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Identification of enterobacteria in free-living nonhuman primates in an urban park in the northern region of the state of Paraná, Brazil

Identificação de enterobactérias em primatas não-humanos de vida livre em um parque urbano na região norte do estado do Paraná, Brasil

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Abstract

Populations of nonhuman primates are often considered to be a link in the chain of emerging infectious diseases, as they are reservoirs for different zoonotic pathogens. The objective of this study was to identify the presence of bacteria from the family Enterobacteriaceae in free-living nonhuman primates. The research was carried out in an urban park located in a city in the northern region of the State of Paraná, Brazil. The animals were captured in Tomahawk-type traps and chemically restrained, being oral and rectal samples collected with sterile swabs. For bacterial isolation, the samples were seeded on MacConkey agar plates and grown under anaerobic conditions. The subsequent identification was conducted using a commercial biochemical kit. Sixteen primates identified as black-capuchin-monkeys (*Sapajus nigritus*) were captured. Seven different enterobacterial species were identified from the oral cavity swabs: six *Escherichia coli* (42.9%), three *Kluyvera* species (21.40%), one *Serratia rubidaea* (7.14%), one *Enterobacter aerogenes* (7.14%), one *Enterobacter cloacae* (7.14%), one *Hafnia alvei* (7.14%), and one *Erwinia herbicola* (7.14%). Seven different species were identified from the rectal swabs: six *Escherichia coli* (40%), three *Kluyvera* species (20%), two *Enterobacter aerogenes* (13.32%), one *Erwinia herbicola* (6.67%), one *Serratia rubidaea* (6.67%), one *Pragia fontium* (6.67%), and one *Edwardsiella tarda* (6.67%). The results indicate that the isolated bacteria belong mainly to the human microbiota and had crossed the interspecific barrier, contaminating the nonhuman primates.

Key words: Gram-negative bacteria. Family Enterobacteriaceae. Monkeys. Microorganisms. *Sapajus nigritus*.

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Resumo

As populações de primatas não humanos frequentemente são consideradas um elo na cadeia de doenças infecciosas emergentes, por constituírem reservatórios que propiciam o surgimento de diferentes patógenos zoonóticos. O objetivo deste trabalho foi identificar a presença de bactérias da família *Enterobacteriaceae* em primatas não humanos de vida livre. O estudo foi realizado em um parque urbano localizado em uma cidade da região norte do Estado do Paraná. Os animais foram capturados em armadilhas do tipo Tomahawk e submetidos a contenção farmacológica para colheita de amostras da microbiota oral e retal com zaragoas estéreis. Para o isolamento bacteriano as amostras foram semeadas pela técnica de esgotamento em placas contendo ágar MacConkey, com posterior identificação por testes bioquímicos utilizando *kit* comercial. Foram capturados 16 primatas não humanos identificados como *Sapajus nigritus* (macaco-prego). Na cavidade oral foi possível identificar sete diferentes espécies de bactérias, sendo seis (42,9%) *Escherichia coli*, três espécies de *Kluyvera* (21,40%), uma (7,14%) *Serratia rubidae*, uma (7,14%) *Enterobacter aerogenes*, uma (7,14%) *Enterobacter cloacae*, uma (7,14%) *Hafnia alvei* e uma (7,14%) *Erwinia herbicola*. No reto foi possível identificar sete diferentes espécies de bactérias, sendo seis (40%) *Escherichia coli*, três espécies de *Kluyvera* (20%), duas (13,32%) *Enterobacter aerogenes*, uma (6,67%) *Erwinia herbicola*, uma (6,67%) *Serratia rubidae*, uma (6,67%) *Pragla fotium* e uma (6,67%) *Edwardsiella tarda*. Os resultados indicam que as bactérias isoladas são pertencentes principalmente à microbiota humana, e estão ultrapassando a barreira interespecífica e contaminando os primatas.

Palavras-chave: Bactérias Gram-negativas. Família Enterobacteriaceae. Macacos. Microrganismos. *Sapajus nigritus*.

Introduction

Species belonging to the order Primates have remarkable behavioral, phenotypic, and genotypic variation, and this includes the endocrine and reproductive aspects. Black-capuchin-monkeys are endemic neotropical primates in the Brazilian Atlantic Forest. They are distributed from the right bank of the Doce River, in Minas Gerais, to the north of Rio Grande do Sul, being bounded on the west by the Paraná River (VILANOVA et al., 2005).

