

# Salmonellosis and Arizonosis in the Reptile Collection at the National Zoological Park

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## SUMMARY

The prevalence of *Salmonella* and *Arizona* organisms in the reptile collection at the National Zoological Park was investigated. Culture of specimens from 317 reptiles, while live or at necropsy, yielded 117 positive results, for an overall infection rate of 37%. Snakes had the highest rate, 55% (69 of 125); lizards had an intermediate rate, 36% (46 of 129); and turtles and tortoises had the lowest rate, 3% (2 of 63). Twenty-four serotypes of *Salmonella enteritidis*, 1 of *S choleraesuis*, and 39 of *Arizona hinshawii* were represented.

While clinical illness was never directly attributed to infection with these organisms, pure cultures of *Salmonella* and *Arizona* were recovered at necropsy from some reptiles with gross and/or histologic lesions in the gastrointestinal tract, liver, spleen, and blood vessels. However, numerous other concurrent diseases and management problems were often considered the immediate cause of death, with *Salmonella* and *Arizona* being ready and significant opportunistic pathogens contributing to the demise of the reptiles.

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A STUDY WAS undertaken to assess the total impact of *Salmonella* and *Arizona*<sup>a</sup> organisms on the reptiles in the collection at the National Zoological Park. Initial impetus for the study stemmed from

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<sup>a</sup>The authors recognize 2 taxonomic designations for this organism. Kauffmann's scheme (Buchanan RE, Gibbons NE(ed): *Bergey's Manual of Determinative Bacteriology*, ed 8. Baltimore, Williams & Wilkins Co, 1974, p 318) classifies *Arizona* as a species of *Salmonella* (*Salmonella arizonae*). The Ewing scheme (Edwards PR, Ewing WH: *Identification of Enterobacteriaceae*, ed 3. Minneapolis, Burgess Publishing Co, 1972, pp 146-147, 259) lists *Arizona* as a separate genus and species (*Arizona hinshawii*). The latter scheme is used by the US Public Health Service, Center for Disease Control, Atlanta, Ga, and by USDA's Veterinary Services Laboratory, *Salmonella* Serotyping Laboratory, Ames, Iowa, and was the basis for classification of organisms recovered in this study. Consequently, we have chosen to utilize the Ewing system in this report.

the finding that these bacteria were being isolated with greater frequency from recent necropsy cases than in the past. It was hoped that the study would define the overall problem and answer the following questions as well.

- 1) Are our reptiles dying of salmonellosis and arizonosis?
- 2) How many reptiles are infected but not clinically ill?
- 3) Which types of *Salmonella* and *Arizona* are prevalent in the collection?
- 4) Can a source of infection be identified?
- 5) Is a treatment/eradication scheme practical or desirable?

## Materials and Methods

A fivefold approach was used during the study period (January through August 1979).

- 1) Vent swab specimens were obtained from new reptiles as soon as possible after their arrival and again about 4 weeks into the quarantine period.
- 2) Vent swab specimens from nonvenomous reptiles already in the collection were taken one time.
- 3) Reptiles submitted for necropsy had at least one specimen cultured.
- 4) Food sources (baby chickens, rats, mice) and water samples from shipments of live amphibians intended as reptile feed were periodically cultured.
- 5) A review of past necropsy records on reptiles was made, and cases involving *Salmonella*- or *Arizona*-positive cultures were reexamined histologically.

Commercially available culture swabs containing modified Stuart's transport medium<sup>b</sup> were used in most instances on live reptiles. Small lizards and snakes required the use of homemade swabs that consisted of a wooden applicator stick, tipped with cotton, wrapped in cellophane tubing, and autoclaved at 49.5 C at 115 lb of pressure for 20 minutes. This system did not include any transport medium. After use, the swab was placed back in the dry cellophane tubing and transported to the laboratory as quickly as possible.

In the laboratory, samples were plated onto Hektoen enteric agar<sup>c</sup> and MacConkey agar<sup>d</sup> and inoculated into gram-negative (GN) broth.<sup>e</sup> Cultures were incubated at 35 C for 18 hours. Colonies from the plate with char-

<sup>b</sup>Culturette, Cepti-seal, Marion Scientific Corp, Rockford, Ill.

