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A LONGITUDINAL STUDY OF *SALMONELLA* FROM SNAKES USED IN A PUBLIC OUTREACH PROGRAM

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Abstract: Snakes are considered to be a source of *Salmonella* infection for humans, but little is known about the actual serotype prevalence in healthy snakes over time. Twelve snakes involved in a public outreach program, representing seven different species, were tested weekly for shedding of *Salmonella* sp. over a period of 10 consecutive weeks. The snakes were housed in close proximity but in separate exhibits. Fresh fecal samples (when available) or cloacal swabs were cultured for *Salmonella* sp., and subsequent *Salmonella* isolates were serotyped. As representatives of the feed source, the feces of two mice and the intestines of one rat were cultured weekly. Fecal samples from 11 of the 12 snakes were positive for *Salmonella* at least once. Seven (58%) of 12 snakes were culture positive five times or more. The weekly prevalence of *Salmonella* shedding varied between 25% and 66%. Two or more different serotypes were isolated from nine snakes over time; however, a predominant serotype was generally isolated from each of these snakes. Altogether 15 different serotypes were identified. Serotypes of public health concern included Newport, Oranienburg, and Muenchen. Two samples from feeder rodents were positive for *Salmonella*. The results are consistent with previous studies showing high intestinal colonization rates with *Salmonella* sp. in snakes. Frequent and intermittent shedding of multiple serotypes was evident. Feeder rodents might serve as a source for intestinal colonization. Appropriate handling protocols should be implemented for all reptiles associated with public outreach programs to minimize risk of *Salmonella* transmission to the public.

Key words: Reptiles, rodent, *Salmonella*, salmonellosis, zoonosis.

INTRODUCTION

It is generally believed that captive reptiles have a high but variable prevalence of intestinal *Salmonella* carriage, ranging from 14% to 95%.[5,11,23,24] Snakes have been shown to have a particularly high prevalence, sometimes reaching 100%.[13] However, few studies have examined prevalence and serotype shedding over time. In the few longitudinal studies that have been performed, snakes have been shown to have intermittent shedding of *Salmonella* and to be carriers of multiple serotypes.[13,19,24] The reasons for these high prevalences in reptiles are unknown, but several hypotheses have been put forth.

One possibility is that *Salmonella* is a normal inhabitant of a reptile's gastrointestinal tract.[16,19] In this case, one would expect a similarly high prevalence in wild reptile populations. These have not been well studied, but isolation rates have been quite variable, from 0% to 95%.[5,14,20,21,23] Potentially, wild snakes carrying *Salmonella* introduced into captive populations could maintain high carriage rates in a population through transmission to offspring and the long survival time of *Salmonella* in the environment.[9,15,25]

Another possibility is that snakes are obtaining *Salmonella* from their food source.[11] Captive snake populations are often fed rodents that are either bred onsite or shipped from an outside facility. *Salmonella* could be introduced to colonies through these sources. A recent outbreak in humans has been attributed to handling rodents used to feed snakes.[10]

The high prevalence of *Salmonella* in snakes and other reptiles are often cited as a source of human infection. It has been estimated that approximately 74,000 cases of reptile-associated salmonellosis occur each year, accounting for 6% of human *Salmonella* infections in the United States.[16] Reptiles have been implicated in numerous, sometimes fatal, infections in children and adults and in an outbreak of human salmonellosis at a zoo.[3,15,19] Hence, captive populations of reptiles infected with *Salmonella* are a potential public health risk, and precautions, such as public education, about the risk are warranted when

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reptiles are used for educational or school programs.

In this study, a healthy population of captive snakes at a zoo was evaluated. The purpose was to characterize *Salmonella* carriage in a cohort of snakes used for educational purposes. An additional goal of this study was to determine the specific serotypes and the carriage of those serotypes over time by individual animals. It was hypothesized that the prevalence would be high and that snakes would intermittently shed multiple serotypes of *Salmonella*. Feeder rodents of these snakes were sampled to determine whether they served as a potential source.

MATERIALS AND METHODS

The captive population of snakes utilized in this study contained a total of approximately 50 snakes, including nine different species. Twelve of these snakes were chosen for inclusion in this study, with the following criteria used for selection: species of snake (to include the majority of species in this collection), specific housing location (to include snakes from all localities used for this collection), contact with the public (i.e., the majority of snakes examined in this study were used for educational purposes), and diet (to include snakes that were fed mice and those that were fed rats). The 12 snakes included in this study represented seven of the nine total species in this collection, including two boas (*Epicrates cenchria cenchria* and *Corallus caninus*) and five colubrids (*Pituophis catenifer sayi*, *Elaphe guttata guttata*, *Elaphe obsoleta rossalleni*, *Lampropeltis triangulum hondurensis*, and *Heterodon nasicus*). These snakes were tested weekly for *Salmonella* infection over a 10-wk period.

