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Original article

Effects of sex, eye-side, diurnal variation on intraocular pressure in calves

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Abstract

The aim of this study was to determine the effects of factors such as sex, eye-side and diurnal variation on intraocular pressure (IOP) values measured by Tono-Pen Vet® in healthy calves. Twenty four (12 males, 12 females) calves were used in this study. IOP measurements were performed in the morning (6:00 a.m.) and at night (8:00 p.m). Average IOP values in all calves were measured as 17.67±2.64 mmHg in the morning and 15.52±2.05 at night and the difference between these two time points was found to be statistically significant ($p<0.0001$). Average IOP values were measured as 16.04±2.82 mmHg in males and 17.15±2.23 mmHg in females with a statistically significant ($p<0.05=0.023$) difference between them. Average IOP values were 16.81±2.85 mmHg in right eyes and 16.37±2.23 mmHg in left eyes and the difference between these values were statistically insignificant ($p>0.05$). At the end of study, overall average IOP in all calves was measured as 16.59±2.59 mmHg. The present data showed a significant difference in terms of sex and diurnal variations and a non-significant difference in eye-side. In addition, this study is the first research article in which the intraocular pressure in calves was measured by Tono-Pen Vet®.

Key words: calves, intraocular pressure, Tono-Pen Vet®, sex, eye-side, diurnal variation

Introduction

Intraocular pressure (IOP), one of various routine systemic eye examination parameters (Giannetto et al. 2009, Park et al. 2011, Pereira et al. 2011), is an important criterion for diagnosing eye diseases such as uveitis and glaucoma that can result in blindness when left untreated (Jeong et al. 2007, Kato 2014). IOP measurement is performed using manometric (direct) and tonometric (indirect) techniques (Andrade et al. 2012). Although manometry, which works on the principle of cannulation /paracentesis of the camera is accepted as the gold standard in IOP measurement, because of its invasiveness it has found just the way in the field of experimental IOP studies rather than in clinical practice (Park et al. 2011, Andrade et al. 2012, Doering et al. 2017). Due to this limitation, for clinical IOP measurement in humans and animals alternative tonometry techniques (identation, applanation, rebound) have been developed (Spiessen et al. 2015, Doering et al.

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2017). Applanation tonometers work by measuring either how much the corneal area flattens with a predetermined force or what is the force required to flatten an area of the cornea that we know beforehand (Andrade et al. 2012). Currently known applanation tonometers include Goldman, Draeger, Perkins, Halberg, Maklakoff, Mackay-Marg, Tono-Pen and pneumotonograph type devices. Among these, the Tono-Pen (Tono-Pen Vet®, Tono-Pen AVIA®, Tono-Pen XL®) varieties, which are easy to use, portable and are minimally affected by the size, position and corneal surface abnormalities of animals are the most preferred ones in veterinary ophthalmology (Andrade et al. 2012, Doering et al. 2017). Normal IOP value in animals ranges between 15-25 mmHg (Gelatt and MacKay 1998, Doering et al. 2017) depending on the breed (Pereira et al. 2011, Pigatto et al. 2011, Ghaffari et al. 2012, Barsotti et al. 2013), age (Pereira et al. 2011, Verboven et al. 2014) and sex (Ofri et al. 1998) of animals, on measurement technique used (Pereira et al. 2011, Pigatto et al. 2011), researcher's experience (Pereira et al. 2011, Pigatto et al. 2011), diurnal variation (Giannetto et al. 2009, Pigatto et al. 2011), stress (Pigatto et al. 2011) and the application of anesthetic agent (Pigatto et al. 2011). To date many experimental and clinical studies (Jeong et al. 2007, Park et al. 2011, Strom et al. 2011, Andrade et al. 2012, Miller and Bentley 2015, Spiessen et al. 2015, Tofflemire et al. 2015, Bauer and Ambros 2016, Meekins et al. 2016, Doering et al. 2017), which have used many different measurement methods, have reported reference IOP values in a large number of animal species. Although it has been mentioned that ophthalmologic diseases cause significant economic losses in farm animals, and the fact that these animals carry experimental and comparative ophthalmologic research values (Ribeiro et al. 2014), relatively few IOP studies on this animal species have been conducted supposedly due to low glaucoma incidence (Gum et al. 1998). Up to now, with regard to reference IOP values in calves, only 3 clinical trials (Woelfel et al 1964, Gum 1991, Tofflemire et al. 2015) have been performed utilizing rebound (Tofflemire et al. 2015), indentation (Woelfel et al 1964) and applanation (Gum 1991) tonometry techniques. However, no studies have shown the extent to which IOP is affected by diurnal variation in these animals and what the reference IOP value would be it one of the new generation tonometric devices, i.e. the Tono-Pen applanation tonometry, is used. The current study, aimed to determine a reference IOP value in healthy calves using Tono-Pen Vet® applanation tonometry and to evaluate this parameter in terms of variables such as sex, eye side and diurnal variation.

