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Major Article

Oral microbiota and their antibiotic susceptibility in free-living monkeys in Goiás State, Brazil: Repercussions for injuries in humans

Elisângela de Albuquerque Sobreira[1], Carla Afonso da Silva Bitencout Braga[2], Sheila Canavese Rahal[3], Evandro Leão Ribeiro[3], Lara Stefânia Netto de Oliveira Leão Vasconcelos[4], Lilian Carla Carneiro[4], Carlos Roberto Teixeira[3], Camila Contin Diniz de Almeida[4] and Vidal Haddad Júnior[5]

[1]. Programa de Pós-Graduação em Animais Selvagens, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brasil.
[2]. Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil.
[3]. Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brasil.
[4]. Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brasil.
[5]. Faculdade de Medicina, Universidade Estadual Paulista, Botucatu, SP, Brasil.

Abstract

Introduction: Goiás State, which is in the midwest region of Brazil, has several urban forests. This fact, along with the expansion of urban areas within the limits of Forest Conservation Units, increases the contact between humans and wildlife, such as capuchin monkeys. The impulsive behavior of these animals and the scarcity of food cause them to vigorously search for food, leading to direct encounters with Park visitors, which can result in scratches and bites and making them potential disseminators of pathogenic microorganisms. **Methods:** Ten specimens of bearded capuchin monkey (*Sapajus libidinosus*) were captured at the Onofre Quinan Environmental Park in Anápolis, Goiás, Brazil. Samples were collected from the monkeys, and the bacteria and fungi present in the samples were isolated and identified. Then, the identified microorganisms were subjected to antibiotic susceptibility testing. **Results:** A total of 111 bacteria and 12 fungi were isolated, including two strict anaerobic bacteria of the genus *Peptostreptococcus*, 109 facultative anaerobic bacteria, and 12 yeasts. Among the facultative bacteria, enterobacteria and *Staphylococcus* were common. Resistance to tetracycline and ampicillin antibiotics was detected in the enterobacteria, and resistance to tetracycline, erythromycin, and clindamycin was detected in the Staphylococci. The other strains were sensitive to all tested antimicrobials. Cefoxitin showed 100% efficacy in all isolated bacteria. **Conclusions:** For bites from capuchin monkeys, we recommend performing complete hygiene and antibiotic therapy, according to medical recommendations. Given the 100% effectiveness of cefoxitin, it should be considered for this type of injury, especially in the study region.

Keywords: Capuchin monkey. Bites and stings. Antibiotic. Bacterial infections. *Sapajus libidinosus.*

INTRODUCTION

The impulsive behavior of capuchin monkeys and the scarcity of food in their habitat cause these primates to search for food and sometimes attack humans to obtain it, leading to bites and scratches. Thus, these monkeys may disseminate potentially pathogenic microorganisms, such as aerobic and anaerobic bacteria, fungi, and even rabies and herpes viruses, to humans[1].

In 2004, the Zoonoses Control Center of Anápolis/GO-Brazil registered 31 injuries caused by capuchin monkeys in the city's parks. In 17 (54.8%) cases, the victims had deep injuries that required medical attention. These attacks were associated with the search for food held by park visitors (EAS, personal communication) (**Figure 1**).

The concern with managing non-human primates is related to not only the risk of transmitting zoonoses but above all the type of traumatic or infectious cutaneous lesions caused by the bites of these animals[2].

Corresponding author: Vidal Haddad Júnior.
e-mail: haddadjr@fmb.unesp.br
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FIGURE 1: A capuchin monkey (*Sapajus libidinosus*; left) and lacerations inflicted by capuchin monkeys (right) after contact with humans in urban areas.

In addition to acute complications, such as large lacerations and bleeding, monkey bites can lead to severe infections. This study aimed to determine the prevalence of bacterial and fungal species in the buccal microbiota of capuchin monkeys, with an aim to understand the possible infectious agents associated with bites inflicted by these animals, develop a care protocol for such injuries, and train medical teams to act in such emergencies.

METHODS

The project was approved by the Ethics Committee for the Use of Animals of the Faculty of Veterinary Medicine and Animal Science of Botucatu, SP (CEUA/FMVZ/UNESP/SP; Protocol No. 170/2015). It was also approved by the Chico Mendes Institute for Biodiversity Conservation (SISBIO Protocol No. 52117-1) of the Ministry of the Environment, Brazil.

Ten capuchin monkeys (*Sapajus libidinosus*) were captured in Onofre Quinan Environmental Park (Anápolis/GO) using traps made of iron and galvanized wire (measuring 1.60 × 0.80 × 0.80 m). The animals were anesthetized with a combination of tiletamine and zolazepam (4.4 mg/kg) administered intramuscularly[3].

