PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 40(9):707-716, September 2020
DOI: 10.1590/1678-5150-PVB-6172

Original Article
Wildlife Medicine

Macroscopic morphological aspects, ecometry and rebound tonometry of the eye bulb in sloth (*Bradypus variegatus*)[1]

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ABSTRACT.- Braz R.S., Amorim M.J.A.A.L., Sá F.B., Albuquerque P.V., Silva E.C.S., Ferri R.C., Fonsêca Filho L.B. & Andrade G.P. 2020. **Macroscopic morphological aspects, ecometry and rebound tonometry of the eye bulb in sloth (*Bradypus variegatus*).** *Pesquisa Veterinária Brasileira 40(9):707-716*. Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: richellebraz@gmail.com

The field related to the visual system of wild animals is deeply scarce. Settling anatomical and physiological parameters for these animals is still a descriptive vision for *Bradypus variegatus* (Schinz, 1825). Thus, our research aimed to determine patterns of normal eye for this species. For this purpose, eight eye bulbs were dissected from the carcasses obtained by natural death, and then performed an overview of ocular anatomical. Rebound tonometry (RBT) and ocular B-mode ultrasonography were also applied for eight eyes in four animals from "Parque Estadual Dois Irmãos", situated in the city of Recife, state of Pernambuco (PE), to estimate the intraocular pressure and ocular ecobiometry. The ocular morphology of sloth is similar as described for other species, however, with some peculiarities. They present a third eyelid emerging in the nasal region of the inferior conjunctival sac and retina and also contain little differentiated blood vessels. Medium the intraocular pressure (IOP) was 4.25mmHg with no difference for both eyes. Ultrasonography of ocular anatomy is also similar regarding other species. Ecobiometric patterns were evaluated to determine the anterior chamber depth, lens width, vitreous chamber depth, and axial length (AL) of ocular globe and the averaged as shown 0.63±1.11mm, 3.73±0.24mm, 6.15±0.41mm, 3.70±0.27mm, and 8.48±0.22mm, respectively. There was no difference between the right and left eyes. The RBT and ocular B-mode ultrasonography are fast exams and easy for animal testing. This study contributed to the characterization of ocular anatomy as well as settling medium values of IOP and intraocular measures; however, further research on physiology and histology is necessary to better understand the visual function of the species.

INDEX TERMS: Macroscopy, morphology, ecometry, rebound tonometry, eye bulb, sloth, *Bradypus variegatus*, ophthalmology, ocular anatomy, ocular ultrasonography, tonometry, xenartros.

RESUMO.- [**Aspectos morfológicos macroscópicos, ecometria e tonometria de rebote do bulbo ocular em bicho-preguiça (*Bradypus variegatus*).**] O campo de estudo relacionado ao sistema visual de animais silvestres é muito escasso. Estabelecer parâmetros anatômicos e fisiológicos para estes animais ainda está restrito a uma visão descritiva, assim ocorre em *Bradypus variegatus* (Schinz, 1825). Diante deste fato, objetivou-se com este estudo determinar padrões de normalidade oftálmica nesta espécie. Para isto foram dissecados oito bulbos oculares de cadáveres obtidos por morte natural e realizada a descrição anatômica ocular. Além disso, foram realizadas tonometria de rebote (TonoVet®) e ultrassonografia em modo B em oito olhos de quatro animais provenientes do Parque Estadual Dois Irmãos, Recife/PE, para avaliação da pressão intraocular e realização da ecobiometria

[1] Received on April 6, 2020.
Accepted for publication on June 23, 2020.
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ocular. A anatomia ocular do bicho-preguiça é semelhante à descrita para outras espécies com algumas particularidades. Apresentam uma terceira pálpebra emergindo na região nasal do saco conjuntival inferior e retina com vasos sanguíneos pouco diferenciados. A pressão intraocular média foi de 4,25mmHg não havendo diferença entre os olhos direito e esquerdo. A anatomia ocular ultrassonográfica é semelhante à encontrada para outras espécies. Os padrões ecobiométricos obtidos foram: profundidade da câmara anterior, espessura do cristalino, diâmetro do cristalino, profundidade da câmara vítrea e comprimento axial do bubo ocular com tamanhos médios de 0,63±1,11mm, 3,73±0,24mm, 6,15±0,41mm, 3,70±0,27mm e 8,48±0,22mm, respectivamente. Não houve diferença entre os olhos direito e esquerdo. A tonometria de rebote e a ultrassonografia ocular em modo B são exames de rápida e fácil execução, sendo bem tolerados pelos animais. Este estudo contribuiu para a caracterização anatômica ocular e para o estabelecimento de valores médios da pressão intraocular e das medidas intraoculares, no entanto são necessárias outras pesquisas na área da fisiologia e histologia para melhor compreensão da função visual da espécie.

TERMOS DE INDEXAÇÃO: Morfologia, macroscopia, ecometria, tonometria de rebote, bulbo ocular, bicho-preguiça, *Bradypus variegatus*, oftalmologia, anatomia ocular, ultrassonografia ocular, xenarthros.

INTRODUCTION

The sloth is an animal that belongs to class Mammalia, superorder Xenarthra, order Pilosa (Britton, 1941), and with two genera *Bradypus* and *Choloepus* (Wetzel & Avila-Pires 1980). In the family Bradypodidae, genus *Bradypus*, includes the three-toed sloth and the species: *Bradypus torquatus*, *Bradypus tridactylus*, and *Bradypus variegatus* (Gardner 2007).

