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Comparison between the rebound (TD - 8000 portable) and applanation tonometer (Tono-Pen Avia™) managed by different evaluators for intraocular pressure measurements in rabbits

Comparação entre o tonômetro de rebote (TD - 8000 portable) e aplanação (Tono-Pen Avia™) manuseados por diferentes avaliadores para mensurações da pressão intraocular em coelhos

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Abstract
This study aimed to compare values of intraocular pressure (IOP) by different tonometers and evaluators (veterinary ophthalmologist specialist and veterinary not a specialist). For this, 30 rabbits were used, and in all (n = 60 eyes), the IOP was initially measured with a rebound tonometer (model TD - 8000 portable, Apramed Indústria e Comércio de Equipamentos Médicos Ltda) and, subsequently, with an applanation tonometer (portable model Tono-Pen Avia™®, Reichert Technologies®, USA). With the two devices, the measurements in mmHg were performed in the central region of the corneas, always performed in the same period, by a professional veterinary ophthalmologist (specialist) and a professional veterinary (not a specialist). Data were statistically compared using the simple analysis of variance test. With the rebound tonometer, IOP ranged from 7 to 14 mmHg when measured by both evaluators; while with the applanation tonometer, from 9 to 15 mmHg by the specialist and from 8 to 16 mmHg by the non-specialist. In the right eyes, the IOP measured by the applanation tonometer by the non-experienced evaluator was statistically lower than the specialist's values; yet, the results of the two evaluators were higher in these same eyes when compared with those of the rebound tonometer. In the left eyes, the IOP measured by the applanation tonometer by the non-experienced evaluator was statistically higher than the specialist's values with the rebound tonometer. Thus, it was possible to infer that, regardless of experience in the area, the applanation tonometer indicated higher mean values of IOP in both eyes and, about the evaluators, the means of the measurements performed by the specialist were higher compared to the non-professional specialist.
Keywords: aqueous humor; glaucoma; veterinary ophthalmology; tonometry; uveitis

Resumo
Este estudo teve como objetivo comparar os valores da pressão intraocular (PIO) por diferentes tonômetros e avaliadores (veterinário oftalmologista especialista e veterinário não especialista). Para isso, foram utilizados 30 coelhos, em todos (n = 60 olhos), a PIO foi medida inicialmente com um tonômetro de rebote (model TD - 8000 portable, Apramed Indústria e Comércio de Equipamentos Médicos Ltda) e, posteriormente, com um tonômetro de aplanação (portable model Tono-Pen Avia™®, Reichert Technologies®, USA). Com os dois aparelhos, as medidas em mmHg foram realizadas na região central das córneas, sempre no mesmo período, por um profissional oftalmologista veterinário (especialista) e um profissional veterinário (não especialista). Os dados foram comparados estatisticamente por meio do teste de análise de variância simples. Com o tonômetro de rebote, a PIO variou de 7 a 14 mmHg quando medida por ambos os avaliadores; enquanto com o tonômetro de aplanação, de 9 a 15 mmHg pelo especilista e de 8 a 16 mmHg pelo não especialista. Nos olhos direitos, a PIO medida pelo tonômetro de aplanação pelo avaliador não experiente foi estatisticamente inferior aos valores do especialista; ainda, os resultados dos dois avaliadores foram maiores nestes mesmos olhos quando comparados com os do tonômetro de rebote. Nos olhos esquerdos, a PIO medida pelo tonômetro de aplanação pelo avaliador não experiente foi estatisticamente superior aos valores do especialista com o tonômetro de rebote. Assim, foi possível inferir que, independente da experiência na área, o tonômetro de aplanação indicou maiores valores médios de PIO em ambos os olhos e, em relação aos avaliadores, as médias das medidas realizadas pelos especialistas foram maiores em relação ao não especialista.
Palavras-chave: humor aquoso; glaucoma; oftalmologia veterinária; tonometria; uveíte

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Introduction

Regardless of the animal species, complementary ophthalmic exams, including the measurement of intraocular pressure (IOP)[1,2] is not commonly performed in clinical routine, making the parameters scarce in the scientific literature, which restricts therapeutic recommendations [3,4,5,6].

