Nasal, oral and rectal microbiota of Black lion tamarins (*Leontopithecus chrysopygus*)

Vania M. Carvalho¹, Ralph E.T. Vanstreels², Cátia D. Paula², Cristiane K.M. Kolesnikovas³, Maria Christina C. Ramos⁴, Selene D. Coutinho¹, Cristiana S. Martins⁵, Alcides Pissinatti⁶,⁷,⁸, José L. Catão-Dias²

¹Laboratório de Biologia Molecular e Celular, Faculdade de Medicina Veterinária, Universidade Paulista, São Paulo, SP, Brazil.
²Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brazil.
³Associação R3 Animal, Florianópolis, SC, Brazil.
⁴Lab&Vet Diagnóstico e Consultoria Veterinária Ltda, São Paulo, SP, Brazil.
⁵Instituto de Pesquisas Ecológicas, Nazaré Paulista, SP, Brazil.
⁶Centro de Primatologia do Rio de Janeiro, Guapimirim, RJ, Brazil.
⁷Centro Universitário Serra dos Órgãos, Teresópolis, RJ, Brazil.
⁸Centro Universitário Plínio Leite, Niterói, RJ, Brazil.

Submitted: November 22, 2013; Approved: April 17, 2014.

Abstract

Black lion tamarins (*Leontopithecus chrysopygus*) are endangered callithrichids. Their conservation may require future translocations or reintroductions; however these approaches involve risks of pathogen introduction in the environment and stress-related opportunistic infections in these animals. In order to screen for opportunistic and potential pathogenic bacterial and fungal microbiota, ten free-ranging and ten captive Black lion tamarins were studied and the results compared. Nasal, oral and rectal swabs were collected and cultured for aerobic and facultative anaerobic bacteria and fungi, and a total 203 bacterial and 84 fungal isolates were obtained. Overall, the most frequent organisms were *Staphylococcus* spp., *Bacillus* spp., *Candida* spp. and *Aspergillus* spp. Microbiota of free-ranging and captive animals were similar in composition. A number of potentially pathogenic organisms were identified, emphasizing the importance of microbiological screening in future translocation or reintroduction conservation management programs.

Key words: microbiota, bacteria, fungi, black lion tamarins, *Leontopithecus chrysopygus*.

Introduction

Black lion tamarins (*Leontopithecus chrysopygus*) are small Neotropical and endangered callithrichids (Kierulf, 2008). This species originally occurred in large Atlantic forest areas of the São Paulo State, Brazil; however, due to factors such as deforestation and fragmentation, the remaining free-ranging animals are now limited to a few forest fragments scattered throughout the state (Coimbra-Filho, 1970, 1976, Valladares-Pádua and Cullen Jr, 1994, Kierulf, 2008, Kleiman & Rylands, 2008).

The total wild population is estimated at about 1000 animals spread through 11 isolated forests, only one population of which is clearly viable, the Morro do Diabo State Park. The remaining 10 isolated populations are too small to be viable in the mid- to long-term, but if managed as a metapopulation they could represent a significant genetic stock for the species conservation (Holst et al., 2006). In parallel, captive propagation is a possibility to establish breeding programs followed by reintroduction in the wild, similarly to the successful reintroduction program devel-
oped for the Golden lion tamarin, *Leontopithecus rosalia* (Beck *et al.*, 1991).

These approaches of population management, however, pose the dilemma of moving individuals among wild populations and from the wild to captivity. The distress from such events has been shown to result in stress-induced immunosuppression that may predispose the animals to infectious and parasitic diseases (Beck *et al.*, 1993, Acevedo-Whitehouse and Duffus, 2009). In addition, moving animals among multiple environments increases the possibility of introducing pathogens in new areas adding risks to the population management process (Cunningham, 1996, Daszak *et al.*, 2000).

Information on the microbiota of callithrichids is scarce and limited to captive animals. To our knowledge the only report on this topic for the genus *Leontopithecus* was made by Moraes *et al.* (2004) who investigated the fungal vaginal microbiota of captive lion tamarins (*L. chrysomelas*, *L. chrysopygus* and *L. rosalia*). Considering the lack of information on the *Leontopithecus* microbiota, the aim of this study was to screen for opportunistic and potential pathogenic aerobic and facultative anaerobic bacterial and fungal microbiota from the oral and nasal cavities and rectum of asymptomatic free-ranging and captive Black lion tamarins.