The holding facilities for the snakes consisted of a room containing multiple aquaria with either individual snakes or multiple snakes housed together ("reptile room"). Additionally, several aquaria contained snakes in an adjoining hallway, and a few additional snakes were housed in a room with various amphibians. For the purposes of this study, only individually housed snakes were chosen. Snakes were chosen from each of the three holding areas, including various locations throughout the "reptile room."

Some of the snakes in this collection were placed on exhibit at the zoo, and many were used for various public education purposes. All snakes within this study population were used for public education purposes, with the exception of the emerald tree boas. These snakes were rotated out on exhibit at the zoo, with the potential for public contact either from direct contact with the snakes or indirect contact with the exhibit. Reptiles have been known to contaminate their environment with *Salmonella,* resulting in human infection.[2]

Typical zoological husbandry practices were maintained in this collection. For the majority of the snakes, with the exception of the emerald tree boas, enclosures contained cypress mulch, one or more hiding locations, and a 50-W spotlight for heat running on a 12-hr on/off cycle. Emerald tree boas were housed on sphagnum moss, with a large pool of water, multiple tree branches, and a 100-W ultraviolet bulb. Fresh water was provided, and spot-cleaning of enclosures was performed daily for all snakes.

To minimize the stress associated with educational use, all snakes had a minimum off-period of 24 hr between public programs. Individual snakes were not used for programs during their shedding period or the 24-hr period after feeding. While not being actively handled during a program, the snakes were placed in a cloth bag in a closed container.

Snakes within this facility were fed either mice raised on site in a separate room of the reptile building or rats that were received frozen from an outside source. Nine of the snakes within this study were fed live mice and three were fed rats. The rat-fed snakes were the Brazilian rainbow boa, bullsnake no. 2, and emerald tree boa no. 2.

Personnel trained to handle snakes safely and appropriately collected fecal samples weekly for a period of 10 consecutive weeks. Fecal samples were placed in individual plastic cups in a refrigeration unit until picked up for transport to the bacteriology laboratory. No samples remained in refrigeration for longer than 3 days. When fecal samples were not available, cloacal swabs were collected with cotton swabs (3M™ Quick Swab, 3M, Saint Paul, Minnesota 55144, USA, or BBL™ CultureSwab™, BD, Franklin Lakes, New Jersey 07417, USA) (Institutional Animal Care and Use Committee approval, University of Minnesota 0705A08882). These samples were transported to the bacteriology laboratory and processed within 2 hr of collection.

Weekly samples of the snakes' diets were also collected. These included two fecal samples from mice and one intestinal sample of a rat. Mice used in this study were isolated in a separate cage before sample collection to ensure fecal samples were from the appropriate mouse. The fecal samples were placed in C & S medium (C&S vials, Fisher Diagnostics, Fisher Scientific Company LLC, Middletown, Virginia 22645, USA) for

Table 1. Weekly *Salmonella* serotypes found in a captive population of snakes.[a]

	ETB No. 1	ETB No. 2	HM No. 1	HM No. 2	BRB	BS No. 1
Week 1	III 57:c:z	Negative	Negative	Negative	Negative	multiple serotypes
Week 2	III 57:c:z	Negative	Florida	Newport	Negative	Oranienburg
Week 3	Negative	Negative	Florida	Negative	Negative	Negative
Week 4	III 57:c:z	ND	Florida	III 48:z52:-	Negative	Negative
Week 5	ND	ND	ND	Newport	III (6),14:z10:z	Oranienburg
Week 6	III 57:c:z	III 57:c:z	Florida	Negative	Negative	Oranienburg
Week 7	Schwarzengrund	III 57:c:z	Florida	Newport	Negative	Oranienburg
Week 8	III 57:c:z	III 58:l,v:z35	Negative	Negative	III (6),14:z10:z	Oranienburg
Week 9	Negative	Muenchen	Florida	Negative	Negative	Oranienburg
Week 10	III 57:c:z	III 57:c:z	Negative	Newport	Negative	Oranienburg

[a] ETB, emerald tree boa; HM, Honduran milksnake; BRB, Brazilian rainbow boa; BS, bullsnake; ER, Everglades ratsnake; CS, cornsnake; WH, western hognose; ND, not done.

transport to the bacteriology laboratory. Sections of the small and large intestines were removed from a thawed rat each week and placed in Whirl-Pak® bags (NASCO, Fort Atkinson, Wisconsin 53538, USA) for transport. The sampled rodents were then fed to snakes in the study population after sampling.

