Intraocular pressure in the smallest primate aging model: the gray mouse lemur

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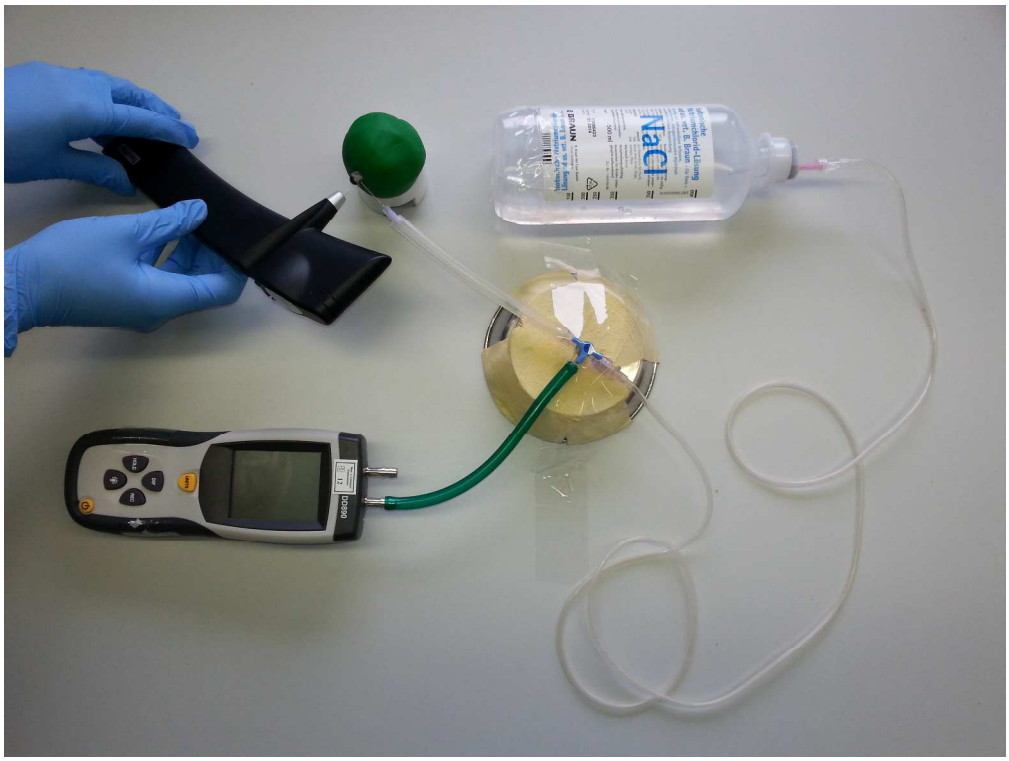

Figure 1. Setup of the manometrical investigation.
1151x863mm (72 x 72 DPI)

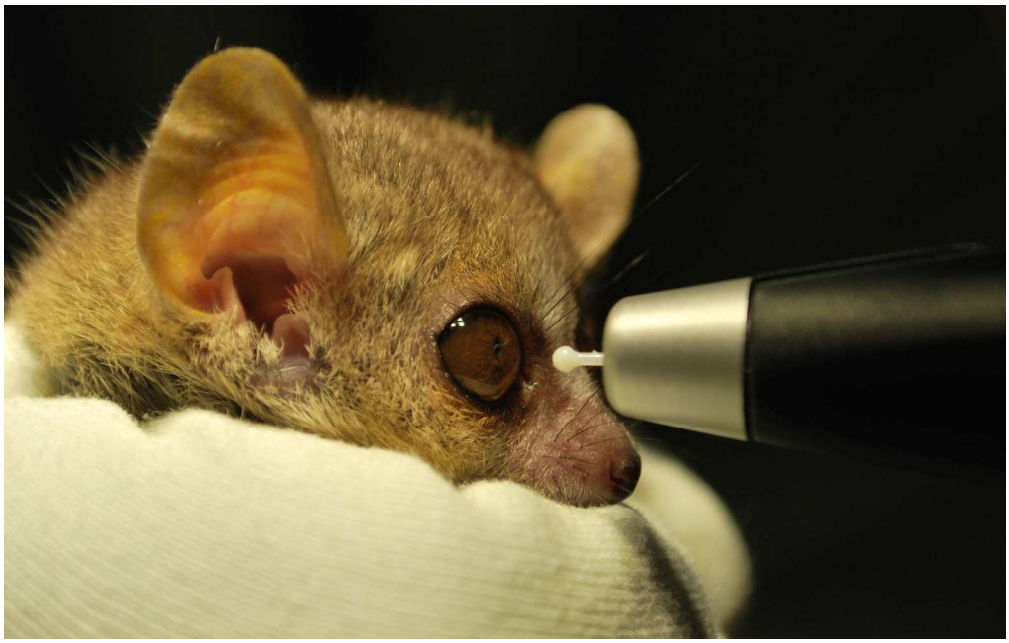

Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.
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Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™
1024x685mm (96 x 96 DPI)

