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Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 19, No. 4, July 1970
pp. 632–638
Printed in United States of America

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE

Vol. 19, No. 4
Printed in U.S.A.

PANAMANIAN AMPHIBIANS AND REPTILES AS CARRIERS OF *SALMONELLA**†

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ABSTRACT: Enteric bacteria pathogenic to man were sought in a total of 548 frogs, toads, and lizards collected throughout the Republic of Panamá. Potential hosts were collected in a variety of habitats, but pathogens were regularly isolated only from species occurring near human habitations, in pasture land, and at a public bathing area. A minimum of 19 species of *Salmonella*, including seven of the Arizona group, was isolated. Most of the species of *Salmonella*, except those of the Arizona group, have been implicated in salmonellosis and diarrheal diseases in man in rural Panamá. Lizards of the genus *Ameiva* showed the highest incidence of infection, with 50.5% of 101 specimens harboring *Salmonella*. Specimens representing three other genera of lizards were also infected, but to a lesser degree: seven (28.0%) of 25 *Sceloporus*, two (18.2%) of 11 *Basiliscus*, and three (7.5%) of 40 *Cnemidophorus*. The giant toad, *Bufo marinus*, generally is more abundant in areas disturbed by man than elsewhere and was infected in moderate numbers: 12 (7.6%) of 185 specimens carried *Salmonella*. *Salmonella* was present in a single frog, *Leptodactylus pentadactylus*, collected in a town, but pathogens were absent in 128 other frogs (13 genera) that were collected mostly in forested areas.

(Accepted 17 December 1969)

Infections from bacteria of the genus *Salmonella* are common and widespread among man's poultry, livestock, and household pets,[1-3] all of which probably are important sources of salmonellosis in human beings. Wild species of homoiothermic vertebrates also are found infected with *Salmonella*.[6] Arizona, one of the groups of *Salmonella*, is known to cause epidemics in turkeys, chickens, canaries, hogs, tortoises, and rattlesnakes, as well as severe diarrhea and gastroenteritis in man.[7] Among the poikilothermic vertebrates, reptiles are frequently carriers of these enteric bacteria,[6,8-10] and pet turtles recently have been implicated as a source of salmonellosis in man, especially children.[11]

Little is known about what kinds of enteric bacteria occur in the poikilothermic vertebrates of tropical America, and so the present study was undertaken to determine whether Panamanian amphibians and reptiles might harbor enteric bacteria that are pathogenic to man. Although the study included amphibians and reptiles from uninhabited regions, we were particularly interested in species from areas frequented by people. In Panamá (and elsewhere) there are certain "edge" animals that have their highest population densities in ecologically disturbed places, such as recent forest clearings and about human habitations. During the course of epidemiologic investigations in rural parts of Panamá, it was observed that large toads and certain lizards were often common on grounds contaminated by wastes from man and his livestock. Under the conditions of inadequate sanitation that prevail in most rural areas, where fecal pollution of ground is the rule, it seemed possible that elements of the amphibian and reptile fauna might constitute a significant reservoir of enteric bacteria, thus contributing to the endemicity of diarrheal diseases caused by *Salmonella*.

MATERIALS AND METHODS

Field Methods

The investigation was begun in January 1965, incidental to other studies, and was terminated in June 1967. Some 548 animals (227 lizards, 193

* This work was supported in part by Grant No. GM 12020 from the National Institutes of Health to the University of Kansas.

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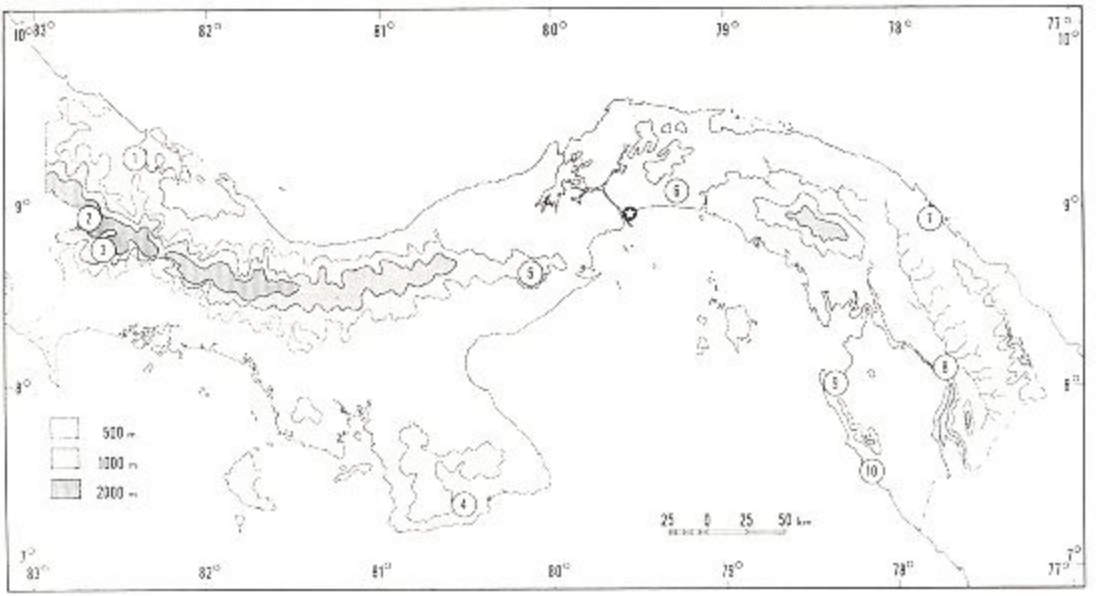

