

Prevalence of *Salmonella* spp in cloacal, fecal, and gastrointestinal mucosal samples from wild North American turtles

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Objective—To determine prevalence of *Salmonella* spp in samples collected from wild North American turtles.

Animals—94 wild North American turtles of 6 species in 2 genera.

Design—Prospective microbiologic study.

Procedures—A convenience sample of wild North Carolina turtles admitted to a veterinary college was evaluated for *Salmonella* spp by use of standard techniques via microbiologic culture of cloacal swab and fecal samples. Gastrointestinal mucosa samples were also collected at necropsy from turtles that died or were euthanized. Cloacal swab samples were also collected from wild pond turtles for bacteriologic culture. Controls were established by use of wild-type *Salmonella* Typhimurium LT2.

Results—94 turtles were tested for *Salmonella* spp; *Salmonella* spp were not detected in any sample. By use of a pathogen-prevalence and sample-size table, the true prevalence of *Salmonella* spp was estimated as < 5%.

Conclusions and Clinical Relevance—Results suggested that wild turtles in central North Carolina may not be active shedders or carriers of *Salmonella* spp. Despite this 0% prevalence of infection, proper hygiene practices should be followed when handling wild turtles. (*J Am Vet Med Assoc* 2006;229:266–268)

Salmonellosis is an important zoonotic infection often associated with contact between pet reptiles and humans in the United States.¹ The CDC estimates that the fecal carriage rate of *Salmonella* spp in pet reptiles is > 90% and that there are approximately 74,000 cases of reptile-associated salmonellosis in the United States annually.¹ The CDC also estimates that since banning the sale of turtles < 4 inches in diameter, 100,000 cases of salmonellosis have been prevented.¹ A number of studies have investigated the risk of salmonellosis associated with the international pet turtle trade.^{2–5} A recent study⁶ found 74% prevalence of *Salmonella* infection in pet reptiles from Japan. A 2002 study of 62 samples (28 cloacal swab specimens and 34

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ABBREVIATIONS

NCSU-CVM	North Carolina State University College of Veterinary Medicine
TRT	Turtle Rescue Team
TSI	Triple sugar iron
CFU	Colony-forming unit

soil samples) from pet store turtles and privately owned turtles identified *Salmonella* spp in 11.3% of samples.⁷ This finding was consistent with other reports^{8–10} of *Salmonella* infection rates of 4.5%, 14%, and 16%, respectively, in a variety of captive chelonian species. Although the prevalence of salmonellosis contracted from pet reptiles in humans is high, it is hypothesized that the risk from wild reptiles, particularly turtles, is low because they are not active shedders or carriers of *Salmonella* spp, as estimated on the basis of previous studies^{11–13} of free-living reptiles. This disparity in reptilian *Salmonella* infection rates may be attributable in part to environmental variations between wild and captive reptiles (captive reptiles may be crowded or subjected to poor hygienic protocol). *Salmonella* studies of wild North American reptiles are limited, compared with the numerous studies conducted on captive reptiles.^{3–10,14} In 2004, a Virginia study¹³ revealed a prevalence of 0% for *Salmonella* infection in 82 free-living reptiles.

The purpose of the study reported here was to determine the prevalence of *Salmonella* spp in samples collected from wild North American turtles.

Materials and Methods

Ninety-four wild turtles, including 46 admitted to the NCSU-CVM TRT¹⁵ and 48 captured from local ponds, were tested for *Salmonella* spp during May, June, and July 2005. There were 14 eastern painted turtles (*Chrysemys picta picta*), 5 common snapping turtles (*Chelydra serpentina*), 13 Florida cooters (*Pseudemys floridana*), 17 eastern box turtles (*Terrapene carolina carolina*), 10 common musk turtles (*Sternotherus odoratus*), and 35 yellowbelly sliders (*Pseudemys concinna concinna*).

