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Lizards in the Ecology of Salmonellosis in Panama

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Enteropathogenic bacteria were isolated from 131 of 447 (29.4%) neotropical Panamanian lizards belonging to 34 species of seven families. Overall, 147 strains of bacteria were isolated comprising 26 *Salmonella* and 10 *Arizona* serotypes. *Gymnopthalmus speciosus* had the highest infection rate, 12 of 13 individuals (92.3%), whereas *Gonatodes fuscus* exhibited the lowest, 1 of 18 (5.6%). The highest infection was detected in lizards whose behavioral patterns were secretive (42.0%) and terrestrial (42.6%), whereas the lowest infection was among the scansorial lizards (17.5%). Rates were highest during the dry season, from January through April. Many neotropical Panamanian lizards were multiply infected by *Salmonella* and *Arizona* strains representing a wide range of serotypes. Infected lizards were distributed in areas varying from remote rural and forested regions to urban developments, offering a potentially important reservoir of enteropathogenic bacteria known to cause infection in man and domestic animals.

During investigations on diarrheal disease in rural Panama, toads and lizards were commonly observed on ground contaminated by human and animal excreta. This suggested that amphibian and reptilian fauna might constitute a potential reservoir of pathogenic enteric agents, thus contributing to the endemicity of diarrheal disease in the area. In a preceding report, *Salmonella* spp. were regularly isolated from lizards inhabiting pasture lands, areas near human habitations, and a public bathing area (9).

In view of the limited literature on *Enterobacteriaceae* in poikilothermic vertebrates of tropical America, a more comprehensive survey of reptilian species of Panama was undertaken. The present study includes findings from lizards seldom associated with humans as well as from species inhabiting dooryards.

MATERIALS AND METHODS

Field methods and collection sites. A total of 447 lizards were collected from 8 January 1966 through 17 November 1969 from various habitats in the Republic of Panama (Fig. 1). Reptiles were captured alive (by hand or noose) and transported in individual polyethylene bags to the laboratory in Panama City within 72 h of collection. Specimens captured in the most remote areas had to be kept for a few days at base camps before transport to the laboratory. Reptiles did not exhibit overt signs of disease.

Most lizards (388; 87%) were captured from six localities, predominantly in central Panama (Fig. 1 and Table 1), as follows: primary and mature second-growth forests in which humans ventured only as occasional hunters (Achiote and Gamboa); along stream beds in pockets of original vegetation scattered around a community of some 200 persons (El Aguacate); an area of 30 scattered small homesteads cleared from the forest (Gaspar Sabanas); tracts of second-growth forest interspersed with old banana plantations and corn fields tended by Cuna Indians (Sasardí); and a residential area in Panama City. Fifty-nine lizards (13%) came from 11 additional sites indicated, but not identified by name, in Fig. 1.

Laboratory methods. Animals were killed by an intracerebral injection of Nembutal given within 60 min after arrival at the laboratory. Portions of their intestines and their contents were transferred aseptically to selenite broth after cutting the intestine longitudinally in a petri dish. After incubation at 37°C for 18 h, MacConkey, bismuth sulfite, and salmonella-shigella agar plates were streaked with portions of the broth and incubated overnight.

All plates were examined after incubation, and as many as 10 non-lactose-fermenting colonies of all types and sizes from each plate were transferred to triple sugar iron agar and lysine iron agar slants. After incubation, isolates were examined biochemically and serologically for the presence of *Salmonella*, *Shigella*, and *Arizona* organisms by conventional methods (3). Enteropathogenic *Escherichia coli* was not investigated.

After preliminary identification, cultures were sent to the Centers for Disease Control, Atlanta, Ga., for final serological determination.

Statistical method. Chi-square (χ^2) values were calculated, by the methods recommended by Bancroft (1), with standard programs in a Hewlitt Packard 9830 computer.

RESULTS

Among 447 lizards representing 34 species and 7 familes (Table 1), 131 (29.4%) harbored *Sal-*

