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PANAMANIAN FOREST MAMMALS AS CARRIERS OF *SALMONELLA**

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Abstract. Enteric bacteria pathogenic to man were sought in a total of 974 forest mammals collected from a variety of sites in rural and jungle areas of Panamá. The highest incidence of infection among the mammals was observed during the Panamanian dry season, which normally extends from January through April. A minimum of 10 *Salmonella* serotypes including, three of the *Arizona* group and *Edwardsiella tarda*, was isolated. Opossums of the genera *Philander*, 11 of 54 (20.1%), and *Didelphis*, 12 of 102 (11.8%) demonstrated high infection rates. One sloth of the genus *Choloepus* and specimens of two genera of rodents also were infected to varying degrees; 1 (11.1%) of 9 *Choloepus*, 8 (1.1%) of 704 *Proechimys* and 1 (16.7%) of 6 *Diplomys*.

Due to its topographic and climatic diversity, and despite its small geographic area (30,000 sq. miles), Panamá has a large mammalian fauna. As many as 214 mammals are known to occur on the Isthmus of Panamá.[1] Studies elsewhere have shown that a large number of species of mammals in many parts of the world are naturally infected by *Salmonella*.[2-4] Previous surveys for enterobacterial pathogens among the wild animal populations in Panamá have recently been carried out to determine what role they might play as reservoirs for enteric pathogens. Those surveys were limited to nonhuman primates,[5] various reptile and amphibian species,[6] and elements of the rodent and marsupial fauna collected along a proposed site for a sea-level canal in eastern Panamá.[7]

The above studies revealed a variety of diarrhea-producing agents such as *Salmonella* and *Arizona* in a number of the more common species of animals found in this country. Such animals were without symptoms of disease, and perhaps may be the principle reservoir for human infections with *Salmonella* in remote rural and jungle communities.

Since rodents, opossums, and other mammals occur throughout Panamá in high population densities, and a number of them proved to be harboring *Salmonella*, it was believed necessary to extend our studies to other geographic areas in this country. Thus, additional species would be included if we were to learn more about the variety and relative frequency of pathogenic enteric bacteria in wild mammals that find their best ecologic conditions in the vicinity of man.

The present investigation was initiated in March 1969, incidental to other studies, and continued through November 1971.

MATERIALS AND METHODS

Field Methods

Mammals were collected over a period of almost 3 years in all seasons and in a variety of habitats in different areas of the Republic of Panamá (Fig. 1 and Table 1). Most were trapped in the Pacific and Atlantic lowlands, and a few were collected along the Panama Canal. Small animals, such as rodents, were captured by setting Sherman traps. Larger animals such as opossums were caught in wire traps of various sizes (National Live Trap Corporation, P. O. Box 302, Tomahawk, Wisconsin 54487). The traps were baited with banana, corn, or toasted coconut. Sloths were captured by hand; bats were netted in free flight. All animals were taken to base camps in the field and kept in 25 × 25 × 35 cm stainless steel cages. Rodents were fed Lab-Blox (Allied Mills, 2205 Lamar Avenue, Memphis, Tenn. 38114). Opossums and other mammals were offered bananas, oranges, and yucca. The length of time they were

