

Zoonotic potential of oral microbiota of capuchins monkeys (*Sapajus libidinosus*)

Potencial zoonótico da microbiota oral de macacos-prego (*Sapajus libidinosus*)

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Abstract

Sapajus libidinosus is a species of primates, popularly known as capuchin monkey. The oral cavity of the monkeys may have pathogenic microbiota for human. Therefore, the study of the microbiota of these species is of great interest in unique health. The objective of the present article was to carry out a survey of the oral microbiota of *Sapajus libidinosus*, kept in captivity, in the Zoobotanical Park of Teresina (PI, Brazil). Ten chemically immobilized primates were used. Samples were collected from the oral mucosa along the maxillary mucogingival transition, and these samples were sent to culture and microbiological identification. Most of the microorganisms found were Gram negative and *E.coli* was the most frequent, followed by *Enterobacter* spp, *Klebsiella* spp, *Proteus mirabilis*, *Citrobacter* spp., *Morganella morganii*, as well as Gram positive bacteria of the type *Staphylococcus* spp. The data obtained demonstrated the presence of bacterial strains with potential risk of transmission to other animals kept in the park as well as humans in contact with these animals.

Keywords: Microorganisms; Oral cavity; Primate; Unique health; Zoonose.

Resumo

Sapajus libidinosus é uma espécie de primata popularmente conhecido como macaco prego. A cavidade oral de macacos pode ser patogênica para o ser humano. Portanto o estudo da microbiota destas espécies é de grande interesse em saúde única. O objetivo do presente artigo foi realizar um levantamento da microbiota oral da espécie *Sapajus libidinosus*, mantidos em cativeiro, no parque Zoobotânico de Teresina. Foram utilizados 10 primatas imobilizados quimicamente. As amostras foram colhidas da mucosa oral junto à transição muco-gengival maxilar dos animais e encaminhadas para cultivo e identificação microbiológica. A maior parte dos microrganismos encontrados foram Gram negativos sendo que a *E. coli* foi a mais freqüente, seguido por *Enterobacter spp*, acompanhado por *Klebsiella spp*, *Proteus mirabilis*, *Citrobacter spp.*, *Morganella morganii*, bem como bactérias Gram positivas do tipo *Staphylococcus spp*. Os dados obtidos demonstraram a presença de cepas bacterianas com potencial risco de transmissão para outros animais mantidos no parque e também para humanos em contato com esses animais.

Palavras-chave: Microrganismos; Mucosa oral; Primatas; Saúde única; Zoonoses.

Resumen

Sapajus libidinosus es una especie de primate conocido popularmente como mono capuchino. La cavidad bucal de los monos puede ser patógena para los humanos. Por tanto, el estudio de la microbiota de estas especies es de gran interés en la salud única. El objetivo del presente artículo es realizar un levantamiento de la microbiota oral de la especie *Sapajus libidinosus*, mantenidos en cautiverio, en el parque Zoobotánico de Teresina. Se utilizaron 10 primates inmovilizados químicamente. Las muestras fueron recolectadas de la mucosa oral junto a la transición muco-gingival maxilar de los animales y encaminados para cultivo e identificación microbiológica. La mayoría de los microorganismos encontrados fueron Gram negativos, siendo *E. coli* fue la más frecuente, seguida de *Enterobacter spp*, acompañado por *Klebsiella spp*, *Proteus mirabilis*, *Citrobacter spp.*, *Morganella morganii*, así como bacterias Gram positivas del tipo *Staphylococcus spp*. Los datos obtenidos demostraron la presencia de cepas bacterianas con riesgo potencial de transmisión a otros animales mantenidos en el parque y también a humanos en contacto con estos animales.

Palabras clave: Microorganismos; Mucosa oral; Primates; Salud única; Zoonosis.

1. Introduction

Nonhuman primates (NHP) constitute an important group among animals submitted to several studies of general interest. Among these studies, many are designed to assess the potential risks of disease transmission to humans, either in natural habitat or in captivity.

Sapajus libidinosus is a species of primates that belongs to the family *Cebidae* (Salles, et al., 2018), popularly known as capuchin monkey, inserted in the genus *Sapajus* (Salles, et al., 2018), distributed in the regions of Savannah and Caatinga (Brazilian bioma) (Ribeiro, et al., 2015a; Salles, et al., 2018). In a natural environment or in captivity, these animals may carry pathogens that cause zoonoses, especially when there is the possibility of direct contact between humans and animals. Hence, nonhuman primates (PNH) are important for public health and safety (Ministério da Saúde, 2017).

