UNIVERSIDADE FEDERAL DA PARAIBA
CENTRO DE CIÊNCIAS AGRÁRIAS

DETERMINAÇÃO DE VALORES DE REFERÊNCIA PARA OS TESTES OFTÁLMICOS DE ROTINA EM MACACOS-PREGO (*Sapajus libidinosus*).

Karla Priscila Garrido Bezerra
Médica Veterinária

2017

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Orientadora: Profa. Dra. Danila Barreiro Campos

Co-orientadora: Profa. Dra. Ivia Carmem Talieri

Dissertação apresentada ao Programa de Pós-Graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba, como parte das exigências para a obtenção do título de Mestre em Ciência Animal.

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KARLA PRISCILA GARRIDO BEZERRA

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KARLA PRISCILA GARRIDO BEZERRA – Nascida em 27 de fevereiro de 1991, na cidade de Mossoró, Estado do Rio Grande do Norte. Ingressou no curso de Medicina Veterinária na Universidade Federal da Paraíba (UFPB), Campus de Areia, em setembro de 2009. Desde a graduação envolveu-se no Projeto de Extensão em Oftalmologia. Obteve o título de Médica Veterinária em setembro de 2014. Em março de 2015, ingressou no curso de mestrado do Programa de Pós Graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba, onde pôde atuar como colaboradora do Projeto de Extensão intitulado “Atendimento clínico e cirúrgico de Oftalmologia Veterinária”.

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CAPÍTULO I - Determinação de valores de referência para os testes oftálmicos de rotina em macacos-prego (*Sapajus libidinosus*)

Página

DETERMINAÇÃO DE VALORES DE REFERÊNCIA PARA OS TESTES OFTÁLMICOS DE ROTINA EM MACACOS-PREGO (*Sapajus libidinosus*)

RESUMO GERAL

Apesar de sua ampla distribuição geográfica e do grande número de exemplares mantidos em cativeiro, estudos básicos sobre a fisiologia ocular de macacos-prego da espécie *Sapajus libidinosus* ainda são incipientes. Diante disto, objetivou-se estabelecer valores de referência para medidas oculares e testes de diagnósticos oftálmicos selecionados e realizar uma análise descritiva da microbiota e da celularidade da conjuntiva ocular da referida espécie. Foram selecionados e utilizados 15 macacos-prego da espécie *Sapajus libidinosus* residentes do CETAS de Cabedelo, Paraíba. Todos os animais foram previamente sedados com cetamina e detomidina por via intramuscular para a coleta de amostras e realização dos testes de diagnósticos oftálmicos, que incluíram: o teste lacrimal de Schirmer 1 (TLS1), tonometria de aplanação, ultrassonografia em modo B, cultura da microbiota bacteriana aeróbica conjuntival e citologia esfoliativa conjuntival. Os valores médios encontrados para os testes oftálmicos e medidas oculares foram: 2,50 ± 2,94 mm/min para o TLS1; 13,3 ± 3,32 mmHg para a PIO; 2,47 ± 0,41 mm para a profundidade da câmara anterior; 2,86 ± 0,96 mm para o comprimento axial da lente; 10,97 ± 0,48 mm para a profundidade da câmara vítrea e 16,32 ± 1,24 mm para o comprimento axial do globo. A pressão intraocular diminuiu linearmente com o aumento do peso (P = 0,0004) e o valor médio da profundidade da câmara anterior foi maior nos machos (P = 0,0004). O gênero bacteriano mais isolado foi o *Staphylococcus* spp., (11 olhos), seguido por *Enterecoccus* (8 olhos), *Escherichia coli* (7 olhos), *Bacillus* sp. (4 olhos) e *Streptococcus* sp. (3 olhos). Na citologia conjuntival, observou-se células epiteliais intermediárias, células epiteliais superficiais escamosas, células ceratinizadas, hemácias, linfócitos, neutrófilos e bactérias. A descrição de dados e a determinação de valores de referência para as medidas oculares e testes oftálmicos obtidos nesta

investigação irão auxiliar o diagnóstico de afecções oculares em macacos-prego (*Sapajus libidinosus*).

Palavras-chave: biometria ocular, citologia conjuntival, microbiota ocular, pressão intraocular, primatas não-humanos, teste lacrimal de Schirmer.

DETERMINATION OF BASELINE VALUES FOR ROUTINE OPHTHALMIC TESTS IN CAPUCHIN MONKEYS (*Sapajus libidinosus*)

ABSTRACT

Notwithstanding its broad geographic distribution and the large number of specimens held in captivity, basic studies on the ocular physiology of capuchin monkeys of the *Sapajus libidinosus* species are still incipient. In face of this, our aim was to establish baseline values for eye measurements and selected tests for ophthalmic diagnosis, and to perform a descriptive analysis of the microbiota and cellularity of the conjunctive of that species. Fifteen (15) capuchin monkeys of the *Sapajus libidinosus* species, hosted at the CETAS of Cabedelo, Paraiba, were selected and used. All animals were previously sedated with ketamine and detomidine via intramuscular injection for collecting samples and performing the ophthalmic diagnosis tests, including: Schirmer tear test 1 (STT1), applanation tonometry, B-mode ultrasound, culture of the aerobic bacterial conjunctival microbiota, and exfoliative conjunctival cytology. The average values found for ophthalmic tests and eye measurements were: 2.50 ± 2.94 mm/min. for the STT1; 13.3 ± 3.32 mmHg for the IOP; 2.47 ± 0.41 mm for the depth of the anterior chamber; 2.86 ± 0.96 mm for the axial length of the lens; 10.97 ± 0.48 mm for the depth of the vitreous chamber, and 16.32 ± 1.24 mm for the axial length of the eyeball. The intraocular pressure decreased linearly as the weight increased (P = 0.0004) and the average depth of the anterior chamber was larger in the male subjects (P = 0.0004). The bacterial genus most frequently found was *Staphylococcus* spp., (11 eyes), followed by *Enterecoccus* (8 eyes), *Escherichia coli* (7 eyes), *Bacillus* sp. (4 eyes), and *Streptococcus* sp. (3 eyes). The conjunctival cytology showed intermediate epithelial cells, squamous superficial epithelial cells, keratinized cells, red blood cells, lymphocytes, neutrophils, and bacteria. The data description and the determination of baseline values for eye measurements and ophthalmic tests obtained in this research will assist the diagnosis of eye diseases in capuchin monkeys (*Sapajus libidinosus*).