FIGURE 1. Map showing major collection sites in the Republic of Panamá. Locality No. 1, Almirante; No. 2, Cerro Pando; No. 3, Llanos del Volcán; No. 4, Guánico Arriba; No. 5, El Valle; No. 6, Pacora area; No. 7, Sasardí; No. 8, El Real; No. 9, Garachiné; and No. 10, Jaqué. Panamá City is shown by the star.

toads, and 128 frogs) were sampled for pathogenic enteric bacteria. The amphibians and reptiles were collected in a variety of habitats in different parts of the Republic of Panamá (Fig. 1 and Table 1). The amphibians were captured by hand, mostly at night with the aid of a head-light; the lizards were caught by hand or shot with 0.22-caliber dustshot, which often only stunned the specimen. Live-caught animals were killed in various ways (sodium pentobarbital injection for lizards; chloroform fumes for large toads and some lizards; or by drowning in chlorobutanol solution for other amphibians).

After death, an abdominal incision was made, and a piece of intestine and its contents were removed. Specimens captured alive in central Panamá (*e.g.*, all lizards from Pacora) were usually taken to the laboratory in Panamá City, where the portion of intestine was cultured for enteric bacteria upon its removal. Collections made in other regions were handled in base camps, where the samples of intestine were placed in separate screw-capped jars containing buffered glycerol-saline solution. The jars were kept at ambient temperature until delivery to the laboratory in Panamá City, where cultures were made usually within 48 hours (never more than 72 hours) of the time that the animals were killed.

Limitations imposed by use of the holding solution necessarily restricted the variety and number of specimens that could be obtained on trips to remote areas, since only animals collected in the last few days of a trip were used in this study. Specimens caught alive sometimes were held for a few days before being killed; intestinal samples were removed in less than an hour after death from specimens that had been shot. Although animals could not always be placed in separate collecting bags, we believe that contamination was avoided by aseptic techniques used in taking the sample of intestine.

Laboratory Methods

The sample of intestine and its contents were transferred aseptically to selenite broth with sterile swabs, after cutting the intestine longitudinally in a Petri dish. After overnight incubation of the broth at 37°C, aliquots were plated on *Salmonella-Shigella* (SS) agar, MacConkey's agar, and bismuth sulfite (BS) agar plates.

All plates were examined after incubation following the procedures recommended by Edwards and Ewing.[12] Six to ten nonlactose-fermenting colonies from each SS and MacConkey plate were transferred to triple-sugar-iron (TSI) slants, and a similar number of suspected colonies were trans-

TABLE 1

Panamanian amphibians and reptiles samples for enteric bacterial pathogens

Species	Number of specimens by locality											
	Almirante	Cerro Pando	El Real	El Valle	Garachiné	Guánico Arriba	Jaqué	Llanos del Volcán	Pacora	Sasardí	Other	Total
Amphibians (frogs & toads)												
Bufo marinus	91		45			25				24		185
Bufo spp.	1								5		2	8
Atelopus chiriquiensis		20										20
Atelopus varius					37							37
Colostethus inguinalis				15							1	16
Eleutherodactylus (8 spp.)	8										11	19
Leptodactylus pentadactylus	1											1
Other (9 genera, 11 spp.)	23										12	35
Total amphibians	124	20	45	15	37	25	—	—	5	24	26	321
Reptiles (lizards)												
Ameiva ameiva						7			87		2	96
Ameiva festiva	1											1
Ameiva leptophrys							4					4
Cnemidophorus lemniscatus			13				27					40
Anolis (9 spp.)	19	1									23	43
Basilicus basilicus									7		4	11
Basilicus vittatus	1											1
Sceloporus malachiticus								25				25
Other (5 genera, 6 spp.)	4								1		1	6
Total reptiles	25	1	13	—	—	7	31	25	95	—	30	227
Grand totals	149	21	58	15	37	32	31	25	100	24	56	548

