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## COMPARATIVE RECTAL BACTERIAL FLORA OF FOUR SPECIES OF FLYING FOX (*PTEROPUS* SP.)

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**Abstract:** The rectal anaerobic and aerobic bacterial flora of four species of flying foxes were determined and compared. Four bacterial species were found in  $\geq 1$  individual from each bat species at a significant ( $\geq 10\%$ ) level of the bacterial population:  $\alpha$ -hemolytic *Streptococcus* sp. (41 of 56 bats), *Enterococcus* sp. (25/56), *Escherichia coli* (21/56), and group D *Streptococcus* sp., not *Enterococcus* sp. (9/56). Five other microbial species were also found in all four flying fox species, but at less significant percentages (found in at least one bat species,  $\geq 5\%$  and  $\leq 10\%$  of the recovered microbial population). These were nonhemolytic *Streptococcus* sp. (30/56), yeast (26/56), *Corynebacterium* sp. (25/56), *Staphylococcus* sp. (25/56), and *Staphylococcus aureus* (22/56). The majority of the species found were gram-positive, and only two obligate anaerobes, a *Lactobacillus* and a *Bacteroides* sp., were recovered from one bat.

**Key words:** Pteropodidae, *Pteropus*, bat, bacteria.

### INTRODUCTION

The suborder Megachiroptera comprises 166 species confined to a single family, the Pteropodidae.<sup>9</sup> This family of fruit- and nectar-feeding bats is found in the tropical and subtropical regions of the Old World, east to Australia and the Caroline and Cook islands.<sup>9</sup> In recent years, Megachiropterans have received increased research interest because of their importance in seed dispersal and pollination in rainforest ecosystems.<sup>9</sup> Further, many species are either threatened or endangered because of habitat loss and over-hunting.<sup>9</sup> Despite this increased interest, little is known about their normal gastrointestinal physiology. The gastrointestinal tract is an important source of bacterial pathogens in mammals.<sup>8</sup> Although several studies have evaluated the bacterial flora of Microchiropterans,<sup>3,4,11,12,15</sup> we are aware of only a single published paper<sup>12</sup> that examines the fecal bacterial flora of Megachiropterans. The aim of this study was to determine and compare the rectal bacterial flora of four captive species of flying fox.

### MATERIALS AND METHODS

#### Animals

Four species of adult ( $>1$ -year-old) flying fox were examined: 13 (4 male, 9 female) giant (*Pteropus vampyrus*), 20 (9 male, 11 female) island (*P. hypomelanus*), 10 (5 male, 5 female) Rodriguez Island (*P. rodricensis*), and 13 (7 male, 6 female) golden-mantled (*P. pumilus*). All bats were as-

essed as healthy on the basis of physical examination and hematologic and plasma biochemical values that were within the reference ranges for each species. The animals were maintained in indoor/outdoor enclosures at the Lube Foundation, a private research and breeding facility in north central Florida. Their diet consisted of a mixture of fruits, vegetables, commercial primate chow, cottage cheese, and a vitamin-mineral supplement. All of the giant and 18 of the island flying foxes were captured in Indonesia and had been in captivity for 42 months. The golden-mantled flying foxes had been captured in the Philippines 19 months earlier. Eight of the Rodriguez Island flying foxes were bred in England and had been at the Foundation for 42 months; the other two were captive-bred at the facility.

#### Bacteriological procedures

Samples for microbiological analysis were collected from the rectum of each bat using a swab (Minitip Culturette, Becton Dickinson and Co., Cockeysville, Maryland 21030, USA) inserted approximately 2–3 cm past the anus. The swab was premoistened with modified Cary-Blair transport medium in the swab container prior to rectal insertion. Immediately after collection, the distal portion of the swab was placed in a sterile serum tube and the air evacuated with a 35-ml syringe attached to a three-way stopcock and needle. The samples were transported within 2 hours of collection to the Department of Clinical Microbiology and Parasitology, College of Veterinary Medicine, University of Florida. In 10 island flying foxes, samples were collected simultaneously using anaerobic swabs (Anaerobic Culturette, Becton Dickinson and Co.) to assess the sensitivity of the collection technique for the detection of anaerobic bacteria.

