

## ORIGINAL ARTICLE

# Conjunctival flora, Schirmer's tear test, intraocular pressure, and conjunctival cytology in neotropical primates from Salvador, Brazil

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## Keywords

*Callithrix jacchus* – *Callithrix penicillata* – *Cebus xanthosternos* – eye – ophthalmic tests

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## Abstract

**Background** This study aimed to establish reference values for selected ophthalmic diagnostic tests in healthy neotropical primates from Salvador, Brazil.

**Methods** A total of 73 intact adults, including *Callithrix jacchus* (n = 31), *Callithrix penicillata* (n = 8), *Cebus* sp. (n = 22), and *Cebus xanthosternos* (n = 9) were used to evaluate the normal conjunctival bacterial flora. *Cebus xanthosternos* (n = 12) were used to evaluate tear production with Schirmer's tear test (STT), intraocular pressure (IOP), and conjunctival cytology.

**Results** For all animals evaluated, Gram-positive bacteria were predominant. Results of the diagnostic tests in *Cebus xanthosternos* were as follows: STT:  $14.92 \pm 5.46$  mm/minutes, IOP:  $19.62 \pm 4.57$  mmHg, and conjunctival cytology revealed intermediate squamous epithelial cells in great quantities.

**Conclusions** These ophthalmic reference values will be particularly useful to diagnose discrete or unusual pathological changes in the neotropical primates eye.

## Introduction

Small- and medium-sized species of primates may adapt easily to anthropomorphic areas, often inhabiting the urban environment, and are commonly found in zoos or in wildlife triage centers [10, 37]. In the wild, they live in groups and inhabit various ecosystems. In Bahia, the marmosets are found inhabiting the coast of the state, tropical savanna and forests, as well as areas of transition between the biomes. Capuchin monkeys are also found in forests and grasslands in the northeast and other regions of Brazil [10].

Reports of eye diseases and determination of parameters for normal ophthalmic tests in primates are scarce. There are reports of a retrobulbar tumor in a squirrel monkey (*Saimiri sciureus*) [3], an odontogenic intraorbital abscess in a capuchin monkey

(*Cebus apella*) [35] and determination of ophthalmic parameters in capuchin monkey (*Cebus apella*) [25], tear production in squirrel monkey (*Saimiri sciureus*) [23], black-tufted marmosets [19], and rhesus monkey (*Macaca mulatta*) [17] and intraocular pressure (IOP) in rhesus monkey (*Macaca mulatta*) [5]. To perform a proper diagnosis in wild and exotic species is necessary to determine normal baseline parameters for ophthalmic tests.

The conjunctival microbiota plays a fundamental role in maintaining the health of the eye, as it prevents the overgrowth of potentially pathogenic agents [36]. When the resident microbiota is altered, the opportunistic pathogens can overlap, leading to the development of diseases [38].

The Schirmer's tear test (STT) evaluates production of the aqueous portion of the tear film and is traditionally

used to diagnose keratoconjunctivitis sicca (KCS) [15]. Tear production has been determined for several species including humans [7, 15, 16, 19, 20, 25, 26, 30, 31, 33, 34].

Tonometry is used to diagnose glaucoma and uveitis, and therefore, it is necessary to know the normal IOP values in different species. However, due to significant differences between species, it is impossible to extrapolate IOP from one species to another [32].

Thus, the objective of the study reported here was to describe the normal conjunctival flora in healthy adult marmosets (*Callithrix jacchus*, *Callithrix penicillata*) and capuchin monkeys (*Cebus* sp. and *Cebus xanthosternos*) as well as establish normal reference values for Schirmer's tear test and IOP and characterize the conjunctival cells of *Cebus xanthosternos*.

## Materials and methods

This study was conducted in two stages with a total 73 intact adults including *Callithrix jacchus* (n = 31), *Callithrix penicillata* (n = 8), *Cebus* sp. (n = 22), and *Cebus xanthosternos* (n = 12).

The ophthalmic evaluation and testing were performed as part of a routine physical examination provided by the local veterinary staff. Thus, physical examination was performed before the ocular examination to exclude animals with indications of systemic disease. The eye and periocular region were examined in normal light for gross abnormalities with a binocular magnifying loupe 3X and a transilluminator. After sampling, fluorescein stain (Ophthalmos, SP, Brazil) was performed to exclude corneal lesions. The ophthalmic examination and the sample collection were all conducted by the same investigator.