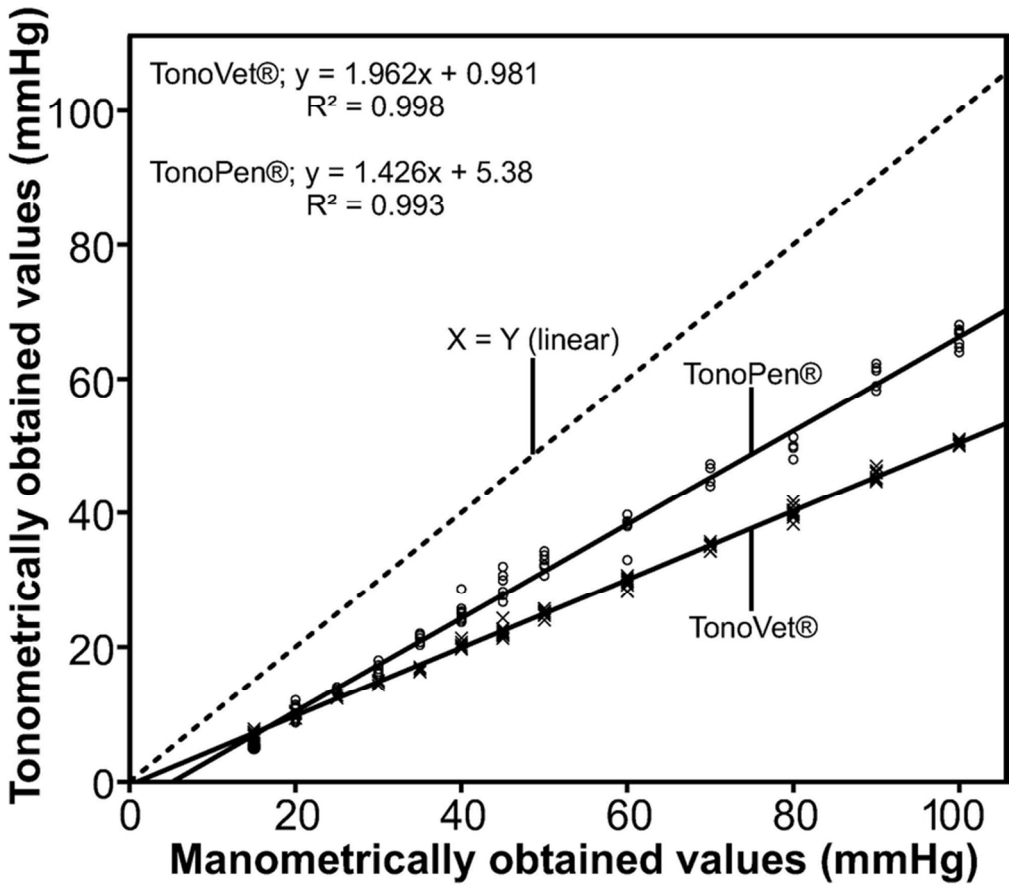

Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical control. Manometrically determined IOP for the eight eyes of four animals are represented on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot represents the mean from three measurements per step/per eye. The dashed line represents the regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.
73x64mm (300 x 300 DPI)