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Each sample was plated onto two blood agar plates (Remel, Lenexa, Kansas 66215, USA) with 5% sheep's blood for nonselective recovery of anaerobic and aerobic organisms, two colistin nalidixic acid plates (Remel) with 5% sheep's blood for recovery of anaerobic and aerobic gram-positive organisms, a MacConkey agar plate (Remel) for the recovery of gram-negative bacteria, a Brucella agar plate (Remel) for recovery of *Campylobacter* sp. incubated under microaerophilic conditions at 42°C, and a selenite enrichment broth (Remel) for recovery of *Salmonella* and *Shigella* sp. One blood agar, one colistin nalidixic acid, and the MAC plates from each sample were incubated at 37°C with 5% carbon dioxide. One blood agar and one colistin nalidixic acid plate from each sample were incubated anaerobically at 37°C for 48 hr using the Bio-Bag Environmental Chamber Type A (Becton Dickinson and Co.). The selenite enrichment broth was incubated at 37°C for 24 hr and then subcultured onto a Hektoen Enteric agar plate (Remel). Depending on growth rate, the organisms were identified after incubation for either 24 or 48 hr using the following commercial systems: API 20E (Biomérieux Vitex Inc., Hazelwood, Missouri 63042, USA) for enteric gram-negative organisms; NFT (Biomérieux Vitex Inc.) for fastidious and nonfermenting gram-negative organisms; RAP ANA II (Innovative Diagnostic Systems, Inc., Norcross, Georgia 30071, USA) for obligate anaerobes, and conventional biochemicals and Gram's stain for gram-positive organisms. Some bacteria were identified only to genus because of the lack of specific identification systems. The  $\alpha$  Streptococci and Enterococci were not speciated.

To semiquantify the bacteria present in each sample, 100 isolated colonies per plated fecal sample were chosen randomly and identified. The proportion of isolated colonies belonging to a specific bacterial genera was assumed to approximate its proportion in the rectal fecal population.

## RESULTS

The bacterial species present in a proportion  $\geq 10\%$  of a sample from  $\geq 1$  animal from any of the four bat species are listed in Table 1. While most bacterial species were shared by at least two bat species, only four were found in all bat populations: an  $\alpha$ -hemolytic *Streptococcus* sp. (41 of 56 bats), an *Enterococcus* sp. (25/56), *Escherichia coli* (21/56), and a group D *Streptococcus* sp., not *Enterococcus* sp. (9/56). Gram-positive organisms predominated in both species number and percentage of rectal population. *Escherichia coli* was the only gram-negative bacteria found consistently in

proportions  $\geq 10\%$ . Table 2 lists microorganisms recovered from at least one bat in proportions  $\geq 5\%$  and  $\leq 10\%$ . When this list is added to Table 1, five more microorganisms become common to all four flying fox species: a nonhemolytic *Streptococcus* sp. (30/56), a yeast (26/56), a *Corynebacterium* sp. (25/56), a *Staphylococcus* sp. (25/56), and *Staphylococcus aureus* (22/56). There were no apparent differences between males and females in their bacterial flora.

No differences in either the type or quantity of recovered organism were observed between the two methods of anaerobic collection. Two obligate anaerobic bacteria, a *Lactobacillus* and a *Bacteroides* sp., were found in low numbers in only one and three of the Rodriguez Island flying foxes, respectively.

## DISCUSSION

The predominant rectal flora of these four flying fox species were found to be gram-positive and aerobic. Gram-positive bacteria are not unexpected, since they are a common component of the intestinal flora of other frugivorous species (e.g., parrots).<sup>1,6</sup> However, the poor recovery of anaerobes is unexpected because they are usually present in large numbers in mammalian intestinal flora.<sup>2,7,8,14,17</sup> Possible reasons for this unexpected finding include 1) inappropriate collection and transportation techniques,<sup>10</sup> or 2) the possibility that the intestinal anatomy of flying foxes is not conducive to anaerobic colonization. The former is unlikely, since two different methods were used, and obligate anaerobic microorganisms were recovered from at least three bats. However, it is possible that anaerobic bacteria are present that require isolation and culture techniques that are more rigorous than those used in this study. The bat intestinal tract is narrow compared to those of mammals of a similar size, and it lacks sacculations that might act as fermentation chambers.<sup>5</sup> Also, in most bat species, ceca and a well-defined ileocecal valve are absent, and a distinguishable colon is not identifiable.<sup>13,16</sup> These anatomical features in association with very rapid ( $\geq 30$  minutes) food transit times<sup>5,13,16</sup> may inhibit anaerobic microbial colonization and proliferation.

In dogs and humans, the fecal bacterial flora reflect flora found in the colon, mainly bacteroides, anaerobic lactobacilli, and coliforms.<sup>2,7</sup> This is in distinct contrast to the mainly gram-positive flora above the ileocecal valve. In flying foxes, which lack a well-defined ileocecal valve, the rectal bacterial flora may resemble flora of the upper intestine.

In contrast to the flying foxes, the gastrointestinal tracts of Microchiropterans appear to be dominated

**Table 1.** Rectal bacterial flora of four flying fox species showing organism frequency (Freq.), and the range and mean percentage (x %) of each organism in the microfloral population. Only those bacteria found in at least one bat at  $\geq 10\%$  of the bacterial colonies are reported.

