RURALS: Review of Undergraduate Research in Agricultural and Life Sciences

Volume 4 | Issue 1 Article 1

February 2009

Incidence of Salmonella on Reptiles in the Pet Trade

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Cover Page Footnote

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1. Introduction

Reptiles are very common carriers of *Salmonella* (Corrente, 2003; CDCP, 1999; CDCP, 2003; Jong et al., 2005). Reptiles can be infected by *Salmonella* strains without showing any symptoms, but if these strains are passed on to warm-blooded animals (including humans), they can be pathogenic (Corrente, 2003). Humans become infected by reptile-associated Salmonellosis (RAS) by ingesting *Salmonella*, which can occur whenever humans handle a reptile or any object contaminated by a reptile then fail to wash their hands properly (CDCP, 1999; ARAVb, 1998).

Between 1991 and 2001, the estimated number of households in the U.S. with pet reptiles doubled from 850,000 to 1.7 million (CDCP, 2003). With reptiles becoming more popular pets, do people have more reason to be concerned about RAS? Human contact with pet reptiles or amphibians is responsible for 74,000 *Salmonella* infections annually in the U.S., making RAS a significant (6%) source of the approximately 1.2 million *Salmonella* infections occurring annually in the U.S. (CDCP, 2003). Increased awareness and the growing number of people harboring reptiles as pets have led to a greater detection of RAS (Pasman et al., 2005; Schroter et al., 2004; Corrente et al., 2006). However, the increasing popularity of exotic reptiles as pets has also led to an increase in the number of RAS cases in the U.S. and Europe (Sanyal et al., 1997; Nakadai et al., 2005).

Jong et al. (2005) suggests that very high percentages of captive reptiles carry at least one strain of *Salmonella*. The prevalence of *Salmonella* in all reptiles (wild or captive) may be as high as 90% (Woodward et al., 1997) to 94% (Mermin et al., 2004). A critical gap in our knowledge about RAS is what proportion of reptiles from commercial pet shops is infected with *Salmonella*. This study seeks to narrow that gap by estimating the prevalence of *Salmonella* in captive (pet) reptiles in Nebraska.

Another area of research that is lacking is the relative contribution of captive reptiles versus wild (non-captive) reptiles to the prevalence of RAS. This study compares data on the prevalence of *Salmonella* in captive reptiles from Nebraska pet shops to previously collected data on the prevalence of *Salmonella* in wild reptiles in Nebraska and highlights the importance of taking precautions when dealing with reptiles from any source.

2. Methods

A total of 80 reptiles (23 snakes, 46 lizards, 11 turtles) were sampled from four commercial pet shops in Omaha, NE, to estimate the prevalence of *Salmonella* in captive reptiles from Nebraska. Depending on the number of reptiles available, between 10 and 30 reptiles were sampled from each pet shop.

For the wild data, 182 snakes (eight species, six genuses) from a wide variety of habitats and geographic locations across the state of Nebraska were sampled for *Salmonella*. All snakes were caught by hand, sampled as described below, then released.

For both the captive and wild reptile *Salmonella* testing, each reptile was sampled at the mouth and at the cloacal opening. A sterile alginate-tipped applicator was passed over the labial scales of the mouth and swabbed along the interior edge of the reptile's mouth if possible. The applicator was then struck on one half of a divided *Salmonella-Shigella* agar plate (Remel) inside a Petri dish. Another sterile alginate-tipped applicator was inserted into the reptile's cloaca approximately 3-5 mm, rotated, and extracted slowly. The applicator was then struck on the other half of the divided agar plate. Date and time, assigned individual number, and side labels of "mouth" and "cloaca" for the corresponding samples in the divided agar plate were recorded on the outer surface of each Petri dish. All Petri dishes were sealed and stored in biohazard safety bags for transport to the laboratory. To standardize the amount of time that passed between sample collection and incubation, no samples were placed in the incubator until one hour after all samples were collected.

