Brazilian Journal of Microbiology 45, 4, 1531-1539 (2014)
ISSN 1678-4405

Research Paper

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

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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 (Kierulff, 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, Kierulff, 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-

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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 immunosupression 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 broths were 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 (Biomerieux). The yeasts also had their filamentation capability tested (Koneman and Roberts, 1990). The identification of mycelian fungi were based on macro and microscopic morphology achieved through 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 mycelian 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 (Actinomicetale 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 mycelian 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

			Nasal cavity		Oral cavity		Rectum	
			FRT	CPT	FRT	CPT	FRT	CPT
Bacteria	Gram +	Actinomicetale	5 [5]	3 [3]	-	2 [2]*	-	-
		Aerococcus	-	-	2 [2]	1 [1]*	1 [1]	1 [1]
		Bacillus	13 [9]	11 [10]	8 [8]	4 [4]*	5 [5]	2 [2]
		Corynebacterium	4 [4]	4 [3]	-	-	-	-
		Enterobacter	-	-	-	-	-	1 [1]
		Enterococcus	-	-	1 [1]	-	1 [1]	3 [3]
		Lactobacillus	-	-	-	-	1 [1]	-
		Lactococcus	-	-	2 [2]	1 [1]*	-	1 [1]
		Micrococcus	1 [1]	1 [1]	-	1 [1]*	-	-
		Staphylococcus	16 [9]	20 [10]	4 [4]	5 [5]*	-	1 [1]
		Streptococcus	1 [1]	-	8 [6]	5 [4]*	2 [2]	1 [1]
		Unidentified	-	-	1 [1]	1 [1]*	1 [1]	-
	Gram -	*Acinetobacter*	-	-	1 [1]	-	-	1 [1]
		Aeromonas	-	-	-	1 [1]*	-	-
		Alcaligenes	-	-	-	1 [1]*	-	-
		Citrobacter	-	-	5 [5]	1 [1]*	-	1 [1]
		Enterobacter	-	-	-	1 [1]*	-	-
		Escherichia	-	-	2 [1]	-	9 [9]	7 [7]
		Klebsiella	-	2 [2]	2 [2]	1 [1]*	1 [1]	3 [3]
		Morganella	-	-	-	-	-	1 [1]
		Ochrobactrum	-	1 [1]	-	-	-	-
		Pantoea	-	-	-	1 [1]*	-	-
		Proteus	-	-	-	-	-	1 [1]
		Pseudomonas	-	-	-	2 [2]*	-	2 [2]
		Serratia	-	-	-	1 [1]*	4 [4]	2 [2]
		Sphingobacterium	-	-	-	-	-	2 [2]
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]
	Mycelian	*Acremonium*	-	-	1 [1]	-	-	-
		Aspergillus	3 [3]	-	3 [3]	5 [5]	-	1 [1]
		Cladosporidium	1 [1]	1 [1]	-	-	1 [1]	-
		Cylindrocapom	-	1 [1]	-	-	-	-
		Fusarium	1 [1]	-	-	1 [1]	-	-
		Glicocladium	-	-	-	1 [1]	-	-
		Helminthosporium	1 [1]	1 [1]	-	-	-	-
		Mycelia sterilia	2 [2]	1 [1]	-	-	-	-
		Mucor	-	-	-	-	1 [1]	-
		Paecilomyces	3 [3]	-	-	-	-	-
		Penicillium	3 [3]	4 [4]	-	2 [2]	-	-
		Pseudoallescheria	1 [1]	-	-	-	-	-
		Trichoderma	2 [2]	3 [3]	-	1 [1]	-	1 [1]
		Verticilium	2 [2]	-	-	-	-	-
Total	Bacteria	Gram +	40 [10] 100% [100%]	39 [10] 93% [100%]	26 [10] 72% [100%]	20 [6]* 59% [100%]	11 [8] 44% [80%]	10 [7] 33% [70%]
		Gram -	0 [0] 0% [0%]	3 [3] 7% [30%]	10 [7] 28% [70%]	9 [4]* 31% [66%]	14 [10] 56% [100%]	20 [9] 67% [90%]
	Fungi	Yeast	8 [8] 30% [80%]	10 [5] 48% [50%]	3 [3] 43% [30%]	1 [1] 9% [10%]	3 [3] 60% [30%]	9 [8] 92% [80%]
		Mycelian	19 [8] 70% [80%]	11 [7] 52% [70%]	4 [4] 57% [40%]	10 [7] 91% [70%]	2 [2] 40% [20%]	2 [2] 18% [20%]