Keywords: ocular biometry, conjunctival cytology, ocular microbiota, intraocular pressure, non-human primates, Schirmer tear test-1.

CONSIDERAÇÕES GERAIS

Os olhos dos primatas não humanos possuem grande semelhança fisiológica e anatômica com o olho humano. Em virtude disto, os macacos do Velho Mundo desempenham uma atividade importante para o meio científico, pois há muitos anos vêm sendo utilizados como modelos experimentais em estudos comparativos para pesquisas voltadas à oftalmologia (GIRARD *et al*., 2011; JAAX *et al*., 1984). No entanto, o uso destes animais têm diminuído devido a algumas questões, tais como as dificuldades no manejo, o alto custo em mantê-los e a baixa fecundidade. Desta forma, os primatas do Novo Mundo vêm se tornando uma opção interessante para estudos de oftalmologia comparativa (MONTIANNI-FERREIRA *et al*., 2008).

A espécie de macacos-prego *Sapajus libidinosus* está introduzida na classificação de primatas do Novo Mundo, sendo pertencentes à família *Cebidae* e gênero *Sapajus*. Esta espécie foi recentemente incluída numa nova classificação taxonômica de macacos-prego, a qual foi sugerida frente às evidências moleculares e biogeográficas associadas às variações morfológicas, ecológicas e comportamentais de cada espécie (LYNCH-ALFARO *et al*., 2012a). Lynch-Alfaro *et al*. (2012a) dividiram os macacos-prego em dois gêneros distintos: o *Cebus* (sem topete), com quatro espécies; e o *Sapajus (com topete)*, com oito espécies, dentre elas, a *S. libidinosus*, estudada nesta investigação.

Os macacos-prego são generalistas e ingerem vários tipos de alimentos, tais como frutos, sementes, flores, ovos, invertebrados e pequenos vertebrados (FRAGAZY *et al.,* 2004a). Estes animais podem viver até 50 anos de idade quando mantidos em cativeiro e chegam a pesar de 2 a 5 kg (FRAGAZY *et al*., 2004a). O gênero *Sapajus* apresenta ampla distribuição na América do Sul, estando disperso na mata atlântica, cerrado e caatinga (LYNCH ALFARO *et al*., 2012 b; RYLANDS *et al*., 2008).

A espécie *Sapajus libidinosus* é uma vítima comum do comércio ilegal de primatas no Brasil em virtude de sua aptidão intelectual e destreza. Em consequência disto e das apreensões e entregas voluntárias ao CETAS (Centro de Triagem de Animais Silvestres) e aos zoológicos, há um aumento no número de exemplares dessa espécie nos poucos cativeiros disponíveis

(LEVACOV *et al*., 2011). Desta forma, é essencial que os veterinários desses locais tenham dados básicos referenciais acerca de vários parâmetros clínicos normais desta espécie, com intuito de realizar um diagnóstico e tratamento adequado diante das afecções de quaisquer sistemas.

Por outro lado, o grande número desses animais mantidos em cativeiros torna-se uma oportunidade para a realização de estudos para a obtenção de dados e fornecimento de informações acerca da fisiologia de vários sistemas, bem como a identificação de modelos experimentais para estudos comparativos.

A oftalmologia vem se tornando uma especialidade essencial no que se refere à manutenção da saúde dos olhos de animais selvagens e espécies exóticas (ORIÁ *et al*., 2015a). Contudo, estudos básicos sobre a fisiologia ocular voltados a algumas espécies de animais selvagens, principalmente no que se refere aos sul-americanos, são incipientes (MONTIANI-FERREIRA *et al*., 2006), sendo, dentre estes, um dos mais pobres em valores de referência, os primatas (SASAKI *et al*., 2009), como é o caso do macaco-prego (*Sapajus libidinosus*).

Essa carência de dados básicos dificulta o diagnóstico e o tratamento adequado das afecções oculares na referida espécie, apesar das contribuições de Montiani-Ferreira *et al.* (2008) e Oriá *et al.* (2013) acerca das espécies de macacos-prego *Cebus apella* e *Cebus xanthosternos*, respectivamente. Os valores basais fisiológicos de testes oftálmicos aplicáveis a estas duas espécies de macacos-prego não subsidiam no diagnóstico das afecções oculares da espécie *Sapajus libidinosus*, visto que, os valores do teste da lágrima de Shirmer (TLS) e pressão intraocular (PIO) variam, consideravelmente, entre as espécies, bem como entre indivíduos da mesma família, não podendo ser extrapolados mesmo entre espécies intimamente correlacionadas (OFRI *et al*., 1998, 2002).

Desta forma, para avaliação da saúde ocular, faz-se necessária a determinação de valores de referência para os testes oftálmicos de rotina, bem como a descrição da flora e celularidade normal da conjuntiva para cada espécie.

A lágrima exerce uma função essencial na manutenção da saúde e função normal da córnea e conjuntiva (OFRI *et al*., 2002). Insuficiência na produção

lacrimal poderá resultar em desordens corneais e conjuntivais (BROOKS *et al*., 2000; OFRI *et al*., 2002), tais como a ceratoconjuntivite seca (CCS) (OFRI *et al*., 2002). Quando há suspeita de deficiência na produção da lágrima, torna-se crucial para o diagnóstico a avaliação da função das glândulas lacrimais, a qual se dá por meio da medição da fração aquosa do filme lacrimal, utilizando o teste lacrimal de Schirmer (TLS) (MONTIANI-FERREIRA *et al*., 2008b), método considerado o padrão-ouro na medicina veterinária (HARTLEY *et al*., 2006; PICCIONE *et al*, 2008a; SWINGER *et al*., 2009).

A tonometria é uma técnica que permite a mensuração indireta da pressão intraocular (PIO) (LAUS, 2009), que é mantida por meio do equilíbrio entre a produção e drenagem do humor aquoso (MCLELLAN; MILLER, 2011). Na medicina veterinária, a tonometria de aplanação é uma das técnicas mais utilizadas para aferição da PIO (VONSPIESSEN *et al*., 2015), auxiliando no diagnóstico e acompanhamento de afecções oculares importantes, como a uveíte e o glaucoma (PIGATTO *et al*., 2011; WANG *et al*., 2013).