All research protocols were in accordance with the Authorization and Information System on Biodiversity of the Ministry of Environment of Brazil (process no 27489-1). In addition, all procedures were conducted in accordance with the humane principles set forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Anesthetic protocols

The primates were captured with dip nets or gloves and then anesthetized. Ketamine (Vetanarcol, König do Brasil, Ltda, SP, Brazil) (20 mg/kg) [29] delivered intramuscularly was used in *Callithrix jacchus*, *Callithrix penicillata*, *Cebus* sp. and a combination of tiletamine and zolazepam (Zoletil 100, Virbac Animal Health, São Paulo, Brazil) (2 mg/kg) [8] delivered intramuscularly, in *Cebus xanthosternos*.

## Stage 1

### Microbiological analysis

Specimens were obtained from the lower conjunctival fornix of both eyes of 70 animals (*Callithrix jacchus* n = 31, *Callithrix penicillata* n = 8, *Cebus* sp. n = 22, and *Cebus xanthosternos* n = 9) by scraping with sterile swabs containing tryptose agar (Bac Swab DME, São Paulo, Brazil) avoiding contact with eyelids and skin. Swabs were kept under refrigeration until arrival at the Laboratory of Bacteriosis of the Federal University of Bahia. The samples were processed within 24 hours after collection by seeding technique for exhaustion in a Petri dish containing sheep blood agar (6%), McConkey agar, and Trypticase broth (Difco™, Sparks, MD, USA) and incubated at 37°C in an aerobic environment chamber gases for 24–48 hours. Bacterial growth, observed after the incubation period, was analyzed for morphotinctorial and biochemical characteristics according to routine laboratory techniques [18]. Yeasts were not surveyed in this study, and no topical anesthetic was used prior to swabbing the conjunctiva for culture.

## Stage 2

A total of six male and six female *Cebus xanthosternos* were used in this phase, and all tests were conducted between 8 and 11AM.

### Schirmer's tear test

Sterile standardized Schirmer's tear test (STT) strips (Ophthalmos, Ribeirão Preto, SP, Brazil) were used to measure the aqueous portion of the tear film prior to instillation of any topical anesthetic to avoid influencing the results (STT 1). Chronologically, this procedure was performed before swabbing the conjunctiva for culture (in 9 of 12 animals). Sterile gloves and sterile standardized strips were used for measurements, and maximum care was taken to avoid contamination.

### Intraocular pressure

Sterile topical anesthetic (proxymetacaine 0.5%, Anestalcon, Alcon Laboratories do Brazil Ltda, São Paulo, Brazil) was instilled before tonometry. During IOP measurements, care was taken to avoid applying any pressure in the neck region during physical restraint. IOP was measured by applanation tonometry using a Tonopen® XL (TonoPen XL, Reichert Technologies, New York, NY, USA). A total of two results, with 5% coefficient of variation (as calculated by the tonometer), were recorded from each eye.

### Conjunctival cytology

A barren interdental brush (interdental brush conical Oral B, Manaus, Amazonas, Brazil) was placed in the conjunctival fornix of the right eye, and samples were obtained using smooth rotational movements. Soon thereafter, the samples were distributed onto glass slides, left to dry, and then stained by the Panoptic fast method.

### Statistical analysis

The Shapiro–Wilk test was used to test data normality for STT and IOP. Comparison of the mean values of all variables was made using the Student's *t*-test.

## Results

### Stage 1

The results for the conjunctival microbiota are shown in Table 1, where a predominance of Gram-positive bacteria can be seen. Of the 140 samples analyzed, 70 presented growths of two or more microorganisms, 58

showed only one microorganism, and in 12 samples no growth was detected.

### Stage 2

The results of IOP and STT measurements are summarized in Table 2. No significant differences were seen between eyes ( $P = 0.4$ ) for STT as well as IOP ( $P = 0.8$ ). There were no significant differences between males and females in STT ( $P = 0.4$ ), or IOP ( $P = 0.5$ ).

### Conjunctival cytology

The same cell pattern was found in all cytology slides. Intermediate squamous epithelial cells were found in great quantities. Some pigmented cells (melanin granules) arranged in clusters or isolated, and fewer superficial keratinized epithelial cells were observed (Fig. 1).