The aplanation tonometer most commonly used in Veterinary Medicine is the Tono-Pen Avia Vet®, considered effective, fast, with reliable and accurate results, and can be used in various animals species because it is portable and less expensive than the rebound one. However, the applanation tonometer Tono-Pen Avia™® (human) is also commonly used in veterinary patients[3,7]. The human and veterinary applanation tonometers uses a microprocessor that coordinates the movement of a piston that advances towards the cornea, flattening it several times; thus, the equipment has a strain gauge at its tip and performs about ten applanations in 15 seconds, creating an IOP graph[7].

Rebound tonometers veterinary (TONOVET®) and human (TD - 8000 portable) are portable, light, easy to handle, effective[8], little invasive due to the speed and subtlety of the measurement (which reduces the eyelid reflex), however, they are costly[9,10,11]. The technology is based on rebound (needle) and wireless thermal printing. The tonometer has a plastic-coated steel ball-tipped projectile that is propelled towards the cornea by a spring and kept floating on the correct axis by a magnetic field; thus, a microprocessor calculates the intraocular pressure according to the deceleration time of the projectile when touching the cornea[9,12,13].

The IOP is the balance between aqueous humor production and drainage[2,4], and in most cases, increased pressure is related to reduced aqueous humor drainage, instead of increasing its production[1]. The measurement of IOP is important, since its increase, considered serious and emergency, can cause glaucoma, or uveitis, inflammation of the uveal tract, which is results in a decrease in IOP[14, 15].

Thus, given the importance of measuring IOP for the diagnosis and early treatment of eye disorders, avoiding eye sequelae and loss of visual acuity[16,17,18], combined with the advantages and disadvantages of the different tonometry devices, the objective of the present study was to compare the IOP values in healthy rabbits when measured by the human rebound (model TD - 8000 portable) and human applanation tonometer (portable model Tono-Pen Avia™®) and, nevertheless, by different evaluators, being a veterinary ophthalmologist (specialist) and a (non-specialist) veterinarian in the area.

Material and methods

The research was carried out in accordance with the international standards of the Association for Research in Vision and Ophthalmology - ARVO (National Institutes of Health Publications number 85-23: Revised 1985), as well as by the Committee on Ethics in the Use of Animals (CEUA) of the University of Franca (UNIFRAN), case nº. 9725071117.

Thirty male, healthy, 3 kg, four-month-old male New Zealand white rabbits (*Oryctolagus cuniculus*) coming from ANILAB - Animal Laboratory Criação e Comércio Ltda, Paulínia (SP) were used. The animals were kept in individual cages at the Vivarium of the University of Franca (UNIFRAN, Franca, São Paulo, Brazil, altitude of 996 m, latitude: 20º 32' 19" S and longitude: 47º 24' 03" W), with water and commercial feed *ad libidum*. Ophthalmological examinations were performed at the same location, avoiding possible transport stress.

Prior to the study, the rabbits were submitted to a rigorous ophthalmological evaluation (clinical evaluation, Schirmer test, fluorescein test and slit lamp biomicroscopy) to verify the absence of ocular alterations. With the animals stationary and physically restrained by the same individual (avoiding excessive pressure on the eyelids and neck and standardizing the positioning of the head and body), IOP measurements, in mmHg, of both eyes of all animals (n = 60 eyes) were performed with the aid of a rebound tonometer (model TD - 8000 portable, Apramed Indústria e Comércio de Equipamentos Médicos Ltda) (Figure 1A), gently touching the central region of the corneas until the device was read. Three consecutive measurements were performed, considering the average of these and the tometer was held in a horizontal position, with the projectile parallel to the ground and starting 4-8 mm perpendicular to the corneal surface.

Subsequently, desensitization of the corneal surfaces of both eyes was performed with a drop of anesthetic eye drops (0.5% proximetacaine hydrochloride - Anestalcon®) for later measurement of IOP, in mmHg, with the applanation tonometer (portable model Tono-Pen Avia™®, Reichert Technologies®, USA) (Figure 1B), gently touching the central region of the corneas until the instrument reads. The Tono-Pen Avia™® was calibrated prior to the measurements of each animal, being performed three consecutive measurements, with a significance value lower than 5%, considering the mean of these measurements. Following the manufacturer's recommendations, the device positioning was not standardized during IOP measurements.

The spherical tip projectile of the rebound tonometer and the protective latex film of the applanation tonometer were for single use, discarded, and replaced for each animal measured. These changes were performed by the same team assistant, avoiding influences on the IOP measurements. IOP measurements were always performed first in the right eye followed by the left, between 8:00 am and 11:00 am, at an ambient temperature of 20° - 23°C, by a veterinary ophthalmologist experienced in the use of the devices (specialist), followed by a veterinary doctor non-specialist in the area (not a specialist) and who never used tonometers.