A superfície ocular é rica em nutrientes, logo, dispõe de muitos microrganismos, os quais compõem a microbiota ocular normal (ARMSTRONG, 2000). A maior parte das bactérias comensais que compõem a microbiota do fórnice conjuntival desempenham uma função essencial no que se refere à manutenção da saúde ocular, impedindo o desenvolvimento demasiado de agentes patogênicos (DUPONT *et al*., 1994; SACK *et al*., 2001).

Os microrganismos que compõem a microbiota ocular normal não são considerados patogênicos, desde que o epitélio corneal esteja íntegro. No entanto, quando há lesão na córnea, a microbiota residente e a transitória podem penetrar no estroma corneal, tendo, como consequência, uma úlcera de córnea infectada, tornando o tratamento dificultoso, podendo resultar em perda da visão (ANDREW *et al*., 2003). Desta forma, torna-se necessária a identificação e descrição da microbiota ocular normal para que o clínico, diante de lesão corneal — especialmente nos casos em que é preciso dar início ao tratamento antes dos resultados da cultura — possa presumir a presença dos microrganismos mais comumente isolados e consiga selecionar o antibiótico ideal (TAMARZADEH; ARAGHI-SOOREH, 2014; WANG *et al*., 2008).

A citologia conjuntival é um método valioso, rotineiramente utilizado para o diagnóstico de alterações conjuntivais (LIMA *et al*., 2005) e trata-se de uma

técnica simples, rápida e de baixo custo (FUJIHARA *et al*., 1997). A citologia possibilita a avaliação da celularidade da superfície ocular, a identificação de agentes infecciosos e células neoplásicas, como também a análise da resposta inflamatória (WILLS *et al*., 1997). Contudo, para a aplicação deste método, é necessário que haja um parâmetro de normalidade para determinar se há ou não alterações (BRANDÃO *et al*., 2002).

A ultrassonografia é um recurso útil para a medição das câmaras e das estruturas oculares (OSUOBENI; HAMIDZADA, 1999). Esta técnica pode ser solicitada em casos de opacificações de meios fisiologicamente transparentes — córnea, humor aquoso, cristalino e humor vítreo. Ademais, esta técnica ainda possibilita a análise de alterações oculares, tais como *phthisis bulbi*, microftalmia, descolamento de retina, glaucoma congênito, neoplasias, pseudoexoftalmia e ectasia escleral (GONZALEZ, 2001).

Ao longo dos anos, têm sido investigados e relatados valores normais de referência para o teste da lágrima de Schirmer, pressão intraocular e medidas anatômicas oculares, bem como a descrição da microbiota ocular e caracterização das células conjuntivais em várias espécies de animais silvestres: herbívoros selvagens (OFRI *et al.,* 1998, 2002), camelo (OSUOBENI; HAMIDZADA, 1999), elefantes asiáticos (BAPODRA *et al*., 2010; TUNTIVANICH *et al*., 2002), furões (MONTIANI-FERREIRA *et al*., 2006), capivaras (MONTIANI-FERREIRA *et al.*, 2008a), macacos-prego *Cebus apella* e *Cebus xanthosternos* (MONTIANI-FERREIRA *et al*., 2008b; ORIÁ *et al*., 2013), chinchila (LIMA *et al*., 2010), saguis (LANGE *et al*., 2012), lhamas (TRBOLOVA *et al*., 2012a), ouriço (GHAFFARI *et al*., 2012), coruja-orelhuda (RODARTE-ALMEIDA *et al*., 2013), veado (ORIÁ *et al*., 2014), rinoceronte (BAPODRA; WOLFE, 2014), jacaré-de-papo-amarelo (ORIÁ *et al*., 2015a), jabuti (ORIÁ *et al*., 2015b), pinguim (BLISS *et al*., 2015) e jacarés-do-pantanal (RUIZ *et al*., 2015). Contudo, ainda não foram relatados dados básicos acerca destes parâmetros em macacos-prego da espécie *Sapajus libidinosus*.

Para a obtenção de um conhecimento mais aprofundado sobre a fisiologia ocular normal de macacos-prego, faz-se necessário reunir dados descritivos e determinar valores normais de referência para testes oftálmicos. Desta forma, objetivou-se determinar valores de referência para os testes oftálmicos de rotina e biometria ocular, descrever a microbiota bacteriana

ocular e caracterizar as células conjuntivais de macacos-prego sadios da espécie *Sapajus libidinosus*.

CAPÍTULO I

Determinação de valores de referência para os testes oftálmicos de rotina em macacos-prego (*Sapajus libidinosus*)

Manuscrito será submetido à revista Veterinary Ophthalmology

Determination of baseline values for routine ophthalmic tests in capuchin monkeys (*Sapajus libidinosus*)

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Title of execution: Ophthalmic diagnosis tests in *Sapajus libidinosus*.

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Summary

Objective To establish baseline values for eye measurements and tests for ophthalmic diagnosis, and to perform a descriptive analysis of the microbiota and cellularity of the conjunctive of capuchin monkeys (*Sapajus libidinosus*).

Materials and methods A total of fifteen (15) healthy capuchin monkeys (*Sapajus libidinosus*) were selected and previously sedated for the collection of samples and to perform ophthalmic diagnosis tests, including: Schirmer tear test 1 (STT-1), applanation tonometry, B-mode ultrasound, culture of the aerobic bacterial conjunctival microbiota, and exfoliative conjunctival cytology.

Results The average values found for ophthalmic tests and eye measurements were: 2.50 ± 2.94 mm/min for STT-1; 13.3 ± 3.32 mmHg for the intraocular pressure (IOP); 2.47 ± 0.41 mm for the depth of the anterior chamber; 2.86 ± 0.96 mm for the axial length of the lens; 10.97 ± 0.48 mm for the depth of the vitreous chamber, and 16.32 ± 1.24 mm for the axial length of the eyeball. The intraocular pressure decreased linearly as the weight increased ($P = 0.0004$) and the average depth of the anterior chamber was larger in the male subjects ($P = 0.0004$). The bacterial genus most frequently found was *Staphylococcus* spp., (11 eyes), followed by *Enterecoccus* (8 eyes), *Escherichia coli* (7 eyes), *Bacillus* sp. (4 eyes), and *Streptococcus* sp. (3 eyes). The conjunctival cytology showed intermediate epithelial cells, squamous superficial epithelial cells, keratinized cells, inflammatory cells, red blood cells, and bacteria.