	<i>Pteropus vampyrus</i>		<i>Pteropus hypomelanus</i>		<i>Pteropus rodricensis</i>		<i>Pteropus pumilus</i>	
	Freq.	x% (range)	Freq.	x% (range)	Freq.	x% (range)	Freq.	x% (range)
Gram-positive rods								
Actinomycete								
<i>Bacillus</i> sp.	9/13	19 (<1-100)	7/20	10 (<1-20)	4/10	11 (<1-20)	2/13	10 (<1-20)
<i>Corynebacterium</i> sp.	4/13	23 (<1-50)	17/20	33 (<1-90)			8/13	14 (<1-100)
Gram-positive cocci								
<i>Enterococcus</i> sp.	8/13	38 (<1-100)	5/20	58 (20-100)	4/10	48 (30-90)	8/13	48 (<1-90)
<i>Micrococcus</i> sp.			2/20	5.0 (<1-10)				
<i>Staphylococcus</i> sp.			10/20	3.7 (<1-20)	8/10	1.7 (<1-10)		
<i>S. aureus</i>	8/13	25 (<1-40)			5/10	8.4 (<1-40)		
<i>Streptococcus</i> sp.								
$\alpha$ -hemolytic	6/13	34 (<1-100)	19/20	24 (<1-90)	8/10	27 (<1-90)	8/13	23 (<1-100)
Group D	2/13	50 (40-60)	2/20	55 (20-90)	3/10	33 (30-40)	1/13	30 (30)
Nonhemolytic			16/20	16 (<1-50)	8/10	11 (<1-30)	4/13	20 (<1-40)
Gram-negative rods								
<i>Enterobacter</i> sp.								
<i>Escherichia coli</i>								
<i>Hafnia alvei</i>	7/13	7.4 (<1-30)	6/20	29 (<1-90)	2/10	70 (70)	2/13	50 (<1-100)
<i>Klebsiella oxytoca</i>							6/13	24 (<1-90)
<i>Morganella morganii</i>	4/13	22 (<1-80)			1/10	60 (60)	2/13	5.3 (<1-10)
<i>Proteus</i> sp.			19/20	2.7 (<1-20)	3/10	10 (<1-20)	5/13	14 (<1-30)
<i>P. mirabilis</i>			2/20	10 (<1-20)				
Yeast	5/13	8.4 (<1-40)	11/20	1.4 (<1-10)				

**Table 2.** Frequency of isolation for rectal microorganisms found in at least one flying fox at  $\geq 5\%$  and  $\leq 10\%$  of the microbial population.

		<i>Pteropus vampyrus</i>	<i>Pteropus hypomelanus</i>	<i>Pteropus rodricensis</i>	<i>Pteropus pumilus</i>
Gram-positive rods	<i>Bacillus</i> sp.			3/10	
	<i>Clostridium septicum</i>	2/13	2/20		
	<i>Corynebacterium</i> sp.			1/10	5/13
Gram-positive cocci	<i>Lactobacillus</i> sp.			1/10	
	<i>Micrococcus</i> sp.	1/13			
	<i>Staphylococcus</i> sp.	5/13			2/13
	<i>S. aureus</i>		6/20		3/13
	<i>Streptococcus</i> sp. Nonhemolytic	2/13			
Gram-negative rods	<i>Bacteroides</i> sp.			1/10	
	<i>Klebsiella-Enterobacter</i> sp. <i>K. pneumoniae</i>			2/10	
	<i>Morganella morganii</i>		1/20	2/10	
	<i>Proteus</i> sp. <i>P. mirabilis</i>			4/10	2/13
				3/10	
				2/10	8/13
Other	Yeast				

by gram-negative bacteria. In a retrospective survey of the bacterial flora of insectivorous Microchiropteras from Great Britain, the predominant bacteria was *E. coli*.<sup>15</sup> Similarly, the aerobic bacterial flora of the insectivorous *Chaerophon pumila* from Madagascar was reported to be diverse, with most species belonging to the Enterobacteriaceae family.<sup>3</sup> A comprehensive study of the enterobacteria from 10 Microchiropteran and four Megachiropteran species showed no specific differences between insectivores and frugivores.<sup>12</sup> Isolates included *E. coli* (15–24%), *Citrobacter* sp. (8–10%), *Enterobacter-Klebsiella* group (40–43%), and *Proteus* sp. group (28–30%).<sup>12</sup> No mention was made of gram-positive bacteria. The blood-drinking vampire bat *Desmodus rotundus* has gram-negative fecal flora, with either *Aeromonas hydrophila*<sup>12</sup> or *E. coli*<sup>11</sup> predominant, depending on the study.

In conclusion, the presence of large numbers of gram-negative and/or anaerobic bacteria in the rectal flora of flying foxes may be considered abnormal and possibly pathogenic, especially when associated with clinical disease (e.g., diarrhea).

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