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Authors: Kalvig, Barbara A., Maggio-Price, Lillian, Tsuji, Joyce, and Giddens, W. Ellis

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SALMONELLOSIS IN LABORATORY-HOUSED IGUANID LIZARDS (*SCELOPORUS* SPP.)

Barbara A. Kalvig,[1] Lillian Maggio-Price,[1] Joyce Tsuji,[2] and W. Ellis Giddens[1]

[1] Department of Comparative Medicine, School of Medicine, University of Washington, Seattle, Washington 98195, USA

[2] Department of Zoology, University of Washington, Seattle, Washington 98195, USA

ABSTRACT: Fifteen wild-caught iguanid lizards (14 *Sceloporus variabilis* and one *S. malachiticus*) were used in a 3 mo study on thermal acclimation. Over a 2 mo period, five of the lizards showed decreased activity, anorexia and enlarged joints, and were either found moribund or were euthanatized due to their poor condition. Specimens taken from lesions in four of the five lizards were cultured and were infected with *Salmonella* spp. *Salmonella* spp. was cultured from cloacal swabs in six of the 10 surviving lizards. Standard metabolic rates of those that were infected did not differ significantly from those that were not infected. We postulate that the lizards were inapparent carriers of *Salmonella* spp. at the time of capture and, as a result of stress, five developed active overwhelming systemic infections.

Key words: *Salmonella* spp., infection, pathology, standard metabolic rate, *Sceloporus* spp., lizards, inapparent carriers.

INTRODUCTION

Salmonellosis has been reported in reptiles since the late 1930's (Mathewson, 1979). Numerous studies have been performed to detect *Salmonella* spp. infections in both wild and captive reptiles (Mathewson, 1979; Kourany et al., 1970; Kourany and Telford, 1982; Cambre et al., 1980; Hoff and White, 1977). In many tropical regions of the world, dense populations of lizards occur; many species have been incriminated as *Salmonella* spp. carriers (Janakiraman and Rajendran, 1974; Dhiraputra and Chavalittamrong, 1979; Kourany and Telford, 1981). There have been few reports of salmonellosis in laboratory-housed reptiles (Boam et al., 1970; Burdick et al., 1984). We isolated *Salmonella* spp. from laboratory-housed lizards in which a variety of clinical syndromes occurred.

MATERIALS AND METHODS

Clinical histories

The experimental group consisted of 14 *Sceloporus variabilis* captured from lowland Costa Rican dry forests (Guanacaste Province; 10°30′N, 85°45′W), and one *S. malachiticus* caught in a Costa Rican highland region (10°15′N, 84°50′W). All lizards appeared healthy at capture. Eleven of the lizards were housed individually in plastic shoebox-type cages within an environmental chamber, and the others were group-housed in aquaria. Cages were lined with foil and sand, and contained a piece of bark or rock. The diet consisted primarily of crickets and a supplement of approximately 5% termites. The crickets were housed in open-mouth jars, fed dog food, lettuce, and potatoes, and were dusted with dog vitamins (Vionate, Rich Health Inc., Irvine, California 92664, USA) prior to being fed to the lizards. Water was provided *ad libitum*. Feces were cleaned off the sand daily, and the water dishes were washed daily. Once each mo, the cages and water dishes were disinfected with a quaternary ammonium compound (T.B.Q., Calgon Vestal Corp., St. Louis, Missouri 63133, USA) and a hypochlorite solution (Georgia Pacific Brand, Leonard Products, Seattle, Washington 98172, USA) rinse. Light was provided by a full-spectrum ultraviolet lamp. The lizards were a subset of those used in a thermal acclimation study (Tsuji, 1988). Those lizards undergoing experimental manipulation were exposed to various temperatures and photoperiod regimes, and their oxygen consumption was measured as an indicator of standard metabolic rate. All lizards were acclimated to the same conditions prior to measurements (see Tsuji, 1988 for methods). Several of the lizards developed clinical disease after entry into the facility as described below.

An 11 g male *S. variabilis* (Case 1), housed separately at the University of Washington, had an enlarged right stifle. Swelling of the right stifle increased progressively, and the left hock region became similarly enlarged. The lizard was found moribund approximately 3 wk after capture and entry into the animal facility and was euthanatized.