Taxonomically, “black-capuchin” is a denomination that includes two genera, *Cebus* and *Sapajus* (LYNCH-ALFARO et al., 2012). Species with a characteristic physical robustness, presence of hair tufts on the top of the head, and a brown or dark gray coat with the ventral parts tending to be more reddish in color were allocated to the genus *Sapajus*, except for the populations of the southern region of Brazil, which are black in coat color and may represent a taxonomic group distinct from their northern counterparts (*Sapajus libidinosus*). Males and females are between 42 and 56 cm and 42 and

48 cm in length, respectively, with a tail of up to 56 cm. The weight of the animals can vary between 2.6 and 4.8 kg (LYNCH-ALFARO et al., 2012).

These animals generally have an omnivorous diet and great behavioral plasticity, giving them the capacity to subsist in anthropic environments (BERNARDO; GALETTI 2004; LUDWIG et al., 2006; ROCHA, 2000).

An important effect observed in the fragmentation of habitats is the high density of primates present in small forest fragments (BERNARDO; GALETTI, 2004; CHIARELLO, 2003; JARDIM, 2005; VAN BELLE, 2006). In the long term, these fragments become insufficient to house and maintain viable populations, compromising the survival of the animals in concert with the deleterious effects of inbreeding and the impossibility of dispersion to other fragments.

Populations of primates are often considered to be a link in the chain of emerging infectious diseases, as they are reservoirs for different zoonotic pathogens (CORTE et al., 2005; DASZAK et al., 2004;

GARCIA et al., 2005). In public health, the concern over nonhuman primates is not only the risk of them transmitting zoonotic infectious diseases to humans, but also the lesions caused by their bites. Because they are animals that are considered intelligent and have quick reflexes and great physical strength, their attacks can be severe (SATTERFIELD; VOSS, 1987). In general, the oral cavity and teeth of monkeys constitute a hyperseptic medium, cultivating highly polymorphic saprophytic and pathogenic microorganisms, as well as enzymes and biological degradation products that are often toxic (DORTU, 1978). Such microorganisms form the natural microbiota and have great importance for the normal functioning of the organs of these animals (MURRAY et al., 2014). These microorganisms are a consequence of the animals' diets, which ranges from varied fruits to small rodents and insects, or of their constant habit of putting their dirty and possibly fecal-contaminated hands in their mouth (ASPIS et al., 2003).

As these animals have direct contact with humans, they can transfer Gram-positive and Gram-negative bacteria through their bite and feces. Fecal materials are dispersed in the environment and found in places where there are wild or domestic animals, human waste, or any surfaces with fecal contamination. Bacteria can survive for a long time in moist soil, water, feces, food, and organic matter surfaces, and therefore, contamination can occur by different means (NUNES et al., 2010).

Of the bacteria found in nonhuman primates, the most important are the enterobacteria belonging to the family Enterobacteriaceae, which are ubiquitous microorganisms found in soil, water, and vegetation in any part of the world. These microorganisms are part of the normal microbiota of most animals, including humans. They cause a variety of diseases in humans, including one third of all cases of bacteremia, more than 70% of urinary tract infections, and many intestinal infections. Some microorganisms such as *Salmonella enterica* serotype Typhi, *Shigella* species, and *Yersinia*

pestis are always associated with human diseases, whereas others such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* are commensals (i.e., members of the normal microbiota) that can cause opportunistic infections (BRITO et al., 2014; CRUZ et al., 2017; LOUREIRO et al., 1985).

Data from population surveys and census of primate populations provide information on the socioecological differences of the species and are an important tool in determining conservation priorities (GANZHORN et al., 1996), supporting debates, and developing species conservation strategies.

The main justification for the conservation or biomedical scientific research of nonhuman primates is their considerable anatomical, physiological, endocrine, and behavioral similarity to humans. In addition to these similarities, they also share the susceptibility to various infectious agents capable to cross interspecific barriers (JOSLIN, 2003). For this reason, the objective of this study was to identify Enterobacteriaceae bacteria in nonhuman primates living in an urban park in the northern region of the State of Paraná, Brazil. The study was carried out in partnership with a research group that had been formed to manage and control this population of nonhuman primates following reports of several occasions of home invasion and food theft committed by these animals, as well as cases of park visitors being bitten that were reported to local health services.