Cloacal swab^a samples were collected from live turtles by inserting a sterile polyester swab into the cloaca and rotating the swab against the inner cloacal wall until fecal material was obtained. Cloacal swabbings were repeated 3 weeks after admittance in 3 eastern box turtles. Cloacal swab samples were also collected from 6 turtles undergoing rehabilitation that were rechecked by the NCSU-CVM TRT. Gastrointestinal mucosa and a cloacal swab sample or fecal material were collected at necropsy from turtles that were dead or euthanized. Gastrointestinal mucosa samples were included to test for nonshedding (carrier-state) turtles.

Each sample was incubated in selenite F broth^b for 18 to 24 hours at 35°C and plated on *Salmonella-Shigella* agar^c for 18 to 24 hours at 35°C. After incubation, the agar plates were examined for colonies resembling those of *Salmonella* spp

(clear colonies with a black center characteristic of hydrogen sulfide production). If there were no suspicious colonies, the agar plates were re-incubated for 24 hours at 35°C and rechecked for growth. Suspicious colonies were plated on Christensen urea slants^c and TSI agar slants^d for 18 to 24 hours at 35°C. *Salmonella* spp were suspected if there was no urease production (no color change to pink on the slant) and the TSI agar slants yielded carbon dioxide and hydrogen sulfide production and the typical reactions of alkaline slant over acidic butt. Suspicious colonies from the urea or TSI agar slants were isolated for biochemical profiling at the NCSU-CVM Clinical Microbiology Laboratory by use of a computerized automated interpretive database.^e

Controls were established with saline (0.9% NaCl) solution–diluted samples and feces (equine)–diluted samples with wild-type *Salmonella* Typhimurium LT2. *Salmonella* organisms were recovered from the saline solution controls at a concentration of 15 CFU/mL and from the fecal controls at a concentration of 150 CFU/mL.

Results

Overall, 97 cloacal swab samples, 1 fecal sample, and 16 mucosal samples were tested. Prevalence of *Salmonella* spp was 0% for all samples from the 94 wild turtles, suggesting that the organism was not being actively shed and/or carried without clinical signs. Most samples yielded negative results on *Salmonella-Shigella* agar^f after 18 to 24 hours and 48 hours of incubation at 35°C in ambient air. In many samples, a lack of color change on Christensen urea agar slants indicated inability of bacteria to hydrolyze urea. Other samples yielded negative results on TSI agar slants because of failure to produce carbon dioxide or hydrogen sulfide and failure to ferment glucose, lactose, or sucrose. Few bacterial isolates required biochemical profiling; these were determined not to be *Salmonella* spp. There were 6 *Citrobacter freundii* isolates.

By interpolation with a pathogen-prevalence and sample-size table (Table 1) used commonly for fish pathogens,¹⁶ and assuming a sensitivity of 65% with an observed prevalence of 0%, the true prevalence of *Salmonella* spp in the turtles was < 5%. If the culture test was 100% sensitive and a large theoretical population was used, a 0% observed prevalence would yield 95% confidence that the true prevalence would be approximately < 3.3%. With a sensitivity of just 50%, the true prevalence would be approximately < 6.6%.

Table 1—Sample sizes required to detect a sample with positive results, assuming test sensitivity of 100%, in populations of various sizes and at various pathogen prevalences.

Population size	Prevalence		
	2%	5%	10%
50	50	35	20
100	75	45	23
250	110	50	25
500	130	55	26
1,000	140	55	27
10,000	145	60	27
> 100,000	150	60	30

Adapted from Ossiander FJ, Wedemeyer G. Computer program for sample size required to determine disease incidence in fish populations. *J Fish Res Board Can* 1973;30:1383–1384. Reprinted with permission.¹⁶

Discussion

The 0% prevalence of *Salmonella* carriers and shedders detected in this study among free-living North Carolina turtles was similar to results of some studies^{11–13} of wild reptiles in North America, but differed from other studies^{1,6,14} that revealed high prevalence of reptile-associated salmonellosis in humans.