### Materials and Methods

Twenty Black lion tamarins were studied in Brazil between 1997 and 1998, of which ten individuals were free-ranging (FRT) in two different governmental conservation areas (three from Fazenda Rio Claro, Lençóis Paulista; seven from Morro do Diabo State Park) and ten individuals were captive (CPT) at the Rio de Janeiro Primate Center (CPRJ). Morro do Diabo State Park is an area with limited human activity or tourism whereas Fazenda Rio Claro is a private reserve. In both areas there is extensive human and farming activity in the surroundings, and it is likely these animals had direct or indirect contact with humans and/or anthropogenic products.

The FRT were regularly monitored by radiotelemetry by the Instituto de Pesquisas Ecológicas (IPÊ - Institute for Ecological Research) as part of an on-going ecological research program. After their localization the tamarins were followed until sunset, when the group chose a tree hollow in which to spend the night. In the following morning before dawn, the tree hollow entrance was blocked and the animals were captured by sawing holes laterally to the resting chamber. The CPT were caught within their enclosure using nets, according to routine procedures established at the institution. All animals showed no evident clinical signs at physical exam and received no medications in the months prior to the study.

Once physically restrained, the tamarins were transferred to individual cages and submitted to an intramuscular association of ketamine 11mg/kg and atropine sulphate 0.044mg/kg. For nasal and oral cavities, sterile urethral swabs were introduced in the nostrils or rubbed in the gum, palate, teeth and tongue. For rectal swabs, the perianal area was cleaned with gauze and sterile saline and a sterile urethral swab was delicately introduced about one centimeter into the rectum through rotation movements. Immediately after sampling, the swab was placed in Stuart transport medium and kept under refrigeration. All procedures were approved by the Brazilian Institute of the Environment and Natural Renewable Resources.

Samples were first cultivated in BHI broth, Blood agar and Mac Conkey agar plates, which were incubated under aerobic conditions at 37 °C. After 24 h, the broth was seeded again in Blood agar and Mac Conkey agar plates. The plates were observed after 24, 48 and 72 h, and disposed after one week of observation. The swabs were also seeded into Sabouraud agar with cloramphenicol 100mg/mL, kept at room temperature and analyzed daily for 30 days. Imprints of isolated bacteria and yeast were Gram-stained and morphologically characterized. Gram positive cocci and Gram negative bacteria were submitted to the catalase and oxidase production tests, respectively. Thereafter, bacteria and yeast were identified biochemically through the use of API®System (Biomérieux). The yeasts also had their filamentation capability tested (Koneman and Roberts, 1990). The identification of mycelial fungi were based on macro and microscopic morphology achieved through the Hiddel method, and according to Barnett and Hunter (1998).

Fisher’s exact test was used to compare the distribution of bacterial groups (Gram positive or Gram negative) and fungal groups (yeast or mycelial fungi) between CPT vs. FRT, as well as between free-ranging tamarins sampled at Fazenda Rio Claro vs. Morro do Diabo State Park. The Kruskal-Wallis test was used to compare the bacterial and fungal recovery rates (isolates retrieved / individuals examined) between different anatomical sites (nasal, oral or rectal; df = 2). Significance level was 0.05 for all tests. It should be clear that whenever the term prevalence is used, it refers to apparent prevalence (total isolates of bacteria or fungi / total individuals examined).

### Results

A total of 203 bacterial isolates were obtained, representing 12 Gram positive and 14 Gram negative genera (*Actinomycetale bacteria were not identified down to genus*); 3 bacterial isolates were not identified successfully. A total 84 fungal isolates were obtained, with 4 genera of yeasts and 14 genera of mycelial fungi (*Mycelia sterilia fungi were not identified down to genus*); one yeast isolate could not be identified. Due to field contamination, oral swabs from 4 captive tamarins were not cultured for bacteria. Figure 1 summarizes the aerobic bacterial and fungal genera isolated from the nasal, oral and rectal swabs of free-ranging (FRT) and captive (CPT) tamarins. Figure 2

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**Figure 1:** Summary of bacterial and fungal genera isolated from nasal, oral and rectal swabs of free-ranging (FRT) and captive (CPT) Black lion tamarins.

