the TPA in our study is consistent with the findings for applanation tonometers in dogs and cats. (11, 15) In the calibration study from Passaglia et al. (15), the TonoPen XL® underestimated the true IOP in cows and sheep mainly at high settings. In a study by Miller et al. (10), the two applanation tonometers (the Mackay-Marg and the TonoPen®) also underestimated IOP significantly compared to direct manometry in enucleated cat eyes in open and closed in vitro systems. In equine eyes, neither the Mackay-Marg nor the TonoPen® calculated IOP accurately compared to the manometric measurements.

(9) The TonoPen® consistently overestimated IOP at lower pressure levels and underestimated IOP at higher pressure levels compared to manometric measurements in a study by Passaglia et al. in cow and sheep eyes (15), whereas in other studies and different species an overall underestimation of the true IOP was found. (10, 11, 15)

In all three species, the rebound tonometer provided more accurate results for IOP than the applanation tonometer. This was also shown in a recent study by McLellan et al. (8) in cat eyes. In contrast to our study, their IOP values measured using the TV were consistently slightly higher than the manometrically controlled IOP in the cat eyes.

Although we found high r^2 values in most cases, it must be recognized that there is a significant difference between the manometrically set IOP and the measured IOP with both tonometers. This is in accordance with a previous study by Görig et al. (11)

To obtain reliable values we calculated regression formulas to correct the measured values. For daily clinical use, a simple correction factor would have been more suitable.

The limitations of our study were the small patient number and the fact that only one breed of every species was examined. Furthermore, we did not measure the central corneal thickness which might be a possible source of error. (32, 33)

In conclusion, our study has established additional reference values for IOP in ruminants. Our results show the importance of calibrating every tonometer for each species. It should always be considered that applanation tonometers tend to underestimate the true IOP, especially at higher pressure levels. For the surveillance of clinical patients, the same type of tonometer should always be used. The TV has offered much more reliable results, but the technique was more difficult to perform in cows.

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TABLES

Table 1: Published values for intraocular pressure (IOP) in ruminants made using different tonometers; SD = standard deviation.

author	species and breed	number of animals	mean IOP ± SD (mmHg)	tonometer
Gum et al. (1998)	cattle (Holstein-Fresian, Jersey)	n = 32 n = 27	27.5 ± 4.8 28.2 ± 4.6	MacKay-Marg
		n = 27	26.9 ± 6.7	Tono-Pen XL®
Kotani et al. (1993)	cattle		23.4 ± 5.9	MacKay-Marg
Andrade et al. (2011)	cattle	n = 10	18.8 ± 1.7	Perkins
Tofflemire et al. (2015)	cattle calves (Holstein)	n = 33	15.2 ± 5.2	TonoVet®
Gerometta et al. (2009)	sheep (Corriedale)	n = 18	10.6 ± 1.4	Perkins
Ribeiro et al. (2014)	sheep (Santa Ines)	n = 10	OS 12.70 ± 1.09 OD 13.90 ± 0.84	Tono-Pen XL®
Broadwater et al. (2007)	goat (Pygmy)	n = 10	11.8 ± 1.5 10.8 ± 1.7	TonoVet® "d" Tono-Pen XL®

Table 2: Results of the intraocular pressure measurements (median, min – max, mean ± standard deviation (SD)) and corresponding *P*-values in cattle, sheep and goats made using the TonoVet® and Tono-Pen AVIA®; * = significant difference (Wilcoxon test, $P \leq 0.05$); OS = oculus sinister, OD = oculus dexter.

	eye	**TonoVet® median (min – max)**	**Tono-Pen AVIA® median (min – max)**	**mean ± SD TonoVet®**	**mean ± SD Tono-Pen AVIA®**
cattle	OS	23 (15 - 37)	15.5 (8 – 27)	23.9 ± 5.0	15.5 ± 3.9
	OD	22 (12 – 40)	16 (9 – 25)	21.5 ± 6.3	15.6 ± 4.3
***P*-value**	OS*	< 0.000			
	OD*	< 0.000			
sheep	OS	11 (8 – 20)	10 (5 – 18)	12.7 ± 3.0	9.8 ± 2.7
	OD	10.5 (7 – 20)	10 (6 – 18)	11.7 ± 3.3	10.5 ± 2.4
***P*-value**	OS*	< 0.000			
	OD*	0.009			
goats	OS	22 (9 – 34)	13 (4 – 25)	21.6 ± 5.4	13.0 ± 4.3
	OD	24 (11 – 37)	13 (6 – 25)	24.3 ± 5.6	14.1 ± 4.6
***P*-value**	OS*	< 0.000			
	OD*	< 0.000			

Table 3: Calculated regression formulas and corresponding r^2 values for the two tonometers used in cattle, sheep and goats to correct the measured values.