Conclusion The data description and the determination of baseline values for eye measurements and ophthalmic tests obtained in this research will assist the diagnosis of eye diseases in capuchin monkeys (*Sapajus libidinosus*).

Keywords: ocular biometry, conjunctival cytology, ocular microbiota, intraocular pressure, non-human primates, Schirmer tear test

INTRODUCTION

The neotropical primate species *Sapajus libidinosus*, popularly known as "Capuchin monkey," is inserted among the New World's monkeys belonging to the Cebidae family.[1] They are omnivores, i.e. consume various types of foods, such as fruits, seeds, flowers, eggs, invertebrates, and small vertebrates. As adults, these animals weigh from 4.5 to 11 pounds (2 to 5 kg), and can live to be 50 years old in captivity.[2] In Brazil, the capuchin monkeys of the *Sapajus libidinosus* species are scattered in the Atlantic Forest and Cerrado, primarily inhabiting the Caatinga. [3,4]

The *Sapajus libidinosus* species has diurnal habits, showing a variable behavioral repertoire,[5] and having intellectual skills and agility. These characteristics aroused the interest of traffickers, making them targets of illegal trade of primates from Brazil. In view of this and of the apprehensions and voluntary deliveries to CETAS (Wild Animals Screening Center)[6] and to zoos, there is an increasing number of animals of this species in the few captivity facilities available. Thus, it is essential for the veterinarians in these locations to have baseline data about various normal clinical parameters for this species in order to perform a proper diagnosis and treatment in face of a disease in any system. On the other hand, the large number of these animals kept in captivity is becoming an opportunity to perform studies for data collection and provision of information about the physiology of multiple systems, as well as to identify experimental models for comparative studies, since New World's primates are becoming an interesting option for compared ophthalmology studies. [7]

Ophthalmology is becoming an essential expertise with regard to the maintenance of the health of the eyes of wild animals and exotic species.[8] However, basic studies on eye physiology of some species of wild animals, especially with regard to the South American animals, are incipient,[9] and the primates represent one of the poorest in terms of baseline values.[10] Over the years, normal baseline values have been investigated and reported for the Schirmer tear test, intraocular pressure, and anatomical eye measurements, as well as the description of the ocular microbiota and characterization of the conjunctival cells in some species of wild, exotic, and savage animals.[11-21] However, baseline data about these parameters have not yet been reported for capuchin monkeys of the *Sapajus libidinosus* species.

This paucity of baseline data makes it difficult to properly diagnose and treat eye diseases in this species, despite already describedreferential data about capuchin monkeys of the *Cebus apella*[7] and *Cebus xanthosternos*[18] species. The basal physiological values of ophthalmic tests applicable to these two species of capuchin monkeys do not subsidize the diagnosis of eye diseases of the *Sapajus libidinosus* species, since the values of the Shirmer tear test (STT) and intraocular pressure (IOP) vary considerably between species, as well as between individuals of the same family, and cannot be extrapolated even between closely related species.[11,12] In this sense, the assessment of eye health of capuchin monkeys requires gathering descriptive data and determining normal reference values for the ophthalmic tests.

In this way, in face of the ophthalmic knowledge gaps about capuchin monkeys of the *Sapajus libidinosus* species, the objective is to determine the baseline values for the

STT, IOP and ocular biometry, as well as to describe the aerobic bacterial microbiota of the eye and to characterize the conjunctival cells in that species.

MATERIALS AND METHODS

Animals

The study was authorized by the Authorization and Biodiversity Information System of the Ministry of the Environment of Brazil under license Nr. 54485-1. In addition, it was approved by the Ethics Committee on the Use of Animals of the Federal University of Paraíba (CEUA-UFPB) under Protocol Nr. 089/2016 and conducted in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and with the human principles established in the ARVO Declaration (*Statement for the Use of Animals in Ophthalmic and Vision Research*) for the Use of Animals in Ophthalmic Research.

Fifteen (15) young, adult and senile capuchin monkeys (10 males and 5 females) of the *Sapajus libidinosus* species, residents of the Wild Animals Screening Center (CETAS), located in Cabedelo, Paraíba, Brazil, were selected and used. The study was performed using the sterilization of animals, which were in a period of adaptation before being reintroduced in their *habitat*.

In order to obtain the clinical data and perform the collection of samples, all animals were submitted to a 12-hour fasting and, after this interval, they were caught with the help of a catching net and leather gloves, weighed, and then submitted to anesthesia with detomidine 1% (Detomidin ®, Syntec, Votuparim, Santana de Parnaíba, São Paulo, Brazil) with a dose of 40 mcg/kg and ketamine 10% (Dopalen ® , Ceva, Paulina, São

Paulo, Brazil) with a dose of 5 mg/kg, via intramuscular injection. The body weight of the animals ranged from 3.1 to 7.5 pounds (1.4 to 3.38 kg). After the onset of the sedative effect, a routine physical examination was performed and then an ophthalmic examination, aimed at pre-screening the animals that do not show clinical signs of systemic and / or eye disease.

For the purpose of eye examination, both eyes and periocular regions were inspected under normal lighting and using a headband magnifier in search of macroscopic changes. From these investigations, fifteen capuchin monkeys were selected and then submitted the following collection of data and samples: i) Schirmer tear test; ii) conjunctival swab for microbiological bacterial analysis; iii) intraocular pressure (IOP) measurement; iv) exfoliative conjunctival cytology; v) ocular ultrasound. To avoid discrepancies in the results, the ophthalmic tests were performed by the same evaluator throughout the collection. The animals were monitored during the anesthetic recovery and returned to their captivity.

Schirmer Tear Test 1 (STT-1)

The Schirmer tear test was performed before other analysis so that tear production was not influenced by eye-drops or clinical maneuvers. The aqueous portion of the tear film was measured in both eyes using standard millimeter-marked sterile absorbent paper strips for STT-1 (Schirmer test ®, Ophthalmos Formulae, São Paulo, SP, Brazil).