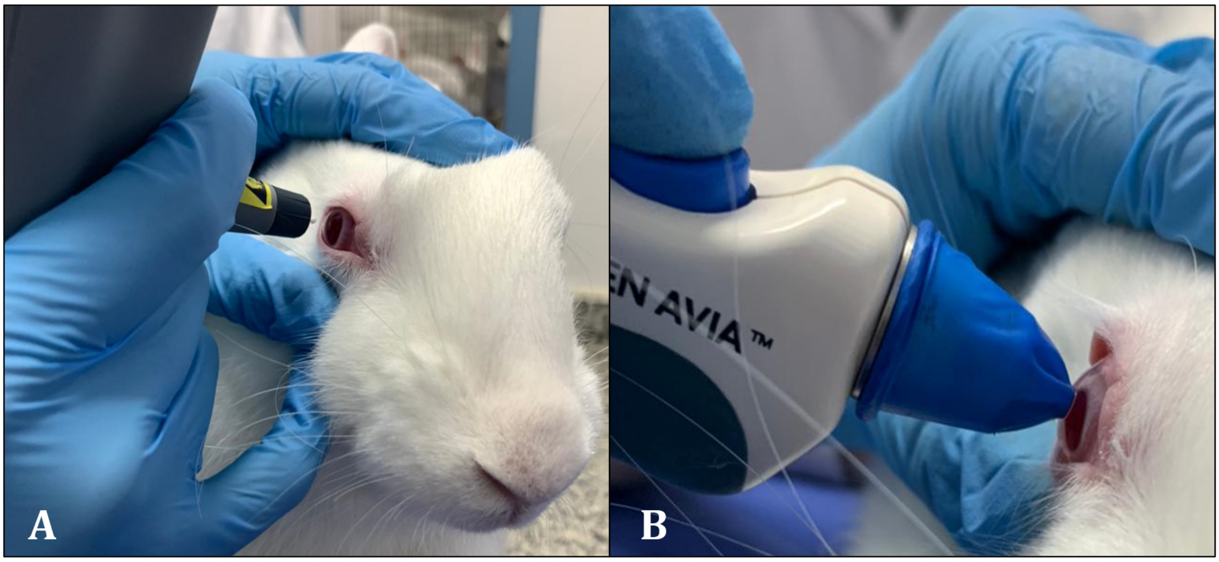

Figure 1. Photographic image of White New Zealand rabbit during measurement of intraocular pressure in the right eye. **A**: rebound tonometer (model TD-8000 portable) and **B**: applanation tonometer (model Tono-Pen Avia™® portable).

The IOP results obtained in mmHg were statistically verified by simple analysis of variance (ANOVA) for entirely randomized experiments, with calculation of the F statistic and its respective "P-value". In cases where $p \leq 0.05$, the treatment means were compared using the Tukey method, with the calculation of the minimum significant difference for $\alpha = 0.05$, using the Graphpad Prism 8.0® program.

Results

The means and standard deviations of the IOP measurements in the 30 rabbits with the different tonometers and evaluators are showed in Table 1. Regardless of the antimere, using the rebound tonometer, the IOP ranged from 7 to 14 mmHg when measured by the specialist; similar results were obtained by the non-specialist. On the other hand, with the applanation tonometer, the variation of this ocular parameter was from 9 to 15 mmHg when performed by the specialist and from 8 to 16 mmHg by the non-specialist.

The IOP measurement in the right eye with the applanation tonometer performed by not a specialist, in a discrete way, was statistically lower compared to specialist, in the same eye and with the same ophthalmic device (Table 1). The IOP value in the left eye obtained with the rebound tonometer by the specialist was significantly lower when compared with the contralateral eye, measured by the same evaluator and device (Table 1).

The IOP of the right eye measured with the applanation tonometer by the specialist was statistically higher compared to the same eye, but obtained with the rebound tonometer by the same evaluator (Table 1). The IOP result of the left eye measured by the applanation tonometer by not a specialist was significantly higher when compared to the same eye, however, with the rebound tonometer by specialist (Table 1).