ferred from the BS plates to lysine-iron-agar (LIA) slants. After incubation, all tubes were examined biochemically and serologically for the presence of *Salmonella*, including Arizona, by conventional methods.[12] Enteropathogenic *Escherichia coli* were not sought. Slide agglutination tests with polyvalent grouping antiserum samples (Difco) were performed on all colonies giving typical *Salmonella* reactions. After preliminary identification, cultures were sent to the National Communicable Disease Center in Atlanta, Georgia, for final determination.

Chi-squares (χ^2) were calculated by methods involving 2×4 and 2×2 tables, and the formula in the latter case contains Yates's correction factor.[13]

RESULTS AND DISCUSSION

Of a total of 128 frogs representing 13 genera, *Salmonella* was isolated only from a single *Leptodactylus pentadactylus*. Nearly all the frogs were collected in forest (*e.g.*, *Eleutherodactylus* spp.) or along forest streams (*e.g.*, *Atelopus* spp., *Colostethus inguinalis*), but the positive specimen of *Leptodactylus* came from within the town limits of Almirante. The situation was different for the giant toad, *Bufo marinus*, in which 12 (6.5%) of 185 specimens were positive for *Salmonella*. The intersample variability in *Bufo marinus* (0 to 16.7% positive, Table 2) is of a low order of statistical significance ($\chi^2 = 8.71$; $p < 0.05 > 0.02$), but reasons for this variability are not evident.

The highest incidences of infection were at the El Real (Darién Province) and Sasardí (San Blas Territory) localities in eastern Panamá, a region of humid forest affected by a winter dry season. Infection was lower in the sample from Almirante (Bocas del Toro Province) in northwestern Panamá, a wet forest region without a dry season (true rain forest). No isolations were

TABLE 2

Incidence of Salmonella *in Panamanian toads (*Bufo marinus*)*

Locality	No. examined	Positive (*No.*)	Positive (%)
Almirante	91	3	3.3
El Real	45	5	11.1
Guánico Arriba	25	0	0.0
Sasardi	24	4	16.7
Total	185	12	6.5

TABLE 3

Incidence of Salmonella *in Panamanian lizards*

Host species	No. examined	Positive (*No.*)	Positive (%)
Ameiva ameiva	96	48	50.0
Ameiva festiva	1	1	—
Ameiva leptophrys	4	2	—
Anolis spp. (9)	43	0	0.0
Basiliscus basiliscus	11	2	18.2
Cnemidophorus lemniscatus	40	3	7.5
Sceloporus malachiticus	25	7	28.0
Other species (7)	7	0	—
Total	227	63	27.8

made from toads collected at Guánico Arriba (Los Santos Province) on the Azuero Peninsula, a region in which the lowland forest has been cleared and where the winter dry season is more pronounced than in eastern Panamá.

The Almirante and El Real collections were made in towns in rainy weather; differences in incidence of infection between these two samples are not statistically significant ($\chi^2 = 2.06$; $p < 0.20 > 0.10$). The Sasardi and Guánico Arriba toads were collected along shallow rivers in the dry season; differences in incidence of infection are not statistically significant ($\chi^2 = 2.59$; $p < 0.20 > 0.10$). Guánico Arriba is in an area of relatively high human population density, but houses are more dispersed than at the El Real and Almirante localities and (because the country is drier and more open on the Pacific side of western Panamá) the toads are likewise more dispersed in the environment. There were no permanent human habitations at the Sasardi locality, but the area is frequented by Cuna Indians who farm in the vicinity. Only at Guánico Arriba was livestock (other than chickens in the towns) present. No enterobacterial pathogens were isolated from forest toads, *Bufo coniferus* and *Bufo typhonius*, but the samples were too small to warrant conclusions (Table 1).

Of 227 lizards sampled, 63 (27.8%) were positive for *Salmonella*. Only one infected lizard, an *Ameiva festiva* from the vicinity of Almirante, was collected in forest. Therefore, the absence of pathogens from the mainly arboreal forest lizards of the genus *Anolis* (Table 3) seems meaningful. Pathogenic enteric bacteria were regularly isolated from *Ameiva ameiva*, a large and common lizard of open, grassy country (including yards and vacant lots in Panamá City) in Pacific-side western Panamá. Samples of this species were collected near houses at Guánico Arriba and in the vicinity of Pacora, near houses and along a river at a beach used for public bathing by holiday and week-end crowds; livestock occurred at all sites. Forty-eight of 87 specimens of *Ameiva ameiva* (55.3%) from the Pacora area were infected with *Salmonella*; seven specimens collected at Guánico Arriba were not infected. Two of four *Ameiva leptophrys* from the outskirts of Jaqué, Darién Province, also were infected with *Salmonella*.