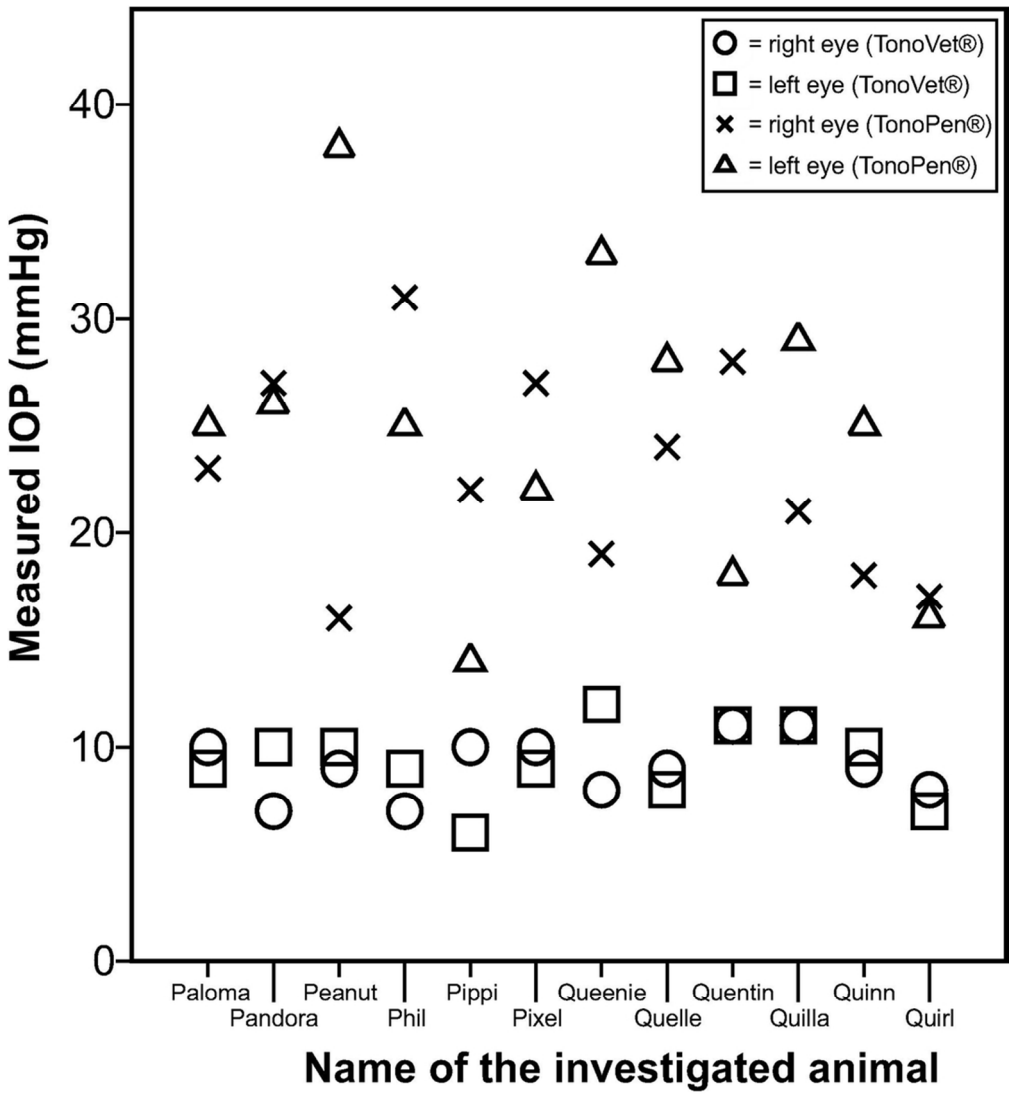

Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in 12 animals. The results show high variation in measurements for the TonoPen™ and much more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab. 1).
91x99mm (300 x 300 DPI)

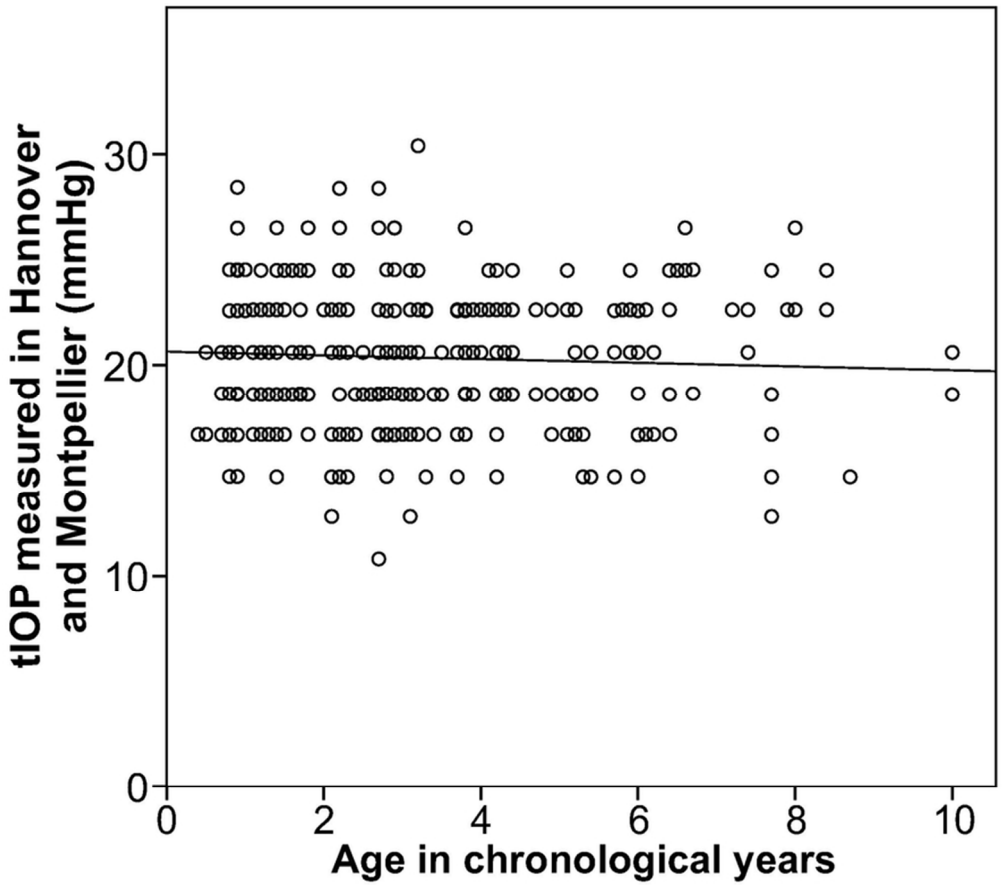

Figure 6. Relation between tIOP values and age in healthy mouse lemur eyes. This scatterplot shows the tIOP values for all measured healthy animals from Hannover and Montpellier (N = 258) on the y-axis in relation to age on the x-axis. The decrease in IOP is statistically not significant (p = 0.077, rs = -0.110).
74x65mm (300 x 300 DPI)

Intraocular pressure in the smallest primate aging model, the gray mouse lemur

Suggested running title: Intraocular pressure in aging mouse lemurs

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Abstract

Objective: The aim of this study was to assess the practicability of common tonometers used in veterinary medicine for rapid IOP screening, to calibrate IOP values gained by the tonometers and to define a reference IOP value for the healthy eye in a new primate model for aging research, the gray mouse lemur.

Studied animals & Procedures: TonoVet® and the TonoPen™ measurements were calibrated manometrically in healthy enucleated eyes of mouse lemurs euthanized for veterinary reasons. For comparison of the practicability of both tonometers as a rapid IOP assessment tool for living mouse lemurs, the IOP of 24 eyes of 12 hand-fixed animals (six males and six females) was measured. To define a standard reference value for IOP in 258 healthy mouse lemurs, two of the largest colonies in the world were examined using the most practicable tonometer.

Results: IOP measurements for the TonoVet® can be corrected by the formula: y = 0.981 + (1.962*TonoVet® value), for the TonoPen™ by y = 5.38 + (1.426*TonoPen™ value). The calibrated IOP for a healthy mouse lemur eye is 20.3 ± 2.85 mmHg. The TonoVet® showed advantages in practicability, e.g. small corneal contact area, short and painless corneal contact and time. IOP measurements of healthy mouse lemur eyes were not affected by age, sex, eye side or colony.