The species *B. variegatus* (Shinz, 1825). is distributed in southern Honduras, northern Argentina, and in more significant numbers in Brazil. The species can also be found in forest areas at low and medium elevations in eastern Central America (Anderson & Handley 2001). In Brazil, it can also be found in the states of Amazonas, Pará, Maranhão, Pernambuco, Alagoas, Espírito Santo, Rio de Janeiro, São Paulo, and Paraná (Gardner 2007). The three-toed sloth can be distinguished by its brown neck (Hayssen 2010). It has a variable size, but most populations weigh from 2.5 to 3.5kg (Dünner & Pastor 2017). Other authors report that the weight can vary from 3.9 to 5.5kg (Aguilar & Superina 2014) and can reach 6.2kg (Hayssen 2010). According to Goffart (1971), species from the genera *Bradypus* and *Choloepus* are incredibly shortsighted and have different senses to obtain food, make contact with other species belonging to their own species, and ensure their protection. Furthermore, the absence of a ciliary muscle and a macula, in addition to the presence of sparse nature of ganglion cells and nerve fibers, indicate a low level of visual acuity (Gilmore et al. 2000).

The eye represents the organ of vision and consists of the eyeball structures and its accessory structures (eyelids, conjunctiva, lacrimal apparatus, and extraocular muscles (EOM)). Most of these structures are located in orbit, where the eyeball is surrounded by a large amount of adipose tissue (Dyce et al. 2010, Reece & Rowe 2017). The eyeball consists of three tunics: the outer layer of the fibrous tunic is composed of the sclera and the cornea, and has the function of shaping and protecting the eye; the middle layer or vascular tunic consists of the choroid, the ciliary body, and the iris; and the inner layer (nerve) or retina is responsible for communications with the brain via the optic nerve (Eurell & Frapppier 2012).

Ultrasonography has been used in Veterinary Medicine in many cases, mainly in the evaluation of abdominal organs. On the other hand, in veterinary ophthalmology, its use is more recent. The eyes are visually accessible to the ophthalmologist; however, in those with opacity in the anterior segment, ultrasound can outline changes that would not otherwise be seen (Dziezyc et al. 1987).

For the ultrasound examination of the eyeball, high frequencies are necessary to delineate the tissues properly. Thus, the 7.5 and 10MHz transducers are used for general examination and retrobulbar portion with or without a cushion indentation (Gonçalves et al. 2009). There are two characteristics of ultrasound that can be associated with its safety and patient's absence of discomfort. It is a technique that provides reliable information in real-time, relatively inexpensive, non-invasive, and that allows the overview of ocular and retrobulbar anatomy (Gelatt-Nicholson et al. 1999).

The knowledge of the ultrasound anatomy and biometry of the ocular structures serves as a basis for ultrasound examination, as this allows the evaluation of abnormalities that cause changes in the dimensions and appearance of the ocular structures, as in cases of retinal detachment, glaucoma, and optic nerve atrophy (ONA) (Ribeiro et al. 2009, Ruiz et al. 2015). In ophthalmology, ocular A-mode and B-mode ultrasounds can be used to assess the eyeball, seeing that both modes allow the assessment of structures (Schiffer et al. 1982). B-mode is the most commonly used; being able to produces a two-dimensional image of the eye that most reflects the ocular anatomy (Moore & Lamb 2007). However, ocular B-mode ultrasonography (USS) allows the measurement of five distances from the eyeball: D1 = from cornea to the anterior lens capsule, D2 = from anterior lens capsule to the posterior lens capsule, D3 = diameter of the lens, D4 = from posterior lens capsule to the posterior wall of the eyeball, and D5 = axial length (AL) of the eyeball, which corresponds to the distance between the cornea and the posterior wall of the eyeball (Hamidzada & Osuobeni 1999, Gonzalez et al. 2001).

Tonometry is the measurement of intraocular pressure (IOP), that is, an actual diagnostic test underused in veterinary practice. Initially, this technique was only used as a diagnostic method for glaucoma, in which the IOP is increased, subsequently became to be used as a diagnostic method for anterior uveitis, where IOP is reduced, and to aid in the diagnosis of other diseases that had a clinical presentation of ocular hyperemia, such as keratitis, conjunctivitis, and scleritis, where the IOP is not compromised (Maggs et al. 2013). Tonometry can be measured indirectly through digital palpation, which is not very accurate and is not recommended, or by using specific instruments. Three techniques are used in veterinary medicine: indentation tonometry, applanation tonometry, and rebound tonometry (RBT) (Featherstone & Heinrich 2013).

For indentation, the Schiötz tonometer is used. The IOP is estimated, considering how far the cornea recedes under the action of a specific force generated by a platform supported on the anesthetized cornea. The result is converted to millimeters of mercury (mmHg) using specific tables for each species.

Flattening is considered one of the most accurate methods, consisting of estimating IOP using the pressure necessary to produce a flattening in a specific cornea area. In Veterinary Medicine, the most used tonometer is the Tonopen®, which has a contact surface of 3mm in diameter with the ceramic center of approximately 1mm that comes into contact with the cornea generating a signal, which is amplified, and sent to a microprocessor. The obtained value is shown on a small screen.

In RBT, the IOP is estimated with the help of the release of a magnetic field action against the cornea, measuring its deceleration after coming into contact with the eyeball. The TonoVet® model has calibration for dogs, cats, horses, and other species. It is necessary to displays the IOP value after obtaining an average of six consecutive measurements. (Slatter & Dietrich 2003, Jeong et al. 2007, McDonald et al. 2017). Recently, a new model has been available to the market; the Tonovet plus contains some updates aimed at improving the accuracy and being easy to use. It has specific calibration for rabbits, cats, dogs, and horses. It also presents audible and on-display indicators informing an incorrect measurement and the corresponding error (Gloe et al. 2019). Comparative IOP studies showed that the value of exam and other factors may vary according to the equipment. Data obtained from measurements with Tonovet and Tonovet Plus did not show statistical differences. However, the second one has greater accuracy in obtaining means (Zhang et al. 2014, Giannakopoulou & Williams 2019, Gloe et al. 2019). The RBT is becoming more popular in Veterinary Medicine as it provides a fast method of estimating IOP values (McDonald et al. 2017). An advantage of RBT is associated with topical anesthesia that is not necessary since contact with the cornea is very brief. In addition, the corneal flattening area is minimal (Takenaka et al. 2011).