This study followed standard protocol for detecting enteric pathogens as explained in Schroter et al. (2004). Forty agar plates at a time (plus four blank agar plates for control) were placed in an incubator at 36° C for 48 hours. Agar plates were then removed from the incubator and read for the presence or absence of *Salmonella.* A dark black coloration indicated a presence of *Salmonella*, and no obvious black coloration indicated an absence of *Salmonella.* Agar plates were then discarded in a biosafety bag for disposal by autoclaving.

The actual prevalence of *Salmonella* infection and 95% confidence intervals for prevalence estimates were calculated for each pet shop, reptile taxonomic group, and wild reptile species. The lower 95% confidence intervals are

$\frac{p}{\left(p+(N-p+1)F_{0.025,2(N-p+1),2p}\right)}$, where p is the number of samples in which *Salmonella* was present and N is the total number of samples tested for a given group (Zar, 1974). Similarly the upper 95% confidence limit is $\frac{(p+1)F_{0.025,2(p+1),2(N-p)}}{N-p+(p+1)F_{0.025,2(p+1),2(N-p)}}$. For samples with no positives, the upper 95% confidence interval is approximately $1-0.025^{1/N}$ (Blythe, 1986); the lower 95% confidence intervals are 0.

We estimated the variation in prevalence among reptile taxonomic groups using a generalized linear model with a logistic link and binomial error distribution. *A priori* we expected prevalence within pet stores to be correlated, so we used a mixed effects approach to account for correlations in prevalence within pet stores. We estimated generalized linear mixed effects models with and without a random effect of pet store on prevalence using the glmmPQL function (Venables and Ripley, 2002) in the R statistics package (R Development Core Team, 2008). We also tested a fixed effect of reptile taxonomic group in both the generalized linear and mixed models.

3. Results

Out of 80 pet store reptile samples collected, 43 (53.75%) yielded *Salmonella* on either the cloacal or mouth portions of the agar plates (Table 1). Cloacal samples yielded a 48.75% (39 of 80) prevalence of *Salmonella*, while mouth samples yielded a 13.75% (11 of 80) prevalence of *Salmonella* (Table 2).

Out of the 182 wild reptiles tested, 12 (6.59%) yielded *Salmonella* (Table 1). Northern water snakes (*Nerodia sipedon*) sampled at one location accounted for 11 of the 12 wild reptiles that tested positive for *Salmonella*, with 61.1% (11 of 18) of all northern water snakes testing positive for *Salmonella* (Figure 3). The only other species to test positive for *Salmonella* was the red-sided garter snake (*Thamnophis sirtalis parietalis*) at a prevalence of 5.88% (1 of 17).

Lizards had the highest probability of testing positive for *Salmonella* in pet stores, while snakes had a lower probability and turtles had the lowest probability. Overall variation in probability of *Salmonella* among reptile taxonomic groups

was large (Figure 1). Probability of testing positive for *Salmonella* was also highly variable among pet shops (Figure 2). Two generalized linear models and two mixed models were created to determine whether variation by taxonomic group or by pet store was more important.

Table 1. Presence or absence of *Salmonella* for pet store and wild data.

Taxonomic Group	**# Positive**	**# Negative**	**# Tested**
Turtles	3	8	11
Snakes	11	12	23
Lizards	29	17	46
Total	43	37	80

Pet Shop	**# Positive**	**# Negative**	**# Tested**
1	11	7	18
2	16	6	22
3	0	10	10
4	16	14	30
Total	43	37	80

Wild Species	**# Positive**	**# Negative**	**# Tested**
Coluber constrictor	0	6	6
Crotalus viridis	0	27	27
Nerodia sipedon	11	7	18
Pantherophis obsoleta	0	4	4
Pantherophis vulpina	0	9	9
Pituophis catenifer	0	12	12
Thamnophis radix	0	89	89
Thamnophis sirtalis	1	16	17
Total	12	170	182

Table 2. Results of pet reptile *Salmonella* testing at both the mouth and cloaca.