Cnemidophorus lemniscatus, a terrestrial lizard closely related to *Ameiva*, occurs in eastern Panamá, where it is most abundant about human habitations and is nearly absent in forest. Incidence of infection was similar for samples from El Real (one of 13 lizards, 7.7%) and Jaqué (two of 27, 7.4%). *Basiliscus basiliscus* is a stream-side lizard whose habitat slightly overlaps that of *Ameiva ameiva* at the public swimming area near Pacora; two of seven specimens from this locality contained *Salmonella* of the Arizona group. *Sceloporus malachiticus* is a highland lizard of extreme western Panamá, where it occurs commonly on and about rural houses. A sample of 25 *Sceloporus* from large boulders on a volcanic outwash plain yielded seven specimens (28.0%) infected with *Salmonella*; few persons travel on this plain, which, however, is used to pasture cattle.

The frequency and host distribution are given in Table 4 for all of the species of *Salmonella* that were isolated. The predominant organisms were *Salmonella miami* (21.8%), *Salmonella glostrup* (20.5%), and *Salmonella arizonae* 28:43 (12.8%). These three species of bacteria accounted for 55% of all pathogens recovered and were isolated exclusively from *Ameiva ameiva*. Of a minimum of 19 species of bacteria, only six

TABLE 4

Frequency and host distribution of species of Salmonella *in Panamanian reptiles and amphibians*

Bacterial species	Number of times strains isolated from host species								Total strains isolated	(%)
	Ameiva ameiva	*Ameiva festiva*	*Ameiva leptophrys*	*Basiliscus basiliscus*	*Sceloporus malachiticus*	*Cnemidophorus lemniscatus*	*Bufo marinus*	*Leptodactylus pentadactylus*		
S. anatum							1		1	1.3
S. miami	17*								17	21.8
S. glostrup	16*†								16	20.5
S. oslo	1								1	1.3
S. panama	1						1		2	2.6
S. madelia	3†								3	3.8
S. oranienburg						1	2		3	3.8
S. rubislaw	1								1	1.3
S. san diego		1	1		1		2	1	6	7.6
S. thompson							1		1	1.3
S. enteritides							1		1	1.3
S. arizonae 1, 3:1, 2, 10					5				5	6.4
S. arizonae 9a, 9b:1, 3, 11							1		1	1.3
S. arizonae 10a, 10b					1				1	1.3
S. arizonae 25:1, 2, 10	1			1					2	2.6
S. arizonae 26:31, 33				1					1	1.3
S. arizonae 28:43	10								10	12.8
S. arizonae						2	2		4	5.1
Salmonella spp.			1				1		2	2.6
Total	50	1	2	2	7	3	12	1	78	100.0

* One *A. ameiva* with *S. miami* and *S. glostrup*.
† One *A. ameiva* with *S. madelia* and *S. glostrup*.

species were found in more than one host species. Only one species, *Salmonella san diego*, was present in more than two species, occurring in several lizards, toads, and a frog.

All but three of the salmonellae serotypes listed in Table 4 (*Salmonella madelia*, *S. thompson*, and *S. enteritidis*) have been implicated in salmonellosis in man in rural parts of Panamá.* Two, apparently healthy human carriers of *Salmonella oslo*, were found at a dairy farm near the Pacora River, where this pathogen was also isolated from a lizard (*Ameiva*). Human infections with *Salmonella* of the Arizona group also have been detected in rural Panamá,* but the serotypes isolated were different from those in Table 4. *Salmonella* infections in man may be asymptomatic but generally produce a food-poisoning syndrome, transient gastroenteritis, or a serious septicemic disease caused by *Salmonella paratyphi* and members of other salmonellae serogroups.[14] *Salmonella* infections conceivably contribute to the death of occasional reptiles,[15] but amphibians and reptiles usually seem to be symptomless carriers; that they may form a reservoir of infection for man and other animals must be considered as a possibility.

Further studies are planned to elucidate the incidence of infection and possible relation of amphibians and reptiles to salmonellosis and diarrheal diseases in man in Panamá, but at present we think that a relation must exist. Salmonellosis is more frequently transmitted from other animals to man than from man to man or from man to other animals,[16] and it is only logical to suspect that "domestic" toads and lizards are a link in the cycle involving man and domestic

* Kourany, M. Unpublished data.

