

## A SURVEY FOR ENTEROPATHOGENIC BACTERIA IN PANAMANIAN PRIMATES<sup>1,2,3</sup>

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**SUMMARY** • Twenty (3.0%) of 671 Panamanian primates, newly arrived at Gorgas Memorial Laboratory, were found to be infected with enteropathogenic bacteria. Six (10.7%) of 56 black howler monkeys (*Alouatta villosa*), 5 (2.5%) of 201 night monkeys (*Aotus trivirgatus*), 4 (5.0%) of 80 black spider monkeys (*Ateles fusciceps*), 5 (1.7%) of 301 Panamanian marmosets (*Saguinus geoffroyi*), and none of 13 red spider monkeys (*Ateles geoffroyi*) or 20 white face monkeys (*Cebus capucinus*) were infected. Thirteen of the 21 isolations were *Salmonella*, 5 were enteropathogenic *Escherichia coli*, and 7 each was an *Arizona*, a *Shigella*, and an *Edwardsiella*. Weekly rectal-swab cultures from 26 monkeys representing black spider monkeys, night monkeys, Panamanian marmosets, and white face monkeys disclosed high infection rates in only black spider monkeys. Six of 7 spider monkeys harbored *Shigella* and/or enteropathogenic *Escherichia coli* organisms. Fourteen of the 16 isolations from spider monkeys were made between the 3rd to the 6th week after arrival at the laboratory. Panamanian primates naturally infected or infected during captivity with enterobacterial pathogens are a potential health hazard to persons associating with these animals. They may serve as sources of infections to other laboratory animals, to scientists and animal handlers in research institutions, and to persons who keep monkeys as pets in their homes.

Infections due to *Shigella* and *Salmonella* organisms may cause diarrheas in captive Old World primates (6, 9, 10, 14). Few surveys have been made of New World primates. Among them, Galton *et al* (7) reported finding *Shigella* and *Salmonella* infections associated with diarrheas in captive spider monkeys (*Ateles geoffroyi*), and Deinhardt *et al* (3) reported finding *Shigella*, *Salmonella*, and coliform organisms, sometimes associated with enteritis, in cotton-top marmosets (*Saguinus oedipus*) and in white-lipped, hairy-faced tamarins

(*Saguinus fuscicollis* and *S. nigricollis*) newly admitted to the laboratory. The latter authors were not able to isolate *Salmonella* and isolated *Shigella* only once from a marmoset maintained in their laboratory. The infected marmoset had been in their laboratory for at least 3 months. Shigellosis and salmonellosis have not been reported in primates in the wild state (1, 10, 15, 16), although Pinkerton (10) did report the occurrence of *Shigella* antibodies in wild baboons, indicating exposure to at least the *Sh. flexneri* group.

Pathogenic enterobacterial infections in captive primates may constitute not only a serious economic problem due to illnesses and deaths, but also may serve as sources of infection to persons associating with these animals (2, 12, 13). The importance of these infections led us to survey a large number of New World (Panamanian) primates being used for malarial studies at Gorgas Memorial Laboratory, Panama, Republic of Panama.

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## MATERIALS AND METHODS

*Source of primates:* Most of the Panamanian marmosets (*Saguinus geoffroyi*), night monkeys (*Aotus trivirgatus*), and black howler monkeys (*Alouatta villosa*) were captured by professional trappers in forested areas and delivered to the laboratory within 48 hours. None of the monkeys exhibited overt signs of infectious disease on arrival. All ages were represented, depending on the season. The young are generally born during the dry season, which extends from January to May.

The black spider monkeys (*Ateles fusciceps*), red spider monkeys (*A. geoffroyi*), and white face monkeys (*Cebus capucinus*) were mostly infants and juveniles which had been cared for by Indians for several weeks prior to arrival at our laboratory. The Indians, who live in distant mountainous areas, captured the young monkeys when they killed their parents for food. Periodically, they brought the young monkeys to the city for sale to our laboratory.

Taxonomic designations of primates are those of Handley (8).