	Mouth	
Cloaca	Positive	Negative
Positive	7	32
Negative	4	37

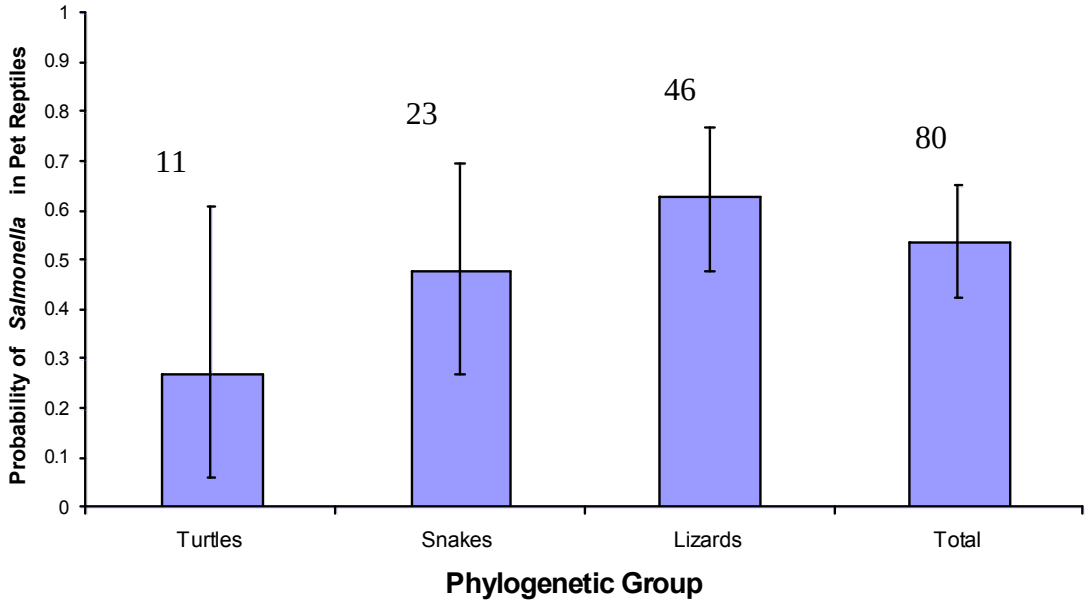

Figure 1. Probability of *Salmonella* in pet reptiles by taxonomic group. Error bars represent upper and lower 95% confidence limits calculated by generalized linear models with binomial response, with number sampled above bars.

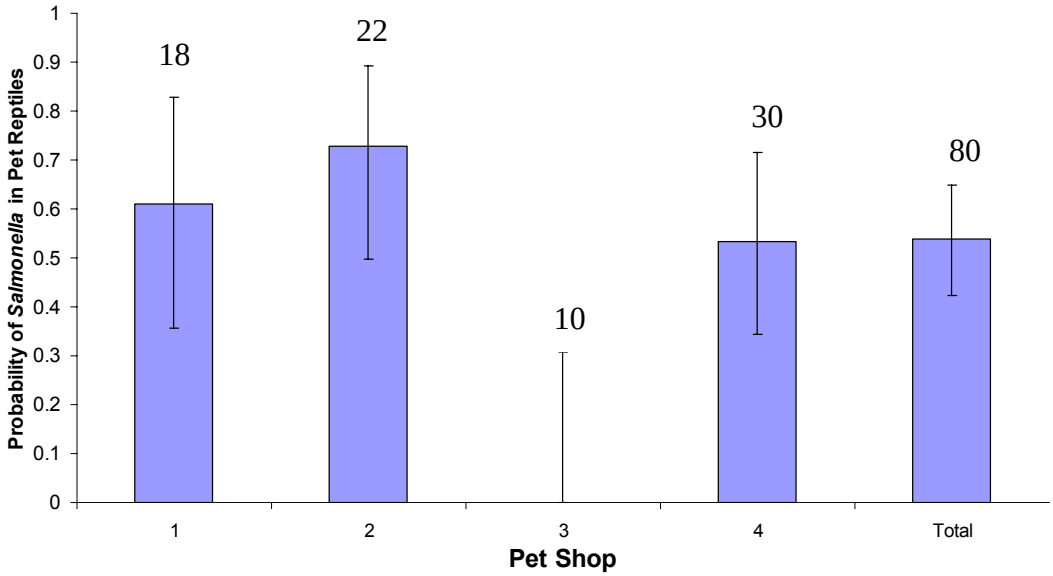

Figure 2. Probability of *Salmonella* in pet reptiles by pet shop. Error bars represent upper and lower 95% confidence limits calculated by generalized linear models with binomial response, with number sampled above bars.