Microbiological Analysis of the Conjunctiva

The samples were obtained by a careful friction of the right eye conjunctival fornix, aseptically, with aid of dry sterile swab, avoiding contact with the fur and eyelid margins.

Immediately after the collections, the swabs were inserted in Stuart transport medium and sent to the Laboratory of Preventive Veterinary Medicine of the Federal University of Paraíba. The samples were grown in BHI enrichment broth (Brain-Heart Infusion) and sheep blood agar at 5% in sequence, and incubated at 37° C in an aerobic environment for 24-48 hours. After the growth of the bacterial colonies, catalase, coagulase and oxidase tests were performed. Enterobacteriaceae were identified using a commercial kit (Bactray®, Laborclin, Pinhais, Brasil).

Intraocular Pressure

The intraocular pressure (IOP) of both eyes was measured with the aid of portable applanation tonometer (Tono-Pen AVIA ®, Reichert Technologies, Buffalo, USA), which was positioned in the center of the cornea after instillation of anesthetic eye-drops based on proxymetacaine hidrochloride at 0.5% (Anestalcon ®, Alcon Laboratórios do Brasil, São Paulo, Brazil).

Exfoliative Conjunctival Cytology

With the topical anesthesia used in the IOP analysis, samples of conjunctiva were collected with the aid of a sterile cytology brush (Kolplast ®, São Paulo, Brazil) through careful scrub of the lower conjunctival fornix of the left eye, as described earlier.[19,20] The samples were then distributed on glass slides, which were dried up at room

temperature, dyed by the Quick Panoptic method, and then evaluated by direct optical microscopy, with a magnification of 40x and 100x. Macroscopically, no lesions were observed in the conjunctiva after the collection.

Ocular Ultrasound

The ocular B-mode ultrasound was performed on both eyes using a M-Turbo® ultrasound (Fujifilm SonoSite Ltda), model HFL 38X, equipped with a linear multi-frequency transducer (6-13 MHz), which was positioned on a thick layer of acoustic gel on the surface of the eyelid.

The biometric measurements were acquired in the axial horizontal plane in the following order: axial length of the eyeball (ALE), depth of the anterior chamber (DAC), axial length of the lens (ALL), and depth of the vitreous chamber (DVC) (Fig. 1).

Statistical Analysis

The data were subjected to the analysis of variance ($P \leq 0.05$) applying the F-test using the software SAS (Statistical Analysis System) version 9.3. The averages were generated by Tukey test.

RESULTS

Schirmer Tear Test 1 (STT-1)

The average value obtained for the STT-1 was 2.50 ± 2.94 mm/min. There was no significant difference in the average values of the STT between males and females (P =

0.53), between the right eye and the left eye ($P = 0.95$), or in relation to body weight ($P = 0.17$).

Intraocular Pressure (IOP)

The average of the IOP was 13,3 ± 3,32 mmHg. There was no significant difference between the average values obtained for males and females ($P = 0,06$), or for the right and left eyes ($P = 0,87$), but the IOP values decreased linearly with the increase of the weight ($P = 0,0004$) (Fig. 2).

Microbiological Analysis

Bacterial organisms have been identified in 100% of the samples. The Gram-positive isolates comprised three different bacterial genera – *Staphylococcus* spp., *Streptococcus* sp. and *Bacillus* sp., while the Gram-negative bacteria comprised two genera – *Escherichia coli* and *Enterococcus* sp. (Table 1).

Out of the fifteen eyes studied, only Gram-positive bacteria were isolated in four (26.67%) and only Gram-negative bacteria in three (20.00%). In addition, five eyes (33.33%) presented growth of a single bacterial genus; three (20.00%) presented growth of two different bacterial genera, and seven (46.67%) presented growth of three different genera.

Bacteria of the genus *Staphylococcus* spp. were present in 11 eyes (73.33%), *Enterecoccus* in eight eyes (53.33%), *Escherichia coli* in seven eyes (46.67%), *Bacillus* sp. in four eyes (26.67%) and *Streptococcus* sp. (20.00%) in three eyes.

Exfoliative Conjunctival Cytology

The conjunctival samples showed high cellularity, with predominance of intermediate polygonal epithelial cells and a smaller number of superficial squamous epithelial cells and keratinized cells. Varying degrees of cytoplasmic melanocytic granulation were observed in all cells.

In addition, a few number of lymphocytes, neutrophils, and free and intracytoplasmic bacteria was observed. Rare erythrocytes were observed, although no conjunctival microtraumas were verified after the collection (Fig. 3).

Ocular Ultrasound

The average value of the DAC was 2.47 ± 0.41 mm. There was no significant difference between the right and left eyes ($P = 0.76$) or in relation to body weight ($P = 0.70$). However, a difference was observed between the average values for males and females ($P = 0.0004$; Table 2).

An average value of 2.86 ± 0.96 mm was obtained for the ALL; 10.97 ± 0.48 mm for the DVC; and 16.32 ± 1.24 mm to the ALE. There was no significant difference between the values of the DVC, ALL and ALE for males and females, for the right and left eyes, or in relation to the weight ($P < 0.05$).

DISCUSSION

The neotropical primate species *Sapajus libidinosus* has been suffering with illegal trade and the loss and fragmentation of its natural *habitat*. These animals arrive weakened to

the recovery centers or zoos, carrying various diseases, including ocular diseases, which are not diagnosed efficiently due to the lack of reference data concerning the ophthalmic diagnosis tests. This fact alerts for the urge to perform descriptive ophthalmic studies for this species of primate. This first description of data and determination of baseline values for IOP, STT-1 and measurements of the eye compartments of the *Sapajus libidinosus* will be useful to face the gaps of ophthalmic knowledge on this species. Furthermore, it was demonstrated that the results found here were similar to those of other species of non-human primates, particularly those used as a biological model in compared ophthalmology studies.

IOP values have already been reported for two species of capuchin monkeys, *Cebus apella* (18.4 ± 3.8 mmHg)[7] and *Cebus xanthosternos* (19.2 ± 4.2 mmHg)[18], which in terms of average are higher than those found in this study. The average IOP for the *Sapajus libidinosus* was significantly closer to that found in rhesus monkeys (*Macaca mulatta,* 15.7 ± 2.0 mmHg)[22], Old World's monkey species accepted as a biological model for glaucoma.[23] Regarding the average IOP for humans (10-20 mmHg)[24], the monkeys in this study had an average value within the normal range.