To isolate strict anaerobic bacteria, the gingival sulcus of the teeth was probed to sample a deeper area. After choosing the site, sterile absorbent paper cones were introduced into the gingival sulcus and left in place for 2 minutes (**Figure 2**). Then, the cones were removed and immersed in 2 mL of pre-reduced anaerobically sterilized Ringer's (PRAS) solution[4], under nitrogen gas. To isolate aerobic bacteria and fungi, sterile swabs containing sterile 0.9% saline solution were rubbed on the teeth, gingival region, and hard palate of the animals **(Figure 3)**.

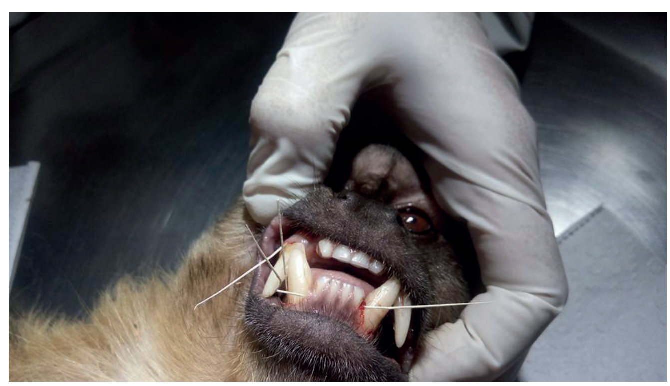

FIGURE 2: Introduction of an absorbent paper cone into the gingival sulcus of *Sapajus libidinosus* to sample anaerobic bacteria.

The swabs were transported at room temperature (20–25°C) to the IPTSP/UFG laboratory and immediately processed[5].

For the isolation and phenotypic characterization of the anaerobic bacteria, the samples were processed in an anaerobic chamber. After homogenization, serial dilutions (10^{-1} to 10^{-4}) were prepared in tubes containing peptone water. Then, 100-µL aliquots of each dilution were plated on *Brucella* agar and *Bacteroides* bile esculin agar. After plating, the media were incubated at 37°C in an anaerobic chamber for 48–72 hours. Bacteria were identified by their biochemical-physiological characteristics according to classification keys[6,7,8].

FIGURE 3: Collection of a sample with a swab from the tooth surface of *Sapajus libidinosus*.

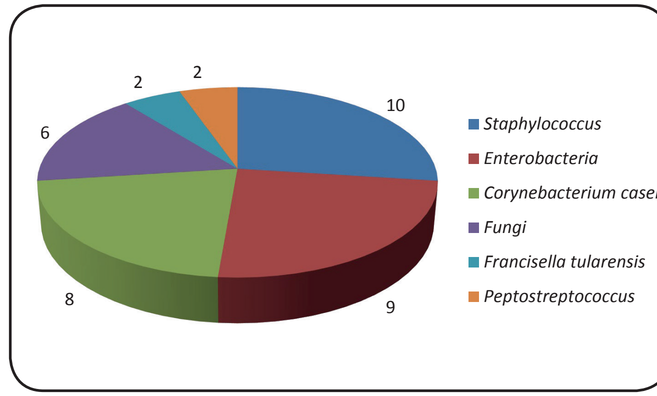

FIGURE 4: Number of animals that were positive for each of the six groups of microorganisms identified in the oral microbiota of *Sapajus libidinosus*.

The isolated bacteria were subjected to antimicrobial susceptibility testing by the agar dilution method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) to obtain the minimum inhibitory concentrations.

For the isolation and phenotypic characterization of aerobic bacteria, the sample contained in the swab was deposited on the surface of the agar and later sowing by exhaustion was performed. Selective media (MacConkey and salted mannitol agar) and enriched culture medium were used, and cultures were incubated at 37°C for 24–48 h. Then, the colonies were identified according to their morphological characteristics and the results of biochemical tests.

All bacteria were subjected to antimicrobial susceptibility testing by the disc diffusion method[9] The isolation and phenotypic characterization of fungi were performed as described previously[10,11,12]. The data were analyzed by Fisher's exact test[13,14].

RESULTS

Several conditions in the oral cavity were observed: the most common was the presence of tartar (80%), tooth wear (60%), and tooth fractures (40%). Using the probe, depths ranging from 0.2 to 0.5 mm were observed, the largest measure was on the vestibular face of the lower left canine.