Materials and Methods

Place of study

This study was carried out in a municipal urban park located in a city in the northern region of the State of Paraná, Brazil, from 2014 to 2015. The park has an area of 47.3 hectares of semi-deciduous seasonal forest, and wild, exotic, and synanthropic animals. The most common species is the free-living *Sapajus nigritus* (MARÓSTICA, 2010).

Animal capture and sample collection

The primates were captured in Tomahawk-type traps distributed inside the park and checked every day. The baits were composed of elements of the animals' usual diet, such as fruits and eggs. Once trapped, the animals were captured with a hand net and anesthetized by a single-dose intramuscular injection of the association called "ZAX-50", prepared with a combination of tiletamine and zolazepam (Zoletil-50®, Virbac do Brasil, São Paulo, Brazil), xylazine (Rompun®, Bayer S.A., São Paulo, Brazil), and atropine (Atropina 1%, Farmagropecuária S.A., Mairiporã, Brazil) in the same syringe or dart. The doses of each item were calculated by interspecific allometric scaling', based on the usual indication for domestic dogs, according to the indication of Pachaly et al. (2011) for Cercopithecinae nonhuman primates.

The animals were submitted to physical examination and therefore the collection of oral and rectal samples was conducted using sterile swabs containing Aimes medium with activated charcoal (Transystem™, Copan Italia, Brescia, Italy). For rectal sampling, the swabs were introduced into the rectal ampulla of each primate, compressing it with rotating movements. For oral sampling, the swabs were inserted into the oral cavity, swabbing gums and tongue with circular and rotating movements.

The collected samples materials were refrigerated and sent to the Laboratory of Preventive Veterinary Medicine and Public Health of the Post-Graduate Program in Animal Science with Emphasis on Bioactive Products of the Universidade Paranaense (UNIPAR, Umuarama, Brazil) for bacterial isolation and identification.

Diagnostic tests

Bacterial isolation

The swabs containing rectal and oral samples were placed in tubes containing 3.0 mL of brain heart infusion (BHI) medium and incubated in an

oven for 24 h at 37°C. After this period, the samples were seeded on MacConkey agar plates and on base agar containing 5% defibrinated sheep blood, and incubated in an oven for 24 h at 37°C under anaerobic condition (QUINN et al., 2005).

The isolated bacterial colonies were inoculated into 3.0 mL of BHI medium and incubated in an oven for 24 h at 37°C. After this period, the cells were centrifuged for 15 min and the supernatant was discarded. The cells were resuspended in 2.0 mL of BHI with 10% glycerol, homogenized, aliquoted into two containers, and stored at -20°C.

Biochemical identification of isolated bacteria

The biochemical identification of bacteria belonging to family Enterobacteriaceae was carried out using a specific enterobacterial identification kit (NewProv®, Paraná, Brazil), according to the manufacturer's recommendations. The samples were subjected to the following tests, recommended by Quinn et al. (2005): L-triphosphane deamination; gas production from glucose; hydrogen sulfide production; lysine and/or ornithine decarboxylation; motility; indole; and rhamnose, citrate, glucose, fructose, and lactose fermentation.

Results

Fourteen male (87.50%) and two female (12.50%) nonhuman primates identified as *Sapajus nigritus* were captured, all of them young adults weighing between 1.7 and 3.8 kg.

Two swab samples (one oral and one rectal) were collected from each animal, totaling 32 samples of which enterobacteria were found on 11 oral and 11 rectal swabs (31.82%). Seven types of bacteria were isolated in five samples (31.82%) (Table 1).

Table 1. Identification of species of the family Enterobacteriaceae in oral cavity and rectal swab samples from 16 *Sapajus nigritus* individuals captured in an urban park in a city in the northern region of the State of Paraná, Brazil, 2017.