	TonoVet®	**Tono-Pen AVIA®**
cattle	y = 1.226x – 5.392	y = 0.7141x – 0.7864
r^2	0.98	0.92
sheep	y = 0.9816x – 2.5601	y = 0.6337x – 1.1840
r^2	0.98	0.98
goats	y = 1.047x – 5.0551	y = 0.6476x – 3.0905
r^2	0.97	0.97

FIGURES

Figure 1: Bland-Altman plots for cattle (a), sheep (b) and goats (c) showing that the TonoVet® tends to underestimate and then overestimate the true intraocular pressure (mmHg).

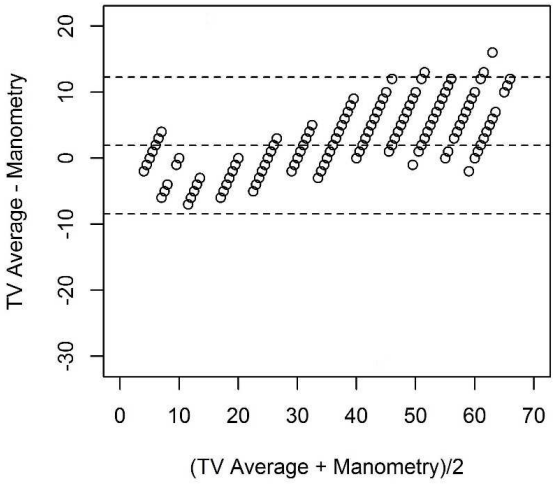

Figure 1a

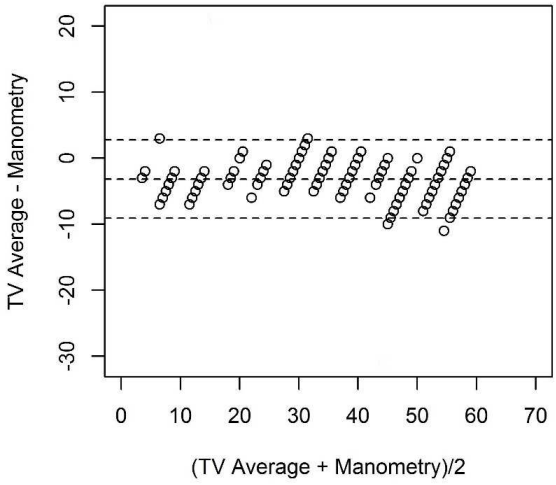

Figure 1b

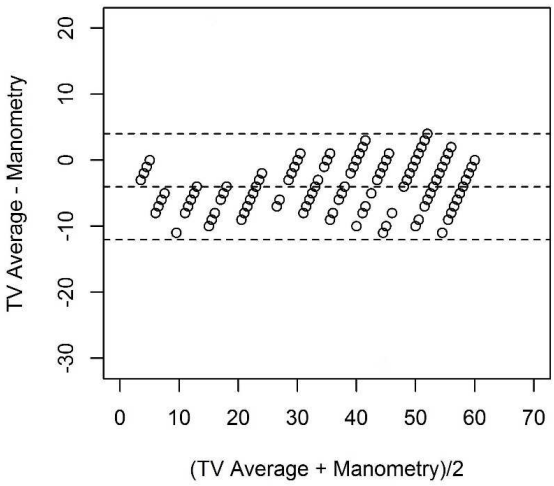

Figure 1c

Figure 2: Bland-Altman plots for cattle (a), sheep (b) and goats (c) showing that the Tono-Pen AVIA® underestimates the true intraocular pressure (mmHg) over the whole pressure range.