A 10 g male *S. variabilis* (Case 2) was noted to be less active than the other lizards upon arrival at the University of Washington. This lizard was housed alone and appeared healthy for 3 wk before swelling of the right hindlimb and hip joint appeared. The lizard was found moribund approximately 4 wk after entry into the animal facility and was euthanatized. No experimental manipulation had been performed.

A 12 g female *S. malachiticus* (Case 3) had been at the University of Washington for 3 yr since its capture in Costa Rica. It had been housed in an aquarium with two *S. malachiticus* and one *S. variabilis*. The four lizards were kept in a room separate from that in which the other specimens of *S. variabilis* were being studied, but for a 4 wk period had been fed crickets that had not been consumed by the study-group lizards.

A 10 g male *S. variabilis* (Case 4) was thin upon entry to the animal facility and showed decreased appetite. It was housed with two *S. variabilis* females, and the three cagemates were used in a sprint speed test over a range of temperatures for 2 to 3 wk after capture. One wk later, this lizard had bilateral swelling of the coxofemoral joints; its overall condition deteriorated and it was euthanatized.

A 12 g female *S. variabilis* (Case 5) had been healthy when separated from the lizard in Case 4. Two wk later, it lost use and normal posture of its hind legs, using only its front legs to ambulate. One wk later, the left thigh and right hock joint were swollen, and the lizard was euthanatized.

Cultures

Cultures were obtained from tissues from four of the five cases. Cloacal swabs were also taken from the 10 remaining *Sceloporus* lizards to detect inapparent *Salmonella* sp. carriers and repeated 1 mo later at the completion of the thermal acclimation study when the lizards were necropsied. Culture swabs were incubated for 18 hr in GN broth (PML Microbiologicals, Tualatin, Oregon 97062, USA), plated to Hektoen agar and streaked for isolation. Biochemical identification was made in our laboratory, and isolates were also sent to the University of Washington Microbiology Laboratory for confirmation of biochemical identification. Serotyping of the isolate obtained from Case 2 was performed by both the University of Washington Microbiology Laboratory and the Seattle-King County Public Health Laboratory (Seattle, Washington 98195, USA).

RESULTS

Necropsy findings

Table 1 summarizes the clinicopathologic findings. Three of the five lizards (Cases 1, 4, and 5) had purulent arthritis or periarthritis involving one or both coxofemoral joints. The inflammatory process also involved the subcutaneous tissue and adjacent skeletal muscle. There was a large periarticular abscess in Case 4 surrounded by granulation tissue and heterophils. Many bacterial colonies were present within the purulent exudate of the abscess and purulent osteomyelitis was also present. Bits of necrotic sequestered bone were noted in some parts of the abscess. Severe purulent myositis with heterophil and lymphocyte infiltration but without joint involvement was observed in one lizard (Case 2). Another lizard (Case 3) had severe purulent peritonitis with extension into the liver parenchyma and focal purulent nephritis. A large, unidentified helminth in the center of this inflammatory exudate might have contributed to the severity of this lesion.

Cultures

Bacterial isolates were identified biochemically as a *Salmonella* spp. in our laboratory. Biochemical analysis of an isolate (Case 2) was confirmed in another laboratory and is shown in Table 2. Serotyping performed by two laboratories used an antibody pool which included the "O" somatic antisera for groups A–E and F–I. In both instances, these were negative, indicating that the isolate was in a further serogroup beyond those commonly tested.

Survey of healthy lizards

Salmonella spp. were isolated from six of the 10 remaining *Sceloporus* lizards. All 10 lizards had undergone experimental manipulation and remained clinically healthy through completion of the thermal acclimation study 3 mo after capture and entry into the animal facility when all were euthanatized and necropsied. In cultures taken at the time of necropsy, *Salmonella* spp. was isolated from the small intestine of two of the lizards which had positive cloacal cultures (Table 3). Three lizards in which *Salmonella* spp. was isolated from

TABLE 1. Clinocopathologic findings in five *Sceloporus* lizards.