Regarding the anatomy, IOP, and ocular ultrasound are not standardized for *B. variegatus*, limiting an adequate ophthalmic evaluation in these animals. Therefore, the need for research related to some information to fill this gap is remarkable. This study had the general objective of describing the macroscopic anatomy and the typical ultrasound aspects of the structures of animal eye bulb, specifying the average data for eye biometrics, and standardizing values for eye pressure to collaborate with scientific studies or assist in the clinical routine of this or other species of the superorder Xenarthra.

MATERIALS AND METHODS

For the ocular anatomy study, eight eye bulbs of four carcasses of the *Bradypus variegatus* species were used, from the collection of the Anatomy Area of the "Departamento de Morfologia e Fisiologia Animal" (DMFA), "Universidade Federal Rural de Pernambuco" (UFRPE), two males and two females, three adults and a young, weighing from 3 to 6kg. The carcasses were previously fixed with 20% formaldehyde and preserved in 30% saline. Subsequently, the eye bulbs were gently removed from the orbit by the transpalpebral enucleation technique proposed by Slatter & Dietrich (2003). After removing them, the eye bulbs were conserved in 70% alcohol and subsequently dissected to perform the anatomical description. Biometric data were measured using pachymetry, and measurements were obtained: AL of the eyeball, lens thickness, length of the vitreous chamber, and horizontal corneal diameter. The photographic images were obtained using a Canon Powershot Sx400is digital camera. For designation purposes, the "International Committee on Veterinary Gross Anatomical Nomenclature" determinations, Nomina Anatomica Veterinaria (2017), were used.

Four animals were assigned for *in vivo* ophthalmic analysis, three females and one male, three adults and one young weighing between 2.5 and 5kg, from the Parque Estadual Dois Irmãos, in the city of Recife. They underwent ophthalmic evaluation using slit-lamp biomicroscopy, and only those considered healthy were included in the study. All animals underwent intraocular pressure assessment of the right and left eyes using RBT (TonoVet®; Icare Finland Oy, Helsinki, Finland) calibrated for the device in P-mode, intended for different species of dogs, cats, and horses. An ultrasound examination was then performed in both eyes using the SonoSite® device, model MTurbo® (Fujifilm SonoSite, Washington, USA), with a linear transducer (6-13MHz) whose frequency was automatically determined by the device. Since these are small structures, the frequency used was close to the maximum allowed by the transducer. The images were obtained by transcorneal and transpalpebral techniques, and the scanning of the eyeball was performed in the vertical and horizontal axial plane. Each animal was placed in the arms of a technical assistant, and its head contained manually. Anesthetic eye drops of 0.5% proxymetacaine hydrochloride (Anestalcon®, Alcon, São Paulo, Brazil) and a layer of aqueous gel (Sterile Aquasonic 100®, Bio-medical instruments inc, Clinton twp, USA) were applied to the cornea for performing the exam in a comfortable way for the animal, without causing injuries. The same operator examined the eyes of all animals.

Biometrics was performed using an image obtained in horizontal section with the transducer positioned in the center of the cornea or eyelid. Positioning was considered satisfactory when it was possible to visualize the eyeball posterior wall as well as other critical intraocular structures such as the cornea, anterior and posterior lens capsules, lens, and vitreous chamber.

Five measurements were obtained from each eye: D1 = length of the anterior chamber, distance between the central point of the corneal image and the anterior lens capsule; D2 = lens thickness, distance between the anterior and posterior lens capsules; D3 = diameter of the lens; D4 = length of the vitreous chamber, distance between the posterior lens capsule and the posterior wall of the eyeball; D5 = AL of the eyeball, distance between the image of the cornea and the posterior wall of the eyeball. The measurements were obtained with the aid of an electronic cursor on the device itself.

The data of biometrics and ocular tonometry were grouped according to the eye and then analyzed. The Tukey test was applied with a significance level of 5%. The research was authorized by the Animal Use and Experimentation Ethics Committee No. 081/2016 and SISBIO No. 46665-3.

RESULTS

Eye anatomy

The sloth's eyeball has a cylindrical shape with an average axial length of 7.5±1.29mm and is fully inserted into the orbit. It is located anteriorly on the animal's head, with a level of rostral extend It is delimited anteriorly by the upper and lower eyelids and posteriorly by the retrobulbar adipose tissue and the orbital cavity; it presents a perforation in the posterior pole for the entry of the optic nerve and blood vessels.

The eyelids of the sloth are thick and have enough tensile strength of occlusion in the live animal. They are covered outside by a short hair layer and inside by the bulbar conjunctiva. Note the presence of the third eyelid, a fold of tissue located between the lower eyelid and the cornea, emerging in the nasal region of the lower conjunctival sac, being covered

by the bulbar conjunctiva. The bulbar conjunctiva near the limbus is thin and has no pigmentation.