Concern about the creation of NHP is not only related to the risk of transmitting infectious diseases to humans, whether zoonotic or not, but mainly due to traumatic or infectious cutaneous lesions caused by bites of the wild animals. Such bites may also occur in the wild in professionals such as biologists, veterinarians, animal handlers and hunters (Haddad Jr, et al., 2013). Because they are animals that are considered intelligent, have quick reflexes and are physically strong, they are capable of severely assaulting their caretakers, inflicting severe injuries (Silveira & Silva, 2016). Given the severity of these accidents and their increasing frequency in urban centers, research has been carried out to evaluate the PNH microbiota (Aspis, et al., 2003). Besides, animal-bite injuries provide a potentially valuable source of surveillance data on rabies viruses that are transmitted primarily by animal bites (Benevides, et al., 2020). Therefore, the study of the oral PNH microbiota is of great interest.

The objective of the present article was to carry out a survey of the oral microbiota of *Sapajus libidinosus* kept in captivity at the Zoobotanical Park of Teresina (PI, Brazil). This survey is of paramount importance for preventive actions aimed at improving Public Health.

2. Materials and Methods

This study was approved by the animal experiment ethics committee of the Federal University of Piauí with protocol CEUA-UFPI 025/2013. It was authorized by Sisbio (System of Authorization and Information in Biodiversity) with the number 39815/2013.

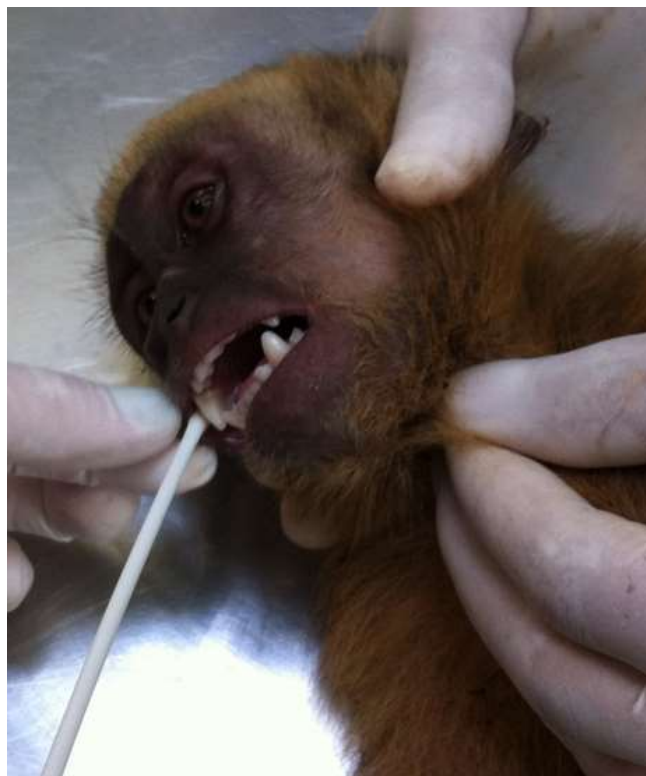
In order to carry out this study, a qualitative field experimentation was carried out as described by Pereira et al. (2018). The data obtained were submitted to descriptive statistics.

In the present research, 10 non-human primates belonging to the *Sapajus libidinosus* species were used (young and adults), kept in the Teresina Zoobotanical Park (State of Piauí, Brazil).

The animals lived in islands and were captured in cat-type cages (used for feral cats). After capture the animals were physically contained using leather gloves. They were weighed and chemically immobilized with anesthetic combination Tiletamine/zolazepan at a dose of 5 mg/kg intramuscularly. Marking of the monkeys was carried out through the implantation of microchips on the dorsal region of the animals (between scapulae).

After the physical examination, the material was collected for microbiological analysis. The procedure consisted of opening the oral cavity of the animals and collecting the sample by means of a swab, with rotating movements for 15 seconds, looking for material of the oral mucosa along the maxillary muco-gingival transition (Figure 1). The presentation of the animals for the collection of the samples was practiced in a random way. The samples were kept refrigerated and sent to the laboratory for culture and microbiological identification.

Figure 1. Specimen of *Sapajus libidinosus* (capuchin monkey), kept in captivity at the Teresina Zoobotanical Park (PI, Brazil), anesthetized to collect of the oral microbiota by swab, looking for material of the oral mucosa along the maxillary muco-gingival transition.



Source: The authors.