Case	Duration in lab (wk)	Experimental manipulation	Clinical signs	Histopathologic findings	Site *Salmonella* spp. recovered
1	2	No	Enlarged stifle and hock	Purulent cellulitis and arthritis	ND[b]
2	4	No	Enlarged hindlimb and coxofemoral joint	Purulent myositis, hepatitis, septicemia	Hindlimb musculature
3[a]	156	No	Moribund	Purulent nephritis and peritonitis	Renal abscess peritoneal fluid
4	6	Yes	Bilateral coxofemoral enlargement	Purulent osteoarthritis; osteomyelitis	Coxofemoral joint
5	8	Yes	Posterior paresis, enlarged thigh and hock joint	Purulent periarthritis and peritonitis	Periarticular abscesses; peritoneal fluid

[a] Contact with crickets fed to lizards numbers 1 and 2.
[b] ND, not done.

cloacal swabs had hepatitis; two lizards had focal lymphocytic hepatitis and one other had marked multifocal necrotizing hepatitis.

Standard metabolic rates of lizards

Standard metabolic rates of nine of the 10 clinically healthy lizards were measured as part of a larger study on thermal acclimation in lizards (Tsuji, 1988). Comparisons of standard metabolic ranges at a range of temperatures (3 to 35 C) showed no significant differences between the six lizards that tested positive for *Salmonella* spp. and the three that were negative (one-way ANOVA, $P = 0.72, 0.18, 0.30$, Table 4).

TABLE 2. Biochemical analysis of isolate from *Sceloporus* sp. lizard (Case 2).

Substrate	Result
Glucose	+ with gas production
Lactose	−
Adonitol	−
Arabinose	+
Inositol	−
Urease	−
Citrate	+
Triple sugar iron	Alkaline/acid with gas production
H_2S	+
Lysine decarboxylase	+
Ornithine decarboxylase	+
Motility	+
Indole	−
ONPG	+
Dulcitol	−
Malonate	−
Methyl red	+
Salicin	+

DISCUSSION

Salmonella spp. is commonly isolated from lizards and other reptiles, and there appears to be a large number of healthy carriers. Burdick (1984) has suggested that *Salmonella* spp. is a normal inhabitant of the reptilian intestinal tract. In a survey of 46 lizards representing seven genera, Onderka and Finlayson (1985) noted that while 22 (48%) were positive for *Salmonella* spp. by intestinal culture, only five (11%) died of *Salmonella* spp. infection. Hence, *Salmonella* spp. may be considered an opportunist rather than a primary reptilian pathogen. Reptiles rarely display clinical signs of disease (Boam et al., 1970; Onderka and Finlayson, 1985) but act as inapparent carriers, shedding the organism in the feces intermittently throughout life (Cambre et al., 1980; Hoff and White, 1977). Boam et al. (1970), isolated *S. mavina* from subcutaneous abscesses in a spiny-tailed iguana (*Ctenosaura acanthura*).

TABLE 3. Cultural and histopathologic findings in 10 clinically normal *Sceloporus variabilis* lizards.

Number	Recovery of *Salmonella* spp.		Histopathologic findings
	Cloaca (pre-mortem)	Small intestine (post-mortem)	
1	+	+	Sarcosporidiosis
2	+	+	Focal lymphocytic hepatitis
3	+	−	Nonsuppurative periarthritis Focal lymphocytic hepatitis
4	+	−	Marked multifocal necrotizing hepatitis
5	+	−	None
6	+	−	None
7	−	−	Granulomatous colitis with dystrophic mineralization*; granulomatous myocarditis with dystrophic mineralization
8	−	−	Gastric nematodiasis
9	−	−	None
10	−	−	None

* Moderate numbers of long filamentous bacteria present within inflammatory foci.

Onderka and Finlayson (1985) summarized five clinical cases in five species, mostly iguanids; a variety of diseases were described which included interstitial nephritis, oophoritis, myocarditis, and aortic valvular endocarditis, and five different serotypes of *Salmonella* spp. were isolated. In a study of laboratory-housed lizards (*Sceloporus occidentalis, Diposaurus dorsalis*), 37% were positive on one or more isolations for *Salmonella* spp. *Salmonella* spp. was consistently isolated from the colon but not from the small intestine (Burdick, 1984). Although not directly comparable, this is consistent with our findings since cloacal cultures (taken 1 mo premortem) were positive in three of four lizards having histologic evidence of bacterial disease, while only one of the four lizards had positive intestinal cultures at necropsy (Table 3).