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

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.

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 mycelian 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	Fungi
Nasal cavity	Free-ranging tamarins	F01 ♀a FRC	*Bacillus* sp., *Staphylococcus intermedius*	*Candida guilliermondii*; *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp.
		F02 ♀a FRC	*Bacillus* sp., *Micrococcus varians*, *Streptococcus oralis*	-
		F03 ♂a FRC	*Bacillus* sp., *Staphylococcus aureus*, *S. intermedius*, *S. xylosus*	*Candida* sp.; *Micelia sterilia*, *Penicillium* sp., *Pseudoallescheria* sp., *Verticilium* sp.
		F04 ♂a MDSP	Actinomicetale, *Corynebacterium* sp., *Bacillus* sp. (2 strains), *Staphylococcus aureus*, *S. capitis S. xylosus*	*Candida parapsilosis*; *Aspergillus* sp., *Helminthosporium* sp., *Verticilium* sp.
		F05 ♀j MDSP	Actinomicetale, *Bacillus* sp. (2 strains), *Staphylococcus xylosus*	*Candida parapsilosis*; *Aspergillus* sp.
		F06 ♂j MDSP	*Staphylococcus aureus*, *S. xylosus*	-
		F07 ♀a MDSP	Actinomicetale, *Bacillus* sp., *Corynebacterium* sp., *Staphylococcus xylosus*	*Candida lusitaniae*; *Paecilomyces* sp., *Trichoderma* sp.
		F08 ♂a MDSP	*Bacillus* sp. (2 strains), *Staphylococcus xylosus*	*Candida guilliermondii*; *Micelia sterilia*
		F09 ♂a MDSP	Actinomicetale, *Bacillus* sp., *Corynebacterium* sp., *Staphylococcus aureus*, *S. sciuri*, *S. xylosus*	*Candida guilliermondii*; *Paecilomyces* sp., *Penicillium* sp.
		F10 ♂a MDSP	Actinomicetale, *Bacillus* sp. (2 strains), *Corynebacterium* sp., *Staphylococcus xylosus*	*Candida tropicalis*; *Aspergillus* sp., *Paecilomyces* sp.
	Captive tamarins	C01 ♂a CPRJ	*Bacillus* sp., *Staphylococcus cohnii*, *S. lentus*, *S. xylosus*	*Trichoderma* sp.
		C02 ♀a CPRJ	Actinomicetale, *Bacillus* sp. (2 strains), *S. xylosus*	-
		C03 ♀a CPRJ	Actinomicetale, *Bacillus* sp., *Staphylococcus lentus*, *S. xylosus*; *Ochrobactrum anthropi*	Micelia sterilia
		C04 ♂a CPRJ	Actinomicetale, *Bacillus* sp., *Corynebacterium* sp., *Micrococcus varians*, *Staphylococcus sciuri*, *S. xylosus*	*Cladosporium* sp., *Penicillium* sp., *Trichoderma* sp.
		C05 ♀a CPRJ	*Bacillus* sp., *Staphylococcus saprophyticus*, *S. xylosus*; *Klebsiella oxytoca*	*Candida famata*, *C. guilliermondii*; *Penicillium* sp.
		C06 ♂a CPRJ	*Bacillus* sp., *Staphylococcus capitis*, *S. cohnii*, *S. sciuri*; *Klebsiella oxytoca*	*Candida famata*, *C. humicola*; *Cylindrocapom* sp., *Penicillium* sp.