Figure 1 shows the collection of material from the oral cavity in the muco-gingival transition region, where bacteria accumulate more frequently.

In the laboratory, in order to investigate aerobic and facultative anaerobic bacteria, the samples were initially inoculated with BHI broth (Brain and heart infusion) incubated at 37°C and, in parallel, at 25°C, with readings in 24-96 hours. The samples cultured in BHI broth were later seeded in 5% sheep blood agar and MacConkey Agar, with incubation of these performed in a similar manner to that described previously for the initial culture.

For the detection of *Staphylococcus* spp, the samples were inoculated into Baird-Parker agar and incubated at 37 ° C for 24-48 hours. In parallel, the samples were also inoculated in tetrathionate broth, followed by sowing in bright green agar and xylose-lysine-tergitol 4 agar (XLT4), with incubating in aerobiose at 37°C with readings in 24-96 hours.

The isolated microorganisms were identified and classified. The microbiological results obtained were not separated in relation to the sex or age group of the animals. The number of bacterial colonies per sample was not quantified. The data obtained were described in an informative table containing the classification and scientific name of the bacteria found.

3. Results and Discussion

The capture of the animals was quiet and safe for the team, and the in cat-type cages used was adequate for such capture. The anesthetic protocol was adequate and sufficient to perform all the necessary procedures for the collection of the microbiota. There were no deaths. This protocol was used by other researchers in the same species, and there were no deaths. However, the authors do not comment on the quality of the anesthesia produced (Ribeiro, et al. 2015a). Dissociative anesthetics such as that used in the animals of the study are undoubtedly the drugs most widely used in the contention of wild animals (Cerejo & Mattos Jr, 2015; Zaniolo, et al., 2018). For this reason, the anesthetic used here was used in other species of wild animals, including monkeys, with good results (Spolti, et al. 2013, Pinheiro, et al., 2014, Dias, et al., 2015; Zaniolo, et al., 2018).

The collect by swab (Figure 1) made it possible to obtain adequate samples of the microbiota, as there was growth of several types of microorganisms (Table 1). In other species the same procedures described herein are used for oral microbiota collection, both in domestic animals (Santos, et al., 2014) and in wild animals (Fornazari, et al., 2007; Silva, et al., 2009; Melo, et al., 2015; Zaniolo, et al., 2018).

After collection of oral material along the maxillary muco-gingival transition of the animals, a microbiological analysis of the samples was performed. Table 1 shows the frequencies of animals colonized by found bacteria, as well as their classification according to Gram.

It was observed that the majority of the microorganisms found were Gram negative and *Escherichia coli* was the most frequent (Table 1). The number of animals that presented this type of bacteria was eight (8/10; 80%). Although it is considered a normal microbiota in the intestine, it is not uncommon to have this microorganism present in the oral cavity of PNH (Zaniolo, et al., 2018; Silva, et al., 2019). In other wild mammals (coatis) this microorganism was also the most frequent in the oral cavity (Fornazari, et al., 2007) and in crocodiles was the second most frequent (Silva, et al., 2009).

Table 1. Microorganisms isolated from the oral mucosa of Capuchin monkeys (*Sapajus libidinosus*) kept in captivity in the Zoobotanic Park of Teresina (PI, Brazil) (N = 10).

	Microorganisms	Number of animals	Percentage of animals
Gram +	<i>Staphylococcus spp</i>	5	50%
Gram -	<i>Escherichia coli</i>	8	80%
	<i>Enterobacter spp</i>	6	60%
	<i>Klebsiella spp</i>	5	50%
	<i>Proteus mirabilis</i>	5	50%
	<i>Citrobacter spp</i>	4	40%
	<i>Morganella morganii</i>	3	30%

Source: The authors

Escherichia coli is one of the microorganisms in which the strains are considered as natural of the intestinal microbiota of the majority of the animals, being considered harmless. However, other types can lead to serious diseases because they are hemorrhagic due to the production of large amounts of toxins, causing severe damage to the intestinal mucosa, especially in young and immunocompromised patients (Ribeiro, et al., 2015b). There is a possibility that the microorganism may reach the urinary tract, causing serious infections, because it is an aggressive pathogen in the urinary tract (Spindola, 2007). Numerous studies have shown that the Gram-negative bacterium *Escherichia coli* is the main agent of urinary tract infection of community origin in humans (infection found or incubated prior to hospital admission), with percentages of isolation ranging from 48.2% to 87.5% of the cases diagnosed of infections urinary tract (IUT) (Resende, et al., 2016). In addition, bacterial

strains of *Escherichia coli* are important agents of enteric diseases, specifically diarrhea, and are often associated with morbidity and mortality in non-human primates kept in captivity. In this way, not only human beings, but also non-human primates can represent a natural reservoir and a source of infection of these bacteria (Carvalho, et al., 2003).