Although reported in humans[25], no relationship between IOP values and gender was observed in this investigation. However, there was a linear decrease of this parameter with weight increase, a correlation that was not observed in rhesus monkeys.[22] Although the IOP difference between genders was not observed, it was observed that the males had higher body weight when compared to females (5.47 x 3.88 pounds or 2.48 x 1.76 kg), which would suggest, considering the correlation and linear regression data obtained, that the males would tend to have a lower IOP due to their higher body

weight. Additionally, it is known that the larger volume of the anterior chamber of the eye is directly related to a higher drainage rate of the aqueous humor and lower IOP.[26] Indeed, it was observed in this work that the depth of the anterior chamber of the *Sapajus libidinosus* was significantly higher in males than in females, reinforcing indication that males with greater weight and larger eyes should present a lower IOP.

The use of chemical restraint can influence the IOP values.[11] It is suggested that the low IOP values in anesthetized animals result from the relaxation of the extraocular muscles and from the decrease of the episcleral blood pressure.[12] However, these effects seem to vary according to the drug. In horses, detomidine reduces the IOP,[27] while ketamine[28] and xylazine,[29] for instance, respectively increases and decreases the IOP in cats. However, no significant changes were observed in this parameter following the use of ketamine in cynomolgus monkeys (*Macaca fascicularis*)[30] and rhesus monkeys (*Macaca mulatta*),[22] being therefore considered the first choice of anesthetic in IOP study in monkeys.[31] The inexistence of IOP values for the *Sapajus libidinosus* without the use of chemical restraint makes it impossible to determine the potential effects of the association of ketamine and detomidine on the IOP. However, the similarity of the average values found in this work with the values found for rhesus monkeys[22] and within the range of values observed for capuchin monkeys[7] and humans[24] suggests that this drug combination may not have changed dramatically the IOP of the *Sapajus libidinosus*.

The measurement of tear production through the STT-1 is an important part of the ophthalmic examination and aims at evaluating the aqueous component of the tear film.[32] The average value of the STT-1 obtained in this study is significantly different

from the value found for other primates, such as rhesus monkeys (*M. mulatta*, 15.1 mm/min),[33] squirrel monkey (*Saimiri sciurueus*, 16.9 ± 1.2 mm/5 min),[34] and two species of capuchin monkeys, *C. apella* (14.9 ± 5.1 mm/ min)[7] and *C. xanthosternos* (14.9 ± 5.5 mm/min).[18] Differences found in the values of the STT-1 between animals of the same family were also discussed in other studies with wild animals,[12,19,35,36] suggesting that some conditions, including specific differences between species, level of stress during the capture, anesthetic protocols, environmental factors, living conditions in the wild or captivity, age, and time of day might influence the values of the STT.

The animals in this study were pre-anesthetized, because the implementation of ocular measurements without the use of chemical restraint would be impracticable, as they were not accustomed to frequent handling. Thus, it is suggested that the STT-1 values should be used taking into account the possible effects of the pre-anesthetics on tear production. The use of ketamine, one of the drugs used in this investigation, caused no changes in the STT results for rhesus monkeys.[33] On the other hand, it was observed in dogs a significant decrease in tear production after an intravenous administration of medetomidine,[37] a drug from the same group as the detomidine used in this study. In eagles, the association of ketamine and medetomidine decreased tear production significantly.[38] Therefore, it is assumed that the low STT-1 values in this investigation may result from the influence of these drugs on tear production, since no ocular alteration consistent with the low values found was observed. In addition, the stress related to the animals' capture might have also have had an influence in the low tear production. Indeed, veterinarians who work with members of the species *Sapajus libidinosus* will need some form of sedation to be able to carry out a full ophthalmic examination, unless these animals are severely debilitated or accustomed to handling.

Thus, the data obtained in this study, even if influenced by factors related to the handling of capture and sedation, will subsidize professionals interested in baseline values obtained after the use of a similar protocol.

In the study of conjunctival microbiota it was observed that the bacteria of the *Staphylococcus* sp. genus were isolated in a greater number of eyes in the species investigated here. This result is consistent with that obtained by Oriá *et al.*[18] about some species of neotropical primates. These authors concluded that bacteria of this genus can be considered as the main residents of the normal ocular microbiota of neotropical primates. In addition, it is known that Gram-positive bacteria are constantly identified and considered normal constituents of the skin surface and mucous membranes microbiota, such as the conjunctiva, but they are opportunists and, under some circumstances, may become pathogenic.[7] On the other hand, in another study with free-living capuchin monkeys (*Cebus apella*),[7] no bacteria of the *Staphylococcus* sp. genus were isolated. However, it is worth mentioning that the bacterial microbiota may vary depending on several aspects, such as the season of the year,[39] geographic location, culture techniques,[40] population density and *habitat* variations.[41]

A significant number of two species of enterobacteria was isolated (*Escherichia coli* and *Enterococcus sp*.) in the *Sapajus libidinosus* monkeys investigated, results consistent with other investigations regarding other non-human primates (*Cebus apella* and *Alouatta caraya*),[42] *(Cebus* sp, *Cebus xanthosternos, Callithrix jacchus,* and *Callithrix penicillata),*[18] in which more than one species of enterobacteria were isolated as well. Bacteria of the *E. coli* species are natural inhabitants of the intestine of mammals, being excreted with the feces, with potential to survive in fecal particles and in water for

months.[43] However, bacteria of this family of Gram-negative bacteria are constantly found in healthy eyes, and may vary between different species.[44] In this way, these findings suggest a possible contamination with fecal matter that can be associated with the characteristic habits and behavior of these animals, especially those who live in large numbers in the same space, like the animals of this study, which facilitates contact and continuous manipulation of objects and water contaminated by feces. In a study with free-living *Cebus apella*,[7] Gram-negative bacteria have not been identified in any of the ocular samples, supporting the hypothesis that *E. coli* can be considered a temporary agent in the conjunctiva, requiring further investigations about the relevance of the presence of this bacteria in the ocular microbiota of capuchin monkeys.