Sample	Identified Species Oral Cavity	Identified Species Rectum
1	<i>Kluyvera</i> species	<i>Serratia rubidaea</i> <i>Enterobacter aerogenes</i>
2	<i>Enterobacter aerogenes</i>	<i>Edwardsiella tarda</i> <i>Kluyvera</i> species
3	<i>Hafnia alvei</i> <i>Escherichia coli</i>	<i>Kluyvera</i> species <i>Enterobacter aerogenes</i>
4	<i>Escherichia coli</i>	<i>Escherichia coli</i>
5	<i>Serratia rubidaea</i> <i>Escherichia coli</i>	<i>Pragia fontium</i> <i>Escherichia coli</i>
6	<i>Escherichia coli</i>	<i>Escherichia coli</i>
7	<i>Erwinia herbicola</i> <i>Enterobacter cloacae</i>	<i>Erwinia herbicola</i>
8	<i>Kluyvera</i> species	<i>Kluyvera</i> species
9	<i>Kluyvera</i> species	<i>Escherichia coli</i>
10	<i>Escherichia coli</i>	<i>Escherichia coli</i>
11	<i>Escherichia coli</i>	<i>Escherichia coli</i>

Seven different species of enterobacteria were identified in the oral cavity swabs: six *Escherichia coli* (42.9%), three *Kluyvera* species (21.40%), one *Serratia rubidaea* (7.14%), one *Enterobacter aerogenes* (7.14%), one *Enterobacter cloacae* (7.14%), one *Hafnia alvei* (7.14%), and one *Erwinia herbicola* (7.14%).

Seven different species of bacteria were identified in the rectal swabs: six *Escherichia coli* (40%), three *Kluyvera* species (20%), two *Enterobacter aerogenes* (13.32%), one *Erwinia herbicola* (6.67%), one *Serratia rubidaea* (6.67%), one *Pragia fontium* (6.67%), and one *Edwardsiella tarda* (6.67%).

The same microorganisms was isolated in both areas in seven individuals (63.63%) (Table 1).

Discussion

There are not many studies related to the investigation of enterobacteria in free-living nonhuman primates. For this reason, the findings in this study have epidemiological relevance in obtaining data on the socioecological differences of the species, which are important for determining conservation priorities (GANZHORN et al., 1996), supporting debates and developing of species conservation strategies.

The bacteria of the Enterobacteriaceae family are grouped into three categories: main pathogens, causing enteric and systemic diseases; opportunistic pathogens, occasionally causing clinical diseases outside the alimentary tract; and nonpathogens (QUINN et al., 2005). Epidemiological studies on different species of wild animals, such as raccoons, collared peccaries, ocelots (GOMES et al., 2011; LIMA et al., 2012; SILVA et al., 2016), and nonhuman primates mostly living in captivity

(RAMALHO et al., 2015; VALE; AGUIAR, 2012), were carried out to investigate the occurrence of enterobacteria, considering their possibility of compromising human, animal, and environmental health, i.e., One Health. Thus, it is important to study the microbiota of the captured monkeys, mainly because of the contact they have with park visitors.

This is the first study of this kind carried out on free-living *Sapajus nigritus* living in an urban park of the northern region of Paraná. The idea of investigating enterobacteria in these animals arose from the possibility of their direct interaction (or indirect contact via the environment) with park visitors, who include public cleaning workers, people who go on walks or take part in zipline or capoeira activities, musicians, elementary and high school students on environment class trips, among others. The possible interaction with these animals, or with the environment where they live, could cause health disorders in humans as well as in other animal species.

This research identified 41.38% of the isolated bacteria as being *Escherichia coli*. A similar result was found by Pinheiro and Rodrigues (2014) in free-living black-striped capuchin monkeys (*Cebus libidinosus*) living at a zoobotanical park in Teresina (PI), where *Escherichia coli* was isolated in 80.00% of the oral swab samples. In another study, Vale and Aguiar (2012) isolated *Escherichia coli* in 41.00% of the rectal swab samples from captive Callitrichid primates from the National Primate Center. Although this microorganism is part of the normal microbiota of the gastrointestinal tract of warm-blooded animals, it is the main cause of urinary tract infections and responsible for hospital infections, including sepsis and meningitis. Its presence in food, water, soil, solutions, and instruments indicate fecal contamination of those sources (JORGE, 2012). Primates feed on fruits, small rodents, and insects, and have the constant habit of putting their dirty and possibly fecal-contaminated hands in their mouth (ASPIS, 2003), a behavior that can justify the

high rate of *Escherichia coli* found in the researched subjects.