		C07 ♀a CPRJ	*Bacillus* sp., *Corynebacterium* sp., *Staphylococcus sciuri*, *S. xylosus*	*Candida famata*; *Trichoderma* sp.
		C08 ♂a CPRJ	*Bacillus* sp., *Corynebacterium* sp. (2 strains), *Staphylococcus sciuri*, *S. xylosus*	*Candida rugosa*; *Helminthosporium* sp., *Penicillium* sp.
		C09 ♀a CPRJ	*Bacillus* sp., *Staphylococcus sciuri*	*Candida famata*, *C. guilliermondii*,*C. humicola*, *C. parapsilosis*
		C10 ♀a CPRJ	*Bacillus* sp., *Staphylococcus sciuri*, *S. xylosus*	-
Oral cavity	Free-ranging tamarins	F01 ♀a FRC	*Bacillus* sp., *Lactococcus lactis cremosis*	*Trichosporon* sp.
		F02 ♀a FRC	*Bacillus* sp., *Enterococcus durans*, *Lactococcus lactis lactis*; *Citrobacter freundii*	*Aspergillus* sp.
		F03 ♂a FRC	*Aerococcus viridans*, *Bacillus* sp.	-
		F04 ♂a MDSP	*Bacillus* sp., *Staphylococus aureus/intermedius*, *Streptococcus adjacens*, *Streptococcus equinus*	*Aspergillus niger*; Unidentified fungus
		F05 ♀j MDSP	*Streptococcus* sp., Unidentified Gram+ bacteria; *Citrobacter diversus/amalonaticus*	*Candida parapsilosis*
		F06 ♂j MDSP	*Aerococcus viridans*, *Bacillus* sp., *Staphylococcus lentus*; *Acinetobacter* sp., *Citrobacter freundii*	*Aspergillus* sp.
		F07 ♀a MDSP	*Bacillus* sp., *Streptococcus equinus*; *Klebsiella pneumoniae pneumoniae*; Unidentified bacteria	*Acremonium* sp.
		F08 ♂a MDSP	*Bacillus* sp., *Streptococcus sanguis*; *Citrobacter diversus/amalonaticus*	Unidentified fungus
		F09 ♂a MDSP	*Bacillus* sp., *Staphylococcus aureus/intermedius*, *Streptococcus adjacens*, *Streptococcus salivarius salivarius*; *Escherichia coli* (2 strains)	*Candida* sp.
		F10 ♂a MDSP	*Staphylococcus aureus/intermedius*, *Streptococcus salivarius salivarius*; *Citrobacter diversus/amalonaticus*, *Klebsiella pneumoniae pneumoniae*	*Trichosporon* sp.
	Captive tamarins	C01 ♂a CPRJ	Not cultured	-
		C02 ♀a CPRJ	Not cultured	*Aspergillus* sp.
		C03 ♀a CPRJ	Actinomicetale, *Aerococcus viridans*, *Staphylococcus xylosus*	-
		C04 ♂a CPRJ	*Staphylococcus xylosus*, *Streptococcus salivarius*, *Streptococcus sanguis*; *Citrobacter diversus/amalonaticus*	-
		C05 ♀a CPRJ	Actinomicetale, *Bacillus* sp., *Staphylococcus xylosus*, *Streptococcus* sp.; *Aeromonas hydrophyla*, *Pseudomonas* sp., *Serratia marcescens*	*Penicillium* sp.
		C06 ♂a CPRJ	*Bacillus* sp., *Staphylococcus xylosus*, *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.