Materials and Methods

The material for the study consisted of 24 calves (12 males and 12 females, 48 eyes) of different breeds and of ages ranging from 1.5 to 6 months (mean 3.2 months) provided from 4 different dairy and beef cattle ranches. Following general physical assessments all animals also underwent thorough direct and indirect ophthalmoscopic examination, slit-lamp biomicroscopic (XL-1®, Shin-Nippon, Japan), Schirmer tear test (Tear Flo®, Rose Stone Enterprises, India), fluorescein staining test, and pupillary light reflex and those found to be physically and visually healthy became candidates for the study. The study was conducted following the approval of the Ministry of Food, Agriculture and Livestock and the Local Ethics Committee of the Firat University Animal Experiments (2018/03-33) and according to the guidelines of the Association for Research in Vision and Ophthalmology. During the study, neither feed and water restrictions nor any agent including sedatives or nerve blockers except for topical anesthetic instilment were applied. The animals were divided into 2 groups, males (Group 1) and female (Group 2) of equal numbers (n= 12). The measurement was performed once 48 eyes, both right and left, of 24 animals, in the morning (6:00 a.m.) and at night (8:00 p.m.) once using the Tono-Pen Vet® applanation tonometry. During measurement, great care was given that no abnormal pressure be applied to the head and neck, to avoid unnecessary physical restraint and sudden movements, to keep the head of the animals above the heart level and to hold the eyelids gently. All the measurements (KK) and the physical restraint (OA) were performed by the same investigators. Prior to each measurement the tonometric device was calibrated and the latex cap on the device tip was changed. One minute after one drop topical anesthetic (Alcaine, 0.5% proparacaine hydrochloride, Alcon, Puurs, Belgium) administration to each eye, the eyelids were slightly retracted and the Tono-Pen Vet® probe was touched gently to the center of the cornea (Fig. 1). In the morning and at night three successive measurements were performed and the maintained value displayed on the device screen was recorded as an average; a case record was for each calf ear tag number. The measurements were repeated until the coefficient of variance associated with the IOP average displayed on the instrument was less than 5%. The measurement order followed in the morning with respect to the case number and eye-side was the same as that for the evening measurement.

Data are given as mean±standard deviation of the mean (SD) and were analyzed using the general linear models (GLM) procedure of SPSS Statistics for

Fig. 1. Intraocular pressure (IOP) measurement with Tono-Pen Vet®.

Table 1. IOP averages of all animals according to gender, eye-side and diurnal variation variables.

Measurement Time Points (Diurnal Variation)	Sex	Eye Sides	IOP (mmHg) Mean±SD
Morning (6:00 a.m.)	Male	Right	17.50±3.34
		Left	16.50±2.90
		Mean	17.00±3.10
	Female	Right	18.67±1.87
		Left	18.00±2.00
		Mean	18.33±1.92
	Male and Female Mean	Right	18.08±2.71
		Left	17.25±2.55
Evening (8:00 p.m.)	Male	Right	14.75±2.63
		Left	15.42±1.62
		Mean	15.08±2.16
	Female	Right	16.33±2.01
		Left	15.58±1.73
		Mean	15.96±1.87
	Male and Female Mean	Right	15.54±2.43
		Left	15.50±1.64
ALL TOTAL MEAN			16.59±2.59

Windows, Version 21.0 (IBM Corp. Armonk, NY: USA.). The Student t-test (Independent samples T test) was used for pairwise comparisons between groups. *P* values less than or equal to 0.05 were considered as statistically significant. Sample size was calculated based on a power of 85%.