All samples were cultured at the University of Minnesota, Veterinary Diagnostic Laboratory. After overnight enrichment in tetrathionate (Hajna) broth at 40°C, samples were inoculated to brilliant green and XLD agar plates. Plates were placed in an O_2 incubator overnight at 37°C. Colonies suspected as *Salmonella* were further identified using the following substrates/biochemical tests: TSI, dextrose, lactose, sucrose, mannitol, maltose, urease, dulcitol, salicin, citrate, lysine iron agar, sorbitol, malonate, motility, indole, ornithine, phenylalanine, and API 20E test kit. After further biochemical identification as *Salmonella*, isolates were serogrouped using an agglutination test. *Salmonella* isolates were then sent to the National Veterinary Services Laboratories for serotyping using standard protocols.[7,12] A "predominant serotype" was defined as a serotype accounting for greater than 50% of all serotypes isolated from an individual snake.

RESULTS

Eleven of the 12 (92%) snakes tested positive for *Salmonella* at least once during the 10-wk period (Table 1). Seven (58%) of these snakes tested positive five or more times. The weekly rate of recovery ranged between 25% and 80% (Figure 1). However, during weeks 1, 4, and 5, not all of the snakes were tested; therefore, some weeks are not representative of the whole sampled population. Excluding these weeks, the weekly prevalence was between 25% and 66%. Nine (75%) of 12 snakes shed multiple serotypes during the study period. Of these snakes, seven had a predominant serotype isolated over the 10-wk period.

Fifteen different serotypes were identified. Ten of these serotypes belonged to *Salmonella enterica* subsp. *arizonae* and five belonged to *Salmonella enterica* subsp. *enterica*. Of all of these serotypes, three have commonly been found in humans and represent a public health concern: Newport, Oranienburg, and Muenchen. A "public health concern" was defined as being listed in the top 20 serotypes identified in human cases of salmonellosis.[4]

Two of the feeder mice were positive for *Salmonella* during the study period. The serotypes identified were identical to serotypes found within the snake population. However, Mouse A from week 7 was fed to everglades ratsnake no. 2, which was consistently negative for *Salmonella*. *Salmonella* Muenchen was isolated from mouse B during week 8 and was fed to corn snake no. 1. *Salmonella* Muenchen was not isolated from corn snake no. 1. None of the feeder rats were positive for *Salmonella* during this study.

DISCUSSION

This study corroborates the high prevalence of *Salmonella* spp. seen in other captive populations of snakes, as well as intermittent shedding.[5,6,11,19,24] This study also confirms that snakes can carry multiple serotypes of *Salmonella,* which has been seen in prior studies.[19,25] Nine snakes carried multiple serotypes, although most of these snakes had a predominant serotype during the study period.

However, it should be noted that only a single colony on each plate was collected for serotyping. Likely, sampling multiple colonies could have

Table 1. Extended.

BS No. 2	ER No. 1	ER No. 2	CS No. 1	CS No. 2	WH
ND	Negative	Negative	III (6),14:z10:z	ND	III 48:z52:z
III 50:r:z	Negative	Negative	III (6),14:z10:z	Oranienburg	Negative
Negative	Negative	Negative	Florida	III 16:z10:e,n,x,z15	Negative
III 16:r:-	III 44:z10:-	Negative	Newport	III 16:z10:e,n,x,z15	Newport
III 6,14:-:z	Negative	ND	ND	ND	ND
Negative	Negative	Negative	Florida	III 16:z10:e,n,x,z15	Negative
Negative	Negative	Negative	III (6),14:z10:z	III 16:z10:e,n,x,z15	III 48:z52:z
Negative	Negative	Negative	Florida	III 16:z10:e,n,x,z15	Negative
Negative	Negative	Negative	III (6),14:z10:z	Oranienburg	III 48:z52:z
Negative	Newport	Negative	III (6),14:z10:z	III 16:z10:e,n,x,z15	Negative

recovered several serotypes per sample. However, determining the possibility of shedding of multiple serotypes at a single time point was beyond the scope and cost of this project. Future studies, in which samples from multiple colonies on each plate are collected, would aid in determining whether a single or multiple serotypes are being shed at any given time. Interestingly, one of the samples was identified as "multiple serotypes," indicating that snake was, in fact, shedding multiple serotypes at that time.

The fact that the prevalence of *Salmonella* varied throughout this study period may help explain the variable recovery rates often seen in other studies. Most studies focused on a single point in time, unlike the present study, which provided a longitudinal perspective. Likely, *Salmonella* is shed in variable numbers and recovery may be based on isolation methods and reptile physiology (i.e. stress). Another possible explanation for the variability of prevalence is the sampling technique. It is generally accepted that fresh fecal samples will have a higher sensitivity than cloacal swabs.[18] Depending on the technique used, one could get a different result. This might contribute to some of the variability in weekly prevalence.