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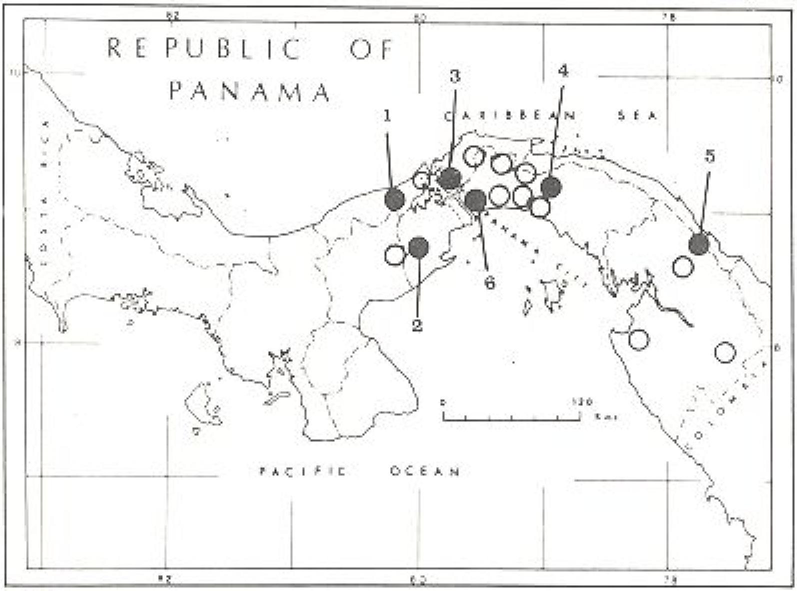

FIG. 1. *Major and minor collection sites in the Republic of Panama. Major sites: (1) Achiote; (2) El Aguacate; (3) Gamboa; (4) Gaspar Sabanas; (5) Sasardí; (6) Panama City. Minor sites are indicated by open circles.*

TABLE 1. *Species of lizards studied, by collection site, Panama, 1969*

Family	Species (location isolated)	No. of lizards in:						
		Achiote	Panama City	Gamboa	Gaspar Sabana	Aguacate	Sasardí	Others
Gekkonidae	*Thecadactylus rapicaudus*				3		1	4
	Lepidodactylus lugubris		8					
Sphaerodactylidae	*Gonatodes albogularis fuscus*	6	3				7	2
	Sphaerodactylus sp.							5
	Lepidoblepharus sanctaemartae	2			2			
Xantusiidae	*Lepidophyma flavimaculata*	16						
Iguanidae	*Anolis capito*	1		1		3		
	A. biporcatus				1	30		3
	A. frenatus			9	1	1		
	A. limifrons	25		9	6	2	26	1
	A. lionatus	15			4	20		
	A. poceilopus			25	19		2	3
	A. auratus	12	5			1		12
	A. tropidogaster		2			6		2
	A. humilis	3			3	2		16
	A. pentaprion					6		2
	Anolis sp. (Chiriqui)							5
	Anolis sp. (Gamboa)			1				
	Anolis sp. (Aguacate)					1		
	Basiliscus basiliscus					4	1	
	Corytophanes cristatus					9		
	Polychrus gutturosus					13		
	Iguana iguana	4						
	Enyalioides heterolepsis	1						
	Sceloporus malachiticus							1
Scincidae	*Mabuya mabuya*	2	1		1	1	3	1
Anguidae	*Diploglossus monotropis*	1						
Teiidae	*Ameiva ameiva*				2	11		1
	A. leptophrys	1			6			
	Ptychoglossus festae	2			3			
	Leposoma rugiceps		14					
	L. southi	3			1			1
	Gymnopthalmus speciosus		13					
	Echinosaura horrida						1	

monella and *Arizona* organisms in their intestinal tracts (Table 2). A total of 286 (64%) of the lizards belonged to the genus *Anolis* of the family *Iguanidae*; the remaining 161 belonged to 19 genera of six other families.

Overall prevalence rates varied in relation to host behavior ($\chi^2_4 = 15.35$; $P < 0.005$) (Table 2). Ground-dwelling and secretive lizards had the highest isolation rates (42.0 to 42.6%) followed by semiaquatic (31.8%), arboreal (27.6%), and scansorial species (17.5%). Individual species-specific rates differed significantly within habitats, but tended to follow the overall pattern. The highest rate (92.3%) was found in *Gymnopthalmus speciosus*, a lizard whose behavior is secretive (i.e., not found openly, often hidden inside holes), whereas high rates (60.0 to 63.6%) were observed in terrestrial (*Anolis auratus*), arboreal (*Anolis frenatus*), and scansorial (*Anolis tropidogaster*; adapted to climbing walls, rocks, and structures) species, suggesting possible acquisition of pathogens from a contaminated environment. The lowest infection rate (5.6%) appeared in another scansorial species (*Gonatodes fuscus*). The remaining rates were intermediate.