<sup>c</sup>Difco Laboratories, Detroit, Mich.

<sup>d</sup>Baltimore Biological Laboratory, Baltimore, Md.

TABLE 1—Culture Results, by Family of Reptile

	No.	No. culture-positive	% culture-positive
Squamata: Serpentes			
Boidae	70	37	53
Colubridae	48	28	58
Viperidae	7	4	57
Total	125	69	55
Squamata: Sauria			
Agamidae	7	1	14
Anguidae	7	6	86
Cordylidae	5	2	40
Gekkonidae	37	10	27
Helodermatidae	3	3	100
Iguanidae	27	9	33
Lacertidae	3	2	67
Scincidae	14	1	7
Teiidae	10	4	40
Varanidae	16	8	50
Total	129	46	36
Chelonia			
Chelidae	4	0	0
Chelydridae	1	0	0
Emydidae	30	1	3
Kinosternidae	7	1	14
Platysternidae	1	0	0
Testudinidae	19	0	0
Trionychidae	1	0	0
Total	63	2	3
Grand total	317	117	37

TABLE 2—Frequency of *Salmonella* Isolation, by Organism

<i>Salmonella</i> serotype	Times isolated			
	Snakes	Lizards	Turtles	Total
<i>bairdii</i>	—	1	—	1
<i>carrara</i>	5	—	—	5
<i>cerro</i>	2	—	—	2
<i>choleraesuis</i> (Kunzendorf)*	1	—	—	1
<i>derby</i>	—	1	—	1
<i>duisburg</i>	—	4	—	4
<i>eastbourne</i>	—	1	—	1
<i>flint</i>	—	2	—	2
<i>gwaai</i>	—	1	—	1
<i>havana</i>	—	1	—	1
<i>manhattan</i>	2	—	1	3
<i>miami</i>	—	3	—	3
<i>newport</i>	1	—	1	2
<i>nima</i>	—	1	—	1
<i>onderstepoort</i>	2	—	—	2
<i>oranienburg</i>	1	2	—	3
<i>orion</i>	—	7	—	7
<i>san-diego</i>	—	1	—	1
<i>seminole</i>	1	—	—	1
<i>tennessee</i>	—	3	—	3
<i>typhimurium</i>	1	—	—	1
6, 7: poorly motile	1	—	—	1
11: poorly motile	2	—	—	2
45: gz 51	—	1	—	1
Untypeable	—	2	—	2
Total (25)	19	31	2	52

\*Separate species. All others are serotypes of *S. enteritidis*.

acteristics of nonlactose fermenters were transferred to triple sugar iron agar slants<sup>c</sup> and incubated another 18 hours. In order to enhance the growth of low concentrations of any nonlactose fermenters, GN broth growth was plated onto Hektoen agar and incubated another 18 hours, and suspect colonies were transferred to triple sugar iron agar slants. Since some *Arizona* serotypes do ferment lactose and saccharose, H<sub>2</sub>S-producing colonies that fermented carbohydrates, in addition to all nonlactose fermenters, were inoculated into a commercially available

TABLE 3—Frequency of *Arizona* Isolation, by Organism

<i>Arizona</i> serotype	Times isolated			Total
	Snakes	Lizards		
1,4:23	1	—	—	1
1,4:29-28	1	—	—	1
1,33:23-21	1	—	—	1
1,33:24-25	2	1	—	3
1,33:26-21	2	—	—	2
1,33:39-24	—	1	—	1
5:1,3,11	2	—	—	2
7A,7B:1,2,5	1	—	—	1
7A,7B:23-31	9	7	—	16
9A,9B:24-21	—	1	—	1
9A,9B:24-31	1	—	—	1
9A,9B:29-31	3	—	—	3
9A,9C:24-31	6	1	—	7
13:1,2,5	1	1	—	2
15:21-37	2	—	—	2
15:23-30	1	—	—	1
15:31-21	1	—	—	1
15:31-28	1	—	—	1
16:23-25	4	1	—	5
16:24-30	1	—	—	1
20:21 monophasic	1	—	—	1
20:21-33	2	—	—	2
20:23-21	3	—	—	3
20:28-24	1	—	—	1
22:1,3,11	—	1	—	1
23:33-25	1	—	—	1
24:24-28	1	—	—	1
24:33-28	3	—	—	3
24: poorly motile	1	—	—	1
26:21-23	1	—	—	1
26:21-26	—	1	—	1
26: poorly motile	1	—	—	1
28:26-30	1	1	—	2
28:29-31	—	1	—	1
29: poorly motile	1	—	—	1
30:23-31	2	—	—	2
30:31-22	1	—	—	1
31:23-30	1	—	—	1
Untypeable	1	1	—	2
Total (39)	62	18	—	80

