

Salmonella Serovars in the Herpetofauna of Indiana County, Pennsylvania

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Herpetofaunal *Salmonella enterica* serovars have not been fully examined in any U.S. region. Thirty-three *Salmonella* serovars were isolated from 156 samples from 34 species, all within Indiana County, Pennsylvania. Results suggest that herpetofaunas could potentially pose a threat to humans. Further understanding of *Salmonella* in herpetofaunas may prevent future human cases.

Herpetofauna (particularly reptiles) has often been implicated as a transmitter of *Salmonella enterica* to humans (4, 5). However, there is a significant lack of systemic evaluation of the incidence of this pathogen in herpetofauna. Consequently, it is still debated whether *Salmonella* is a natural member of the intestinal flora of herpetofauna (19). The first reported *Salmonella* isolation from reptiles occurred in 1939 in the Gila monster, the common chuckwalla, and the regal horned lizard (3). *Salmonella* in amphibians was first reported in 1958 (2), wherein 15 of 27 cane toads tested positive. Since these reports, relatively few studies have examined *Salmonella* in herpetofaunas, especially amphibians. With only a few frog and toad species (10, 23, 24) and no salamanders being examined, investigation is warranted to determine occurrence rates.

While *Salmonella* presence within herpetofauna is often asymptomatic (11), symptomatic conditions in humans can arise as a result of transmission from herpetofauna (17). Some common conditions associated with salmonellosis include bacteremia, gastroenteritis, meningitis, osteomyelitis, peritonitis, and pleuritis (13, 17). In rare instances, human infection from herpetofauna has been fatal (17). Therefore, the potential risks associated with herpetofaunal contact need to be assessed. Despite this need, literature pertaining to *Salmonella* in herpetofauna is scant, with most species having never been sampled. The objective of this study was to isolate and identify *Salmonella enterica* serovars present within the free-living herpetofauna of Indiana County, Pennsylvania.

Most free-living individuals were hand caught. Some turtles were caught via hooded traps baited with sardines in oil (8). All were within Indiana County, Pennsylvania, borders (40°54'N/40°21'N, 79°27'W/78°48'W). Indiana County is located in western Pennsylvania and covers ~214,700 ha, including 1,295 ha in water coverage. There are numerous vegetative types present, including deciduous and coniferous forests and old fields coupled with agricultural and urbanized areas. All provide habitats suitable for herpetofauna. Sampling occurred in areas that would yield the most species discovered, using our previous observations and the Pennsylvania Herpetological Atlas Project data set.

Dry cloacal swabs were obtained using sterilized cotton-

tipped swabbing sticks. Once obtained, the stick was placed in an individual vial, sealed, labeled, and returned to the laboratory. Prior to sample enrichment, 4.6 g of tetrathionate broth base was resuspended in 100 ml autoclaved water and 2 ml iodine solution (6.0 g iodine crystals and 5.0 g potassium iodine in 20 ml autoclaved water). Samples were enriched with ~10 ml of tetrathionate broth and incubated at 37°C for 48 h (12). Culturing occurred by streaking ~1 ml of broth on brilliant green (BG) agar plates containing sulfadiazine, with plates being incubated at 37°C for 24 h (26). Subculturing occurred after initial BG plating to obtain a pure culture. Biochemical tests were conducted on BG suspect isolates to ensure *Salmonella* presence prior to serotyping. These tests included glucose, lactose, sucrose, maltose, and mannitol (9). In-house serotyping was conducted using the Kauffmann-White schema (14, 15).

A homogeneity chi-square analysis was conducted using SPSS for Windows (version 11.0.0) in order to determine whether the sampled amphibian and reptile populations were homogenous with respect to *Salmonella* occurrence. The same approach was utilized when we determined the homogeneity of *Salmonella* occurrence among the sampled families within the classes Amphibia and Reptilia.