Results

In the morning measurements, when the mean IOP value of the animals was evaluated in terms of sex and eye-side variables, the differences between the average IOP of the right and left eye of (R: 17.50±3.34 mmHg, L: 16.50±2.90 mmHg, $p>0.05$, Table 1) male animals and that between the mean IOP of the left and right eye (R: 18.67±1.87 mmHg, L: 18.00±2.00 mmHg, $p>0.05$, Table 1) of female animals were found to be statistically insignificant within themselves. A statistical significant difference was determined to exist between average IOP of male animals and that of female animals (M: 17.00±3.10 mmHg, F: 18.33±1.92 mmHg, $p<0.05$, Table 1) when the sex variable was considered. With regard to the eye side variable the difference between the mean IOP values of the right and the left eyes in all animals was not statistically significant (R: 18.08±2.71

Table 2. Statistical analysis of IOP averages of all animals according to gender, eye-side and diurnal variation variables (* p<0.05, *** p<0.0001).

Measurement Time Points (Diurnal Variation)	Sex	Eye Sides	IOP (mmHg) Mean± SD
Morning (6:00 a.m.)			17.67±2.64
Evening (8:00 p.m.)			15.52±2.05
	Male		16.04±2.82
	Female		17.15±2.23
		Right	16.81±2.85
		Left	16.37±2.23
ANOVA			*P*
Measurement Time Points			0.0001***
Sex			0.0360*
Eye Sides			0.410
Eye x Measurement Time Points			0.409
Eye x Sex			0.572
Measurement Time Points x Sex			0.632
Eye Sides x Measurement Time Points x Sex			0.362

mmHg, L: 17.25±2.55 mmHg, p>0.05, Table 1). A mean IOP of all animals was determined to be 17.67±2.64 mmHg when the data in the morning were evaluated in total (Table 2).

In the night measurement no statistically significant difference was found between the right and left eye IOP means (R: 14.75±2.63 mmHg, L: 15.42±1.62 mmHg, p>0.05, Table 1) of male animals and also between the right and left eye (R: 16.33±2.01 mmHg, L: 15.58±1.73 mmHg, p>0.05, Table 1) of female animals. Independent evaluation of the sex variable showed that the difference between average IOP of male animals and that of female animals was statistically significant (M: 15.08±2.16 mmHg, F: 15.96±1.87 mmHg, p<0.05, Table 1). This difference was found to be statistically insignificant when the eye side variable (R: 15.54±2.43, L: 15.50±1.64 mmHg, p>0.05, Table 1) was taken into account. Overall mean IOP for the night measurements was 15.52±2.05 mmHg (Table 2).

From measurement time variable (morning: 17.67±2.64 mmHg, evening: 15.52±2.05 mmHg) the difference was significant (p<0.0001, Table 2, Fig. 2). Cumulative evaluation of both morning and night data with regard to sex variable showed that mean IOP (16.04±2.82 mmHg) of male animals was significantly lower (p = 0.023) than that (17.15±2.23 mmHg) of female animals (Table 2, Fig. 3).

Overall data (R: 16.81±2.85 mmHg, L: 16.37±2.30 mmHg) indicate a non-significant difference (p = 0.410) between mean IOP of right (16.81±2.85 mmHg) and left (16.37±2.30 mmHg) eyes of all animals measured (Table 2, Fig. 4).

It was determined that the reference mean IOP for the calf breeds used in the study was 16.59±2.59 mmHg when the data, regardless of the measurement time, sex, and eye side variables, were assessed in total (Table 1).