**Figure 2:** Distribution of bacterial and fungal isolates between free-ranging (FRT) and captive (CPT) Black lion tamarins.
presents details on the combination of bacterial and fungal isolates found in each individual.

In none of the three anatomical sites there were significant differences (all \( p > 0.05 \)) on the proportion of bacterial groups (Gram positive or Gram negative) or fungal groups (yeast or mycelial fungi) between the isolates from CPT and FRT, nor between tamarins from Fazenda Rio Claro (FRC) and from Morro do Diabo State Park (MDSP).

| Bacteria         | Gram + | FRT  | CPT  | FRT  | CPT  |
|------------------|--------|------|------|------|------|
| Actinomycetale   | 5 [5]  | 3 [3]| -    | -    | 2 [2]*|
| Aerococcus       | -      | 2 [2]| 1 [1]*| 1 [1]|
| Bacillus         | 13 [9] | 11 [10]| 8 [8]| 4 [4]*| 5 [5]|
| Corynebacterium  | 4 [4]  | 4 [3]| -    | -    | -    |
| Enterobacter      | -      | -    | -    | 1 [1]|
| Enterococcus     | -      | -    | 1 [1]|
| Lactobacillus    | -      | -    | -    | 1 [1]|
| Lactococcus      | -      | -    | 2 [2]| 1 [1]*|
| Micrococcus      | 1 [1]  | 1 [1]| -    | 1 [1]*|
| Staphylococcus   | 16 [9] | 20 [10]| 4 [4]| 5 [5]*| -    |
| Streptococcus    | 1 [1]  | 6 [6]| 5 [4]*| 2 [2]|
| Unidentified     | -      | -    | 1 [1]| 1 [1]*|

| Fungi Yeast      | Candida| 8 [8]| 10 [5]| 2 [2]| 1 [1]| 1 [1]*| 7 [7]|
|------------------|--------|------|------|------|------|-------|-------|
| Geotrichum       | -      | -    | -    | -    | 2 [2]|
| Kloeckera        | -      | -    | -    | -    | 1 [1]|
| Trichosporon     | -      | -    | 1 [1]| -    | -    |
| Unidentified     | -      | -    | -    | -    | 1 [1]|

| Mycelium         | Acremonium | 3 [3]| 3 [3]| 5 [5]| -    | 1 [1]|
|------------------|------------|------|------|------|------|-------|
| Aspergillus      | 1 [1]      | 1 [1]| -    | -    | -    | -     |
| Cladosporidium   | -          | -    | 1 [1]| -    | -    | -     |
| Cylindrocarpon   | -          | -    | -    | -    | -    | -     |
| Fusarium         | 1 [1]      | -    | -    | 1 [1]| -    | -     |
| Glicolodium      | -          | -    | 1 [1]| -    | -    | -     |
| Helminthosporium | 1 [1]      | -    | -    | -    | -    | -     |
| Mycelia sterilis | 2 [2]      | 1 [1]| -    | -    | -    | -     |
| Macor            | -          | -    | -    | 1 [1]| -    | -     |
| Paeosollomyces    | 3 [3]      | -    | -    | -    | -    | -     |
| Penicillium       | 3 [3]      | 4 [4]| -    | 2 [2]|
| Pseudallescheria  | 1 [1]      | -    | -    | -    | -    | -     |
| Trichoderma      | 2 [2]      | 3 [3]| -    | 1 [1]| -    | -     |
| Verticillium     | 2 [2]      | -    | -    | -    | -    | -     |

| Total Bacteria   | Gram +   | 40 [10]| 39 [10]| 26 [10]| 20 [6]*| 11 [8]| 10 [7]| 100% [100%]| 93% [100%]| 72% [100%]| 59% [100%]| 44% [80%]| 33% [70%]|
|------------------|----------|--------|--------|--------|--------|--------|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Gram -           | 0 [0]    | 3 [3]| 10 [7]| 9 [4]*| 14 [10]| 20 [9]|
| Fungi Yeast      | Candida  | 8 [8]| 10 [5]| 3 [3]| 1 [1]| 3 [3]| 9 [8]| 30% [80%]| 48% [50%]| 43% [30%]| 9% [10%]| 60% [30%]| 92% [80%]|
| Mycelium         | 19 [8]   | 11 [7]| 4 [4]| 10 [7]| 2 [2]| 2 [2]| 70% [80%]| 52% [70%]| 57% [40%]| 91% [70%]| 40% [20%]| 18% [20%]|

* Due to field contaminations, samples from only six individuals were cultivated.