enteric identification system.<sup>c</sup> Organisms with a *Salmonella* or *Arizona* biochemical profile were sent to the Salmonella Serotyping Laboratory at Ames, Iowa.<sup>f</sup> Necropsy and food and water specimens were handled in the same manner.

## Results

Of 317 reptiles studied during the designated period, 117 were positive for 1 or more *Salmonella* or *Arizona* serotype, for an overall infection rate of 37%. Snakes proved to have the highest infection rate, 55% (69 of 125); lizards had an intermediate rate, 36% (46 of 129); and turtles and tortoises had the lowest rate, 3% (2 of 63; Table 1).

*Salmonella* was isolated 52 times, representing 24 serotypes of *S. enteritidis* and 1 of *S. choleraesuis*. Lizards yielded most of the isolates (31); snakes yielded 19, and turtles yielded 2 (Table 2). *Arizona* was isolated 80 times, representing 39 serotypes. Snakes provided 62 *Arizona*, and lizards, 18 (Table 3). *Arizona* isolates were not recovered from turtles or tortoises. Seven reptiles had more than 1 *Arizona*

<sup>c</sup>Analytical Profile Enteric 20 Index, Analytab Products, Div of Ayerst Laboratories, Plainview, NJ.

<sup>f</sup>Salmonella Serotyping Laboratory, Veterinary Services Laboratory, APHIS, Ames, Iowa.

serotype, and 5 had a mixed *Salmonella-Arizona* infection.

Most of the isolates were recovered from live animals, with necropsy cases accounting for only 9 isolations. Of 10 reptiles that died during the study period, 1 (an Eastern Kingsnake, *Lampropeltis getulus*) had a negative antemortem culture but yielded *S typhimurium* from the heart blood at necropsy approximately 1 month later. The reverse situation occurred with a Green Tree Monitor, *Varanus prasinus*, which had *Arizona* when alive but was culture-negative at necropsy 2 months later. Four animals maintained their antemortem positive culture status at necropsy; but in 2 instances (a Rosy Boa, *Lichanura trivirgata* and a Green Tree Python, *Chondropython viridis*), different *Arizona* isolates were recovered after death.

Nine snakes, 12 lizards, and 8 turtles were new accessions to the collection during the study period. Three of the snakes (33%) and 5 of the lizards (42%) were positive on 1st culture, with 7 of them potentially bringing new *Salmonella* or *Arizona* serotypes into the collection. Turtles were consistently negative for the organisms while in quarantine.

Three sets of rats, mice, and baby chickens were killed and colon-cultured during the study period in an effort to determine whether they could be a potential source of infection, but neither *Salmonella* nor *Arizona* was ever isolated from them. Additionally, water from shipping bags of amphibians intended as reptile feed was negative for these organisms.

### Necropsy Review

Necropsy records dating back to 1975 were reviewed to determine the past history and prevalence of *Salmonella* and *Arizona* in the reptile collection. A total of 20 snakes and lizards were culture-positive at necropsy during this time. Only 3 of these were found in a 36-month period (from January 1975 through December 1977), whereas the remaining 17 were found in the 20-month period from January 1978 through August 1979. Five reptiles were culture-positive for more than 1 tissue, 8 were culture-positive for only 1 of multiple tissues sampled, and 7 were culture-positive for the single organ sampled. Gastrointestinal tract (stomach, small intestine, colon, cloaca), liver, bile, heart blood, and yolk sac were the tissues yielding positive cultures.

Histologic reexamination of 14 of the 20 culture-positive necropsy cases was made. Lesions were most commonly encountered in the gastrointestinal tract, liver, spleen, and arterioles from several different organs. There was a spectrum from acute fibrinonecrotic to granulomatous changes. Sometimes, acute to chronic processes were evident within the same tissue.