Discussion

IOP is one of the important signs of ocular health (Jeong et al. 2007, Pereira et al. 2011). The measurement of this parameter in pet animals is very important in terms of the diagnosis and follow-up procedures for some important eye diseases such as uveitis characterized by intraocular hypertension and glaucoma with increased IOP (Doering et al. 2017). It is important to know the physiological IOP value in animals when a tendency for the IOP to increase or decrease in these diseases needs to be followed (Doering et al. 2017). IOP can be readily influenced by factors such as animal species (Ghaffari et al. 2012), race (Pereira et al. 2011, Pigatto et al. 2011, Ghaffari et al. 2012, Barsotti et al. 2013), age (Pereira et al. 2011, Verboven et al. 2014),

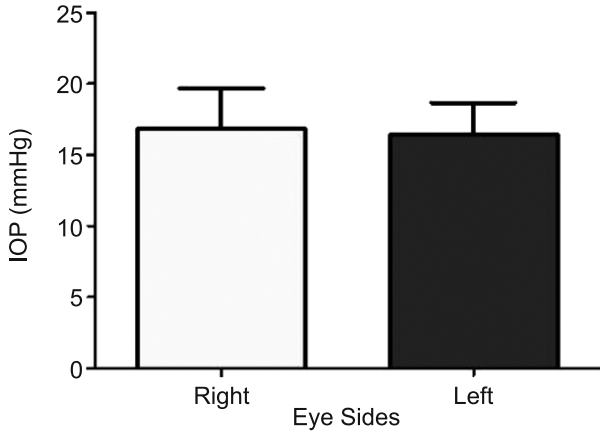

Fig. 2. Bar graph of statistical analysis of IOP averages of all animals according to eye-side variability ($p>0.05$).

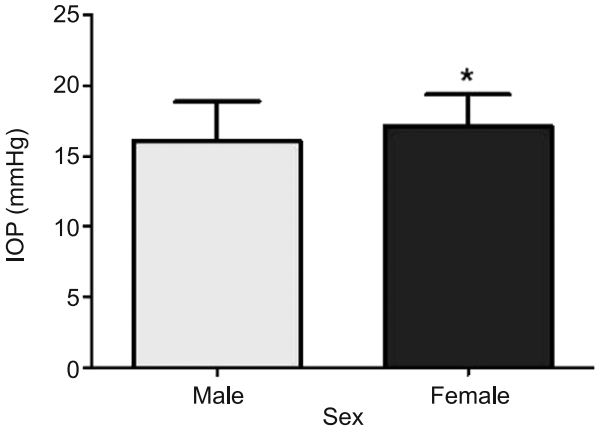

Fig. 3. Bar graph of statistical analysis of IOP averages of all animals according to sex variability (*$p<0.05$).

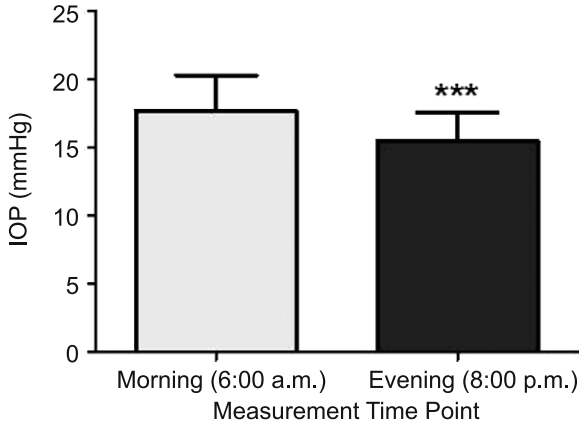

Fig. 4. Bar graph of statistical analysis of IOP averages of all animals according to diurnal variation variable (*** $p<0.0001$).