Figure 1 - Aerobic and facultative anaerobic bacteria and fungi genera retrieved from the nasal, oral and rectal cavities (number of isolates [number of individuals with the isolates]) of ten free-ranging (FRT) and ten captive (CPT) Black lion tamarins.
| Bacteria | Fungi |
|----------|-------|
| **Nasal cavity** | |
| F01 7a FRC | Bacillus sp., Staphylococcus intermedius | Candida guilliermondii; Cladosporium sp., Fusarium sp., Penicillium sp., Trichoderma sp. |
| F02 7a FRC | Bacillus sp., Micrococcus varians, Strepococcus oralis | Candida sp.; Micaela sterilis, Penicillium sp., Pseudoallescheria sp., Verticillium sp. |
| F03 7a FRC | Bacillus sp., Staphylococcus aureus, S. intermedius, S. xylosus | Candida parapsilosis; Aspergillus sp. |
| F04 7a MDSP | Actinomycetace, Corynebacterium sp., Bacillus sp. (2 strains), Staphylococcus aureus, S. capitis S. xylosus | Candida lusitaniae; Paecilomyces sp., Trichoderma sp. |
| F05 7a MDSP | Actinomycetace, Bacillus sp. (2 strains), Staphylococcus xylous | Candida guilliermondii; Micella sterilis |
| F06 7a MDSP | Staphylococcus aureus, S. xylosus | Candida guilliermondii; Paecilomyces sp., Penicillium sp. |
| F07 7a MDSP | Actinomycetace, Bacillus sp., Corynebacterium sp., Staphylococcus xylous | Candida tropicalis; Aspergillus sp., Paecilomyces sp. |
| F08 7a MDSP | Bacillus sp. (2 strains), Staphylococcus xylous | |
| F09 7a MDSP | Actinomycetace, Bacillus sp., Corynebacterium sp., Staphylococcus xylous, S. scirri, S. xylosus | |
| F10 7a MDSP | Actinomycetace, Bacillus sp. (2 strains), Corynebacterium sp., Staphylococcus xylous | |
| C01 7a CPRJ | Bacillus sp., Staphylococcus cohnii, S. lentus, S. xylosus | Trichoderma sp. |
| C02 7a CPRJ | Actinomycetace, Bacillus sp. (2 strains), S. xylosus | Micella sterilis |
| C03 7a CPRJ | Actinomycetace, Bacillus sp., Staphylococcus lentus, S. xylosus; Octobactrum anthrophi | Cladosporium sp., Penicillium sp., Trichoderma sp. |
| C04 7a CPRJ | Actinomycetace, Bacillus sp., Corynebacterium sp., Micrococcus varians, Staphylococcus scirri, S. xylosus | Candida fatamata, C. guilliermondii; Penicillium sp. |
| C05 7a CPRJ | Bacillus sp., Staphylococcus saprophythicus, S. xylosus; Klebsiella oxytocca | Candida fatamata, C. humicola, Cylindrocarpon sp., Penicillium sp. |
| C06 7a CPRJ | Bacillus sp., Staphylococcus capitis, S. cohnii, S. scirri; Klebsiella oxytocca | Candida fatamata, C. guilliermondii; C. humicola; C. parapsilosis |
| C07 7a CPRJ | Bacillus sp., Corynebacterium sp., Staphylococcus scirri, S. xylosus | |
| C08 7a CPRJ | Bacillus sp., Corynebacterium sp. (2 strains), Staphylococcus scirri, S. xylosus | |
| C09 7a CPRJ | Bacillus sp., Staphylococcus scirri | |
| C10 7a CPRJ | Bacillus sp., Staphylococcus scirri, S. xylosus | |
| **Oral cavity** | |
| F01 7a FRC | Bacillus sp., Lactococcus lactis cremosum | Trichosporon sp. |
| F02 7a FRC | Bacillus sp., Enterococcus durans, Lactococcus lactis lactis; Citrobacter freundii | Aspergillus sp. |
| F03 7a FRC | Aerococcus viridans, Bacillus sp. | |
| F04 7a MDSP | Bacillus sp., Staphylococcus aureus/intermedius, Streptococcus adhaerens, Streptococcus equinus | Aspergillus niger; Unidentified fungus |
| F05 7a MDSP | Streptococcus spp., Unidentified Gram+ bacteria; | Candida parapsilosis |
| F06 7a MDSP | Citrobacter diversus/amalonicatus | |
| F07 7a MDSP | Aerococcus viridans, Bacillus sp., Staphylococcus lentus; Actinobacter sp., Citrobacter freundii | Aspergillus sp. |
| F08 7a MDSP | Bacillus sp., Streptococcus equinus; | Acremonium sp. |
| F09 7a MDSP | Klebsiella pneumoniae pneumoniae; Unidentified bacteria | |
| F10 7a MDSP | Bacillus sp., Streptococcus sanguis; | Unidentified fungus |
| **Rectal cavity** | |
| F01 7a FRC | Not cultured | |
| F02 7a FRC | Not cultured | |
| F03 7a FRC | Actinomycetace, Aerococcus viridans, Staphylococcus xylous | Aspergillus sp. |
| F04 7a FRC | Staphylococcus xylous, Streptococcus salivarius, Streptococcus sanguis; | |
| F05 7a FRC | Actinomycetace, Bacillus sp., Staphylococcus xylous, Streptococcus sp.; Aeromonas hydrophila, Pseudomonas sp., Serratia marcescens | Penicillium sp. |
| F06 7a FRC | Bacillus sp., Staphylococcus xylous, Streptococcus salivarius | Aspergillus sp. |