A total of 111 bacteria and 12 fungi were isolated, including two strict anaerobic bacteria of the genus *Peptostreptococcus*, 109 facultative anaerobic bacteria, and 12 yeasts. Among the isolated microorganisms, six different groups were detected. The number of animals positive for each group of microorganisms is shown in **Figure 4**.

Of the 109 facultative anaerobic bacteria, 38 were enterobacteria, and the commonly isolated species were *Escherichia coli* and *Proteus vulgaris*. Of the 38 bacterial isolates in the genus *Staphylococcus*, *S. cohnii* was the most common species.

For the antibiotic testing, we observed that the tested antibiotics were effective for most enterobacteria, although resistance to tetracycline and ampicillin was detected. *Staphylococci* showed higher resistance to tetracycline, erythromycin, and clindamycin. The other strains were sensitive to the tested antimicrobials. All isolated bacteria were susceptible to cefoxitin.

DISCUSSION

Among the 10 *Sapajus libidinosus* studied in the present study, we noted the presence of tartar (80%) and gingivitis (10%). Due to the diet the monkeys receive in captivity, they appear to be more predisposed to plaque buildup than free-living animals[15]. Although the animals in the Onofre Quinan Environmental Park are free-living, they have an inadequate diet; they feed on not only fruits, insects, and the eggs of wild birds but also food deposited in the trash or taken from the hands of park visitors, such as popcorn, snack foods, bread, soft drinks, and others.

The teeth most affected by tartar were the upper and lower premolars, the upper molars, and the lower and upper canines. No tartar was observed on the incisors. Tartar accumulation on the teeth is mainly due to its proximity to the salivary ducts. A study of tartar in German Shepherd dogs showed that the minerals in saliva are deposited on bacterial plaque, mineralizing it[16]. Then, the surface of the plaque becomes rough, which facilitates greater bacterial accumulation.

The fractures found in the four male animals were most frequently observed in the canine teeth. This could be related to several factors, such as fights in dispute of hierarchical conditions in the troop as well as the longer canine teeth in males than in females, which facilitates dental fracture[17]. The presence of a scar on the lower lip of one male could also be related to fighting.

Anaerobic bacteria were only isolated from animals presenting gingivitis in the lower left canine with a larger gingival sulcus (~0.5 mm). Anaerobic bacteria in orangutans (*Pongo pygmaeus*) was also isolated from subgingival plaque originating from both affected and unaffected regions[18]. However, the study did not report the depth of the gingival sulcus in the sample sites. In the present study, most of the probed grooves were 0.2 mm deep, with no clinical signs of periodontal disease, which may have hindered the isolation of anaerobic bacteria.

Enterobacteria were present in 90% of the evaluated animals, and *E. coli* and *P. vulgaris* were the most common species. The presence of these bacteria is a consequence of the habits of *Sapajus libidinosus*, as they constantly put their contaminated hands, which may contain fecal material, in their mouths, and they often lick each other. *E. coli* are important agents of enteric diseases, specifically diarrhea, and they have been isolated from black lion tamarins (*Leontopithecus chrysopygus*) in captivity and are associated with morbidity and mortality[19]. In addition to the impact that *E. coli* can have on the health of *Sapajus libidinosus*, there is also the risk of transmission to humans in cases of bites.

The isolation of *Staphylococcus* spp. in the present study (in 100% of the animals) presupposes a risk of infection in bites from *Sapajus libidinosus*. Data on the incidence rates of bites inflicted by these animals are scarce, as well as data related to the bacteriological studies of these lesions and the prevalence of injuries caused by non-human primates.

The antibiotic resistance evaluation demonstrates that, in cases of primate bites, it is important to identify any contaminating bacteria as well as to perform antibiotic susceptibility testing, so that the most appropriate treatment can be administered. Because of the high infection rates, all bites should be treated presumptively. For animal bites, the recommendations for prophylactic antibiotics are amoxicillin + clavulanic acid or cephalexin for three to five days[2].

The obtained results identify the possible infectious agents associated with bites from *Sapajus libidinosus*, which are useful for the development of a treatment protocol for infections caused by animal bites in the study region.

The recommended protocol for treating bites from capuchin monkeys includes leaving the lesion open (i.e., without primary closure), whether infected or infected, and to irrigate them copiously. Removal of foreign bodies should also be performed, and the dead tissue should be debrided. Late primary closure can be performed after a few days, if the risk of infection has decreased. Small, uninfected wounds can be allowed to close by secondary intention[3]. In our sample, cefoxitin was the most effective antibiotic for the isolates (100% efficacy); thus, it should be used as the first treatment option for capuchin monkey bites in the study region.

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Conflict of interest: The authors declare that there is no conflict of interest.

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