Lesions in the gastrointestinal tract varied. Grossly, acute lesions in the intestines were ulcerative and hemorrhagic; chronic changes appeared as diffuse thickenings of the gut wall, with dry

caseonecrotic debris on the mucosal surface. Histologically, the acute lesions showed fibrinohemorrhagic necrosis, with heterophilic reactions. Necrosis was either multifocal and restricted to the mucosa or diffuse with involvement of the entire wall. More chronic lesions had granulomas with heterophils in their centers, with ulceration of luminal epithelium and an abundance of mononuclear cells in the lamina propria.

Grossly, the livers of some of the culture-positive reptiles had white to tan spots 2 mm or less in diameter scattered throughout the parenchyma. Microscopically, there were multifocal areas of necrosis and infiltrates of heterophils, with early granuloma formation. The centers of some of the inflammatory lesions contained gram-negative coccobacilli. Lesions in the spleen generally were acute fibrinonecrotic and rarely granulomatous.

Fibrinoid necrosis was observed in the arterioles of mainly the digestive tract but also the kidneys, adrenal glands, and several other organs. The necrosis involved the intima, with occasional hemorrhage into the media and adventitia. In addition, 3 reptiles had proliferative vasculitis, with fibrous thickening of the media and adventitia and reduction of vascular lumens.

The gross and histologic lesions observed in the gastrointestinal tract and liver were often associated with pure cultures of *Salmonella* or *Arizona*, but mixed cultures containing *Pseudomonas* spp, *Proteus* spp, and *Citrobacter freundii* also were encountered. Vascular and splenic lesions were not observed grossly; hence, cultures of these tissues were not attempted.

### Discussion

Similar studies on populations of captive reptiles have been reported worldwide.<sup>1-10</sup> Wide variation in culture techniques and manner of reporting make comparison difficult. In our study, GN broth was chosen as the enrichment medium. Using this broth, increased numbers of salmonellae have been recovered, whereas the more commonly used selenite F and tetrathionate broths are known to be toxic for *S choleraesuis* and certain serotypes of *S enteritidis*.<sup>11,12</sup> It is the opinion of one of the authors of this report (EES), after years of culturing specimens from reptiles in a zoological collection, that many of the *Arizona* serotypes will be missed if the more selective enrichment broths are used. The same reasoning was used in the selection of Hektoen enteric agar as the selective plating medium for this study.

The high percentage of culture-positive reptiles arriving into quarantine, coupled with the consistently negative cultures from feed and water sources, would suggest that it is the animals themselves that constitute the greatest potential point source of infection for other inhabitants of the collection. One important consideration is that the rate of infection based on 1 culture alone may not reflect the true rate, since it is possible that many of

the reptiles cultured negative could be intermittent shedders of organisms.

Reptiles infected with *Salmonella* and *Arizona* are generally found to be free of overt signs of disease. This was believed to be the case in the present study also. Some investigators believe that the reptiles and the bacteria have evolved together over millions of years into the ideal host-parasite relationship.<sup>5,13</sup> In this report, we were never able to attribute clinical illness in reptiles directly to infection with these organisms, despite the fact that pure cultures were obtained from lesions at necropsy in some cases. In only 2 cases, both Blood Pythons (*Python curtus*), were lesions found in all 4 sites described (gastrointestinal tract, liver, spleen, and vessels). More than half of the reptiles with lesions attributed to *Salmonella* or *Arizona* had underlying conditions such as cryptosporidiosis (2 snakes), mycobacteriosis (1 snake), hepatocellular carcinoma (1 snake), trauma from cagemates, hyperthermia after thermostat malfunction, and eggbound oviduct. Four reptiles were hatchlings less than 2 weeks old at death. It would appear from the pathologic findings that *Salmonella* and *Arizona* tend to be opportunistic pathogens.

Treatment for or complete elimination of *Salmonella* and *Arizona* from a reptile collection or from specimens newly entering a collection does not seem feasible on the basis of our findings and the fact that there has been little success in eliminating these organisms with antibiotics.<sup>14</sup> Antibiotic therapy in fact might create more resistant strains, with the possibility of increased virulence.

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