The cornea is smooth, transparent, convex, on its anterior face, and circular, making up the anterior region of fibrous tunic. It is covered above by the eyelids, and its posterior external limit is the limbus, a place of transition between the cornea and sclera. It has an average horizontal diameter of 4±0.80mm. The sclera forms the outer layer of the eyeball; and it is variably white with shades of gray. It is located posterior to the limbus, immediately after the cornea. The sclera provides a site for the insertion of the musculature responsible for the ocular movement. Four muscles could be differentiated in this study: the rectus dorsal and rectus ventralis which were inserted in the upper and lower pole of the eyeball, respectively, and the medial rectus and lateral rectus muscle inserted in the nasal and temporal plane of the eyeball, respectively. These four muscles are inserted in the sclera posterior to the limbus through a small tendon. The identification of the dorsal and ventral oblique muscles was compromised due to limited technique and samples. They were located in some bulbs, but they could not be well differentiated at all. These are also inserted in the sclera in the region posterior to the limbus (Fig.1).

Inside the eyeball, posterior to the cornea, a small space is filled with aqueous humor, the anterior chamber that is delimited later by the iris. The iris is located between the cornea and the lens and in which is located the site of separation between the anterior and posterior chambers. It has brown pigmentation in all individuals analyzed. The sloth has a circular pupil; *in vivo*, the pupil remains circular when dilated or contracted. Immediately after the iris, the lens is surrounded by the anterior and posterior capsules and has a biconvex and circular shape with an average 4.5±0.57mm thickness.

After the posterior lens capsule, the vitreous chamber can be differentiated, filled by the vitreous body, a dense and slightly watery structure. This chamber has an average of 5.0±0.81mm in length. The retina-choroid-sclera complex (RCSC) forms the posterior wall of the eyeball. This region is

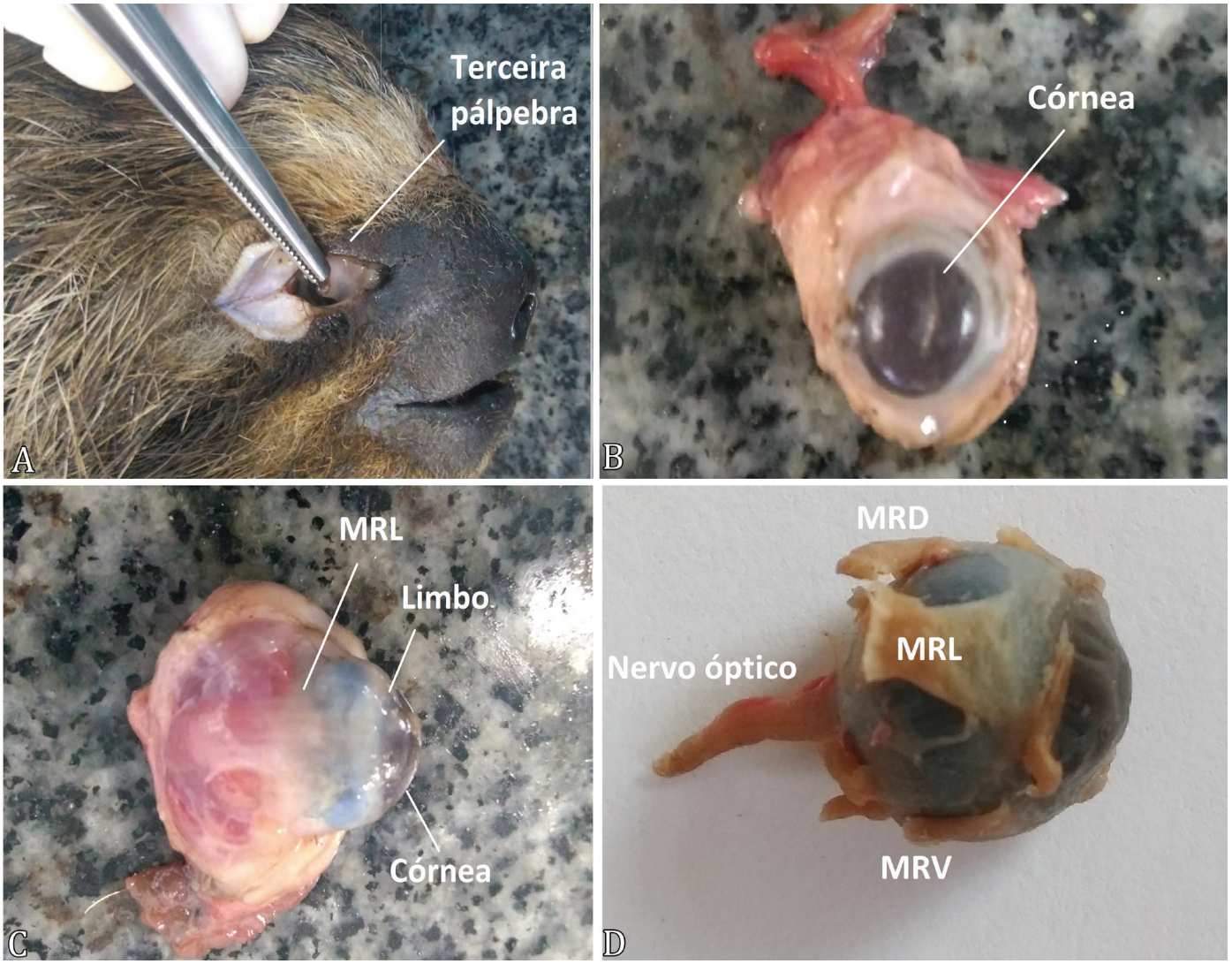

Fig.1. Sloth eye. (**A**) Traction of the third eyelid, (**B**) anterior view of the eyeball, (**C**) lateral view of the eyeball, immediately after enucleation, (**D**) side view of the eyeball. After dissection of the musculature. Lateral rectus muscle (LRM), dorsal rectus muscle (DRM), ventral rectus muscle (VRM).

completely pigmented with no apparent *tapetum lucidum*. In the macroscopic evaluation of the retina, it was not possible to identify the blood vessels in all ocular bulbs especially in some vascular fragments seen in the central region. The separation of these three segments could not be carried out, given the close communication between them and the visualization of the optical disc. At the posterior pole of the eyeball, the optic nerve enters into the orbit through the optic foramen and is accompanied by some blood vessels that provide nutrition for the eyeball.