The IOP measurement in the right eye with the applanation tonometer performed by not a specialist was statistically higher compared to the same evaluator and eye, but with the rebound tonometer (Table 1). The IOP of the left eye measured with the applanation tonometer by the specialist was statistically higher compared to that of the same eye, but obtained with the rebound tonometer by the non-specialist (Table 1).

Table 1. Means and standard deviations of intraocular pressure measurements (in mmHg) of 30 healthy male New Zealand White rabbits (*Oryctolagus cuniculus*) with the aid of a rebound tonometer followed by an applanation tonometer, in the right eyes (RE) and left eyes (LE), totaling 60 eyes, performed by a professional veterinary ophthalmologist (specialist) and a professional veterinary (not a specialist).

Rebound tonometer (mmHg)				Applanation tonometer (mmHg)			
Specialist RE (Means ± SD)	Specialist LE (Means ± SD)	Not a specialist RE (Means ± SD)	Not a specialist LE (Means ± SD)	Specialist RE (Means ± SD)	Specialist LE (Means ± SD)	Not a specialist RE (Means ± SD)	Not a specialist LE (Means ± SD)
11.2±1.4	10.5±1.5[b]	10.9±1.4	10.4±1.5	12.2±1.2[c]	12.2±1.32[f]	11.5±1.3[a,e]	12.6±1.4[d]

[a] Significantly different from the RE of specialist when measured by the applanation tonometer ($p \leq 0.05$).
[b] Significantly different from the RE of specialist when measured by the rebound tonometer ($p \leq 0.05$).
[c] Significantly different from the RE of specialist when compared to the rebound tonometer ($p \leq 0.05$).
[d] Significantly different from specialist LE when compared to the rebound tonometer ($p \leq 0.05$).
[e] Significantly different from the RE of not a specialist when compared with the rebound tonometer ($p \leq 0.05$).
[f] Significantly different from not a specialist LE when compared to the rebound tonometer ($p \leq 0.05$).

Discussion

The admission of rabbits is in accordance with the literature based on guidelines and other researchers[19,20]. In addition to the ease of obtaining, maintaining and handling, the use of these animals in scientific works allows increasing the number of animals tested and also, it is considered that the physiology, orbital and corneal anatomy is similar to that of dogs, cats and humans, being considered reliable species for experiments in the field of human and veterinary ophthalmology [21,22].

The comparison of IOP values in healthy rabbits, using the humans rebound and applanation tonometer, was based on the precision of these devices, in addition to the speed, ease of performing the techniques, costs when compared to veterinary devices and importance of the results for the definitive diagnosis of some ocular and correct therapeutic institution[11,22]. However, the scarcity of scientific data regarding the standardization of IOP values in this animal species secondary to the differences between methodologies (breeds of rabbits, types of tonometers used, times, frequencies and seasons of the year in which the measurements were made and measurement methods), made the discussion restricted.

Complementary tonometry ophthalmic exams did not cause discomfort and ocular lesions, and only mechanical restraint was enough to perform them, similar to what is described in the scientific literature in other animal species[11,23], which can be routinely used in rabbits. In this theme, the applanation tonometer was effective in measuring IOP and, despite Pigatto et al.[24] reported the need to use anesthetic eye drops prior to measurement as a disadvantage, there were no intercurrences regarding their use in rabbits, with no ocular reactions such as conjunctival congestion and ocular discomfort.

Following the recommendations of Andrade et al.[13] and Martín-Suárez et al.[25], the age and race of the rabbits were standardized, as well as the brightness of the place and the measurement period, as these factors contribute to the variation in IOP, regardless of the device and experience of the evaluator. In addition, prior to the experiment, all animals underwent a rigorous ophthalmological evaluation to rule out ocular changes, since scarred or damaged corneas can predispose to failures in the IOP measurement, since they increase the central thickness of the organ[19]. Also, to minimize oscillations in the IOP values, the correct positioning of the rebound device was recommended during measurements, preventing gravitational forces from affecting the speed and deceleration of the ball tip[26,27,28,29,30], although Zhang et al.[31] denote indifference between the vertical or horizontal positioning of the device during measurements in young rabbits.