Conclusion: Tonometry using TonoVet® is the more practicable assessement tool for IOP measurement of the tiny eyes of living mouse lemurs. Pathological deviations can be identified based on the described reference value.

Key words: intraocular pressure, tonometer, reference value, mouse lemur, primate, aging

Introduction

Mouse lemurs belong to the smallest living primates worldwide. (1) Due to this fact, their maintenance and breeding is more cost-efficient than in larger primate species and they are also not known for spreading zoonotic diseases which makes them an extraordinary primate model for research. Additionally mouse lemurs have a life expectancy which is much shorter than in other non-human primate aging models with about 8 years in the wild and up to 18.5 years in captivity. (2, 3) The genome of mouse lemurs has recently also been sequenced by the Broad Institute (GenBank accession number ABDC00000000). Besides of the importance of mouse lemurs for biomedical as well as aging research, (4-9) they are also important for evolutionary research, based on their high and cryptic species diversity, their uneven distribution and their flexible adaptations to their natural habitats. (10)

Mouse lemurs are nocturnal and have relative small absolute eye sizes with 9.4 mm in diameter. (11, 12) Aged gray mouse lemurs were reported to suffer from different eye diseases such as cataract, retinal atrophy and buphthalmia, an abnormal enlargement of the eyeball. (13) Whether this malformation was due to glaucoma, which causes ocular hypertension, still need to be clarified though. Because of the difficulty in handling non-anesthetized animals, however, IOP was never studied in mouse lemurs before. Applanation and rebound tonometry are commonly used in veterinary medicine to determine IOP in domestic animals such as dogs, (14, 15) cats, (16-18) and birds (19, 20) as well as in laboratory animals such as rats, (21, 22) rabbits (23) and macaques. (24, 25) Since factory settings for TonoPen™ and TonoVet® are only available for common species in the veterinary clinics such as dogs, cats and horses, for uncommon species it is necessary to calibrate measurements by manometry to get a true IOP (tIOP) before defining standard reference IOP values for a given species. (see e.g. rabbits, (26) birds (20) and macaques. (25))

The TonoPen™ is an applanation tonometer often used for intraocular measurements in veterinary medicine. (14, 16, 21) IOP measurement gives an indirect assessment of the IOP by using the Imbert-Fick law. (27) It measures the counter pressure that is necessary to flatten a thin membrane surrounding a sphere filled with liquid. Its use in dogs and cats is easy and fast but requires a local anaesthesia of the cornea.

The TonoVet® is a rebound tonometer based on a patented measurement system which uses a small, disposable probe which is brought into contact with the cornea. (17, 18, 20, 24, 28) The probe is rebounding with a determined speed, correlating to the IOP. The higher the IOP is, the higher the speed of the return-bounce. Its use is easy and fast and requires no local anaesthesia.

In this study we applied TonoPen™ and TonoVet® as rapid IOP assessment tools to the gray mouse lemur, to

1. calibrate IOP measurements of the tonometers by manometry,
2. assess the practicability of the tonometers to measure the IOP of mouse lemurs´ eyes in-vivo to screen colonies,
3. apply the most practicable technique for screening IOP in two of the world's largest colonies, to investigate the effect of eye position, sex, colony and age on IOP and establish a reference value for IOP.

Methods

Animals and maintenance

Mouse lemurs (*Microcebus murinus*) tested in this study belonged to two licensed breeding colonies housed at the Institute of Zoology at the University of Veterinary Medicine Hannover (for details in housing conditions see; (29) Hannover breeding licence number 42500/1H) and the University of Montpellier 2 (Agreement $N^{br} \neq$ C-34-172-23). Out of 349 investigated animals 258 animals, which showed no ocular pathologies, have been used for analysis, 75 (38 females; 37 males) from Hannover and 183 (101 females; 82 males) from Montpellier, ranging from 0.5 to 10 years. All animals were born in captivity. Since mouse lemurs are nocturnal, the captive animals were maintained under artificial light conditions with a reversed light cycle. Additionally, animals in Montpellier are maintained under an accelerated photoperiodic regime. This means that the photoperiodically triggered reproductive "year" lasted 8 instead of 12 months. It has been shown that these conditions accelerate aging processes in gray mouse lemurs by the factor 1.5. (30-32)

Ophthalmologic investigation

Handling for ophthalmological examinations was similar to the weekly caretaker handling of the animals resulting in reduced stress for the lemurs. All examinations were conducted at the end of the sleeping period/beginning of the activity period to minimize disturbances of the animal's activity. All procedures applied in this study were licenced by the respective authorities (Hannover licence number, 33.9-42502-05-11A200, LAVES to Elke Zimmermann; Montpellier licence number, 34-124 to Jean-Michel Verdier).

Both eyes of a lemur were investigated with a slit-lamp bio-microscope (SL-14; Kowa, Eickemeyer, Germany) and indirect ophthalmoscope (Omega 100; Heine, Ettenheim, Germany) to determine potential eye pathologies with a possible effect on IOP or corneal consistence. To get a view on the lens and retina, mydriatic eye-drops (Mydrum®, Chauvin ankerpharm GmbH, Berlin, Germany) were used to widen the pupil.

1. Manometry

We determined the IOP value of an enucleated eye with the TonoVet® and TonoPen®, respectively, at manometrically defined IOP pressure steps (DD-890, ATP Messtechnik GmbH, Ettenheim, Germany). The used manometer was calibrated by the bureau of standards in Hannover (Mess- und Eichwesen Niedersachsen Betriebsstelle Eichamt Hannover, Goethestraße 44, 30169 Hannover). The pressure measured by the manometer in this setup (including the pressure in the examined eye) will be labelled as the true IOP (tIOP). The values in the more relevant sector for clinical use between 5 mmHg and 50 mmHg have been taken in steps of 5 mmHg ± 0.1 mmHg. Between 50 mmHg and 100 mmHg measurements were taken in steps of 10 mmHg ± 0.1 mmHg.