Eye tonometry

There was no significant difference ($p>0.05$) in intraocular pressure between the right and left eyes. The mean IOP was 4.25±0.70mmHg (Table 1).

Ultrasonographic aspects and ocular biometrics

The ultrasound examination in B-mode was performed, as shown in Figure 2. According to this technique, the sloth's eyeball is characterized by a cylindrical structure, well delimited, presenting anechoic content (Fig.3) and composed of the anterior and posterior chambers and the vitreous chamber. The three chambers are presented as an anechoic structure with few reflective elements.

The structure closest to the transducer is the cornea, represented by a convex, hyperechoic, semicircular line. A space delimited previously by the cornea and posteriorly by the anterior capsule of the lens, the anterior chamber, filled with anechoic content, could be observed. The lens was visualized as an anechoic structure located between two concave and convex hyperechoic lines that corresponded to the anterior lens and posterior lens capsules, respectively. It was not possible to observe the iris and pupil by ultrasound examination. After the posterior lens capsule that extends to the posterior wall of eyeball, the vitreous chamber was located, characterized by an utterly anechoic area. The posterior wall of the eyeball was represented by a thin hyperechoic convex line, forming the scleral chorioretinal tissue layer. The optic nerve is represented by a thin hypoechoic area outlined by the retrobulbar space with moderate echogenicity. The average values of measurements of intraocular structures are shown in Table 2. Statistical analyzes using the Tukey's test at 5% significance showed no difference between the right and left eyes. The mean depth of the anterior chamber (length of the anterior chamber) was 0.63±1.11mm. The average lens thickness (lens length) was 3.73±0.24mm. The average lens diameter was 6.15±0.41mm, thus occupying 44% of the eye length. The average depth of the vitreous chamber (length of the vitreous chamber) was 3.70±0.27mm. The mean axial length of the eyeball was 8.48±0.22mm.

Table 1. Averages of intraocular pressure (IOP) in the right and left eyes in *Bradypus variegatus*

IOP	Animal A	Animal B	Animal C	Animal D	Mean	SD
RE (mmHg)	5	3	4	4	4	1.9
LE (mmHg)	5	4	4	5	4.5	0.5

IOP = Intraocular pressure, RE = right eye, LE = left eye, SD = standard deviation.

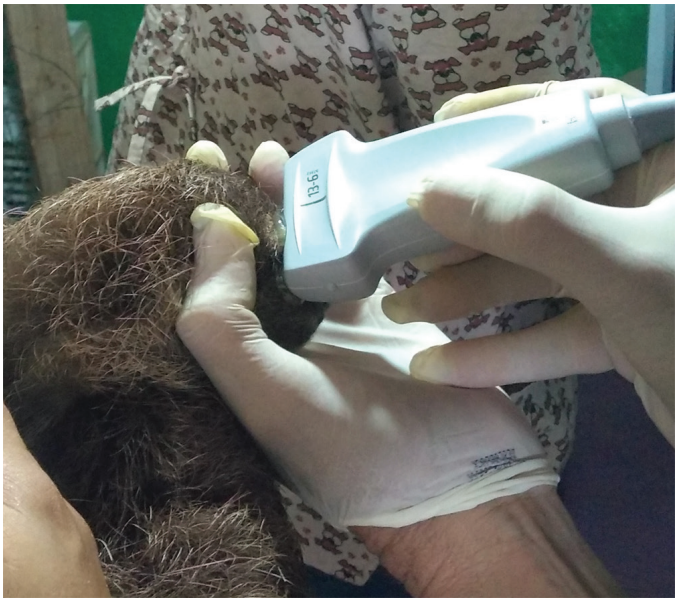

Fig.2. Performing an ocular B-mode ultrasonography in *Bradypus variegatus*.