			salivarius, Unidentified Gram+ rod	
		C07 ♀a CPRJ	Not cultured	*Aspergillus* sp., *Fusarium* sp.
		C08 ♂a CPRJ	Not cultured	*Aspergillus* sp.
		C09 ♀a CPRJ	*Bacillus* sp., *Staphylococcus xylosus*, *Streptococcus equines*; *Enterobacter cloacae*, *Klebsiella pneumoniae pneumoniae*, *Pantoea* sp.	*Penicilium* sp., *Glicocladium* sp.
		C10 ♀a CPRJ	*Bacillus* sp., *Lactococcus lactis cremosis*, *Micrococcus* sp.; *Alcaligenes* sp., *Pseudomonas* sp.	*Candida humicola*; *Aspergillus* sp., *Trichoderma* sp.
	Free-ranging tamarins	F01 ♀a FRC	*Bacillus* sp., *Streptococcus* sp.; *Escherichia coli*	-
		F02 ♀a FRC	*Bacillus* sp., *Streptococcus* sp.; *Escherichia coli*	*Cladosporium* sp.
		F03 ♂a FRC	*Aerococcus viridans*; *Escherichia coli*	-
		F04 ♂a MDSP	*Bacillus* sp.; *Klebsiella oxytoca*, *Serratia marcescens*	*Candida colliculosa*
		F05 ♀j MDSP	*Bacillus* sp.; *Lactobacillus lactis lactis*; *Escherichia coli*, *Serratia marcescens*	*Geotrichum penicillatum*
		F06 ♂j MDSP	*Escherichia coli*, *Serratia marcescens*	*Geotrichum penicillatum*
		F07 ♀a MDSP	*Enterococcus* sp.; *Escherichia coli*, *Serratia* sp.	*Mucor* sp.
		F08 ♂a MDSP	Unidentified Gram+ Catalase- cocci; *Escherichia coli*	-
		F09 ♂a MDSP	*Bacillus* sp.; *Escherichia coli*	-
		F10 ♂a MDSP	*Escherichia coli*	-
	Captive tamarins	C01 ♂a CPRJ	*Enterococcus faecium*; *Escherichia coli*	*Candida albicans*
		C02 ♀a CPRJ	*Streptococcus* sp., *Bacillus* sp.	*Candida magnoliae*
		C03 ♀a CPRJ	*Aerococcus viridans*; *Escherichia coli*, *Morganella morganii*	*Aspergillus* sp.
		C04 ♂a CPRJ	*Escherichia coli*, *Proteus vulgaris*	-
		C05 ♀a CPRJ	*Bacillus* sp., *Enterobacter aglomerans*; *Klebsiella pneumoniae*, *Serratia odorifera*	*Candida humicola*
		C06 ♂a CPRJ	*Lactococcus lactis lactis*; *Escherichia coli*, *Pseudomonas cepacia*	Unidentified yeast
		C07 ♀a CPRJ	*Enterococcus faecium*; *Klebsiella oxytoca*, *Acinetobacter* sp., *Serratia odorifera*	*Candida* sp.
		C08 ♂a CPRJ	*Escherichia coli*, *Klebsiella oxytoca*	*Candida* sp.
		C09 ♀a CPRJ	*Escherichia coli*, *Pseudomonas* sp., sp.*hingobacterium multivorum*	*Candida krusei*, *Kloeckera apis/aspiculata*
Rectum		C10 ♀a CPRJ	*Enterococcus faecium*, *Staphylococcus xylosus*; *Citrobacter diversus*, *Escherichia coli*, sp.*hingobacterium multivorum*	*Candida krusei*; *Trichoderma* sp.

♂ = male, ♀ = female; j = juvenile, a = adult. FRC = Fazenda Rio Claro, MDSP. = Morro do Diabo State Park; CPRJ = Rio de Janeiro Primatology Center.

Figure 2 cont -

However, a few considerations can be made: Actinomicetale 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*, *Corynnebacterium* 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 callitrichid 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 celulitis (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 centres, 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 immunosupression 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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