There are 2 general hypotheses regarding the reason captive reptiles shed *Salmonella* spp at a higher rate than wild reptiles. The first hypothesis is that wild turtles are natural carriers of *Salmonella* spp but do not actively shed the organism unless relocated to a stressful environment, such as captivity. Our results suggested that wild turtles in North Carolina are not natural carriers of *Salmonella* organisms. Three turtles admitted to the NCSU-CVM TRT yielded negative results for *Salmonella* spp upon entrance to the facility and again 3 weeks later, after undergoing the stress of hospitalization. Six wild turtles that were admitted to the NCSU-CVM TRT, and cared for by a turtle rehabilitator, all yielded negative results for *Salmonella* spp. The second hypothesis is that free-living turtles are not natural carriers of *Salmonella* spp, but rather acquire the organism and start shedding when brought into the pet industry. The study reported here did not address that issue, but did suggest that wild turtles may not be carriers of the organisms before capture.

One possible explanation for the lack of detectable *Salmonella* spp from cloacal swabs is a lack of appreciable fecal material, although *Salmonella* organisms were detected at 150 CFU/mL in the fecal control samples. Lack of detection of *Salmonella* organisms that may have been in the carrier state could have resulted from poor technique in gastrointestinal scrapings. Another possible explanation is that in some cases, multiple samples may be required to detect *Salmonella* spp in feces via bacterial culture, perhaps because of intermittent shedding of the organism. In 1 report,¹⁷ a PCR assay was more sensitive than ELISA and bacterial culture in detecting *Salmonella* spp from the cloacal-colon samples of green iguanas (*Iguana iguana*). The authors estimated that from 30% to 45% of infected animals could go undetected with a culture-only *Salmonella* screening protocol based on test sensitivities of 55% and 70% in 2 populations of 120 animals each. A study¹⁸ of captive lizards revealed that use of single bacterial cultures of cloacal swab samples or feces indicated a prevalence of 62.5% for *Salmonella* infection in clinically normal lizards and that this method was 75.8% sensitive. Nevertheless, it seems highly unlikely that the present study would have failed to detect *Salmonella* spp in 94 turtles if the organisms were present.

Results of this study suggest that further investigation of the pet reptile industry and the differences in the environment between captive and wild reptiles are needed. Despite the findings reported here, proper hygiene practices should be followed when handling and maintaining all reptiles.^{19,20}

a. Solon 6-inch Dacron tipped plastic applicator, Solon Manufacturing Co, Solon, Me.

- b. Selenite F broth, VWR International, West Chester, Pa.
- c. Christensen's urea agar slant, Remel Inc, Lenexa, Kan.
- d. Triple sugar iron agar slant, Remel Inc, Lenexa, Kan.
- e. Vitek32, bioMérieux Inc, Hazelwood, Mo.
- f. *Salmonella-Shigella* agar plate, Remel Inc, Lenexa, Kan.