After *E. coli* the most commonly found bacteria were *Enterobacter* spp, seen in six animals (6/10; 60%) (Table 1) Such bacteria are common in oral microbiota of wild animals (Melo, et al., 2015, Carvalho, et al., 2014; Zaniolo, et al., 2018; Silva, et al., 2019; Albuquerque, et al., 2020), including monkeys (Carvalho, et al., 2014; Zaniolo, et al., 2018; Silva, et al., 2019; Albuquerque, et al., 2020). The *Enterobacteriaceae* family includes some of the most important pathogens for humans and animals. With regard to men, these pathogens are among the main agents of hospital infection and undoubtedly constitute the main cause of nosocomial infection in many countries (Trabulsi & Alterthum, 2005). In urocultures of community origin enterobacteria are a frequent cause of IUT in humans (Resende, et al., 2016).

Gram-positive bacteria *Staphylococcus* spp. were observed in five animals (5/10; 50%). These microorganisms are identified in samples from the oral cavity of other monkey species (Silva, et al., 2019; Albuquerque, et al., 2020). Besides, the genus *Staphylococcus* usually occurs on the skin and mucous membranes of mammals and birds. Some researchers have emphasized the function of skin hygiene by *Sapajus apella* monkeys. Probably, the oral aid in cleansing and removal of ectoparasites may interfere with the composition of the oral mucosal microbiota of this species of non-human primates (Parr, et al., 1997, Aspis, et al., 2003). The presence of these microorganisms presupposes risk of an infectious inflammatory process in case of bite by these animals. Bites by monkeys are seen frequently (Haddad Jr, et al., 2013; Benevides, et al., 2020). Many of these bites are due to the fact that these animals are kept as pets even illegally (Haddad Jr, et al., 2013). However, data on the occurrence of bite produced by wild animals are poorly reported, those related to the bacteriological study of the lesions also are in small quantity, and in relation to accidents with non-human primates, there are few records (Aspis, et al., 2003; Benevides, et al., 2020). Bacteria of this group (*Staphylococcus* spp) can also cause IUT mainly in young women (Resende, et al., 2016).

Bacteria from the *Klebsiella* spp group were present in five animals (5/10; 50%). Such microorganisms are isolated in the oral cavity of other species of monkeys (Carvalho, et al., 2014; Silva, et al., 2019; Albuquerque, et al., 2020). *Klebsiella pneumoniae* is frequently associated with lung diseases, meningitis, and other pathological processes in primates. Outbreaks of severe purulent peritonitis and sepsis caused by these bacteria have occurred in

calitriquídeos (small monkeys) (Carvalho, et al., 2014). In humans they may also be responsible for IUT (Resende, et al., 2016).

The bacterium *Proteus mirabilis* was recorded in five animals (5/10; 50%) (Table 1). Such a microorganism has already been isolated from the oral cavity (Albuquerque, et al., 2020) and rectal ampoule of other monkeys (Silva, et al., 2019) and is associated with IUT in humans (Resende, et al., 2016).

In four animals, *Citrobacter* spp were identified. These bacteria are found in the oral microbiota of another species of monkeys (Albuquerque, et al., 2020) and are considered to cause diarrhea, pneumonia, sepsis, nephritis and cystitis in non-human primates (Parr, et al., 1997).

Morganella morganii was identified in three animals (3/10; 30%) (table 1). This microorganism is most commonly found in the oral cavity of snakes (Melo, et al., 2015) and is capable of causing sepsis in crocodiles (Silva, et al., 2009) and subcutaneous abscess in boas (*Boa constrictor*) (Ferreira, et al., 2012).

4. Conclusion

In the present study, the zoonotic potential of the main bacteria belonging to the oral microbiota in capuchin monkeys (*Sapajus libidinosus*) captive in the State of Piauí (Brazil) was evidenced. The research is unpublished in the State and contributes with data still scarce in the national and international literature regarding the specie.

The data generated demonstrated the presence of bacterial strains with potential risk of transmission to other animals kept in the park as well as humans in contact with these animals.

The research demonstrates the need to raise awareness of this problem to all those who live with these animals, in order to influence care management and self-protection.

Other studies may demonstrate the presence of these bacteria in other animals kept in the park, and in humans who have had contact with these animals.

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