Figure 2 - Details on the aerobic and facultative anaerobic bacteria and fungi retrieved from the nasal, oral and rectal cavities of ten free-ranging and ten captive Black lion tamarins.
However, a few considerations can be made: Actinomycetale bacteria were retrieved from the nasal swabs of 6/7 MDSP, but were absent in 3 FRC; Lactococcus was absent in the oral swabs of 7 MDSP, but present in 2/3 FRC; Streptococcus was absent in the oral swabs of 7 MDSP, but present in 2/3 FRC. Bacterial recovery rates were different among anatomical sites (K = 12.88, df = 2, p = 0.002), with recovery rates of rectal samples being lower than those of oral and nasal samples (nasal = 4.10±1.37 isolates retrieved / examined individual; oral = 4.12±1.41; rectal = 2.75±0.97). Similarly, fungal recovery rates were also different among anatomical sites (K = 12.67, df = 2, p = 0.002), with recovery rates of nasal samples being higher than those of oral and rectal samples (nasal = 2.40±1.63; oral = 1.00±0.79; rectal = 0.80±0.62).

In the nasal cavity, Gram positive bacteria were predominant over Gram negative bacteria (G+ 96.3% of bacterial isolates, G- 3.7%) with Staphylococcus (43.9% of bacterial isolates) and Bacillus (29.3%) recognized as the most frequent Gram positive genera. Mycelian fungi dominated the fungal microbiota of the nasal cavity (Yeast 37.5% of fungal isolates, Mycelian 62.5%).

In the oral cavity, Gram positive bacteria had an overall frequency higher than Gram negative bacteria (G+ 70.8% of bacterial isolates, G- 29.2%). Streptococcus (28.3% of bacterial isolates), Bacillus (26.1%) and Staphylococcus (19.6%) were the most frequent Gram positive genera. Mycelian fungi were more frequent than yeast in the oral fungal microbiota (Yeast 22.2% of fungal isolates, Mycelian 77.8%).

Unlike in the nasal and oral microbiota, the rectum revealed Gram negative bacteria as being more frequent than Gram positive bacteria (G+ 38.2% of bacterial isolates, G- 61.8%). Escherichia coli was the most frequent bacteria (29.1% of bacterial isolates), followed by Serratia spp. (10.9%). Yeasts were more frequent than mycelian fungi in the rectal samples (Yeast 75% of fungal isolates, Mycelian 25%).