## Discussion

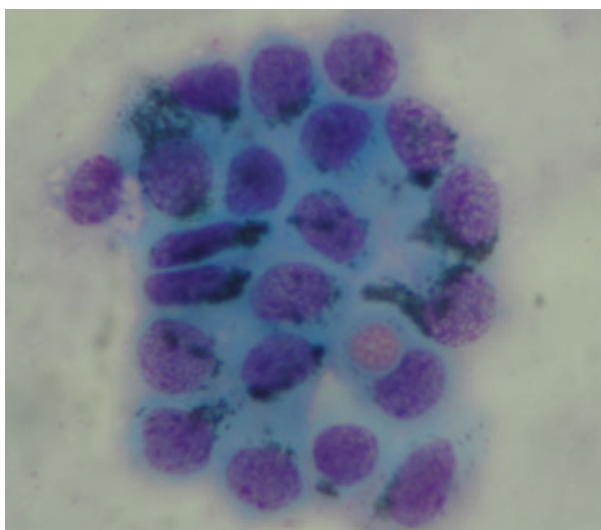
Bacteria growth was observed in the present study in 87.2% and 98% of samples from marmosets and

**Table 1** Conjunctival flora isolated from healthy *Callithrix jacchus* (n = 31, 62 eyes), *Callithrix penicillata* (n = 8, 16 eyes), *Cebus* sp. (n = 22, 44 eyes), and *Cebus xanthosternos* (n = 9, 18 eyes)

Bacterial type	<i>Callithrix jacchus</i> and <i>penicillata</i>		<i>Cebus</i> sp.		<i>Cebus xanthosternos</i>	
	No. of isolations	%	No. of isolations	%	No. of isolations	%
Gram-positive						
<i>Staphylococcus</i> spp.	48	50.0	23	17.5	14	29.1
<i>Staphylococcus epidermidis</i>	18	8.9	13	9.9	2	4.2
<i>Staphylococcus aureus</i>	11	1.5	5	3.8	10	20.8
<i>Bacillus</i> spp.	4	4.2	10	7.6	6	12.5
Diphtheroids	2	2.1	9	6.9	9	18.7
<i>Staphylococcus</i> spp.	–	–	10	7.6	–	–
<i>Streptococcus B hemolytic</i>	3	3.1	3	2.3	–	–
<i>Micrococcus</i> spp.	–	–	1	0.8	2	4.2
<i>Streptococcus</i> spp.	1	1.0	–	–	1	2.1
<i>Staphylococcus intermedius</i>	1	1.0	–	–	–	–
Gram-negative						
<i>Escherichia coli</i>	1	1.0	25	19.1	1	2.1
<i>Enterobacter</i> spp.	–	–	11	8.4	–	–
<i>Pseudomonas</i> spp.	2	2.1	6	4.6	–	–
<i>Alcaligenes</i> spp.	1	1.0	6	4.6	–	–
<i>Klebsiella</i> spp.	1	1.0	4	3.0	–	–
<i>Enterobacter</i> spp.	2	2.1	–	–	2	4.2
<i>Enterobacter harfnia</i>	1	1.0	–	–	1	2.1
<i>Citrobacter</i> spp.	–	–	2	1.5	–	–
<i>Proteus vulgaris</i>	–	–	1	0.8	–	–
<i>Proteus mirabilis</i>	–	–	1	0.8	–	–
Total	96	100	131	100	48	100

**Table 2** Results obtained for Schirmer's tear test and intraocular pressure from healthy *Cebus xanthosternos* (n = 12, 24 eyes)

Test	Gender	n	Mean value	Standard deviation	95% Confidence interval
STT	Female	6	13.9	6.1	10.0–17.8
	Male	6	15.9	4.8	12.9–19.0
	Overall group		14.9	5.5	12.6–17.2
IOP	Female	6	19.8	5.7	16.1–23.4
	Male	6	18.5	2.0	17.3–19.8
	Overall group		19.2	4.2	17.4–20.9

**Fig. 1** Photomicrograph of the main cells found in the conjunctiva of *Cebus xanthosternos* – Panoptic fast (1000×). Note a cluster of squamous epithelial cells with melanotic pigment in the cytoplasm.

capuchin monkeys, respectively, with a significant presence of Gram-positive bacteria comparatively to Gram-negative bacteria. This was due to the high prevalence of *Staphylococcus* in the samples, which can be attributed to the presence of such bacteria in the normal flora of the skin and mucous membranes [27].