The cytological analysis showed predominance of intermediate epithelial cells and, in a smaller number, superficial squamous epithelial cells and superficial keratinized epithelial cells, similar to the results for other species of neotropical primates.[18] EAlthough conjunctival pigmentation has not been visualized macroscopically, varying degrees of melanocytic cytoplasmic granulation have been observed. This finding is consistent with the one found in other wild animals and exotic species, such as neotropical primates,[18] capybara,[13] deers,[19] and broad-snouted caiman[20]. Some authors suggest that the cytoplasmic pigmentation in dogs and cats is associated with the presence of chronic eye diseases, such as keratoconjunctivitis sicca.[45] This hypothesis cannot be applied according to the results of this investigation, because no signs of eye diseases or conjunctival pigmentation were found in the animals of this study. Besides, the observation of a few number of lymphocytes, neutrophils and bacteria can be considered normal even in the absence of clinical signs of eye diseases.[46,47]

Ocular ultrasound has been reported in some species of wild and exotic animals.[14,15,48,49] It was observed that the average of the measurements obtained for the depth of the anterior chamber, for the depth of the vitreous chamber, for the lens thickness, and for the axial length of the eyeball were remarkably similar to those found for rhesus monkeys (*Macaca mulatta*).[49] In the same study, it was observed that male rhesus monkeys had higher values in the measurements of the lens, vitreous chamber, and axial length of the eye compared to the females, as already discussed, the only difference observed in the *Sapajus libidinosus* was about the measurement of the depth of the anterior chamber, which was significantly larger in males.

The baseline values of the ophthalmic tests and optical components' dimensions established in this study, as well as the description of the aerobic bacterial microbiota and the characterization of conjunctival cells may subsidize the diagnosis and appropriate treatment of eye disorders in capuchin monkeys (*Sapajus libidinosus*). Moreover, in the scientific field, these baseline data have a great value with regard to the compared ophthalmology, since it is believed that these animals may be used as biological models for the study of eye diseases.

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Table 1. Bacteria isolated from the right conjunctival fornix of 15 healthy eyes of capuchin monkeys (*Sapajus libidinosus*) held captive in the Wild Animals Screening Center of Cabedelo-PB.

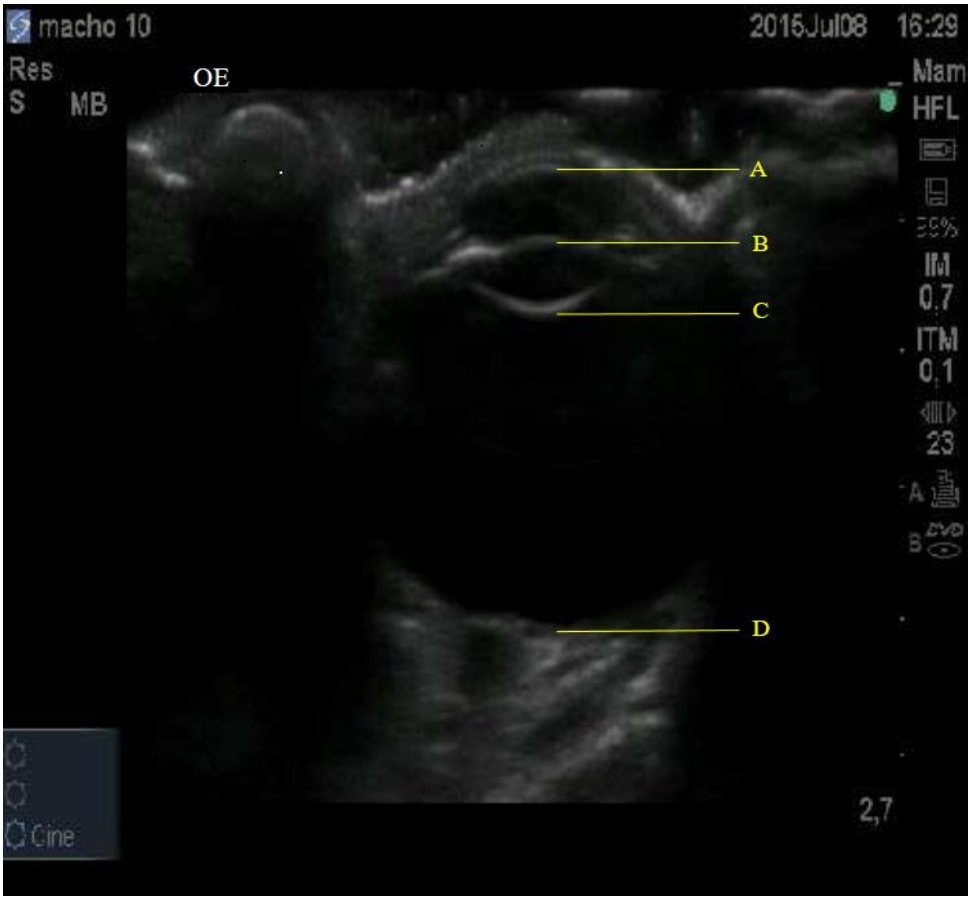
	Bacteria isolated	*n*
Gram-positive	*Staphylococcus hyicus*	2/15
	Coagulase-Positive *Staphylococcus*	3/15
	Streptococcus dysgalactiae	3/15
	Bacillus sp.	4/15
	Coagulase-Negative *Staphylococcus*	6/15
Gram-negative	*Enterococcus sp.*	8/15
	Escherichia coli	7/15

n – number of bacteria

Table 2. Ocular biometry in capuchin monkeys (*Sapajus libidinosus*) held captive in the Wild Animals Screening Center of Cabedelo - PB

Biometric data (mm)	Female Mean ± SD	Male Mean ± SD	Right eye Mean ± SD	Left eye Mean ± SD
DAC	2.15 ± 0.22[a]	2.68 ± 0.36[b]	2.45 ± 0.37	2.5 ± 0.47
ALL	2.83 ± 0.35	2.88 ± 1.21	2.90 ± 1.04	2.82 ± 0.91
DVC	10.98 ± 0.34	10.96 ± 0.55	11.00 ± 0.46	10.93 ± 0.50
ALE	15.99 ± 0.48	16.52 ± 1.51	16.38 ± 1.28	16.25 ± 1.23