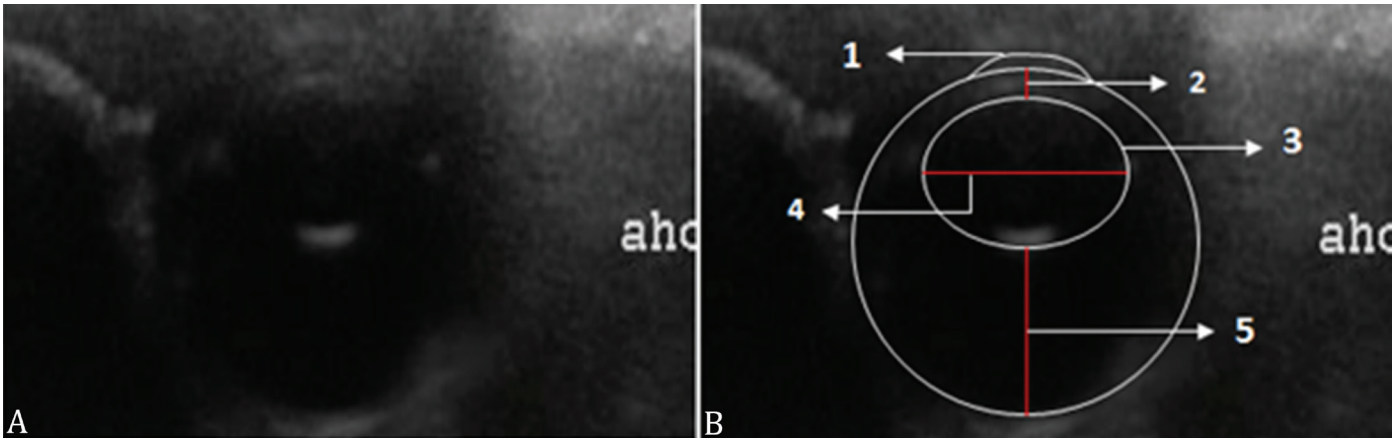

Fig.3. Image of ocular B-mode ultrasound for (**A**) normal sloth eye, and (**B**) schematic drawing of Figure 3A. Cornea (1), anterior segment (2), lens (3), lens diameter (4), glass chamber (5).

Table 2. Descriptive analysis of the results obtained by means of ocular ultrasonography in *Bradypus variegatus*

Eye data	Mean	SD	Maximum	Minimum	CV	CI (95%)
D1 (mm)	0.63	0.11	0.80	0.50	18.60	0.53 - 0.72
D2 (mm)	3.72	0.24	4.10	3.40	6.53	3.51 - 3.92
D3 (mm)	6.15	0.41	6.90	5.80	6.78	5.80 - 6.49
D4 (mm)	3.70	0.27	3.90	3.10	7.50	3.47 - 3.92
D5 (mm)	8.48	0.22	8.70	8.00	2.62	8.29 - 8.66

SD = Standard deviation, CV = coefficient of variation, CI = confidence interval, D1 = anterior chamber, D2 = lens thickness, D3 = lens diameter, D4 = glassy chamber length, D5 = axial length of eyeball.

DISCUSSION

To date, no descriptive studies on the anatomy of the ocular bulb of the sloth (*Bradypus variegatus*) have been found, so all the discussion on this subject were related from existing references to other mammals. The ocular morphology of *B. variegatus* is similar to that described for other domestic and wild animals, but with some peculiarities (Reece & Rowe 2017).

The eyeball is fully inserted into the orbit. The orbit is located on the anterior face of the skull - a similar finding was reported by Piggins & Muntz (1985) in the genus *Bradypus*. The front-facing eyes provide a wide binocular field of view that allows the animal to focus on nearby objects (Dyce et al. 2010). According to Samuelson (2013), the orbit depth contributes to the protection of the eyes and has a typical eye appearance. Its location in the skull largely influences the visual field extension and depth for certain species or breed. The position of the orbit varies between species. In cattle and horses, the eyes are located laterally, which provides a panoramic view. In dogs and cats, the eyes are located more anteriorly, emphasizing the binocular overlap between the two eyes. (Maggs et al. 2013). Piggins & Muntz (1985) reported that the angle between the diverging optical axis of each eye in *Bradypus* is estimated at 50 degrees and the monocular and binocular visual fields at 70 degrees with a binocular overlapping 35 degrees.

Four muscles inserted into the sclera could be identified. These muscles are responsible for eyeball movement in the vertical and horizontal directions (Dyce et al. 2010). As the dorsal and ventral obliques had limited identification, other muscles were associated with the quality of the samples used and the thickness of these tissues.

The third eyelid was found in the anatomical dissection. It is noted that its use could be identified in a few moments in the live animal in association with a stimulation of the cornea during the RBT performance. According to Johnson (1901), the third eyelid is absent in the family Bradypodidae, and this absence would be related to an evolutionary characteristic of the species. We consider that the non-visualization of the Johnson (1901) structure was associated with the type of procedure performed. It is used direct ophthalmoscopy in live animals for eye evaluation, however, an anatomical study on carcass was not performed and corneal stimulation was not used. The anatomical positioning of the third eyelid in the sloth can be compared to the positioning described for some domestic species such as dogs and cats, where it is also located in the medial angle of the eye, between the lower eyelid and the eyeball (Dyce et al. 2010). This structure is absent or is reported as vestigial in some species, such as the capybara (*Hydrochaeris hydrochaeris*) and chinchillas (*Chinchilla lanigera*) (Montiani-Ferreira et al. 2008, Lima et al. 2010, Montiani-Ferreira & Lima 2014).

The circular pupil in mydriasis or miosis was described by Johnson (1901) in armadillos (*Dasypus villosus*). The shape of the pupil varies between species. In primates, canines, and large cats, it has a circular shape, while in small cats, as in the domestic cat, it appears as a vertical-slit pupil when contracted. Animals with slit-shaped pupils are more sensitive to light than those with circular pupils (Samuelson 2013). According to Goffart (1971), sloths are animals with eyes that are mainly adapted for low-intensity light vision.

An anatomical description of the eyeball was performed by Piggins & Muntz (1985) in the species *Bradypus tridactylus*. According to the authors, this eye type has an average axial length of 8.4mm and a spherical shape. Moreover, it described a wide and quite convex cornea. Findings for the species *B. variegatus* are similar to the *B. tridactylus*, it has an average axial length of 7.5mm, an exposed ocular surface between the eyelids composed of the cornea that is broad and convex, but with an ocular bulb presenting a cylindrical shape. The sloth has a shallow anterior chamber when compared to the AL of the eyeball. It has a lens that occupies about 40% of the ocular volume; a similar proportion has been described in chinchillas (Lima et al. 2010). These characteristics may be related to the visual acuity of the species. A lens that occupies a large space in the eye causes the anterior chamber to approach the cornea, and its posterior segment is closer to the retina. These anatomical characteristics can be related to a balance connected with the low visual acuity of the species. According to Goffart (1971) and Hayssen (2010), animals of the genus *Bradypus* are incredibly myopic, do not have ciliary muscle or macula, their retina comprises many thin stem cells with few retinal ganglion cells (RGCs), and cones are rare or absent.