Studies in humans, dogs, birds of prey, Canadian beavers, brocket deer, coates and crab-eating raccoon, cats, horses, sheep, and capybaras showed similar results regarding the predominance of Gram-positive bacteria [2, 9, 21, 24, 26].

However, some authors [6] reported 57% growth of Gram-negative bacteria in domestic ducks under the water cycle of the species, while others [13] reported growth of only Gram-negative bacteria in howler monkeys. Even though it is the most common etiologic agent of bacterial conjunctivitis in domestic animals [39], *Staphylococcus* sp. stands out as the most common

bacteria isolated from healthy eyes, thus contributing to the prevalence of Gram-positive organisms among the isolates.

Gram-negative bacteria were isolated more frequently in capuchin monkeys than in marmosets, which can be attributed to the behavior and social habits characteristic of these animals, such as manipulation of the genitals, hand contact with the ground, and water contaminated by feces and urine, in addition to preexisting environmental factors. These factors reinforce the hypothesis of direct influence of environment on the composition of the microbiota of normal conjunctiva.

There was a relatively high prevalence of Gram-negative bacteria cultured from the conjunctival sac of *Cebus* sp. Because it is enterobacteria, the high prevalence of *Escherichia coli* (19.1%) in this species can be attributed to the habits of animals that constantly touch their eyes.

Studies identified several factors that may influence the prevalence of certain microorganisms, such as geography, climate, season, species, and environment of the individual [27]. It is emphasized that the sampling method, type of swab, and laboratory procedures can also significantly influence the results.

Schirmer's tear test is an important component of the mammalian ophthalmic examination and is used to evaluate the aqueous component of the tear film [14]. The values found in this study were similar to those found for capuchin monkey (*Cebus apella*) (14.9 mm/minutes) [25] and rhesus monkey (*Macaca mullata*) (15.1 mm/minutes) [17]. However, lower values were determined for squirrel monkey (*Saimiri sciureus*) (5.6 mm/minutes) [23]. These differences could be attributed to factors such as species-specific differences, environmental factors, age of animals, and level of stress during capture, anesthetic protocols, and the living conditions of wild or captive animals.

The mean IOP value of capuchin monkeys was close to that of human beings (10–20 mmHg) [28] and similar to those found in *Cebus apella* ( $18.4 \pm 3.8$ ) [25]. Even with chemical restraint, the IOP values did not differ much from the values found in *Cebus apella* that were restrained manually. However, because the IOP of *Cebus xanthosternos* without chemical restraint is not known, it is not possible to establish the influence of the anesthetic protocol used in this study. The mean IOP value in this study was higher than the values reported in rhesus monkeys (*Macaca mulatta*) ( $15.7 \pm 2.0$  mmHg), a species accepted as an experimental model for the study of glaucoma [5, 11]. Nevertheless, further studies are needed to investigate the potential of *Cebus xanthosternos* as an experimental model for

the study of glaucoma and the influence of different anesthetic protocols in this species.

Cytological evaluation showed a single-cell pattern composed of intermediate squamous cells and superficial keratinized epithelial cells. In deer (*Mazama gouazoubira*), some authors found a large number of goblet and intermediate cells [24], and others observed in horses a majority of polyhedral and cylindrical cells and a few squamous cells [1]. In our study, melanin granules were observed in the cytoplasm, and this finding is in agreement with what was found in normal conjunctival cytology in other species such as deer, horses, cattle, and sheep [22, 24]. However, it is worth mentioning that we did not observe pigmentation macroscopically. Studies of impression and exfoliative normal human conjunctival cytology demonstrated the presence of epithelial and goblet cells [4, 12], while goblet cells were not evident in the conjunctiva of *Cebus xanthosternos*.

## Conclusion

In conclusion, members of *Staphylococcus* sp. genus, mainly coagulase-negative strains, may be considered as the main inhabitants of the normal ocular microbiota of neotropical primates, although Gram-negative bacteria can be found in a minor proportion of samples. We believe that the results found in this study could help wildlife veterinarians to recognize and diagnose ophthalmic changes in these species.

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