In this study, *Escherichia coli* (71.44%), *Erwinia herbicola* (14.28%), and *Kluyvera* species (14.28%) were detected in oral and rectal swabs of seven individuals (63.33%). This can be explained by the grooming habits of primates, which is a natural behavior involving the cleaning of different body surfaces. Its primary function is hygiene, but it can also be equally important as a social display behavior, and these animals spend most of their time in grooming activities (HUTCHINS; BARASH, 1976; STAMMBACH KUMMER, 1982; SCHINO, 1988; SUSSMAN et al., 2005). This behavior can be displayed on themselves (self-grooming) or on others in their group (alogrooming). Therefore, it is possible that the researched animals had oral \times rectal or rectal \times oral self-cross-contaminations, or bacterial transmission during alogrooming, resulting in the same bacterial species being detected in both oral and rectal swabs (HUTCHINS; BARASH, 1976).

The second most prevalent microorganisms were *Kluyvera* species, detected in 20.69% of the oral and rectal swab samples. Andrade et al. (2010) detected different microorganisms, including *Kluyvera* species, in primates of the species *Macaca fascicularis* at the Center for Laboratory Animal Breeding (CECAL/FIOCRUZ), in Rio de Janeiro (RJ). These bacteria are part of the normal human digestive tract microbiota and can be found in the environment as free-living organisms in water, soil, sewage, hospital sinks, and food products of animal origin (POSSUELO; RENNER 2016). Our results demonstrate that humans and primates are sharing susceptibility to different pathogens that can cross interspecific barriers (JOSLIN, 2003), most likely caused by inadequate sanitary management (CYSNE, 2007) in the studied area.

Microorganisms of the genus *Enterobacter* (*E. aerogenes* and *E. cloacae*) are classified as opportunistic (QUINN et al., 2005). These bacteria

were already detected by Loureiro et al. (1985) and Ramalho et al. (2015) in captive *Sapajus apella* and *Sapajus flavius* primates, respectively, their presence being possibly due to the hygiene and social habits of the animals.

Serratia rubidaea is detected mainly in humans with severe trauma or underlying diseases, including sepsis, bacteremia, and urinary tract infections (BONNIN et al., 2015), but it has also been found in black-lion-tamarins in Rio de Janeiro (CARVALHO et al., 2014).

The other enterobacteria isolated in this study (*Edwardsiella tarda*, *Hafnia alvei*, *Erwinia herbicola*, and *Pragia fontium*) are not commonly related to the normal microbiota of *Sapajus nigritus*. However, the appearance of these species in the researched animals can possibly be related to park visitors feeding them foods that are not part of their natural diets. This factor may have contributed to the appearance of metabolic and nutritional diseases in local wildlife animals, such as diabetes, obesity, and caries, indicating changes in the microbiota as a possible cause (OLIVEIRA et al., 2015).

Another concern related to microorganisms is bites that visitors can sustain while feeding the primates. These lesions may be the gateway to infections caused by microorganisms found in the oral microbiota of these animals. As there are few studies on this microbiota, they are suspected to carry antimicrobial-resistant bacteria, which would result in serious economic and social consequences to humans.

Conclusions

The results indicate that the isolated bacteria belong mostly to the human microbiota and that they can cross the interspecific barrier and contaminate nonhuman primates. These primates have hygiene habits that facilitate their interaction with bacteria from the environment and from their excreta, contaminating their oral cavity with fecal and environmental residues.

Environmental education measures should be implemented for the park visitors and the local population who live near the area, such as advising visitors to not feed the animals, keeping garbage containers closed, avoiding closer contact with these animals, and, most importantly, keeping these animals from having contact with human residues. Future studies would be needed to elucidate the possible bacterial contamination among different animal species, humans, and the environment, to propose more effective strategies for human and environmental health preservation.

Ethical Aspects

This project was approved by the Committee for Ethics in Animal Research (CEPEEA) of the Universidade Paranaense (UNIPAR) under protocol number 28437, and by the Chico Mendes Institute for Biodiversity Conservation (ICMbio) under protocol number 44345-1.

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