Eight healthy eyes of four animals euthanized for veterinary reasons (incurable pathologies) were enucleated transconjunctivally immediately after euthanasia. These eyes were called healthy since they were found inconspicuous and showed no signs of pathological disease according to an ophthalmological investigation performed not more than 6 months before. After enucleation, the eyes were stored in 0.9% NaCl solution at 6°C for up to a maximum of 4 hours before measurement. A small bowl of dough was adjusted to ensure the fixation of the enucleated eye. The cannula (24 G, length 25 mm, B. Braun Melsungen AG, D-34209) was inserted transsclerally into the vitreous and was not moved or reinserted while taking the measurement. The pressure was constant and measured values showed no fluctuation.

Minimal leakages were observed sporadically, but sealed by themselves with higher pressure. A three-way stopcock was connected with the cannula, the manometer and a NaCL solution reservoir via three silicon tubes. Pressure was adjusted by changing the height of the NaCl solution reservoir. The whole system was opened all the time to avoid fluctuations. (see Fig. 1) Once the manometer displayed a constant pressure, the pressure was measured using the TonoVet® and TonoPen™. Each complete measurement by the TonoVet® represented the mean of four single values, for the TonoPen™ the mean consists out of four to five single values. The measurement was carried out until three complete measurements per eye were successfully obtained.

2. In-vivo application of TonoPen™ and TonoVet®

The tonometers TonoPen™ and TonoVet® were used to measure IOP in the eyes of 12 young mouse lemurs (6 males, 6 females; aged between 2 and 3 years; Colony Hannover). Both eyes of these mouse lemurs were inconspicuous and showed no signs of pathological diseases according to an ophthalmological investigation performed one day before. Measurements of the TonoVet® and TonoPen™ have been taken 24 hours after the ophthalmological investigation to minimize influences of the mydriatic eye-drops on the IOP. Each animal was investigated on both eyes until a successful measurement with the respective tonometer has been achieved. The successful measurement is indicated by an acoustical signal.

2.a Rebound tonometry (TonoVet®)

Six single values of the IOP were taken per eye by the TonoVet® tonometer (TonoVet®; ICare, Finland Oy). The TonoVet® then automatically deleted the lowest and highest value and calculated the mean out of the remaining four values. Only successfully completed measurements displaying a mean on the TonoVet® were recorded. One successfully completed measurement per animal was taken. For all 12 animals no anaesthesia and no forced fixation of the eyelids were necessary (see Fig. 2).

2.b Applanation tonometry (TonoPen™)

The TonoPen™ tonometer (TonoPen™; Reichert® Technologies, Eickemeyer, Germany) was used to measure IOP of the same 12 animals as for the TonoVet®. Before measurements were taken, the eyes were locally anaesthetized with eye-drops (Proparakain-POS® 0.5%). To prevent a reflective wink of the eyelids of an animal, the examiner had to fix the eyelids with his fingers in open position (see Fig. 3). Between four and five single values per eye were necessary for the TonoPen™ to calculate a mean. Only successfully completed measurements displaying a mean on the TonoPen™ were recorded. One successfully completed measurement per animal was taken.

3. Determination of IOP in two colonies

To determine a reference value for the healthy mouse lemur eye, a large sample size is required.

241 animals in Montpellier and 108 animals in Hannover have been investigated. For the determination of the IOP the TonoVet® was used. The animals were investigated at the end of the sleeping/beginning of the activity period. For Montpellier the time for investigation ranged from 09:00 a.m. – 03:00 p.m. (beginning of activity period at 12:00 a.m. for all

animals), for Hannover from 09:00 a.m. – 05:00 p.m. (beginning of activity period at 10:00 a.m., 12:00 a.m. or 02:00 p.m. respectively, according to the room). After IOP measurement, an ophthalmological investigation was performed for each animal as described in the paragraph ophthalmologic investigation to select animals with healthy eyes for this study. 58 animals for Montpellier and 33 animals for Hannover showing eye malformations were thereby excluded from further analysis.

Data analysis

1. Manometric calibration of the IOP measured by TonoVet® and TonoPen™ in enucleated eyes

For the manometric calibration of the TonoVet® and TonoPen™ IOP measurements, we performed a regression analysis out of the eight means (= eight eyes) per step mmHg per instrument.

2. Comparison of IOP between TonoPen™ and TonoVet®

For the in-vivo comparison of applanation (TonoPen™) and rebound (TonoVet®) tonometry, we calculated the mean, range, standard deviation and median IOP for each instrument out of the measurements of the 24 eyes of the 12 animals. For the TonoVet® tonometer and the TonoPen™ tonometer, we compared measured IOP values between left and right eyes using the paired t-test and measured IOP values between sexes using the unpaired t-test since the values followed a normal distribution. The IOP values obtained by the TonoPen™ and TonoVet® were compared using the Wilcoxon-signed-rank test.

3. Comparison of IOP across colonies

To assess the effect of sex and eye position, IOP values (using TonoVet®; $N_{totalanimals}$ = 258, $n_{totaleyes}$ = 516; Hannover, N = 75; Montpellier, N = 183) obtained for each eye and animal per colony were compared between the left and right eye and between sexes using the Wilcoxon-signed-rank test and Mann-Whitney-U test, respectively.