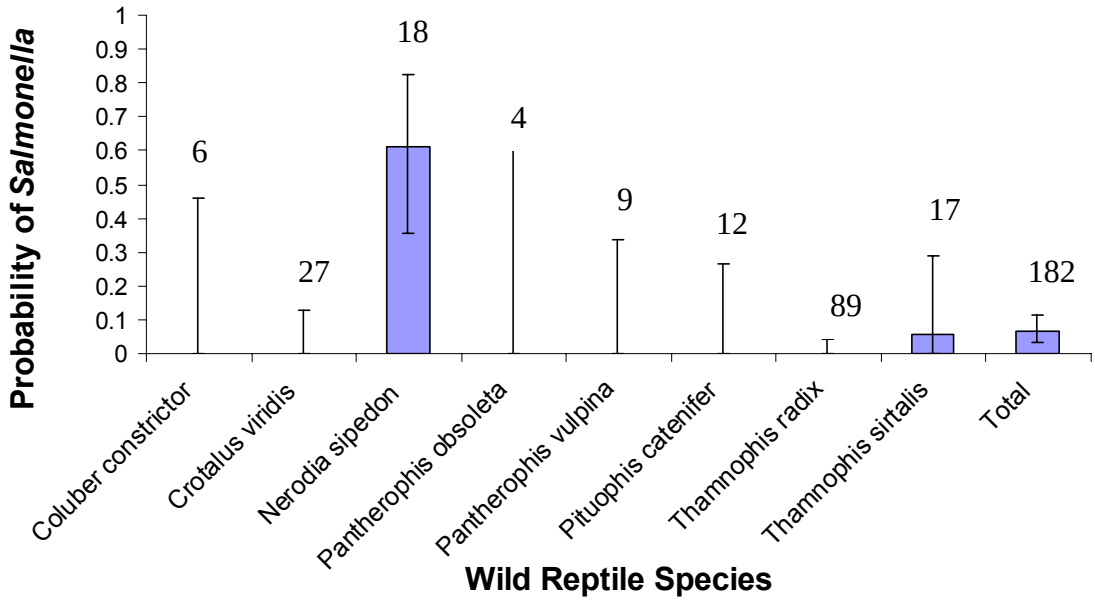

Figure 3. Probability of *Salmonella* in wild reptiles by species. Error bars represent upper and lower 95% confidence limits calculated by generalized linear models with binomial response, with number sampled above bars.

The model with a residual variation closest to 1.0 should be considered the most appropriate model. The intercept values shown for each model in Table 3 correspond directly to the estimated prevalences of *Salmonella* via the log odds scale, in which a value of 0 is a prevalence of 0.5, negative values correspond to a prevalence less than 0.5, and positive values correspond to a prevalence greater than 0.5. The actual estimated prevalence value is 1/(1+exp^(-intercept)).

The generalized linear model with no fixed or random effects (glm.0) tested the null hypothesis in which neither taxonomic group nor pet shop were responsible for the variation in prevalence of *Salmonella*. A residual deviance of 110.45 divided by 79 degrees of freedom yields a residual variation of 1.40, suggesting that something is varying among reptiles in prevalence of *Salmonella*.

The generalized linear model with a fixed effect of taxonomic group (glm.1) tested whether or not taxonomic group alone was responsible for the variation in prevalence of *Salmonella*. A residual deviance of 107.87 divided by 77 degrees of freedom yields a residual variation of 1.40 again, suggesting that taxonomic group alone does not account for the variation in prevalence of *Salmonella* in pet reptiles.

Table 3. Output results from R for the generalized linear models glm.0 and glm.1 and mixed models mm.0 and mm.1 for the pet reptile dataset. Model values from estimate column are intercept values for the log odds scale, where actual estimated prevalence is 1/(1+exp^(-intercept)). Taxon values from estimate column must be added to the model intercept to calculate the prevalence.