In addition, intergeneric variations in infection existed ($\chi^2_6 = 32.8$; $P < 0.005$), and, within the genus *Anolis*, species-specific characteristics were significantly related to infection. Terrestrial *A. auratus* supported a significantly higher rate than *Anolis humilis* ($\chi^2_1 = 4.0$; $P < 0.04$); the rate in arboreal *A. frenatus* was considerably greater than that in *Anolis biporcatus* ($\chi^2_1 = 7.30$; $P < 0.008$), as was the rate in scansorial *A. tropidogaster* as compared with that in *A. limifrons* ($\chi^2_1 = 11.43$; $P < 0.001$).

Table 3 shows the frequency of occurrence of *Salmonella* and *Arizona* spp. serotypes and the species of lizards from which they were recovered. Overall, 147 strains were isolated, including 26 serotypes of *Salmonella* and 10 serotypes of *Arizona* spp. The predominant serotypes were of *Salmonella* spp. and were serotypes bonaire (21.1%), rubislaw (10.9%), miami (8.8%), and wassenaar (8.2%); they accounted for 49% of the strains recovered. Of the 26 *Salmonella* serotypes, 16 were found in more than one host species. Six serotypes were present in at least five species of lizards and occurred most frequently in *Anolis*, which lived in all types of perching sites in the forest, from floor to canopy.

Infections by more than one serotype simultaneously were frequent. Thirteen multiple infections involving two, three, and even four different *Salmonella* serotypes were detected in individual hosts (Table 3). Multiple infections of *Salmonella* and *Arizona* were detected on four occasions.

TABLE 2. *Prevalence of Salmonella and Arizona organisms in captured lizards, by species and its behavior*

Host species, by behavior	No. of specimens examined	Positive no.	Positive %	No. of serotypes isolated
Terrestrial				
Anolis auratus	30	19	63.3	9
A. humilis	24	4	16.7	4
Ameiva ameiva	14	6	42.7	9
Subtotal	68	29	42.6	
Secretive				
Lepidophyma flavimaculatum	16	4	25.0	5
Leposoma rugiceps	14	2	14.3	2
Gymnopthalmus speciosus	13	12	92.3	3
Subtotal	43	18	42.0	
Semiaquatic				
Anolis lionatus	39	9	23.1	6
A. poecilopus	49	19	38.8	9
Subtotal	88	28	31.8	
Arboreal				
Anolis biporcatus	34	7	20.6	7
A. frenatus	11	7	63.6	3
Polycrus gutturosus	13	2	15.4	2
Subtotal	58	16	27.6	
Scansorial				
Gonatodes fuscus	18	1	5.6	1
Anolis limifrons	69	10	14.5	6
A. tropidogaster	10	6	60.0	5
Subtotal	97	17	17.5	
Total	354	108	30.5[a]	
Varied				
20 spp. of 16 genera	93	23	24.8[a]	11
Grand total	447	131	29.4[a]	

[a] Average percent of specimens positive.

Rare serotypes of *Salmonella* belonging to subgenus IV, such as bonaire, houten, parera, and wassenaar, and some of the commonly occurring ones belonging to subgenus I, such as rubislaw, san diego, panama, and miami, were among those isolated. In general, lizards with diverse behavioral patterns were found with infections involving these serotypes.

Infected lizards were found at all major localities (Table 4). Excluding Panama City, human