Values with different letters have significant differences (P = 0.0004). SD: standard deviation; DAC: depth of the anterior chamber; ALL: axial length of the lens; DVC: depth of the vitreous chamber; ALE: axial length of the eyeball.


macho 10
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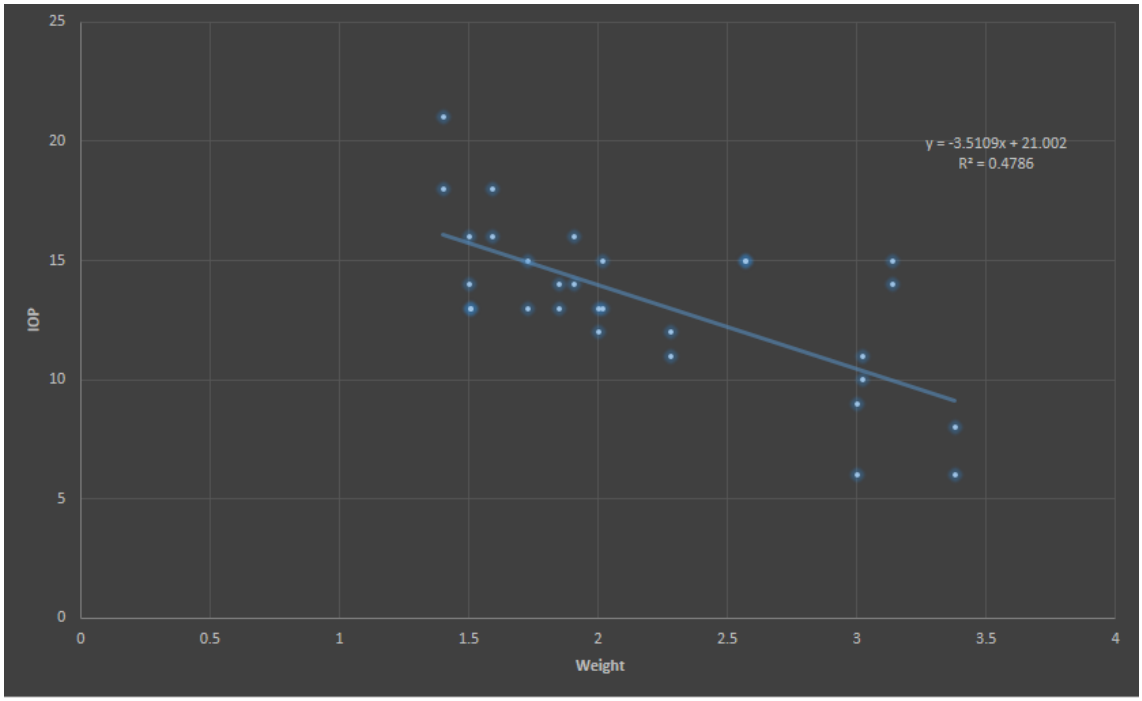
y = -3.5109x + 21.002
R² = 0.4786
IOP
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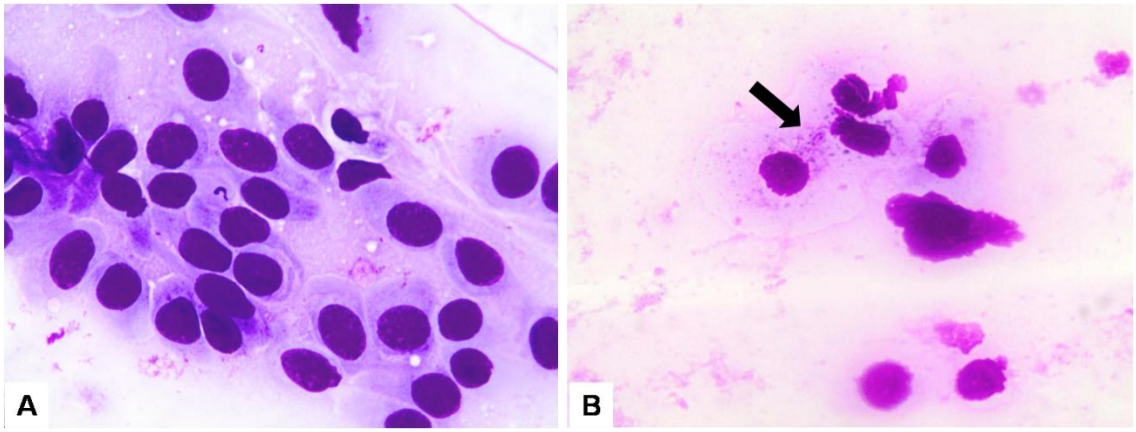
A
B

LEGENDAS PARA FIGURAS

Fig. 1 Mode-B ultrasound image of the eye of a capuchin monkey (*Sapajus libidinosus*). (A-B) Depth of the anterior chamber; (B-C) axial length of the lens; (C-D) depth of the vitreous chamber; (A-D) axial length of the eyeball.

Fig. 2 Linear regression graph for the intraocular pressure (IOP) and weight of capuchin monkeys (*Sapajus libidinosus*).

Fig. 3 Cytological examination of conjunctiva from normal monkey (*Sapajus libidinosus*). A) Normal conjunctival smears show sheets of polygonal intermediary cells. (Panotic,1000x). B) Note macrophage showing phagocytosis of cocci (arrow). (Panotic 1000x).

CONSIDERAÇÕES FINAIS

Estudos básicos acerca da fisiologia ocular em macacos-prego (*Sapajus libidinosus*) são incipientes, tornando difícil o diagnóstico e o tratamento da doença ocular nesta espécie. Frente a esta lacuna, os valores normais de referência dos testes oftálmicos e das dimensões dos componentes ópticos estabelecidos neste estudo, bem como a descrição da microbiota da conjuntiva e a caracterização das células conjuntivais poderão subsidiar o diagnóstico e o tratamento adequado diante de afecções oculares na referida espécie.

Adicionalmente, diante da analogia da fisiologia ocular e da aparência geral dos olhos dos macacos-prego com a de outras espécies de macacos utilizadas em estudos de oftalmologia comparada, acredita-se que esses animais possam ser utilizados como modelo biológico para estudo de doenças oculares.

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