*Care and housing of primates:* The care and handling of primates at this laboratory has been described elsewhere (11). The marmosets or night monkeys were kept in groups of 2-4 in cages having wire bottoms. The other species of primates were usually maintained in large groups within concrete-floored gang cages. These latter groups were separated by species, except that the black and the red spider monkeys were housed together.

*Laboratory methods:* Rectal swabs were collected from monkeys upon arrival at the laboratory and sent immediately to the bacteriology department in screw-capped tubes containing a solution of buffered glycerol-saline. MacConkey's and SS agar plates (Baltimore Biological Laboratory, Baltimore, Maryland) were streaked with each rectal swab within minutes after the samples were collected, and the swab was then placed in a tube of selenite F enrichment

medium. After incubation, aliquots of the enrichment medium were streaked on MacConkey's and SS agar plates. Following overnight incubation, the plates were examined according to the procedures of Edwards and Ewing (4).

Up to 10 lactose-fermenting colonies were picked from MacConkey's medium and transferred to heart infusion agar slants. These were individually tested after incubation against enteropathogenic *Escherichia coli* specific OB and O antisera (Difeo Laboratories, Detroit, Mich.). Viable and heat-killed suspensions from each slant were used in the slide agglutination tests. All culture suspensions agglutinating in polyvalent and OB antisera were further tested with O antisera in the tube agglutination test. Enteropathogenic *E. coli* cultures, agglutinating at a titer of 1:320 or greater, were confirmed biochemically.

Up to 6 non-lactose fermenting colonies from each streaked plate were examined

TABLE 1  
*Enterobacterial pathogens isolated from Panamanian primates on arrival at Gorgas Memorial Laboratory 1965-1967*

Species	Number examined	Number positive	Percent positive	Enterobacterial pathogens isolated
<i>Alouatta villosa</i> (Black howler monkey)	56	6	10.7	<i>Salm., E.E.C.</i>
<i>Aotus trivirgatus</i> (Night monkey)	201	5	2.5	<i>Salm., E.E.C.,</i> <i>Ariz., E. tarda</i>
<i>Ateles fusciceps</i> (Black spider monkey)	80	4	5.0	<i>Salm., Shig., E.E.C.</i>
<i>Ateles geoffroyi</i> (Red spider monkey)	13	0	0.0	None
<i>Cebus capucinus</i> (White face monkey)	20	0	0.0	None
<i>Saguinus geoffroyi</i> (Panamanian marmoset)	301	5*	1.7	<i>Salm., E.E.C.</i>
TOTALS	671	20	3.0	

\* One marmoset was infected with 2 species of *Salmonella*. *Salm.* = *Salmonella*; *E.E.C.* = enteropathogenic *E. coli*; *Ariz.* = *Arizona*; *E. tarda* = *Edwardsiella tarda*; *Shig.* = *Shigella*

biochemically and serologically for the presence of *Shigella*, *Salmonella*, and *Arizona* by the methods already mentioned. Following identification and serological grouping, tentatively identified organisms were submitted to the Enteric Bacteriology Unit, National Communicable Disease Center, Atlanta, Georgia, for species identification.

## RESULTS

Table 1 summarizes the results obtained from single rectal swab examinations on 671 newly arrived primates. A total of 21 enterobacterial pathogens were isolated from 20 primates representing 4 species of Panamanian monkeys. Isolations were most frequent from black howler monkeys; 6 (10.7%) of 56 individuals harbored either enteropathogenic *E. coli* or *Salmonella*. Infections due to enterobacterial pathogens were not detected in either red spider monkeys or white face monkeys. Of 301 Panamanian marmosets, 5 (1.7%) harbored *Salmonella* or enteropathogenic *E. coli*. Four (5.0%) of 80 black spider monkeys and 5 (2.5%) of 201 night monkeys were infected

with specific enterobacterial pathogens.