An additional noteworthy finding in this study was the isolation of *Salmonella* from mice, although the serotypes isolated from the mice

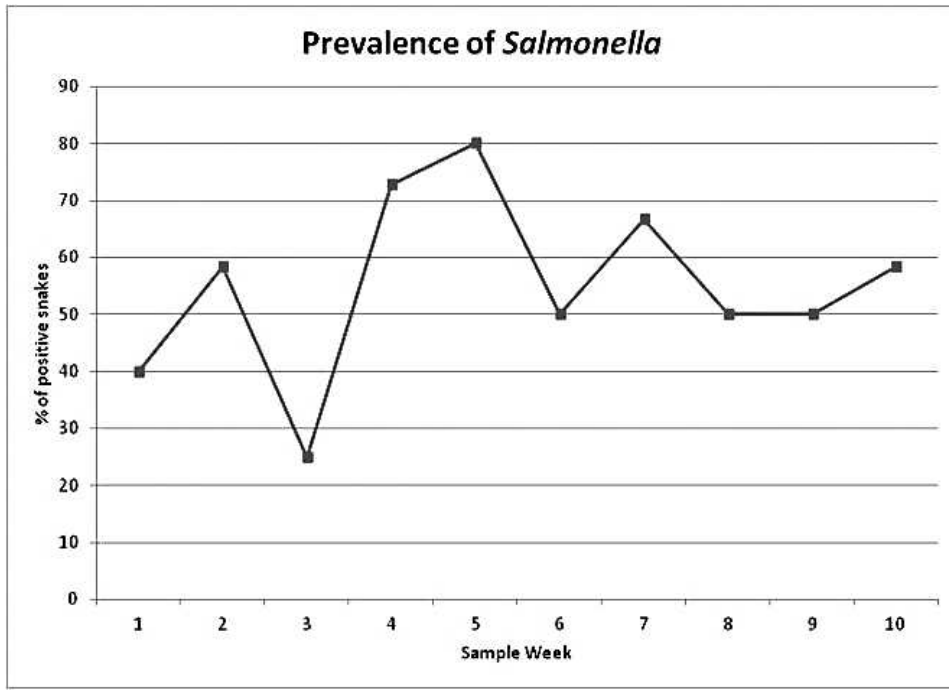

Figure 1. Weekly recovery of intestinal *Salmonella* in a captive population of snakes.

Table 1. Extended. Continued.

Mouse A	Mouse B	Rat
ND	ND	ND
Negative	Negative	Negative
Negative	Negative	Negative
Negative	Negative	Negative
ND	ND	ND
Negative	Negative	Negative
III 16:z10:e,n,x,z15	Negative	Negative
Negative	Muenchen	Negative
Negative	Negative	Negative
Negative	Negative	Negative

were not isolated from the snakes to which they were fed. Thus, these results were inconclusive regarding feed as a source of *Salmonella* infection in this population. Nevertheless, the recovery of *Salmonella* from feed sources does highlight a potential on-going source of *Salmonella* to captive reptiles, potentially allowing for long-term carriage and environmental contamination.

This study highlights the expected public health risk from handling snakes. As discussed, there were several serotypes in this study known to be public health risks (Newport, Oranienburg, and Muenchen). However, it should be pointed out that although these were identified as "public health risks," all serotypes of *Salmonella* have the potential to infect humans and result in salmonellosis.[18] In fact, the *Salmonella enterica* subsp. *arizonae*, generally associated with "cold-blooded" animals, have been shown to cause disease in humans as well.[8,22,26]

The snakes in this study are part of a public outreach effort. These settings are valuable educational programs to stimulate interest in animals, understand their ecology, and prepare the next generation of scientists. The educational value of allowing the public to interact with animals needs to be balanced with appropriate public health precautions.

There is an occupational risk to handlers and keepers. In this outreach program, direct public contact with these snakes was allowed before the study. Handlers are aware of the *Salmonella* risk and encourage the public to wash their hands after reptile contact. The Centers for Disease Control and Prevention have published a list of recommendations to help prevent infection of *Salmonella* from reptiles and amphibians.[3] Additionally, both the National Association of State Public Health Veterinarians and the Association of Zoos and Aquariums have provided guidelines for reducing risk from animals in public contact settings such as petting zoos or zoological parks.[1,17] Handlers and keepers need to be informed of the risk and take appropriate precautions. The key public and occupational recommendations include providing adequate hand-washing facilities and supervision, especially when children are involved.

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