The vitreous chamber is nearly filled by the vitreous body, with its posterior wall composed of the RCSC. In the retina and choroid coat, the blood vessels are poorly differentiated macroscopically. Johnson (1901) considers this characteristic common to species of Xenartros. Goffart (1971) used the classification of pseudoangiotics.

In previous years, several investigations were carried out in order to determine the normal IOP of several species (Leiva et al. 2006, Broadwater et al. 2007, Blackwood et al. 2010, Rusanen et al. 2010, Pereira et al. 2011, Falcão et al. 2017, McDonald et al. 2017, Hibbs et al. 2018), however, there is no report describing normal values for sloths.

The RBT has been increasingly used in veterinary ophthalmology. TonoVet® was designed its use in animals with small eyes (Falcão et al. 2017). Its software is pre-programmed for six measurements, and the IOP is displayed as an average value of six consecutive central corneal rings. It is calibrated for dogs and cats as described for D-mode, horses for H-mode, and other species for P-mode (Jeong et al. 2007). As the RBT does not present an internal calibration table to measure IOP in sloths, the calibration table, that is not specific to any species, was used in these animals, that is, the P-mode.

Some factors can alter the final IOP value, such as stress, anesthesia, and external eye pressure (Pereira et al. 2011). Manual restraint was performed subtly to reduce the stress of the animals, so that, there was no interference in IOP measurement. According to Corrêa et al. (2014), the use

of mydriatic drugs can affect intraocular pressure leading to hypertension; therefore, no agent or any type of ocular medication was performed until IOP measurement. The use of TonoVet® to measure IOP did not cause discomfort to animals, considered a fast and well-tolerated technique, as has also been described by other authors (Leiva et al. 2006, Jeong et al. 2007, Rusanen et al. 2010, McDonald et al. 2017).

The measurement of IOP by using RBT has been carried out in recent years, and standards for different species have been established with the use of TonoVet®. Studies have been carried out on various domestic and wild species such as parrots (11.4mmHg), alpacas (14.2mmHg), donkey (25.5mmHg), cats (20.74mmHg), dogs (9.15mmHg), goats (7, 9mmHg), rabbits (9.51mmHg), bats (19.3mmHg) (Leiva et al. 2006, Broadwater et al. 2007, Blackwood et al. 2010, Rusanen et al. 2010, Pereira et al. 2011, Falcão et al. 2017, McDonald et al. 2017, Hibbs et al. 2018).

The values of IOP were similar to those observed in *B. variegatus* (4.25mmHg) and have also been described in chinchillas (*C. lanigera*) (2.49mmHg) and bullfrog (*Rana catesbeiana*) (4mmHg) (ChacaltanaI el al. 2016, Cannizzo et al. 2017). Cannizzo et al. (2017) related the low IOP in bullfrogs using TonoVet® in P-mode, according to this study, the P-mode tends to underestimate IOP measurements when compared to D-mode. Previous studies have shown that RBT can underestimate the IOPs less than 25mmHg in cats (Rusanen et al. 2010) and more significant than 70mgHg in horses (Knollinger et al. 2005). Löbler et al. (2011) carried out a study that aimed to calibrate the RBT using the Icare TAO1 tonometer, calibrated for humans, pigs and rabbits. They concluded that RBT tends to underestimate the true values of IOP in these animals. It can be used to determine IOP as long as the measured values are corrected with the appropriate linear function.

We consider that the mean obtained for *B. variegatus* by using TonoVet® may be linked to a physiological value of the species. However, further studies comparing the results obtained with the aid of TonoVet® with measures obtained by directly measuring IOP (manometry) are necessary to establish RBT accuracy in sloths. The discrepancies in the IOP values described significant interspecific variation. These occur due to the specific anatomical and physiological peculiarities of each species, such as the shape of the eyeball, the circadian rhythm, formation and drainage of aqueous humor flow, cornea size or the examiner's experience (Pereira et al. 2011, Rodarte-Almeida et al. 2013, Falcão et al. 2017), which makes the comparison between unfeasible or perhaps different species and emphasizes the need to establish reference standards as well as the calibration of TonoVet® for each species.

As it is a non-invasive and painless technique, the ultrasonography was a well-tolerated test in all animals. Manual restraint associated with only one drop of anesthetic eye drops of 0.5% proxymetacaine hydrochloride was sufficient to allow an adequate assessment, excluding the need for anesthetic drugs as described by Dziezyc et al. (1987) and Mattoon & Nyland (2015). According to Gonzalez et al. (2001), it is preferable to avoid the patient's sedation, so that the rotation and retraction of the eye are minimized. There were no complications, such as ocular trauma seen after the ultrasound. Such factors make ocular ultrasound a feasible diagnostic method for the routine of ophthalmic evaluation in *B. variegatus*.