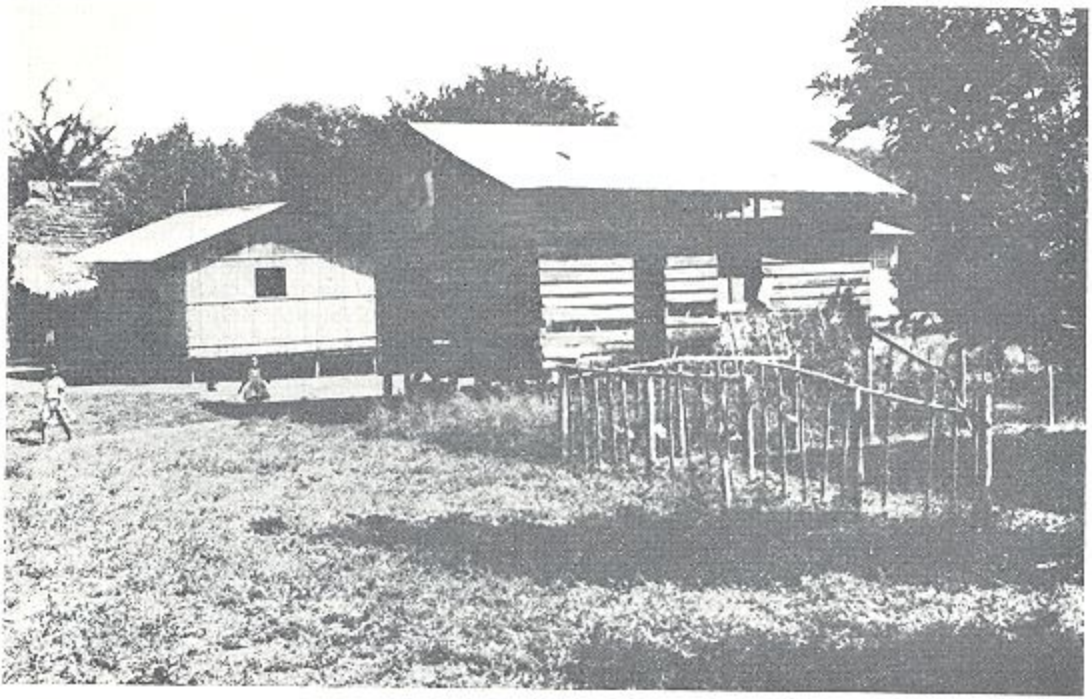

FIGURE 2. Jaqué, a rural town in Darién Province, eastern Panamá. The children are playing on the same ground where specimens of an abundant lizard (*Cnemidophorus lemniscatus*) were found infected with *Salmonella*. Giant toads (*Bufo marinus*) forage about the houses at night and are known carriers of enteric bacterial pathogens, although the toad population was not sampled at this locality.

animals.[17] This also is suggested by our preliminary data indicating lower rates of infection among forest species than those occurring in the vicinity of man. Human beings usually are infected by ingesting contaminated food or water. Water sources may be contaminated by salmonellae from the feces of lizards,[18] unprotected food may be contaminated by the excreta of lizards in houses,* or insects such as flies may serve as mechanical carriers of pathogens from the wastes of any infected animal. Children, who seldom seem inclined to wash their hands, coexist on the same playgrounds (Fig. 2) with infected toads or lizards and possibly transmit pathogens from the ground directly to the mouth.

Several species of amphibians and reptiles that were found infected by *Salmonella* are very common dooryard animals that are not restrained by fences. These species (*Bufo marinus*, *Ameiva ameiva*, and *Cnemidophorus lemniscatus*) are widely distributed in the American Tropics, each part of which has still additional species that find their best ecologic conditions in the vicinity of man. Thus, the chronically inadequate sanitation that prevails in the rural Tropics seems further complicated by the high population densities of such animals, which merit more attention as potentially important carriers and reservoirs of pathogenic enteric bacteria.

* This possibility is mentioned by Fiennes.[19] Several kinds of lizard occur in houses in Panamá but were not included in the present study. Species of the genera *Gonatodes*, *Lepidodactylus*, *Mabuya*, and *Sphaerodactylus* deserve consideration.

ACKNOWLEDGMENTS

The authors are deeply grateful to Dr. W. H. Ewing of the National Communicable Disease Center, Atlanta, Georgia, for the determination of the species of *Salmonella* and the strains of *S. arizonae*.

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