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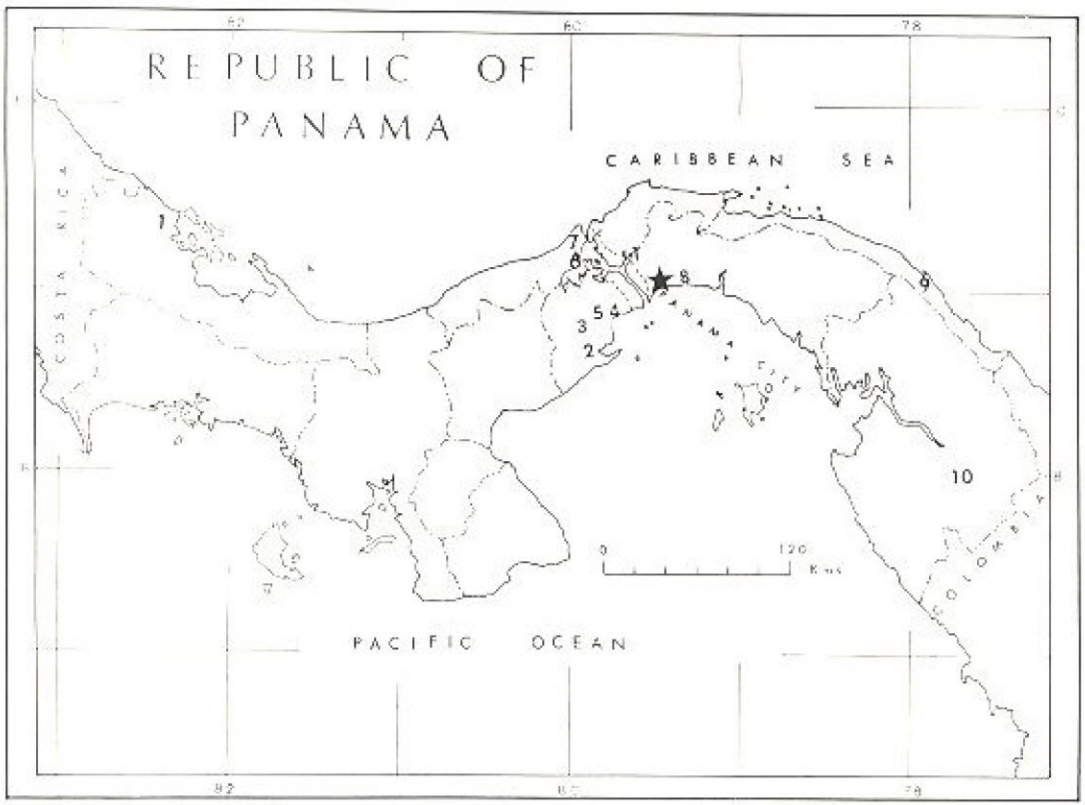

FIGURE 1. Map showing major collection sites in the Republic of Panamá. Locality No. 1, Almirante; No. 2, Cerro Campana; No. 3, Aguacate; No. 4, La Chorrera; No. 5, Santa Rita; No. 6, Achiote; No. 7, Las Tablitas; No. 8, Juan Diaz; No. 9, Sasardi; No. 10, Rio Mono; and No. 11, Madden Lake. Panama City is shown by the star.

held in the field never exceeded 1 week. None of the animals exhibited overt signs of disease on arrival at the Gorgas Memorial Laboratory in Panama City, where they were kept individually in cages for a variable length of time.

Laboratory Methods

Small animals (rodents and marsupials) were killed by chloroform, or by cardiac exsanguination after ether anesthesia. The large mammals (edentates and carnivores) after being tranquilized with Sernylan (Bio-Ceutic Laboratories Inc., Saint Joseph, Missouri 64502) were bled from the heart.

Aseptic techniques were used to remove portions of intestine and its contents from each animal immediately after death. The specimens were transferred with sterile swabs to selenite broth. Following incubation for 18 hours, aliquots of the selenite broth were streaked on *Salmonella–Shigella* (SS) agar, Mac Conkey's (MC) agar, and bismuth sulfite (BS) agar plates. All plates were examined after incubation, according to the procedures recommended by Edwards and Ewing.[8]

Up to 10 nonlactose-fermenting colonies from each SS and MC plate were streaked on triple-sugar-iron (TSI) agar slants, and at least five suspected colonies were transferred from the BS plates to TSI agar and to lysine-iron-agar (LIA) slants. After overnight incubation, the cultures were studied by biochemical and serological methods described elsewhere.[8] Examination was done only for *Salmonella*, *Shigella*, *Arizona*, and *Edwardsiella*. Following preliminary identification and serological grouping, cultures were submitted to the Enteric Bacteriology Unit, Center for Disease Control, Atlanta, Georgia for serotype identification.