**Discussion**

Investigation of clinically healthy black lion tamarins showed Gram positive bacteria as dominant in the nasal and oral microbiota, while the rectal microbiota was predominantly composed of Gram negative bacteria. Nasal, oral and rectal microbiota shared many microorganisms but in different proportions. The nasal microbiota had the highest recovery rate, being dominated by Gram positive bacteria and presenting a large variety of mycelian fungi and a high frequency of Candida yeast. The oral microbiota had inter-
mediary recovery rate and was predominantly composed of Gram positive bacteria and mycelian fungi (note that the higher relative frequency of mycelian fungi in the oral cavity when compared to the nasal cavity was more due to a decreased recovery of yeasts than to an increased recovery of filamentous fungi). The rectal microbiota presented poor recovery rates, being composed by a majority of Gram negative bacteria and yeast fungi. These findings are consistent with those described for other primates including humans (Brown et al., 1973, Hill et al., 1978, Nordstrom et al., 1989, Bailey and Coe 2002, Moreira et al., 2003, 2004).

Overall, the most frequent bacteria were Staphylococcus spp. (22% of bacterial isolates) and Bacillus spp. (21%), followed by Escherichia coli, Streptococcus spp., Actinomicetale, Klebsiella pneumoniae, Corynebacterium spp. and Citrobacter spp. Gram positive cocci as Aerococcus, Staphylococcus and Streptococcus were previously reported in the intestinal tract, skin and mucosa of healthy nonhuman primates (Daniel et al., 1976, Hill et al., 1978, Swindle et al., 1982, Lewis et al., 1987), however in some conditions have also been implied as the cause of pneumonia, nephritis and cystitis in this group of animals (Hunt et al., 1978, Boever and Wallach 1983, McClure et al., 1986). Streptococcus pneumoniae is an important pathogen leading to pneumonia in primates, but it is not usually considered as an important callithrichid pathogen (McClure et al., 1986, Chi et al., 2007); while we did not identify this specific organism in the studied animals, there were five Streptococcus isolates that could not be identified down to species. Aerococcus viridans is frequent in the reproductive tract of primates, but is also known to cause abortion and natimortality (Swindle et al., 1982). Bacillus, as a sporeforming bacteria, has the soil as its primary reservoir and is widespread in water and air (Nicholson, 2002). As a consequence it is encountered also in the microbiota of animals (Jungle et al., 2005, Lima et al., 2012, Souza et al., 2012), meantime, its role as spontaneous nonhuman primate pathogen is unknown.

Escherichia coli is known to be one of the most abundant saprophytic bacteria in the gastrointestinal tract of warm-blooded animals. However, pathogenic strains are important cause of diarrhoea and can also cause other pathologic conditions, including septicaemias, meningitis, urinary infections, abscesses and cellulitis (McClure et al., 1986, Carvalho et al., 2003, Blanco et al., 2004, Carvalho et al., 2012). Indeed, E. coli seems to be a potential pathogen for callithrichids (Mansfield et al., 2001). In the present study E. coli was isolated most commonly from rectal samples being demonstrated in 80% of animals sampled. Carvalho et al. (2003), studying Neotropical monkeys (56% of which were callithrichids) from rehabilitation centers, zoos and private breeders in Brazil, found enteropathogenic Escherichia coli (EPEC) strains in 29% of the studied animals, all of which were callithrichids. Although 37% of the individual with these pathogenic strains were clinically healthy, colon’s histopathological evaluation of animals submitted for necropsy and from which EPEC was isolated revealed distortion and reduction in crypt size and an inflammatory infiltrate. Furthermore, the isolates revealed a genetic relationship with human EPEC (Carvalho et al., 2007).

*Klebsiella pneumoniae* has been frequently implied in pulmonary disease, septicaemia, meningitis and other pathologic processes in primates (Fox and Rohovsky 1975, Hunt et al., 1978, Chalmers et al., 1983, Gozalo et al., 1991, Pisharath et al., 2005). Outbreaks in callithrichids breeding colonies have revealed *K. pneumoniae* infection causing severe purulent peritonitis and sepsis (Gozalo et al., 1991, Pisharath et al., 2005). It is interesting to note that in this study, although recovered from free-ranging and captive animals, *Klebsiella* was present more frequently in the rectum and nasal cavity of animals’ kept in captivity.