If findings within colonies did not reveal a significant effect of eye position or sex, we used the median value of an animal per colony for further statistical analysis. To explore the effect of colony, we compared IOP values between colonies using the Mann-Whitney-U test.

The effect of age on IOP was analysed using a Spearman-Rank correlation. For the colony of Montpellier, the cycle age was multiplied by the factor 1.5 to calculate the chronological age in years. To define the reference IOP value for a healthy mouse lemur eye, we calculated the mean, range, standard deviation and median of the IOP of the healthy eye of both colonies.

Results

1. *In vitro calibration of the rebound (TonoVet®) and applanation (TonoPen™) tonometry*

We found a linear correlation between either the rebound tonometry and direct manometry or applanation tonometry and direct manometry (see Fig. 4; n = 8 eyes; N = 4 mouse lemurs). The regression analysis showed consistent linear underestimation of IOP by the TonoVet® and TonoPen™. From 15 mmHg up to 100 mmHg, the regression analysis showed that the measured IOP (mIOP) for TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) can be corrected by using the function tIOP = 0.981 + (1.962 * mIOP) and for TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) by using the function tIOP = 5.38 + (1.426 * mIOP). In both tonometers it was not possible to obtain values below 15 mmHg. From 15 mmHg up to 100 mmHg the TonoVet® constantly underestimated the tIOP by half. While the TonoPen™ almost measured the same values at 20 mmHg as the TonoVet®, the measured values start slightly to increase compared to the TonoVet® reaching around 66% of the tIOP at 100 mmHg.

2. *In-vivo IOP measurements with TonoPen™ and TonoVet®*

A comparison of IOP values between the left and right eye for each instrument showed no significant difference between eye side (TonoVet®, paired t-test, N = 12, t = -0.538, p = 0.601; TonoPen™, paired t-test, N = 12, t = -0.794, p = 0.444).

No difference between sexes was found for IOP neither for the TonoVet® (unpaired t-test, $N_{total} = 24$, $N_{males}=N_{females} = 6$, $t = -0.130$, $p = 0.292$) nor for the TonoPen™ (unpaired t-test, $N_{total} = 24$, $N_{males}=N_{females} = 6$, $t = -0.340$, $p = 0.198$). Thus, we have not differentiated between sexes in all further analyses.

The mean IOP for the TonoVet® tonometer was 9.21 ± 1.53 mmHg, the median was 9.0 mmHg and the range 6-12 mmHg, the tIOP was 19.03 ± 2.24 mmHg.

For the TonoPen™ tonometer the mean was 23.83 ± 5.89 mmHg, the median 24.5 mmHg and the range 14-38 mmHg with a tIOP of 39.36 ± 5.1 mmHg. (see Tab. 1)

IOP values measured by the TonoVet® and the TonoPen™ differed significantly (Wilcoxon-test, N = 24, T = 0.00, n = 24, p = < 0.001). The comparison of measured values between instruments showed a higher estimation of the average IOP for the TonoPen™. The average value for the TonoPen™ is more than twice as high as the average value estimated by the TonoVet®. Measurements with the TonoPen™ in the same animal for the left and right eye show high variability, while the values obtained with the TonoVet® are much more consistent e.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). (see Fig. 5)

Several problems occurred in the application of the TonoPen™ in the examiner-animal context. The tip of the TonoPen™ was large in comparison to the small eyes of the mouse lemurs causing reflective winks of the animal because of contact to the animals' eyelashes. It was not possible to fixate the animals' eye-lids without causing indirect pressure on the eye-bulb. Besides, the examiner had to protect himself against bites since the hand which was fixating the animals' eye-lids was hazardously close to the animals' mouth and sharp teeth. The time that was necessary to complete one successful measurement per animal varied between 5 and 10 minutes (without waiting time for the applied anaesthetizing eye-drops).

When using the TonoVet® for IOP measurement, the animals showed no visible reaction when the probe touched the animals' cornea, furthermore almost no reflective wink was visible. Since no forced fixation of the animals' eye-lids was necessary, bites never occurred. The investigation lasted on average only 30 seconds.

3. Comparison of IOP between two mouse lemur colonies and the definition of a reference IOP value for the gray mouse lemur

Both colonies have been investigated using the TonoVet®. A comparison between measured IOP values in the left and right eye for each colony showed no significant differences (Hannover, Wilcoxon-test, N = 75, T = 22.05, n = 47, p = 0.361; Montpellier, Wilcoxon-test, N = 183, T = 55.27, n = 113, p = 0.336).

No difference between sexes was found for IOP (Hannover, Mann-Whitney-test, N_{total} = 150, N_{males} = 37 eye-pairs, $N_{females}$ = 38 eye-pairs, U = -1.314, p = 0.189; Montpellier, Mann-Whitney-test, N_{total} = 366, N_{males} = 82 eye-pairs, $N_{females}$ = 101 eye-pairs, U = -1.552, p = 0.121). Thus, sexes were not further differentiated in further analyses.

The comparison of IOP values between both colonies showed no significant difference (Mann-Whitney-test, N_{total} = 516, $N_{Hannover}$ = 75 eye-pairs, $N_{Montpellier}$ = 183 eye-pairs, U = -0.230, p = 0.818). Based on that, we did not further differentiate between colonies for subsequent analysis.

The effect of age on IOP was assessed by a Spearman-Rank correlation. No significant correlation between chronological age and IOP was revealed (p = 0.418, r_s = -0.036, N = 516, see Fig. 6). Thus, age has not affected IOP in the healthy eye for the investigated age-span.