References

1. CDC Healthy Pets Web site. Spotlight on turtles. Available at: www.cdc.gov/healthypets/spotlight_an_turtles.htm. Accessed Sep 1, 2005.
2. Baker EF, Anderson HW, Allard J. Epidemiological aspects of turtle-associated salmonellosis. *Arch Environ Health* 1972;24:1-9.
3. Chassis G, Gross EM, Greenberg Z, et al. *Salmonella* in turtles imported to Israel from Louisiana (lett). *JAMA* 1986;256:1003.
4. Shane SM, Gilbert R, Huntington KS. *Salmonella* colonization in commercial pet turtles. *Epidemiol Infect* 1990;105:307-316.
5. Tauxe RV, Rigau-Perez JG, Wells JG, et al. Turtle-associated salmonellosis in Puerto Rico. Hazards of the global turtle trade. *JAMA* 1985;254:237-239.
6. Nakadai A, Kuroki T, Kato Y, et al. Prevalence of *Salmonella* spp in pet reptiles in Japan. *J Vet Med Sci* 2005;1:97-101.
7. Pasmans F, De Herdt P, Haesebrouck F. Presence of *Salmonella* infections in freshwater turtles. *Vet Rec* 2002;150:692-693.
8. Cambre RC, Green DE, Smith EE, et al. Salmonellosis and arizonosis in the reptile collection at the National Zoological Park. *J Am Vet Med Assoc* 1980;177:800-803.
9. Greenberg Z, Sechter I. *Salmonella* serotypes isolated from snakes and other reptiles—Israel, 1953-1989. *Isr J Vet Med* 1992;47:49-60.
10. Gopee NV, Adesyun AA, Caesar K. Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *J Wildl Dis* 2000;36:284-293.
11. Brenner D, Lewbart G, Stebbins M, et al. Health survey of wild and captive bog turtles (*Clemmys muhlenbergii*) in North Carolina and Virginia. *J Zoo Wildl Med* 2002;33:311-316.
12. Mitchell JC, McAvoy BV. Enteric bacteria in natural populations of freshwater turtles in Virginia. *Va J Sci* 1990;41:233-242.
13. Richards JM, Brown JD, Kelly TR, et al. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the wildlife center of Virginia. *J Zoo Wildl Med* 2004;35:562-563.
14. Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Vet Microbiol* 2002;84:79-91.
15. Lewbart GA, Kishimori J, Christian LS. The North Carolina State University College of Veterinary Medicine Turtle Rescue Team: a model for a successful wild-reptile clinic. *J Vet Med Educ* 2005;32:377-381.
16. Ossiander FJ, Wedemeyer G. Computer program for sample size required to determine disease incidence in fish populations. *J Fish Res Board Can* 1973;30:1383-1384.
17. Mitchell MA, Shane SM, Orr K, et al. *Salmonella* diagnostic testing in the absence of a gold standard, in *Proceedings. Annu Meet Assoc Reptilian Amphib Vet* 2000;143-144.
18. Pasmans F, Martel A, Boyen F, et al. Characterization of *Salmonella* isolates from captive lizards. *Vet Microbiol* 2005;110:285-291.
19. Chin JC. *Control of communicable diseases manual*. 17th ed. Washington, DC: American Public Health Organization, 2000.
20. Johnson-Delaney CA. Reptile zoonoses and threats to public health. In: Mader DM, ed. *Reptile medicine and surgery*. 2nd ed. Philadelphia: WB Saunders Co, 2006;1017-1030.



Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Effects of dexamethasone and isoflupredone acetate on plasma potassium concentrations and other biochemical measurements in dairy cows in early lactation

Natalie J. Coffey et al

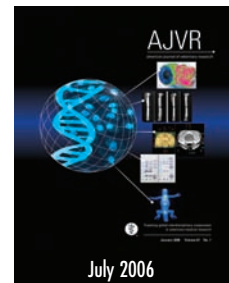
Objective—To determine whether administration of isoflupredone acetate (ISO) to healthy cows increases the frequency of severe hypokalemia and whether dexamethasone (DEX) has detectable mineralocorticoid properties.

Animals—33 cows at 20 to 25 days of lactation.

Procedures—Cows were randomly allocated to 5 treatment groups and received 2 IM injections (on days 0 and 2) of sterile saline (0.9% NaCl) solution (10 mL each), an injection of ISO (20 mg) or DEX (20 mg) followed by 10 mL of saline solution, or 2 injections of ISO or DEX. Milk production was measured, physical examinations were performed, and blood and urine samples were collected daily on days 0 through 7.

Results—Physical examination variables did not differ among groups; however, 1 cow developed atrial fibrillation on day 4. Both corticosteroids significantly increased plasma glucose concentrations, and ISO significantly decreased plasma potassium concentrations and increased total carbon dioxide concentrations with time. One dose of ISO decreased mean plasma potassium concentration by 25% on day 2, compared with day 0, and severe hypokalemia (serum potassium concentration < 2.3 mEq/L) developed in 1 of 6 cows. Mean plasma potassium concentration was 46% lower on day 3 than on day 0 in cows receiving 2 doses of ISO, and 5 of 7 cows became severely hypokalemic. Mean urinary fractional excretion of potassium significantly increased from that on day 0 in cows receiving 2 doses of ISO.

Conclusions and Clinical Relevance—Both corticosteroids had glucocorticoid activity; however, only ISO had mineralocorticoid activity. Compared with saline solution, administration of 2 doses of ISO significantly increased the frequency of severe hypokalemia. (*Am J Vet Res* 2006;67:1244-1251)



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