According to Dziezyc et al. (1987), ocular imaging via ultrasound is easy to obtain, in which the eye is an excellent organ for the exam. In *B. variegatus*, there was some difficulty to obtain the images due to the serene membrane of the small corneal surface concerning the size effect of transducers and due to the relatively thick eyelids, thus making manual retraction difficult for adequate corneal exposure. This anatomical feature influenced the technique used to obtain the images. In some animals, it was not possible to perform the transcorneal and transpalpebral techniques, even being the most commonly used. According to Mattoon & Nyland (2015), the transpalpebral technique is easier to be performed. However, the image quality is inferior compared to the positioning of the transducer directly in the cornea. It is described in the literature that the transcorneal technique is the method of choice for evaluating intraocular structures, as it allows obtaining the most distinctive images of the eyeball and orbit and better visualization of the vitreoretinal and retrobulbar structures (Gonzalez et al. 2001). Whereas the transpalpebral technique allows an adequate assessment of the vitreous chamber, retina, and deeper orbital structures; on the other hand, the lens and the anterior chamber cannot be satisfactorily evaluated, even if a cushion indentation is used (Mattoon & Nyland 2015). In addition, it is reported that the trichotomy of the eyelid improves the quality of the ultrasound image, as it reduces the amount of air between the skin and the transducer (Mattoon & Nyland 2015). However, because these animals were in the process of rehabilitation, trichotomy was not performed. Despite these facts, in the present study, obtaining ocular images for ocular biometrics was not impaired with this technique. However, a more detailed assessment of the anterior segment was impaired with the transpalpebral technique.

Ultrasound gel was used for comfort and better assessment of intraocular structures, which allowed the transducer to be distanced a few millimeters from the ocular surface, as described by Soares et al. (1998), and served as a cushion indentation for better visualization of the cornea when a thicker layer was used.

The ocular A-mode ultrasonography is described as the method of choice for performing eye biometrics. However, the ocular B-mode ultrasonography allows a more accurate assessment of eye structures, and biometrics can be performed safely, thus being a preferred method. It is currently the most used in veterinary ophthalmology, and its use is recommended by many authors (Gonçalves et al. 2009, Merlini et al. 2011, Mattoon & Nyland 2015, Ruiz et al. 2015, Morais et al. 2019).

The use of a 6-13MHz transducer in the ocular B-mode ultrasound examination was considered adequate for a general assessment of the eyeball since it was possible to visualize and measure the most critical internal structures. A 10 or 12.5MHz transducer is ideal for a more detailed assessment of the bulb. However, orbit and bulb can be visualized with a 7.5MHz transducer, but it is impossible to differentiate the retina from the choroid or sclera with transducers less than 10MHz (Petersen-Jones & Crispin 2002).

In ocular ultrasound of animals, in general, the cornea is represented by a curvilinear hyperechogenic line in the proximal field of the screen (Dudea 2011). The anterior

chamber is then filled with anechoic material, outlined later by the iris (Mattoon & Nyland 2015). The lens is represented as an anechoic structure located between two curved echogenic lines, the anterior and posterior lens capsules (Merlini et al. 2011). The vitreous chamber is represented by the anechoic region posterior to the lens (Gonzalez et al. 2001), and the posterior wall of the eyeball is seen as a single echogenic line being called the RCSC, as the three layers cannot be identified separately (Gonzalez et al. 2001). All the above statements were compatible with those found in the ocular ultrasounds of *B. variegatus*, making the ocular ultrasound anatomy of the species similar to those described for other wild and domestic species (Gonzalez et al. 2001, Montiani-Ferreira et al. 2008, Mattoon & Nyland 2015).

The cornea and the anterior segment could not be adequately evaluated with a 6-13MHz transducer. For better image resolution and more detailed discrimination of small structures, 20MHz transducers should be used (Dietrich 2013).

Ocular biometrics can be used to determine ocular change, shape, or size as it can occur in conditions such as glaucoma. The biometric measurements obtained in this study were the same as those defined in previous studies (D1 = anterior chamber, D2 = lens thickness, D3 = lens diameter, D4 = vitreous chamber length, D5 = AL of the eyeball). However, the mean values differed, concluding that measurements vary according to each species due to their eye anatomy. Therefore, the values of such measurements are not compared between species. No study on ocular biometrics in the species *B. variegatus* was found in the literature.

In a statistical analysis at 5% of significance, no significant difference was found between the biometrics of the left and right eyes, which is in agreement with the findings of other authors for other species that used ocular B-mode ultrasound to perform eye biometry (Hernández-Guerra et al. 2007, Squarzoni et al. 2010, Toni et al. 2010). All measures of sloth eye presented a remarkable homogeneity, especially compared to ocular biometric values.

Some values obtained with ultrasound biometrics could be compared to the values obtained with the study of macroscopic anatomy. The compared parameters were the AL of the eyeball, the lens thickness, and the length of the vitreous chamber. There was a difference of ±1mm between the measurements, but all were compatible with the ultrasound examination measurements, with no statistical difference between means. However, *post mortem* changes can affect measurements due to variation in muscle tone and fluid volume that can lead to changes in the eyeball dimensions.

CONCLUSIONS

The ocular anatomy of the species *Bradypus variegatus* is similar to those features described for other species with some peculiarities. They present a retina with poorly differentiated blood vessels.

By using the RBT, it was possible to measure the IOP in this species, being easy to perform and well accepted by the animals.

The ocular B-mode ultrasound is a fast, safe, reliable, and well-tolerated procedure for sloths. Its ocular ultrasound anatomy was similar to that observed in other species. Biometric measurements such as depth of the anterior chamber, the lens thickness, the diameter of the lens, depth of the vitreous chamber, and AL of the eyeball could be performed by this method.

The techniques employed in this study can be routinely used in medical practice. The data obtained can assist in diagnosing eye diseases and favor the management and preservation of the species. Further research in histology and physiology is necessary for a better understanding of the species' visual function.

Acknowledgments.- To the anatomy area of the Departamento de Morfologia e Fisiologia Animal of the Universidade Federal Rural de Pernambuco for making specimens available for research. The team at Parque Estadual Dois Irmãos for their availability in the management of animals in captivity. Professor Marleyne Amorim and Professor Fabrício Bezerra for their guidance.

Conflict of interest statement.- The authors have no conflict of interests.

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