Two or more different salmonellas were recovered from each of the 4 primate species shown in Table 2. Eight *Salmonella* serotypes were represented. Only 1 recovery of *Arizona* and a single *Shigella* infection, due to *Sh. sonnei*, were detected. *Edwardsiella tarda*, a recently described species (5) whose relationship to diarrhea is still unknown, was also isolated once. Enteropathogenic *E. coli* were harbored by 4 of the 6 species of primates studied.

To provide some information on the extent of intracolony transmission of enteropathogenic bacteria, a group of monkeys was followed by weekly rectal swab cultures for a period of 3-20 weeks after arrival at the laboratory. Because of insufficient time and the secondary nature of this project, only 26 monkeys representing 4 species were selected for study. The care and handling of these monkeys was the same as that given other monkeys maintained at the laboratory. The period of examination for a particular monkey was terminated when it entered a malarial experiment or died. The results are shown in Table 3.

Cultures during the first 2 weeks after arrival revealed no infections by enterobacterial pathogens. Black spider monkeys experienced more infections than the other species of primates. *Shigella* organisms, representing 3 different species, were the predominant pathogens. They were recovered 11 times from 5-7 spider monkeys between the 3rd and 6th week of examination.

The occurrence of *Sh. flexneri* 6 in 3 spider monkeys during the 3rd week, but not in the preceding 2 weeks (Table 3), suggests a common source of infection. Two of these primates continued excreting the same *Shigella* serotype for another week. One additional isolation of *Shigella*, *Sh. flexneri* 2a, was made in the 9th week from monkey 46B, indicating a persistence of this infection for at least 5 weeks. Three recoveries of *E. coli* 0128:B12 were made between the 4th and 6th weeks of examination, each from a different spider monkey. An addi-

TABLE 2

Host distribution and frequency of enterobacterial pathogens isolated from Panamanian primates on arrival at Gorges Memorial Laboratory 1965-1967

Bacterial species	Number of isolations from host species				Total
	<i>Alouatta villosa</i>	<i>Aotus trivirgatus</i>	<i>Atelos fusciceps</i>	<i>Saguinus geoffroyi</i>	
<i>S. bredeney</i>				1	1
<i>S. infantis</i>			1		1
<i>S. javiana</i>	1				1
<i>S. muenster</i>		1		1*	2
<i>S. oranienburg</i>	2				2
<i>S. panama</i>				1	1
<i>S. poona</i>	2	1		1*	4
<i>Salmonella</i> Group H			1		1
<i>Arizona</i> 29: 33: 31		1			1
<i>Shig. sonnei</i>			1		1
<i>E. coli</i> 026:B6		1	1		2
<i>E. coli</i> 055:B5	1			2	3
<i>Edwardsiella tarda</i>		1			1
TOTALS	6	5	4	6	21

\* One *Saguinus geoffroyi* infected with both *S. muenster* and *S. poona*. *S.* = *Salmonella*; *Shig.* = *Shigella*

TABLE 3

Results of weekly rectal-swab cultures for enterobacterial pathogens from Panamanian primates at Gorgas Memorial Laboratory May to September 1967