TABLE 1

Panamanian forest mammals sampled for enterobacterial pathogens

Species	Number of specimens by locality												
	Archúte	Aguacate	Almirante	Cerro Campana	Chorrera	Juan Diaz	Las Tablitas	Río Mono	Santa Rita	Sasardi	Madden Lake	Other	Total
Marsupialia													
Caluromys derbianus	–	–	–	–	–	–	–	–	5	–	–	1	6
Marmosa robinsoni	–	–	–	–	–	–	–	–	1	16	–	–	17
Philander opossum	2	5	–	3	–	–	–	–	42	–	2	–	54
Metachirus nudicaudatus	1	–	–	–	–	–	–	–	1	14	–	–	16
Didelphis marsupialis	7	–	–	3	4	–	1	–	85	–	1	1	102
Chiroptera													
Phyllostomus hastatus	–	–	–	–	–	–	–	–	–	–	2	2	4
Edentata													
Bradypus infuscatus	–	–	–	–	–	–	10	–	–	–	–	2	12
Choloepus hoffmanni	–	8	–	–	–	–	–	–	–	–	–	1	9
Dasypus novemcinctus	–	–	–	–	–	–	–	–	–	–	–	1	1
Rodentia													
Heteromys desmarestianus	–	–	–	–	–	–	–	–	–	–	1	–	1
Oryzomys caliginosus	–	–	–	–	–	–	–	–	–	3	–	–	3
Oryzomys capito	–	–	–	–	–	–	–	–	–	1	–	–	1
Tylomys panamensis	4	–	–	–	–	–	–	–	–	–	–	–	4
Sigmodon hispidus	–	–	14	–	–	–	–	–	6	–	–	–	20
Rattus rattus	–	–	–	–	3	–	–	–	7	–	1	–	11
Proechimys semispinosus	28	5	99	–	–	7	8	5	516	25	10	1	704
Diplomys labilis	–	–	–	–	–	–	–	–	6	–	–	–	6
Carnivora													
Potos flavus	–	–	–	–	–	–	–	–	–	2	–	–	2
Bassaricyon gabbii	–	–	–	–	–	–	–	–	–	1	–	–	1
Totals	42	18	113	6	7	7	19	5	669	62	17	9	974

TABLE 2

Incidence of enterobacterial pathogens in Panamanian forest mammals

Scientific name	Common name	No. examined	Positive	
			No.	%
Marsupialia				
Caluromys derbianus	Woolly opposum	6	0	–
Marmosa robinsoni	Brown-murine opossum	17	0	–
Philander opossum	Four-eyed opossum	54	11	20.1
Metachirus nudicaudatus	Brown-masked opossum	16	0	–
Didelphis marsupialis	Common opossum	102	12	11.8
Total Marsupialia		195	23	11.8
Edentata				
Bradypus infuscatus	Three-toed sloth	12	0	–
Choloepus hoffmanni	Two-toed sloth	9	1	11.1
Dasypus novemcinctus	Nine-banded armadillo	1	0	–
Total Edentata		22	1	4.5
Rodentia				
Heteromys desmarestianus	Spiny pocket mouse	1	0	–
Oryzomys caliginosus	Rice rat	3	0	–
Oryzomys capito	Rice rat	1	0	–
Tylomys panamensis	White-tailed rat	4	0	–
Sigmodon hispidus	Cotton rat	20	0	–
Rattus rattus	Roof rat	11	0	–
Proechimys semispinosus	Spiny rat	704	8	1.1
Diplomys labilis	Arboreal spiny rat	6	1	16.7
Total Rodentia		750	9	1.2
Others				
Bassaricyon gabbii	Olingo	2	0	–
Potos flavus	Kinkajou	1	0	–
Phyllostomus hastatus	Spear-nosed bat	4	0	–
Total others		7	0	–
GRAND TOTALS		974	33	3.4

RESULTS

A total of 974 forest mammals were trapped, and represented five orders: Rodentia, Marsupialia, Edentata, Carnivora and Chiroptera (Table 1). A rodent (*Proechimys semispinosus*) and two marsupial species (*Philander opossum* and *Didelphis marsupialis*) comprised 88.5% of the sample, and 16 different species represented the remaining 11.5%. Other important species examined included sloths and carnivores, which were not as abundant or were not successfully trapped during our studies. The order Chiroptera was represented by four specimens of *Phyllostomus hastatus*.