Other bacteria isolated in the studied animals included *Pseudomonas aeruginosa* and *Citrobacter freundii*, which have been reported to cause diarrhoea, pneumonia, septicaemia, nephritis and cystitis in nonhuman primates (Hunt et al., 1978, Boever and Wallach 1983, Lausen et al., 1986, McClure et al., 1986, Ocholi et al., 1989). Zoonotic bacterial pathogens associated with diseases of captive nonhuman primates, as *Salmonella*, *Shigella*, *Bordetella*, *Pasteurella* and *Yersinia* (Baskerville et al., 1983; Taffs et al., 1983; Cooper et al., 1976) were not isolated in this study, indicating that the sanitary management is an important approach to maintaining colonies’ health (Daszak et al., 2000).

Mycelian fungi were the most frequently retrieved fungal organisms from nasal and oral swabs of the studied tamarins, whilst yeasts were most frequently recovered from rectal samples. Overall, the most frequent fungal genera were *Candida* (35% of fungal isolates) and *Aspergillus* (15%), followed by *Penicillium* and *Trichoderma*. Fungal infections are generally considered less common than those caused by bacteria, but also have an important role as opportunistic agents and may cause significant impairment or death (Chalmers et al., 1983, Megaki 1986, Kalter 1989, Nordstrom et al., 1989). *Candida* is well known to be an important inhabitant of the intestinal tract of captive primates, and has been reported to cause glossitis, esophagitis, gastritis and septicaemia (Stone et al., 1974, Chalmers et al., 1983, Nordstrom et al., 1989); it has also been identified in the vaginal microbiota of clinically healthy Black lion tamarins (Moraes et al., 2004). Mycelian fungi are known to be occasionally present in the skin, vagina and intestinal microbiota of free-ranging and captive primates without causing disease [Daniel et al., 1976, Nordstrom et al., 1989, Benno et al., 1987, Moraes et al., 2004], but *Aspergillus* is recognized to cause respiratory disease in immunologically impaired primates (Migaki, 1986, Haustein et al., 2008).
Little is known about the microbiotal differences among free-ranging and captive primates of the same species, but with different habitats and behaviours there could be differences between the two groups. Diet and habitat, exposure to vaccinations and antibiotics, contact with humans and other non-human primates, increased density of individuals, and poor welfare or stress are also important factors that may affect the microbiotal composition in captive animals (McClure et al., 1980, Rolland et al., 1985, Benno et al., 1987, Lewis et al., 1987, Costa et al., 1989, Bruorton et al., 1991, Bailey and Coe, 2002). In the present study relatively few differences were found in the microbiotal composition of the studied free-ranging and captive lion tamarins; it is unclear if this is occurred because there are no real differences, or if it was only due to small sampling size. The nasal, oral and rectal samples of these animals were similar in the relative proportions of the major bacterial and fungal groups, and had similar composition in terms of most prevalent genera. On the other hand, organisms that are known to be frequently pathogenic such as *Aeromonas*, *Pseudomonas* or *Klebsiella* were either recorded only in captive animals, or were found to be more frequent in those animals. It is not clear, however, if those potential pathogens were human-borne, and future molecular studies should attempt to clarify the phylogenetic origin of these organisms.

The small sample size, limited by the rarity of specimens in captivity and the logistic difficulties of collecting and processing samples from these free-ranging animals, may have restricted the detection of relevant differences among captive and free-ranging animals, as well as among free-ranging tamarins from different conservation areas. Translocations and reintroductions are management strategies that require intensive animal handling and movement, which are stressful conditions that might lead to stress-induced immunosuppression and increased occurrence of opportunistic infectious diseases (Fox and Rohovsky, 1975, Ocholi et al., 1989, Bush et al., 1991). Because most of the organisms identified are opportunistically pathogenic under conditions of stress, minimizing stress during all steps of the translocation and reintroduction processes may be relevant. Finally, it is important that a long-term health-monitoring program is established post-release, and any deaths during or after the release process are thoroughly evaluated with pathologic and microbiologic examinations.

Acknowledgments

We are thankful to the field assistants involved in the captures, José Aparecido de Oliveira and José Maria Aragão, and to Dr. Marcos Amaku for the contributions to the manuscript. This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 1996/12176-9, 1999/09459-7), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, E-26/171.271/2006). We are also thankful to the Greater Los Angeles Zoo Association (GLAZA), the American Society of Primatologist (ASP) and the Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA). José Luiz Catão-Dias is a recipient of a scholarship by the CNPq (301517/2006-1) and Ralph E. T. Vanstreels has a scholarship by FAPESP (2009/53956-9).

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