Based on that a reference value for IOP measured with the TonoVet® was calculated with a mean of 9.87 ± 1.56 mmHg, a median of 10 mmHg and a range of 5-15 mmHg. Using the regression function the calculated mean for healthy mouse lemur eyes is tIOP = 20.3 ± 2.85 mmHg.

Discussion

This is the first study measuring the IOP of mouse lemurs. Our findings suggest that the TonoVet® is the most suitable tool (compared to the TonoPen™) for rapid IOP screening of the tiny eyes of this smallest bodied primate. Results showed that IOP in the clinically healthy eye is not affected by age, sex, eye side (left or right). Thus, a reference value for IOP could be defined based on a large sample-size of more than 250 individuals for this novel primate model for aging research.

Calibration of IOP using manometry

Every tonometer has to be calibrated for each species specifically due to the fact of different corneal attributes, especially corneal thickness, to get the tIOP values. (33, 34) IOP measurements using eyes persisting in the eye socket in ex-vivo as well as enucleated eyes revealed no significant differences between these procedures. (35) Manometric in-vivo measurement of IOP is, however, known to potentially cause damage at intraocular structures and pain, thus for ethical reasons we decided to base our manometric investigation on enucleated eyes of animals which died for natural reasons or were euthanized due to incurable diseases which had no impact on IOP.

Our manometric calibration for the TonoVet® and TonoPen™ showed a consequent linear underestimation of the tIOP for both instruments which can be corrected by the established regression functions. For a fast clinical interpretation of the measurements displayed on the TonoVet® the values can be multiplied by 2. For the TonoPen™ a similar but not as easy to use formula is: tIOP = (1.5*measured value) + 5.

A comparable underestimation of the IOP measured by the TonoVet® and TonoPen™ was reported for dog eyes. (15) This underestimation was explained by corneal specification

especially corneal thickness, calibration-standards and use by different examiners. For our study, the same examiner performed the measurements so that the effect of the examiner on the measured IOP values was minimized whereas both corneal specification and different calibration standards between TonoVet® and TonoPen™ are likely to explain the underestimation of IOP values. In humans a significant effect on IOP measurements caused by different thickness in different corneal areas using the rebound tonometry has already been shown: higher values were determined when corneal thickness was higher. (33, 34) Our measurements were taken at the center of the cornea to minimize this effect. We expect that central corneal thickness in mouse lemurs is relatively thin, which would explain the linear underestimation. Further investigations on corneal thickness of mouse lemurs e.g. with an ultrasound pachymeter are necessary to investigate this in more detail.

Practicability of TonoPen™ or TonoVet® to screen the IOP of mouse lemur eyes

The practical value of a tonometer is as important as the calibration. Thus, which of the commonly used tonometers in veterinary science, TonoPen™ or TonoVet®, is the most suitable tool to screen tiny eyes of a large number of mouse lemurs in colonies on a regular basis? The eyes of the gray mouse lemur have only a diameter of 9.4 mm. (11) Thus, a previous ophthalmological study in the gray mouse lemur had difficulties in using applanation tonometry in non-anesthetized animals. (13) We applied both the TonoPen™ and the TonoVet® for a subgroup of non-anesthetized animals to assess the practicability of these instruments. We showed that both tonometers can be applied, but that there are huge differences in practicability and ethical justifiability. The investigation with the TonoPen™ required a much longer time (up to 10 minutes) to assess the IOP of an animal compared to TonoVet® (up to 30 seconds). The most time consuming issue emerged by the high number of failed IOP measurements (indicated by an alarm-signal of the TonoPen™). The failure in

measurement was due to the fact that the veterinarian had to pay attention to exerted pressure, contact-area and animal position while measuring the IOP. In contrast, unsuccessful measurements (indicated by an alarm-signal) were quite rare for the TonoVet®. Furthermore, the small eye-bulbs of the mouse lemurs make it necessary to fixate the eye-lids manually and eventually causing pressure on the eye-bulb whereas no fixations of the animal's eye-lids were necessary for the TonoVet®. The animal itself must be fixated much stronger when using the TonoPen™ causing systemical hypertension and consecutively higher IOP. Consequently, our experience using the TonoPen™ supports Beltran et al. (13) that the use of this instrument to measure IOP in the tiny eyes of mouse lemurs is problematic. The extensive manipulation which is necessary prevents the determination of physiological expected IOP values. Usually physiological IOP ranges from 15-23 mmHg e.g. in humans, (36) dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) (see Tab. 2) Therefore non-physiologically high IOP as measured with the TonoPen™ in mouse lemurs (tIOP = 39.36 ± 5.1) may be the result of stress, high systemic blood pressure and unintended pressure on the bulbus questioning the ethical justifiability of this method.

Other positive effects of the TonoVet® were that IOP of non-anesthetized animals can be measured rapidly and without any visible harm for the measured animal. Furthermore a veterinarian can standardize the measurement quickly and get fast routinisation. The TonoVet® showed satisfying results concerning reproducibility with a relatively small variation comparable to those of other studies using larger mammals such as rhesus macaques (25) or rabbits. (26) All in all, based on these findings we recommend the TonoVet® as a suitable IOP assessment tool for rapid screening of the eyes in non-anaesthetized mouse lemurs.