These *Sceloporus* spp. lizards were probably natural carriers of *Salmonella* spp. when they arrived at the University of Washington from Costa Rica. Most of the lizards were housed alone, and sanitation measures appeared quite adequate. Since none of the lizards displayed evidence of lacerations or abrasions, and examination of the cages did not reveal any sharp objects, transmission of infection from one or two sick animals via ingestion or skin trauma after arrival was considered unlikely. Insects have been incriminated as carriers of *Salmonella* spp. to lizards (Dhiraputra and Chavalittamrong, 1979). While the insects used as feed were not cultured directly, illness in the *S. malachit-*

TABLE 4. Mean (SD) of standard metabolic rates (ml O_2/h)* of carriers and non-carriers of *Salmonella* spp. measured at three temperatures.

Carrier status	Measurement Temperature (C)		
	10	16	35
+	0.16 (0.01)	0.31 (0.04)	1.83 (0.21)
−	0.16 (0.01)	0.28 (0.03)	1.66 (0.30)
ANOVA results	$F = 0.14$, $P = 0.72$	$F = 2.28$, $P = 0.18$	$F = 1.27$, $P = 0.30$

* Standard metabolic rates are corrected to an average body mass of 12.3 g using the relationship, Log(SMR) = 0.602 Log (body mass). Statistics were performed on log transformed values.

icus which had been healthy since its entry in 1982 (Case 3) might have been attributable to consumption of crickets previously exposed to affected lizards. However, the cagemates of this lizard remained healthy.

The five lizards in this report probably developed clinical salmonellosis secondary to stress-induced activation of inapparent infection with subsequent development of septicemia. It is likely that Case 1 arrived with joint disease due to *Salmonella* spp. infections with exacerbation and spread of infection upon confinement. We feel certain that the organism producing disease in these lizards was a *Salmonella* spp. even though it was not typeable. The biochemical analysis of isolates suggested that the organism might fit into Subspecies 4 of *Salmonella enterica* (Ewing, 1986). Neither malonate, ducitol, nor inositol is utilized in this group, whereas salicin may be utilized. Many of the Subspecies 4 are in further serogroups (beyond A–I). Considering that this isolate was obtained from a wild lizard caught in Costa Rica, it would not be unusual to find a non-typeable isolate analyzed in a laboratory testing for serotypes most commonly associated with human disease.

This study is also one of the first to examine the effects of *Salmonella* spp. on a physiological measure of whole-animal functioning. Standard metabolic rates of carriers and non-carries of *Salmonella* spp. were not significantly different for the limited number of individuals tested. The metabolic rates of these nine lizards also did not differ significantly from previous measurements of *S. variabilis* lizards (n = 12) that were clinically healthy (data not shown). These results suggest that lizards that are carriers of *Salmonella* spp. may be physiologically unaffected unless their immunocompetence is compromised. Another previous group of these lizards were subjected to the same experimental conditions, but none showed the ill effects noted in this report. These lizards were from a different location in Guanacaste Province, Costa Rica. Therefore, populations of *S. variabilis* may differ in their resistance or exposure to *Salmonella* spp.

Lizards and other ectothermic animals thermoregulate behaviorally in accordance with an environmental temperature range. This would be difficult to simulate in a laboratory environment where temperature is usually maintained at more constant ranges. Consequently, even laboratory conditions intended to mimic natural conditions may not, thus placing lizards under substantial stress. Capture, transport, artificial diet, laboratory conditions, experimental manipulation, and parasitism may all be sources of stress. Any one or combination of these factors may have immunocompromised the lizards and resulted in expression of clinical disease. The occurrence of subclinical or overt disease should be considered in those experimental studies using wild-caught reptiles housed in captivity; necropsy evaluation of animals used in such studies is advised.

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