The results of bacteriological cultures for enteric pathogens in these animals are summarized in Table 2; mainly *Salmonella* and *Arizona* were recovered from 33 (3.4%) of the 974 animals examined. Organisms were demonstrated in the orders Marsupialia (11.8%), Edentata (4.5%) and Rodentia (1.2%). Among these orders, the incidence of infection varied considerably in relation with the species of animals studied and the season of the year. The highest infection rates (Table 2) were determined in *P. opossum* (20.1%) and *D. marsupialis* (11.8%), the two most common marsupials captured. The most numerous of the animals surveyed was the spiny rat, *P. semispinosus*, in which 8 (1.1%) of 704 specimens harbored enterobacterial pathogens. Infected *Proechimys* and *Diplomys* came from the Central Pacific side of Panamá.

The host distribution and frequency of the

TABLE 3

Frequency and host distribution of enterobacterial pathogens in Panamanian forest mammals

Bacterial species	Number of times strains isolated from host species					Total (%) strains isolated
	Choloepus hoffmanni (1)*	*Didelphis marsupialis* (12)	*Diplomys labilis* (11)	*Philander opossum* (11)	*Proechimys semispinosus* (8)	
S. newport	–	3	1	5	2	11 (31.3)
S. san diego	–	4	–	1	–	5 (14.2)
S. sundsvall	–	1†	–	–	2	3 (8.5)
S. bonair	–	1†	–	–	–	1 (2.9)
S. oslo	–	1	–	–	–	1 (2.9)
S. miami	–	–	–	1	–	1 (2.9)
S. panama	–	–	–	–	1	1 (2.9)
S. wassenaar	–	–	–	1	1	2 (5.7)
S. rubislaw	–	–	–	1‡	–	1 (2.9)
Salmonella, group H	–	1	–	–	1	2 (5.7)
A. kinsawii 16: 22, 31	–	–	–	–	1	1 (2.9)
A. kinsawii 26: 33, 31	1	–	–	–	–	1 (2.9)
A. kinsawii 30: 23, 25	–	1	–	–	–	1 (2.9)
E. tarda	–	1	–	3‡	–	4 (11.4)
TOTAL	1	13	1	12	8	35 (100.0)

* Number of animals found infected.
† One *Didelphis* with *S. bonair* and *S. sundsvall*.
‡ One *Philander* with *S. rubislaw* and *E. tarda*.

bacterial species isolated are given in Table 3. In general, of the 35 strains recovered, *Salmonella newport* (31.4%), *S. san diego* (14.2%), and *E. tarda* (11.4%) were the most prevalent and accounted for 57% of all strains recovered. Ten *Salmonella* and three *Arizona* serotypes were represented. *Edwardsiella tarda*, whose relationship to diarrhea in humans is under investigation (Kourany, M., unpublished data), was isolated on four different occasions from opossum (*Didelphis* and *Philander*) hosts.

Nine of the 11 infected *Philander* were captured in central Panamá, in the dry season, while the other two were taken in the wet season. Two of these opossums harbored *Edwardsiella* alone while another was infected with both *Salmonella* and *Edwardsiella* (Table 3); the remaining eight harbored *Salmonella* only. Among the 102 *Didelphis* examined, 12 were infected with either *Salmonella*, *Arizona*, or *Edwardsiella*. The majority, however, harbored salmonellae (Table 3) and were collected in the dry season; only 3 of the infected 12 were caught in the wet season (Table 4). Opossums belonging to other genera (*Caluromys*, *Marmosa*, and *Metachirus*) were not found infected with pathogens.

Of the 750 rodents sampled, 9 (1.2%) were positive for enterobacterial pathogens. Among the eight genera tested, infected rodents were found in only two (Table 2). Only one infected *Diplomys* and eight infected *Proechimys* from central Panamá were collected. The infected *Diplomys* rat was captured in the dry season; all but one of the *Proechimys* which harbored pathogens were trapped in the wet season (Table 4).