TABLE 3. *Frequency of enterobacterial pathogens in lizards by host*

Serotype of:	No. of times strains were isolated from the following host species (no. of lizards examined):															Total strains	%
	Gonatodes (18)	*Lepidophyma* (16)	*Anolis biporcatus* (34)	*Anolis frenatus* (11)	*Anolis limifrons* (69)	*Anolis lionotus* (39)	*Anolis poecilopus* (49)	*Anolis auratus* (30)	*Anolis tropidogaster* (10)	*Anolis humilis* (24)	*Polychrus* (13)	*Ameiva ameiva* (14)	*Leposoma rugiceps* (14)	*Gymnopthalmus* (13)	Others (93)		
Salmonella spp.																	
rubislaw				1							1	1[a]	2[m]	7	4	16	10.9
san diego		1						4[c]		1				1	3[d,e]	10	6.8
panama							1	2	2			1		4		10	6.8
thompson		1														1	0.7
madelia												2[f,b]			1	3	2.0
bonaire			1	5	4	2	7[g]	3		1		1[b]			7[d,h]	31	21.1
houten											1	1[f]			2	4	2.7
carrau												1[f]				1	0.7
miami		1[i]	1[j]			2	5	1	1[k]			2[f,b]				13	8.8
chameleon	1		1[j]					1								3	2.0
parera					1		1[g]	5								7	4.8
glostrup								1[c]	1							2	1.4
java						2										2	1.4
nagoya							1									1	0.7
lohbrugge				1			1									2	1.4
wassenaar			2		3[l]		1		1[k]	1					4	12	8.2
bern					1											1	0.7
montevideo													1[m]		1[h,m]	2	1.4
degania			1													1	0.7
denver										1					1	2	1.4
newport						1										1	0.7
4,5,12,eh:−								2								2	1.4
45ah:(g)Z_{51}:−								1								1	0.7
−:Z_4,Z_{32}:−					1		2									3	2.0
11:Z_4,Z_{32}:−[n]							1								1	2	1.4
$50_{1,2,3}$:Z_4,Z_{24}:−[n]			1													1	0.7
Arizona hinshawii																	
26:23:31		1														1	0.7
29:33:31												2[a]				2	1.4
26:33:31												1				1	0.7
24:24:28						1										1	0.7
26:23:30						1										1	0.7
20:24:28			1													1	0.7
15:22:21															1	1	0.7
12:27:28		1[i]														1	0.7
26:31:33															1[e]	1	0.7
9a9b:29:31					1[l]				2							3	2.0

[a] One lizard with *Arizona* serotype 29:33:31 and *Salmonella* serotype rubislaw.
[b] One lizard with *Salmonella* serotypes madelia, miami, and bonaire.
[c] One lizard with *Salmonella* serotypes glostrup and san diego.
[d] One lizard with *Salmonella* serotypes san diego and bonaire.
[e] One lizard with *Arizona* serotype 26:31:33 and *Salmonella* serotype san diego.
[f] One lizard with *Salmonella* serotypes houten, carrau, madelia, and miami.
[g] One lizard with *Salmonella* serotypes bonaire and parera.
[h] One lizard with *Salmonella* serotypes montevideo and bonaire.
[i] One lizard with *Arizona* serotype 12:27:28 and *Salmonella* serotype miami.
[j] One lizard with *Salmonella* serotypes miami and chameleon.
[k] One lizard with *Salmonella* serotypes miami and wassenaar.
[l] One lizard with *Arizona* serotype 9a9b:29:31 and *Salmonella* serotype wassenaar.
[m] One lizard with *Salmonella* serotypes rubislaw and montevideo.
[n] New serotype described by us.

activity within these localities was sparse or absent, limited to hunters, settlers, or Indians who lived in small hamlets or individual huts scattered through the forest. The highest rates (48.9% and 43.5%, respectively) occurred at the Gamboa and Panama City sites, near the center

TABLE 4. *Prevalence of Salmonella and Arizona spp. in lizards by locality*

Locality	No. examined	Positives no.	Positives %[a]	No. of serotypes isolated
Achiote	94	25	26.6	9
Sasardi	41	6	14.6	3
Gamboa	45	22	48.9	6
Gaspar Sabanas	52	12	23.1	8
Aquacate	110	31	18.2	18
Panama City	46	20	43.5	5
Others	59	15	25.4	8

[a] Average of column entries was 29.4.

of the Isthmus, two localities with little ecological similarity. The rate was lowest (14.6%) in the reptiles from Sasardí. The intersample variability in lizards grouped by major localities (14.6% to 48.9%) was highly significant ($\chi^2_6 = 18.74$; $P < 0.006$) of infection. Table 5 shows the seasonal distribution of infection in lizards. Infection was heavier during the dry season from January through April. The difference between the late dry season (37.3%) and the early wet season (23.3%) was significant ($\chi^2_1 = 4.400$; $P < 0.05$). There was no difference in infected lizards between the early (23.3%) and the late wet seasons (14.9%) ($\chi^2_1 = 1.753$; $P > 0.15$), but the difference between the late wet (14.9%) and the early dry seasons (38.8%) was significant ($\chi^2_1 = 8.924$; $P < 0.005$).

DISCUSSION

An estimated 4% of the species of amphibians and reptiles of the world are found on the Isthmus of Panama (15). Of the 357 species, 60% are reptiles, and 19% of these are lizards. Panamanian lizards belong to seven families containing 29 genera and 67 species (15), many of which are represented in the material examined in this study.

Many neotropical lizards were found infected by a diversity of *Salmonella* and *Arizona* serotypes; infection was ubiquitous, from remote rural and forested regions to urban developments. Infection among lizards was highest during the dry season, especially March and April, possibly due to greater activity among some species during the dry season in their search for food and, consequently, to greater contact with sources of infection.