gender (Ofri et al. 1998), measurement technique used (Pereira et al. 2011, Pigatto et al. 2011), diurnal variation (Giannetto et al. 2009, Pereira et al. 2011, Pigatto et al. 2011), stress (Pigatto et al. 2011) and anesthetic agent application (Pigatto et al. 2011). There are a large number of clinical trials measuring IOP parameters in pet animals, especially dogs; however, in this respect the number of studies performed in large and small ruminants is limited. In one of these studies, it was found that the use of 0.5% topical prednisolone 3 times a day in cows elevated IOP to peak level after 4 weeks and the average IOP difference between the treated and untreated eyes was 15 mmHg (Gerometta et al. 2004). Some researchers (Tektas et al. 2010) have conducted a study in humans based on the results of this study (Tektas et al. 2010) and determined that approximately 30-40% of the patients treated with topical and systemic glucocorticoids developed ocular hypertension and concluded that glucocorticoid-induced glaucoma in cattle may be used as a model for human primary wide angle glaucoma studies. Another study (Ribeiro et al. 2014) reported that such ocular conditions may cause significant economic losses in livestock, and also the ocular studies performed in these animals may have research value in comparative ophthalmology. To date, the physiological mean for IOP in adult cattle has been reported as 26.9±6.7 mmHg with Tono-Pen XL (Gum et al. 1998), 18.8±1.7 mmHg with Perkins applanation tonometry (Andrade et al. 2011) and 16.1±1.0 mmHg (right eyes) and 16.5±1.2 mmHg (left eyes) with Perkins applanation tonometry (Gerometta et al. 2004). There have been only 3 clinical trials in calves in which physiological IOP average was determined. Woelfel et al. (1964), who conducted the first study in this particular animal group, determined physiological IOP averages with an indentation tonometry as 16.4±5.5 mmHg. In another study (Gum 1991) using Mackay-Marg applanation tonometry, mean IOP in healthy calves was found to be 20.0±5.5 mmHg. In the most recent study (Tofflemire et al. 2015) on calf IOP it was found that this mean was 15.2±5.2 mmHg using TonoVet® rebound tonometry. The present study used Tono-Pen Vet® applanation tonometry in 24 healthy calves, different from the previous studies, and determined the physiological mean IOP as 16.59±2.59 mmHg, which appears to be higher than the average IOP value of Tofflemire et al. (2015), but lower than that of Gum (1991). In fact, many researchers (Pereira et al. 2011, Pigatto et al. 2011) have mentioned that the difference in measurements can be attributed to the use of a different method or to the use of different tonometric devices even with the same working principles. It is further suggested that the IOP parameter may even vary by 1-2 mmHg among the tonometric devices working with the same principle but manufactured differently and this variation may increase up to 3-4 mmHg when devices with different operating principles are compared. For these reasons, it has been emphasized that for the same animal species as well as for each tonome-

ter with a different working principle a separate physiological IOP average should be established (Miller and Bentley 2015). In one of the studies supporting this view, Pereira et al. (2011) measured mean IOP as 15.44±2.16 mmHg with Tono-Pen Avia® applanation tonometry and as 9.51±2.62 mmHg with TonoVet® rebound tonometry in healthy rabbits and found a statistically significant difference between the two means. In another study comparing two tonometric devices in healthy dogs, it was found that the mean IOP value of 16.9±3.7 mmHg measured by TonoVet® rebound tonometry decreased to 11.6±2.7 mmHg when Tono-Pen XL® applanation tonometry was used (Park et al. 2011). Similar results were obtained in a comparison of Tono-Pen Vet® applanation tonometry with TonoVet® rebound tonometry (Spiessen et al. 2015) in glaucomatous cases and TonoVet® rebound tonometer with TonoPen XL® applanation tonometry in normal Eurasian Eagle owls (Jeong et al. 2007). The rebound tonometer is superior to applanation tonometers in that quick measurement is performed, it is repeatable and highly reliable and can be used with no need for topical anesthesia (Verboven et al. 2014); among the disadvantages are that the measurement is affected negatively by the position of the animal and sudden movements of the probe, and it also needs recalibration after each use (Pereira et al. 2011). Taking into account the impossibility of using the same type of tonometric device in every clinical practice, it is rational to establish a physiological IOP value in calves for every tonometric device. In the present study, a Tono-Pen Vet® applanation tonometer was used for the first time to determine the physiological IOP average in healthy calves and the average IOP value in these particular animals was found to be 16.59±2.59 mmHg.

Tofflimire et al. (2015) mentioned that taking no account of the diurnal variation variable in their study while determining the physiological mean IOP parameters was a limitation in their work. Indeed, many studies (Jaén-Díaz et al. 2007, Giannetto et al. 2009, Pereira et al. 2011, Martín-Suárez et al. 2014, Ribeiro et al. 2014, Garzón-Ariza et al. 2017) have shown that IOP is not a constant parameter and can change according to different measurement times during the day. Similar to the results of these studies (Giannetto et al. 2009, Pereira et al. 2011, Martín-Suárez et al. 2014, Garzón-Ariza et al. 2017), in our study we observed that the IOP is high in early in the day and low in the evening. Since these results indicate that the IOP may vary according to measurement time, it is recommended (Pereira et al. 2011) that while IOP measurement is conducted the transition phase from the dark to the light should be especially taken into account.