Model/Taxon	**Estimate**	**Standard Error**	**Standard Deviation**
glm.0	0.1503	0.2242	
glm.1	0.4626	0.3096	
Snakes	-0.5496	0.5197	
Turtles	-0.9326	0.6487	
mm.0	-0.1810	0.6753	1.2205
mm.1	0.1530	0.7488	1.2839
Snakes	-0.8424	0.5630	
Turtles	-0.6546	0.6856	

The mixed model with a random effect of pet store (mm.0) tested whether or not pet shops accounted for the variation in the prevalence of *Salmonella* infection. This model had a residual variation of 0.954 for each pet shop's prevalence of *Salmonella* infection, implying that *Salmonella* infection in captive reptiles may be dependent upon pet shop.

The mixed model with a random effect of pet store and a fixed effect of taxonomic group (mm.1) tested whether or not pet shops and taxonomic group accounted for the variation in the prevalence of *Salmonella* infection. This model had a residual variation of 0.951 for each pet shop's prevalence of *Salmonella* infection, implying that adding the fixed effect of taxonomic group does not make the model more accurate.

4. Discussion

Together, the four generalized linear and mixed models suggest that pet shops may account for the variation in prevalence of *Salmonella*, while reptile taxonomic groups do not. Models glm.1 and mm.1 show that prevalence of *Salmonella* decreases from lizards to snakes and turtles (Table 3), because the estimate value

decreases from the intercept value on the log odds scale. However, the standard errors on these estimates are large and the confidence limits include zero, so the reptile taxonomic group does not help explain the variability in the probability of testing positive for *Salmonella*.

While the probability of *Salmonella* by reptile taxonomic groups may not reveal much, the breakdown of probability of *Salmonella* by pet shop provides very conclusive results. The prevalence of *Salmonella* in pet shops 1, 2, and 4 were all above 50%, while pet shop 3 had a 0% prevalence rate. Therefore the presence of *Salmonella* was equivalent to "all-or-none" in pet shops. In the wild reptiles tested, presence of *Salmonella* was localized as well, with positive samples only being found in one area from Sarpy County in addition to one specimen from Richardson County.

The presence of *Salmonella* in Nebraska reptiles appears to be mainly dependent upon location—geographic proximity to other reptiles with *Salmonella* in the wild, or the presence of *Salmonella* in other pet reptiles within a pet shop. If one reptile from an area (or pet shop) tests positive for *Salmonella*, other reptiles in that area are more likely to be infected with *Salmonella* if in contact with other infected reptiles, or within areas contaminated with *Salmonella*. This stems from the fact that once *Salmonella* contamination occurs, it is easily transmitted between organisms that share an environment. The high localized prevalence of *Salmonella* means that any humans coming into contact with reptiles from a high-prevalence area will have a higher chance of infection with *Salmonella* if proper precautions are not taken.

Most *Salmonella* infections in humans cause a moderate gastrointestinal disorder characterized by diarrhea, fever, vomiting, and abdominal cramps, but in severe cases the bacteria can spread to the bloodstream, bone marrow, or nervous system to cause severe or even fatal illnesses such as septicemia, bacteremia, or meningitis (ARAVb, 1998; Ebani et al., 2005; Jong et al., 2005; Nakadai et al., 2005; Schroter et al., 2004). Severe infections are more likely in infants and immuno-compromised individuals (e.g. people with diabetes mellitus, chemotherapy patients, people infected with HIV, and bone marrow transplant recipients) (ARAVb, 1998).

Salmonella survives in the environment for long periods of time, allowing it to be transmitted by environmental surfaces long after a reptile carrier is gone; *Sal-*

monella can survive in contaminated water for extended periods (Mermin et al., 2004). Indirect contact, such as drinking from the same body of water as a reptile shedding *Salmonella*, can lead to *Salmonella* transmission (Mermin et al., 2004).