No. of primate	Species of primate	Sex	Age	Week of observation after arrival at laboratory															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Ateles fusciceps</i> (Black spider monkey)																			
46B		M <sup>1</sup>	J <sup>2</sup>	— <sup>3</sup>	—	—	a <sup>4</sup>	a	f	—	—	a							
47B		M	J	—	—	—	f	—	—	—	—	—							
48B		F	J	—	—	—	—	—	—	—	—	—							
715A		F	J	—	—	b	b	b	—	—	—	—							
716A		F	J	—	—	c	b	c	—	—	—	—							
717A		F	J	—	—	b	b	—	—	—	—	—							
718A		M	J	—	—	b	—	—	f	—	—	—	—	d	—	—	—	—	— <sup>5</sup>
<i>Aotus trivirgatus</i> (Night monkey)																			
719A		F	A	—	—	—	—	—	—	—	—	—	—						
721A		M	A	—	—	—	—	—	—	g	—	—	—						
746A		F	A	—	—	—	—	—	—	—	—	—	—						
755A		F	A	—	—	—	—	—	—	—	—	—	—						
756A		M	A	—	—	—	—	—	—	—	—	—	—	—					
757A		M	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— <sup>5</sup>
758A		M	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	g	—
759A		F	A	—	—	—	—	—	—	e	—	—	—	—	—	—	—	—	—
770A		F	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
771A		M	A	—	—	—	—	—	f	—	—	—	—	—	—	—	—	—	—
<i>Saguinus geoffroyi</i> (Panamanian marmoset)																			
726A		M	A	—	—	—	—	—	—	—	—	—	—	—					
731A		F	A	—	—	—	—	—	—	—	—	—	—	—					
744A		M	I	—	—	—	—	—	—	—	—	—	—	—					
747A		M	A	—	—	—	—	—	—	—	—	—	—	—					
760A		M	A	—	—	—	—	—	—	—	—	—	—	—					
762A		M	I	—	—	—	—	—	—	—	—	—	—	—					
<i>Cebus capucinus</i> (White face monkey)																			
714A		F	J	—	—	—	—	—	—	—	—	—	—	—					— <sup>5</sup>
720A		M	J	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	a <sup>5</sup>
745A		F	J	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

1 M = male; F = female

2 J = juvenile; A = adult; I = infant

3 — indicates negative result.

4 a = *Shigella flexneri* 2ab = *Shig. flexneri* 6c = *Shig. sonnei*d = *Escherichia coli* 026:B6e = *E. coli* 0111:B4f = *E. coli* 0128:B12g = *Salmonella typhimurium*

5 Cultures were attempted for 1 to 4 additional weeks, but no further isolations were made.

tional isolation of *E. coli* 026:B6 from a spider monkey was made during the 11th week.

Enteropathogens were present in 3 of 10 night monkeys; 1 monkey had 2 separate infections. No isolations were made from cultures prior to the 6th week. The pathogens isolated were *E. coli* 0111:B4, *E. coli* 0128:B12, and *S. typhimurium*. The latter was isolated twice.

Pathogens were not demonstrated in Panamanian marmosets during the study. One of 3 white face monkeys experienced an infection with *Sh. flexneri* 2a during the 16th week.

Nine animal handlers and technicians who cared for or worked with the monkeys were negative for enteropathogenic bacteria on fecal cultures made at the initiation of the weekly surveys and on a subsequent examination.

#### DISCUSSION

Single examinations of 671 newly arrived and apparently healthy primates showed that only 20 (3.0%) harbored enterobacterial pathogens. Black howler monkeys had the highest infection rate, but only 6 (10.7%) of 56 monkeys of this species were infected. This indicates that these pathogens are not a major problem in any of the primate species on arrival at our laboratory.

*Salmonella* was the dominant organism in the primates examined on arrival. Thirteen of the 21 isolates represented by 8 different serotypes were *Salmonella*. We do not know whether the monkeys are natural reservoirs of this organism or acquired the infection between the time of capture and arrival at the laboratory.

*Shigella* was encountered only once, in a black spider monkey which had probably been held in an Indian home for several weeks prior to arrival at the laboratory. This is consistent with reports noted previously that *Shigella* species have been isolated only from captive primates (1, 6, 10, 14-16).

By contrast, 5 of 7 black spider monkeys kept at our laboratory were found positive for *Shigella*. These infections possibly originated directly or indirectly from their Indian captors. Six of these 7 spider monkeys had one or more positive cultures of *Shigella* and/or enteropathogenic *E. coli*, most were isolated between the 3rd to the 6th week after arrival at the laboratory. This is consistent with the epidemic pattern of shigellosis (8, 14). The *Shigella* rate in the spider monkeys was probably due to their food being placed on the concrete floors of their cages, where contamination by feces was likely.

Panamanian monkeys may be naturally infected or can be infected during captivity with enterobacterial pathogens. These infections pose a potential health hazard to other laboratory primates and to their human handlers. Further studies are necessary to clarify intracolony transmission of these agents, colony control measures, and problems arising from such infections, especially those of economic and public health importance.

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