One hundred fifty-six (92 amphibian and 64 reptilian) samples were collected during 2004 from 34 species (14 salamanders, 6 frogs and toads, 3 turtles, and 11 snakes). Total field work effort equaled ~273 man-hours. Overall, 97 of 156 (62.2%) tested positive, with 33 *S. enterica* serovars being isolated (Tables 1 and 2). More specifically, 61 of 64 (95.3%) reptilian samples tested positive, while 36 of 92 (39.1%) amphibian samples tested positive.

Salmonella occurred significantly more in reptiles than amphibians ($\chi^2 = 50.660$, $P < 0.0001$, $df = 1$). However, within each vertebrate class, there was no variation in *Salmonella* occurrence between the different families that were examined. Five amphibian families that were represented included Ambystomatidae, Salamandridae, Plethodontidae, Bufonidae, and Ranidae ($\chi^2 = 8.549$, $P = 0.073$, $df = 4$). Three reptilian families that were represented included Chelydridae, Emydidae, and Colubridae ($\chi^2 = 0.387$, $P = 0.824$, $df = 2$).

Reptiles had a 95.3% occurrence rate. The reported incidences of *Salmonella* in reptiles have varied from low to high (16) and most likely vary among all wildlife populations (25). For example, a recent study (22) reported a 0% occurrence rate in nine species of reptiles, five of which were examined in

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TABLE 1. Occurrence rate and list of *Salmonella* serovars in amphibian species

Order	Species (common name) ^a	No. tested	No. (%) testing positive	<i>Salmonella enterica</i> serovar(s)
Caudata	<i>Ambystoma maculatum</i> (spotted salamander)	10 larvae	0 (0)	
	<i>Notophthalmus viridescens viridescens</i> (red-spotted newt)	3	2 (66.7)	Give, Tennessee
	<i>Desmognathus fuscus fuscus</i> (Northern dusky salamander)	5	1 (20)	Typhimurium
	<i>Desmognathus monticola</i> (seal salamander)	6	2 (33.3)	Enteritidis, Stanley
	<i>Desmognathus ochrophaeus</i> (Allegheny Mountain dusky salamander)	7	5 (71.4)	Enteritidis (2), Muenchen, Newport, Thompson
	<i>Eurycea bislineata bislineata</i> (Northern two-lined salamander)	3	1 (33.3)	Senftenberg
	<i>Eurycea longicauda longicauda</i> (long-tailed salamander)	4	3 (75)	Montevideo, Muenster, Typhimurium
	<i>Gyrinophilus porphyriticus porphyriticus</i> (Northern spring salamander)	3	3 (100)	Montevideo, Muenchen, Typhimurium
	<i>Hemidactylum scutatum</i> (four-toed salamander)	1	1 (100)	Enteritidis
	<i>Plethodon cinereus</i> (Eastern red-backed salamander)	10	0 (0)	
	<i>Plethodon glutinosus</i> (Northern slimy salamander)	4	1 (25)	Infantis
	<i>Plethodon hoffmani</i> (Eastern red-backed salamander)	1	1 (100)	Muenchen
	<i>Plethodon wehrlei</i> (Wehrle's salamander)	3	0 (0)	
	<i>Pseudotriton ruber ruber</i> (Northern red salamander)	4	3 (75)	Infantis, Manhattan, Muenchen
Anura	<i>Bufo americanus americanus</i> (Eastern American toad)	5	3 (60)	Give, Ohio, Potsdam
	<i>Bufo fowleri</i> (Fowler's toad)	2	1 (50)	Senftenberg
	<i>Rana catesbeiana</i> (American bullfrog)	8 adults, 8 tadpoles	6 (37.5)	Blockley, Jacksonville, Miami, Montevideo, Newport, Senftenberg
	<i>Rana clamitans melanota</i> (Northern green frog)	2	2 (100)	Montevideo, Typhimurium
	<i>Rana palustris</i> (pickerel frog)	2	0 (0)	
	<i>Rana sylvatica</i> (wood frog)	1	1 (100)	Bellville

^a Common names as described by Crother et al. (6, 7).