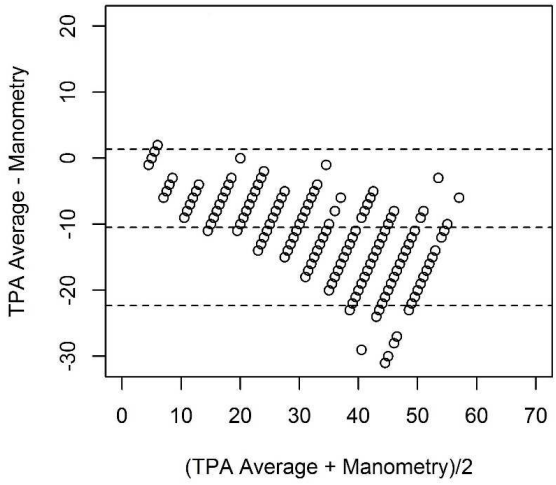

Figure 2a

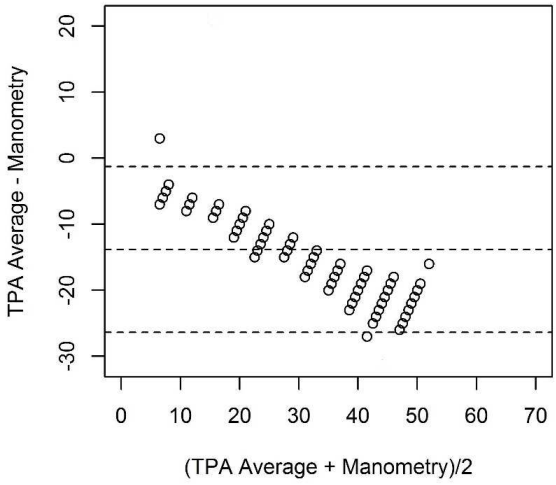

Figure 2b

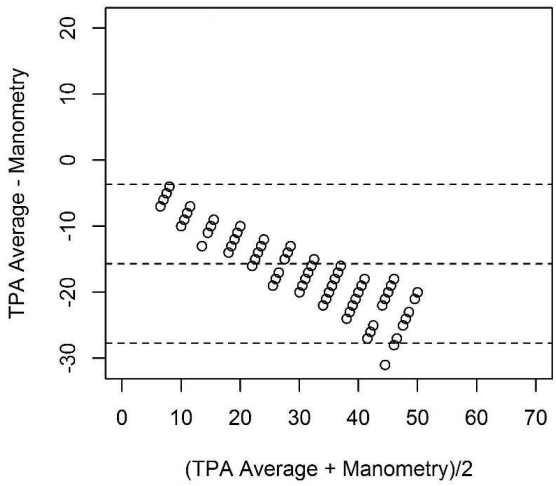

Figure 2c

Figure 3: Regression analysis for the TonoVet® in cattle (a), sheep (b) and goats (c): calculated regression line (solid line) and ideal regression line (interrupted line).