When comparing, throughout the day, the IOP values in 38 healthy and young male and female rabbits (New Zealand White) with different devices, Pereira et al.[12] reported that the mean was lower with the Tonovet® veterinary rebound tonometer (9.51±2.62 mmHg) compared to the Tono-Pen Avia® (15.44±2.16 mmHg) when measured by the same specialist ophthalmologist. Corroborating these findings, in the present study, statistical reductions in IOP values were observed when measured by the human rebound tonometer compared to the applanation tonometer, even when handled by the same examiners, with and without experience in the area, especially in the right eyes. According to Grandin[32] and Wang, Dong and Wu[33], this can be explained by the stress caused by the first measurements with the rebound tonometer, because even though they were painless, the animals were not used to and adapted to the procedure considered new, overestimating the later measurements with the applanation tonometer.

Differing from the results found in this research, in 46 evaluated humans, Fernandes et al.[34] described higher IOP values when measured by a rebound tonometer (ICare) compared to applanation (Goldmann).

Studies conducted in human patients[35,36] and animals[11,37] showed that variations in animal behavior during measurement, well the tear film, eyeball size and the rigidity, curvature and central thickness of the cornea can change tonometry measurements in the same individual. These factors may explain the significant reduction in IOP in the left eye of the rabbits in this experiment compared to the right eye when measured by the rebound tonometer by the same ophthalmologist. Furthermore, Pereira et al.[12] argued that a difference of 2.0 mmHg between the eyes of the same animal can be considered normal, in addition to individual variability.

Still, with regard to the IOP variations found in this experiment, the significant reduction in the value of the right eye when measured by different evaluators with the applanation tonometer can be attributed to different pressures exerted on the corneal surface, as described by Tofflemire et al.[38]. Ma et al.[22] also discussed that corneal hysteresis (corneal response to rapid deformation) can influence IOP measurements, as it involves different corneal biomechanical properties such as viscoelasticity and resistance.

In this research, the differences in IOP found in the left eyes between measurements with different tonometers and evaluators, coincided with the reports by Liu et al.[39] and Andrade et al.[13] when investigating other animal species, that the values may change due to the device model and the examiner's skill/experience. In this way, the importance of the ophthalmologist specialist during the performance of complementary exams is emphasized, as he has knowledge about the correct positioning of the device[14], interpretation of results, as well as possible variations influenced by age, race, corneal status and eye disorders [22]. Oliveira et al.[14] elucidated that incorrect positioning of the rebound tonometer can underestimate or overestimate IOP values, unlike the applanation tonometer that can be used in any position. Still in relation to the different measurements between tonometry devices, when measuring IOP in young and female New Zealand rabbits, Ma et al.[22] reported that the TonoVet® was significantly superior to the Tono-Pen® in terms of intra-session repeatability and interoperator reproducibility, thus suggesting more first-device accuracy.

Regardless of the type of tonometry device and examiner, the IOP data found in rabbits in the current study were lower than those described by Zhong and Desai[7], Pereira et al.[12] and Vareilles et al.[40], and however, according to the scientific literature, the IOP of healthy rabbits varies due to several factors, including individual ocular characteristics, types and placement of tonometry devices, time of day and examiner experience[11,12]. Thus, the comparison between normality results of this ocular parameter must be cautious when considering the different methodologies recommended. More future studies are necessary to determine the response of each ophthalmic device on the biomechanical properties of the rabbit cornea.

Conclusion

Based on the established methodology and the results obtained, it is assumed that intraocular pressure was easily measured in healthy rabbits with both the rebound and applanation tonometers, with only physical restraint. Furthermore, regardless of the evaluator's experience, the applanation tonometer showed, statistically, higher mean values of intraocular pressure in both eyes, however, clinically there were discret variations. As for the evaluators, the averages of the measurements performed by the specialist were statistically higher compared to the not a specialist in the area, even though they were clinically subtle.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Author contributions

Conceptualization: A.T. Jorge, D. Paulino Júnior and F.G.G. Dias. *Formal analysis*: V.T.S. Almeida and S. Almeida Júnior. *Acquisition of financing*: V.T.S. Almeida, D. Paulino Júnior and F.G.G. Dias. *Investigation*: V.T.S. Almeida, C.B. Garcia, A.T. Jorge, D. Paulino Júnior, J.G. Martins, P.M. Pereira, S. Almeida Júnior and F.G.G. Dias. *Methodology*: A.T. Jorge, D. Paulino Júnior, S. Almeida Júnior and F.G.G. Dias. *Project management*: D. Paulino Júnior and F.G.G. Dias. *Supervision*: D. Paulino Júnior and F.G.G. Dias. *Writing (review & editing)*: V.T.S. Almeida and F.G.G. Dias.

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