No enterobacterial pathogens were isolated from bats (*Phyllostomus*) or carnivores (*Bassaricyon* and *Potos*), but the number of specimens studied was small. Among the edentates examined, *Arizona* was isolated only from a single two-toed sloth (*Choloepus*) collected in central Panamá at El Aguacate in the dry season (Tables 2 and 4).

TABLE 4

Infection rates for enterobacterial pathogens in forest mammals according to season

Host mammal	Wet season			Dry season		
	No. examined	Positive		No. examined	Positive	
		No.	%		No.	%
Philander	17	2	11.8	37	9	24.3
Didelphis	45	3	6.7	57	9	15.8
Choloepus	6	0	–	5	1	
Proechimys	472	7	1.5	232	1	0.4
Diplomys	4	0	–	2	1	
TOTAL	544	12	2.2	331	21	6.3

FIGURE 2. Extensive deforestation in Panamá and the opening of large tracts of forest for cattle raising and agriculture is disturbing the ecological balance by limiting the natural cover and altering the food habits of many mammalian species. Notice hut (center) amidst what once was jungle.

DISCUSSION

This study indicated that diarrheal producing agents, such as *Salmonella*, are present among some elements of the wild mammalian fauna of Panamá and that they might serve as important reservoirs. *Salmonella* was the most prevalent of the pathogens isolated and at least 10 different serotypes were identified. *Arizona* as well as *Edwardsiella* was found, but was not as common as *Salmonella*, pointing to a diversity of harbored enteric bacterial species.

The mammals with the highest infection rates are common scavengers and are widely distributed in rural and jungle areas. They seem to thrive best in the vicinity of man, thus emphasizing their probable role as incidental reservoirs of *Salmonella* organisms. Since the distribution of these organisms in man is closely linked with that in the animal population of man's environment, these organisms are more frequently transmitted from animals to man than from man to man or from man to other animals.[9] All of the serotypes listed in Table 3, except *S. bonair* and *S. wassenaar*, have been implicated in salmonellosis of man in rural or urban parts of Panamá.[9, 10–12] Human infections with *E. tarda* and with *Arizona* organisms also have been detected, but the *Arizona* serotypes isolated were different from those found in the present study (Kourany, M., unpublished data).

The highest incidence of infection among the mammals was observed during the dry season, which normally extends from January through April. Overall infection rates for the enterobacterial pathogens were about three times as high in animals trapped during the dry months (Table 4) than in those collected in the wet months ($p < 0.003 > 0.002$). This is particularly true for the two opossum species most commonly infected, *P. opossum* and *D. marsupialis*. It should be pointed out that these species are much more predatory and active during the dry season when they are constantly in search of food and this could account for the higher infection rates due to greater contact with sources of infection.

Most of the infected animals in the present study were caught in or near areas of human activity and could serve as a source of infection for man, his livestock, poultry, and other domestic animals. For example, the foraging habits of *D. marsupialis* bring this animal into closer contact with humans. This may pose an important public health problem since those species of animals capable of harboring enteric pathogens occur throughout Panamá in high densities and may contribute to health hazards in the rural areas.

With a high potential for social and economic growth, Panamá is developing at a fast pace. Roads and communication networks rapidly are being expanded through jungle and rural areas. In many regions, extensive deforestation is taking place. Large tracts of forest are being destroyed by traditional slash-and-burn methods (Fig. 2) as individual farms are opened for cattle raising and agriculture.

The chronically inadequate sanitation that prevails in these regions is most likely to be aggravated in the near future as large segments of the human population from central and western Panamá migrate to settle and colonize the newly opened lands. This situation is upsetting the ecological balance of the forest and is most likely to affect the stability of the wild mammalian fauna. Their natural cover and food habits may be affected and may bring man in closer contact with them, thus increasing many of the diseases, such as salmonellosis, that are transmitted from animals to man. Problems of potable water are compounded by a general lack of safe disposal of feces. The result is that animals harboring and shedding enterobacterial pathogens in close proximity to man, like the common and the four-eyed opossums, may conceivably be contributing fac-

tors in maintaining the endemicity of diarrheal disease of man in Panamá.

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