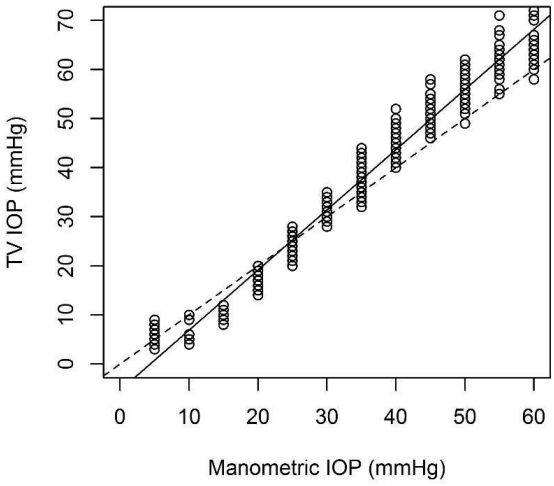

Figure 3a

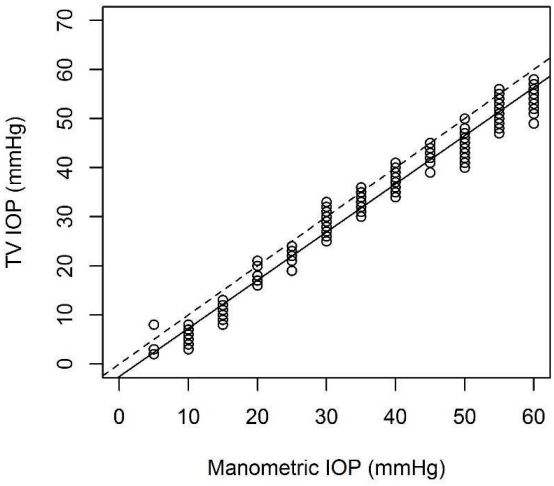

Figure 3b

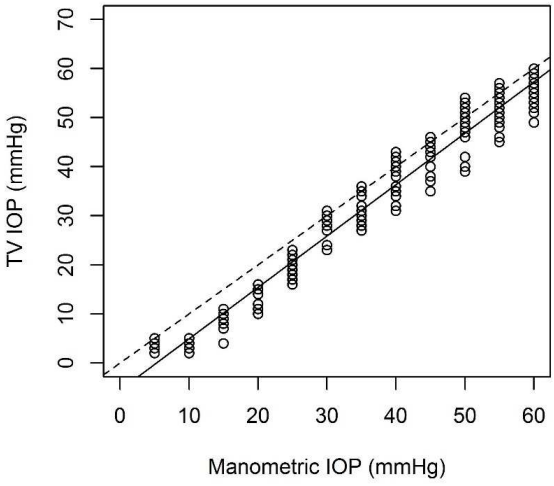

Figure 3c

Figure 4: Regression analysis for the Tono-Pen AVIA® in cattle (a), sheep (b) and goats (c): calculated regression line (solid line) and ideal regression line (interrupted line).

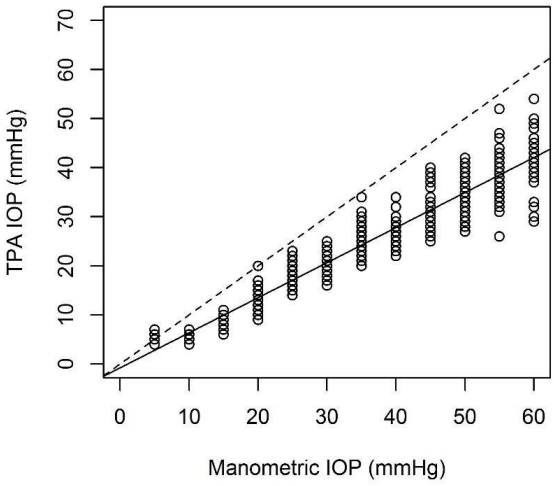

Figure 4a

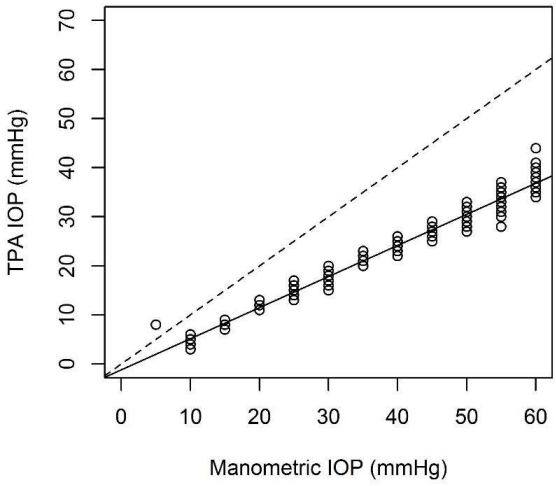

Figure 4b

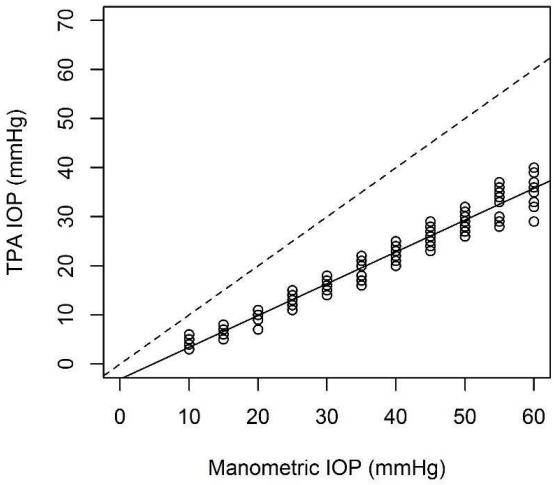

Figure 4c