Definition of a reference IOP for the healthy gray mouse lemur eye

A reliable mean for IOP in mouse lemurs requires a large sample-size. To enlarge our sample-size we have analyzed if eye side (left or right), sex or age had any influence on IOP in mouse lemurs. Our study showed no significant differences between eye side and sex and no statistically significant correlation between age and IOP. The slight decrease of IOP between the age of 0.5 and 10 years was even smaller than the value of the determined standard deviation. Animals of ten years and older were excluded due to diagnosed pathologies.

Our screening analysis of the two colonies, Hannover and Montpellier, included 258 animals with 516 healthy eyes in total and showed a tIOP of 20.3 ± 2.85 mmHg. This value matches quite well to the IOP range of 15-23 mmHg reported from humans (36) and mammals of veterinary and biomedical importance (see Tab. 2) such as dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) Since these mammals differ largely in size, activity and phylogeny, IOP seems to be independent from these factors.

Circadian rhythm is also described to affect IOP, e.g. in cats, rabbits and Tibetan monkey. (23),(38),(39) To minimize this effect, we always determined IOP at the beginning of the animal's activity period.

Our study showed no correlation between age and IOP in the healthy mouse lemur eye comparable to e.g. Tibetan monkeys. (39) Age-effects on IOP in animals and humans are ambiguous. Whereas investigations performed in rhesus monkeys and dogs showed a decrease in IOP with age (37),(14) studies in humans revealed both an increase and a decrease depending on the tested populations. (40),(36),(41) High blood pressure, obesity and other vascular deficiencies were discussed as explanations. (42-44) Consequently we postulate that physiological status and vascular conditions were healthy in our studied mouse lemur population.

It has to be taken into account that for our study we considered only ophthalmological inconspicuous animals. Pathologies influencing the state of health of the eye and leading to glaucoma or higher intraocular pressure have to be considered in follow-up studies.

Conclusion

To conclude, we demonstrated the practicability, usefulness and reliability of the TonoVet® as a powerful tool for screening IOP in mouse lemurs, a novel primate model for human aging research. Average IOP of healthy mouse lemur eyes is not affected by eye side, sex and colony and does not correlate with age. Furthermore, the value of IOP of mouse lemurs coincides with those of other mammals. For future studies using this smallest-bodied primate aging model, our findings are an important foundation to disentangle peripheral from central pathologies.

Software for statistical analysis

The statistical analysis was performed using SPSS 22.0 for Windows. Significance level was set at $P = 0.05$.

List of Abbreviations

IOP: Intraocular pressure; tIOP: true intraocular pressure; mIOP: measured intraocular pressure

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MD, MJ, EZ, IN, JS have conceived, coordinated and designed the study. Manometrical data was acquired by MD. Data from the screening of both colonies was acquired by MD and MJ. Statistical analysis was conducted by MD. All authors contributed in drafting, reading and approving the final manuscript.

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Figure 1. Setup of the manometrical investigation.

Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.

Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™

Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical control. Manometrically determined IOP for the eight eyes of four animals are represented on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot represents the mean from three measurements per step/per eye. The dashed line represents the regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.

Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in 12 animals. The results show high variation in measurements for the TonoPen™ and much more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab. 1).

Figure 6. **Relation between tIOP values and age in healthy mouse lemur eyes.** This scatterplot shows the tIOP values for all measured healthy animals from Hannover and Montpellier ($N = 258$) on the y-axis in relation to age on the x-axis. The decrease in IOP is statistically not significant ($p = 0.077$, $r_s = -0.110$).

Table 1. Overview of intraocular pressure (IOP) values in 12 animals for TonoVet® and TonoPen™.

The median, range, mean and standard deviation have been calculated for each instrument using the median from each animal's right and left eye.

	TonoVet® IOP results (mmHg)		**TonoPen™ IOP results in mmHg**	
Animal name	Right eye	Left eye	Right eye	Left eye
Pixel ♂ (2 years)	**10**	**9**	**27**	**22**
Quilla ♀(3 years)	**11**	**11**	**21**	**29**
Quinn ♂(3 years)	**9**	**10**	**18**	**25**
Quelle ♀(3 years)	**9**	**8**	**24**	**28**
Quirl ♂(3 years)	**8**	**7**	**17**	**16**
Pippi ♀(2 years)	**10**	**6**	**22**	**14**
Paloma ♀(2 years)	**10**	**9**	**23**	**25**
Peanut ♂(2 years)	**9**	**10**	**16**	**38**
Quentin ♂(3 years)	**11**	**11**	**28**	**18**
Queenie ♀(3 years)	**8**	**12**	**19**	**33**
Pandora ♀(2 years)	**7**	**10**	**27**	**26**
Phil ♂(2 years)	**7**	**9**	**31**	**25**
Median (mmHg)	**9**		**24.5**	
Range (mmHg)	**6-12**		**14-38**	
Mean ± SD (mmHg)	**9.2 ± 1.53**		**23.83 ± 5.89**	

Table 2. Variation of calibrated intraocular pressure (IOP) values in different species

Species	Reference	Mean IOP or range	Standard deviation
Human (N > 100.000)	[43]	15-16 mmHg	± 2.5-3 mmHg
Macaques (N= 102)	[44]	14.9 mmHg	± 2.1 mmHg
Gray mouse lemur (N = 516)	**this study**	**20.3 mmHg**	**± 2.85 mmHg**
Cats (N = 57)	[29]	20.74 mmHg	± 0.49 mmHg
Horses (N = 35)	[39]	22.1 mmHg	± 5.9 mmHg
Rabbits (N = 38)	[34]	15-23 mmHg	
Lewis rats (N = 115)	[32]	17.3 mmHg	± 5.25 mmHg
Dogs (N > 900)	[25]	19.0 mmHg	± 5.7 mmHg