Reptiles are asymptomatic carriers of *Salmonella* and probably become infected during birth or through contaminated food, water, or soil (Mermin et al., 2004; Sanyal et al., 1997). In rare cases, pet reptiles can acquire *Salmonella* from being fed undercooked chicken or from contact with household dust (Mermin et al., 2004). But the high rate of colonization seen in most reptiles suggests that *Salmonella* is a natural commensal organism in reptiles' gastrointestinal tract (Mermin et al., 2004).

The results of this study support the notion that *Salmonella* testing is not always conclusive. The present study found an 11.5% chance of a reptile testing negative yet actually being positive for *Salmonella*, based on the number of reptiles that tested positive for *Salmonella* at either the mouth or cloaca, but not at the other end (Table 2). In addition, *Salmonella* may be shed intermittently in reptile feces (Sa, 2001; Sanyal et al., 1997), making it impossible to determine indefinitely whether an individual reptile is free of *Salmonella* or not (ARAVa, 1998). Therefore, as a safety precaution all reptiles should be presumed to be infected with *Salmonella*, irregardless of previous bacterial culture results.

Treating reptiles with antibiotics to eradicate *Salmonella* from their intestinal tract is not effective and increases the risk of the emergence of antimicrobial-resistant *Salmonella* serotypes (ARAVa, 1998; Mermin et al., 2004). If transmitted to humans, these resistant serotypes would be a greater health risk as antibiotic treatment would become far less effective (ARAVa, 1998). Also, reptile owners have not been successful in efforts to raise reptiles free of *Salmonella* (ARAVa, 1998). Since the eradication of *Salmonella* and prevention of transmission to reptiles are not viable options, the effort to reduce RAS in humans should focus instead on preventing reptile-to-human transmission of *Salmonella*. The most inclusive list of guidelines to follow regarding human precautionary measures, domestic reptile care, and who should avoid reptiles can be found in the ARAV Client Education Handout (ARAVb, 1998).

Prevalence of RAS in humans is the sum of the relative contributions of pet reptiles versus wild reptiles to the reptile-to-human transmission of *Salmonella*. These relative contributions can be determined by comparing between pet and

wild reptile groups (1) the prevalence of *Salmonella* in each group, (2) the virulence of *Salmonella* serotypes carried by each group, and (3) the percentage of people with RAS who may have been exposed to each group.

For the first component, the present study determined the prevalence of *Salmonella* in Nebraska to be 53.75% in pet reptiles and 6.49% in wild reptiles. Therefore, this study suggests that the relative risk of contracting *Salmonella* from a randomly encountered reptile is greater if that reptile is from a pet store than from the wild.

For the second component, Pasmans et al. (2005) examined 44 serotypes (subspecies I, II, IIIb, and IV) of *Salmonella enterica* from captive (pet) reptiles and found that all were able to invade human intestinal epithelial cells, which agrees with the consensus view that every *Salmonella enterica* serotype can invade the human intestine and cause disease, depending on the patient's age and immune status. Ebani et al. (2005) claims that all *Salmonella* serotypes are potentially pathogenic. The simplest conclusion from these studies is that *Salmonella* serotypes from wild reptiles would be equally pathogenic to humans, however the virulence of serotypes found in wild reptiles has not been directly evaluated.

For the third component, Mermin et al. (2004) conducted case-control studies of human salmonellosis from 1996-1997 and found an association between illness from *Salmonella* infection and "any reptile or amphibian contact." The study categorized RAS cases into "reptile or amphibian in home" versus "visited place with reptile," yet the second category does not specify between captive (pet) or wild reptile contact. Therefore, the percentage of people with RAS exposed to wild reptiles is not clear in this study. On a broader scale, still no research has compared the rates of exposure to pet versus wild reptiles in people with RAS, which was suggested by Thomas et al. (2001).

The present study provides limited evidence that the presence of *Salmonella* in reptiles is mainly dependent upon location. The study sampled from only four pet shops in Nebraska, and the previously collected wild reptile data only tested wild snakes. A more comprehensive study needs to be conducted in which a broader variety of wild reptiles are tested and compared with captive reptiles from many more pet stores in (1) different geographic areas and (2) sizes of cities, as well as with different (3) sources of reptiles, (4) management (private vs. corporate) and (5) levels of sanitation.

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