Except for the study of Ofri et al. (1998) in lions and that of Wu et al. (2006) in humans, where they found that IOP was higher in males compared to females, no other studies have investigated the effect of sex on IOP. However, in the present study, the mean IOPs of male and female calves were determined as 16.04±2.82 mmHg and 17.15±2.23 mmHg, respectively. When these means were evaluated statistically a significant difference was found between them. This result supports the study of Ofri et al. (1998) and Wu et al. (2006) with one difference which was that the average IOP of the females was higher rather than lower than that of males as noted by these authors.

There are many studies carried out in animals to investigate age-related IOP involvement. Gelatt and MacKay (1998) found that in healthy dogs grouped as young, adult, and elderly, IOP tended to decline with age, about 2-4 mmHg between those older than 6 and dogs younger than 2 years. Similar results have been reported by Kroll et al. (2001) and Kato (2014) in clinical studies on cats and dogs, respectively. Unlike these researchers (Ekesten and Narfström 1992, Gelatt and MacKay 1998, Kroll et al. 2001, Kato 2014) Ofri et al. (1998) in their studies on lions, Montiani-Ferreira et al. (2006) in mountain ferrets, and Montiani-Ferreira et al. (2008) in capuchin monkeys, reported no effect of age on IOP. The age of the calves used in the current study varied between 1.5 and 6 months. This age range was not wide enough to group animals by age and to indicate the association of IOP within this age variation. Indeed, Ekesten and Narfström (1992) and Gelatt and Mackay (1998) evaluated the IOP-age relationship at a broad age range and found that IOP declined significantly in the later years.

In the present study, regardless of sex and diurnal variations, the mean IOP of the right and left eyes was found to be 16.81±2.85 and 16.37±2.23 mmHg, respectively, with a resultant of no significant difference between them. The lack of a significant effect of eye side variability on IOP has also been previously reported (Gum et al. 1998, Ghaffari et al. 2011, Ghaffari et al. 2012, Tofflemire et al. 2015, Garzón-Ariza et al. 2017).

IOP measurements can be affected by a number of factors, i.e. abnormal pressure on the head and neck of animals, inappropriate physical constraints, abnormal body posture, researcher's measuring experience, use of anesthesia, choroidal blood flow, vitreous contents, sclera rigidity and orbicularis oculi muscle tension (Komáromy et al. 2006, Broadwater et al. 2007, Rusanen et al. 2010, Pereira et al. 2011, Pigatto et al. 2011). Prior to starting the study, all animals underwent a thorough systematic and ophthalmological examination and those physically and visually healthy were included in the study. To avoid the researchers' depen-

dent measurement difference all tonometer applications (KK) and physical restraint of animals (OA) were performed by the same persons. Great care was taken to avoid sudden movements during physical restraint, so that no abnormal pressure was applied to the animal's head, eyelids and neck during restraint, to ensure that the measurements were made at the standing position where the animal's head was above the level of the heart and to ensure that the eyelids were gently opened. Except for topical anesthetic instillation prior to IOP measurement, no other agent, including sedatives or nerve blockers that may affect IOP parameters was included in the study.

This study investigated the relation between IOP parameters and variabilities such as sex, eye-side and diurnal variation in healthy calves. To our knowledge, this is the first time a Tono-Pen Vet® applanation tonometer has been used for IOP measurement in calves, and a new reference IOP average relating to this device has been established. This study was conducted on different calf breeds, with the use of low numbers of the calves for a particular breed, and therefore breeds related IOP variability failed to be investigated which was accepted as a limitation of this study. As a conclusion, the results of this study may be useful to researchers working in the field of clinical ophthalmology and to clinicians in terms of diagnosis. In addition, this study may fill the gap in the literature in relation to IOP parameter and the diurnal variation in calves.

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