Natural infection of lizards and other cold-blooded animals by *Salmonella* spp. has been reported by others (7, 13, 17, 18). Although these animals are frequently found to be carriers of these agents, their epidemiological role remains unclear. However, lizards should not be underestimated as potential sources of *Salmonella* infection, particularly in tropical and developing countries in which conditions of inadequate sanitation still prevail. The extensive distribution of lizards and the habits of many species (geckos; *Ameiva* spp.) which live in close association with man may offer opportunities for human and domestic animal infection, through direct or indirect contact. Even in urban dwellings, the behavior of geckos may lead to contamination of food or utensils with their excreta (12). Several species of lizards, such as *Gonatodes*, *Gymnopthalmus*, and *Ameiva*, seen in or near homes of humans were found positive for *Salmonella* in this study. In rural and jungle areas of Panama, *Iguana*, a genus of primarily herbivorous arboreal lizards, are eaten regularly by local people. Two specimens of this lizard harbored enteric pathogens. Enteric bacteria were regularly isolated from *Ameiva ameiva*, commonly found in open, grassy country, including yards and vacant lots in Panama City.

Of the *Salmonella* serotypes listed in Table 3, at least five have been implicated in salmonellosis of man in Panama. These include serotypes rubislaw, san diego, panama, newport and miami, the last four having been isolated also from humans in remote jungle areas (10, 11). Information on the incidence of *Salmonella* infection in Panama is lacking. However, results of studies on infants with acute diarrheal disease in Panama City revealed a prevalence of 1.2% for *Salmonella* infection among 1,819 cases studied (10), whereas in four rural communities, 9.1% of 591 persons of all ages, and irrespective of the occurrence of diarrhea, harbored *Salmonella* organisms (6). In another study undertaken among local indigenous laborers and their families and nonresident personnel hired to work on feasibility studies for a proposed sea-level canal in the jungles of eastern Panama, *Salmonella* organisms were isolated from 2 of 29 diarrheal cases

TABLE 5. *Seasonal distribution of infection in lizards*

Season in:	No. of lizards examined	Positives no.	Positives %[a]
1968			
Early dry (Jan.–Feb.)	81	21	26.9
Late dry (Mar.–Apr.)	110	41	37.3
Early wet (May–Sept.)	86	20	23.3
Late wet (Oct.–Dec.)	74	11	14.9
1969			
Early dry (Jan.–Feb.)	49	19	38.8
Late dry (Mar.–Apr.)	34	16	47.1

[a] Average of column entries was 29.5.

(6.9%) and from 10 of 244 asymptomatic persons (4.1%) (11). Human infections with *Arizona* organisms have also been found infrequently in rural populations of Panama (5, 6). These organisms are closely related to salmonellae and have been known to cause severe outbreaks of infection in animals as well as diarrhea and gastroenteritis in humans (4). Outbreaks of *Arizona* in humans are often associated with contaminated food, indicating a pathogenic potential similar to that observed in salmonellosis (14).

Numerous *Salmonella* serotypes similar to those recovered during this study have been associated with human illnesses in a number of countries throughout the world. For example, in Africa (Madagascar, Nigeria, and Senegal, among other countries), serotypes rubislaw, san diego, panama, thompson, nagoya, wassenaar, montevideo, and newport have frequently been isolated from humans (2). In Israel, serotypes montevideo, glostrup, and degania have been prevalent in humans (16). In Mexico, serotype carrau, in addition to others, has caused illness in humans (19). In various countries of eastern Europe, *Salmonella* organisms have played a varying role in the bacterial etiology of infant diarrheal infections in different areas and at different times. Many serotypes were encountered as a cause of diarrhea, among which are included java and madelia (8).

Although it is not convincingly clear that the organisms recovered from lizards can all cause disease in humans, it is reasonable to believe they may contribute significantly to health problems of humans. In Panama, more and more of the jungles are being cleared and human populations are moving in to colonize the newly opened lands in a national goal for development of the country by the year 2000. Roads and communication networks are rapidly being expanded through jungle and rural areas, upsetting the ecological balance of the forest and most likely affecting the stability of the sylvatic fauna. Their natural cover and habits may be affected and may bring man in closer contact with them, thus increasing the incidence of many diseases, including salmonellosis.

From the high prevalence of infection in lizards which have little direct contact with humans, it can be assumed that the reptile-salmonellae associations are independent of the human host. It would be desirable to investigate whether or not lizards are potential reservoirs of enteric infection for humans.

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