our study; yet, we report a 95.3% rate in reptiles. Further examination of *Salmonella* in the reptile community is needed to establish the cause(s) of variation in occurrence both within and between reptile species. Meanwhile, amphibians had a 39.1% occurrence rate, which is lower than that reported by previous studies (2, 10). However, there is a significant lack of data concerning *Salmonella* occurrence in the amphibian species sampled in this study, making comparisons difficult, if not impossible. For example, this study is one of the first to examine *Salmonella* in salamanders. The analysis of salamanders may have lowered the *Salmonella* occurrence rate in the sampled amphibian population. For instance, only 2 of 18 (11.1%) individuals of the genus *Plethodon* ($n = 18$) and no *Ambystoma* ($n = 10$) tested positive for *Salmonella*. This trend is suggestive

of a relatively low occurrence rate among salamanders. Further examination of salamander species should be conducted to more fully clarify incidence rates.

The two most common *Salmonella* serovars isolated from Indiana County herpetofauna, comprising 36.1% of all isolates, were *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis. These two serovars are also the most common in the United States, accounting for ~50% of all human infections (18). Our data suggest that both amphibians and reptiles could pose a threat to humans due to the zoonotic capabilities of *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis.

While no antibiograms were conducted, they have been reported for some of the serovars isolated from our sampled

TABLE 2. Occurrence rate and list of *Salmonella* serovars in reptilian species

Order	Species (common name) ^a	No. tested	No. (%) testing positive	<i>Salmonella enterica</i> serovar(s)
Testudines	<i>Chelydra serpentina serpentina</i> (Eastern snapping turtle)	3	3 (100)	Anatum, Galiema, Typhimurium
	<i>Chrysemys picta</i> (painted turtle)	3	3 (100)	Give, Hull (2)
	<i>Glyptemys insculpta</i> (wood turtle)	1	1 (100)	Galiema
	<i>Terrapene carolina carolina</i> (Eastern box turtle)	3	3 (100)	Hadar, Muenster, Saint-Paul
Squamata suborder Serpentes	<i>Coluber constrictor constrictor</i> (Northern black racer)	1	1 (100)	Enteritidis
	<i>Diadophis punctatus edwardsii</i> (Northern ring-necked snake)	11	10 (90.9)	Enteritidis, Florida, Luciana, Manila, Muenster, Oranienburg, Rubislaw, Saint-Paul, Typhimurium (2)
	<i>Elaphe alleghaniensis</i> (Eastern ratsnake)	3	3 (100)	Enteritidis, Muenchen, Newport
	<i>Lampropeltis triangulum triangulum</i> (Eastern milksnake)	4	3 (75)	Montevideo, Typhimurium (2)
	<i>Nerodia sipedon sipedon</i> (Northern watersnake)	7	6 (85.7)	Berta, Enteritidis, Manhattan, Montevideo, Thompson, Typhimurium
	<i>Ophedrys vernalis</i> (smooth greensnake)	2 adults, 5 neonates	7 (100)	Agona (6), Enteritidis
	<i>Regina septemvittata</i> (queen snake)	2	2 (100)	Enteritidis, Ohio
	<i>Storeria dekayi dekayi</i> (Northern brownsnake)	2 adults, 13 neonates	15 (100)	Bredeney, Typhimurium (14)
	<i>Storeria occipitomaculata occipitomaculata</i> (Northern red-bellied snake)	1	1 (100)	Enteritidis
	<i>Thamnophis sirtalis sirtalis</i> (Eastern gartersnake)	3	3 (100)	Jacksonville, Newport, Rissen

^a Common names as described by Crother et al. (6, 7).

species (1, 20, 21). However, comparisons are impractical due to different host animal species, the locations of these studies, and possible antibiotic resistance differences among identical serovars. Therefore, future studies could include antibiogram analysis of *Salmonella* serovars collected from herpetofauna in order to more accurately assess both the antibiotic resistance of *Salmonella* serovars and the subsequent risk to humans.

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