Evaluating the Efficacy of Baquacil® Against *Salmonella* sp. in the Aquatic Habitat of the Red-Eared Slider, *Trachemys scripta elegans*

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ABSTRACT: Turtle-associated salmonellosis (TAS) in humans has been a concern of public health officials since the 1960's. The rising incidence of TAS in young children during the late 1960's and early 1970's eventually led to the implementation of inter- and intrastate regulations on the sale of chelonians less than 10.2-cm in total length in the United States of America. Although attempts to eliminate *Salmonella* spp. in chelonians using antibiotics have been made, they have not been successful in reducing prevalence to levels that would reverse current regulations. Baquacil® is a commercial algistat and microbicide. Fifty-five red-eared slider turtles, *Trachemys scripta elegans*, were used to evaluate the efficacy of Baquacil® as a method to suppress *Salmonella* sp. in the sliders habitat. The sliders were maintained individually in plastic containers that contained either chlorinated tap water, dechlorinated tap water and 25 ppm Baquacil®, or dechlorinated tap water and 50 ppm Baquacil®. Water samples were collected from each slider container three times per week for one month and cultured for *Salmonella* sp. Water samples collected from the sliders housed in Baquacil® were less likely to be *Salmonella*-positive than those in the control group ($p<0.001$). There was no difference in the *Salmonella* status of the water samples between the 25 and 50 ppm treatment groups. At the conclusion of the study, the intestinal tracts of the sliders were cultured for *Salmonella* sp. There was no difference in the *Salmonella* status of the intestinal cultures collected from any of the sliders at necropsy ($p=0.8$). No pathological lesions were found to be associated with swimming the sliders in 50 ppm Baquacil® for one month. Baquacil® may be used to suppress *Salmonella* sp. in the water column of sliders.

KEY WORDS: *Trachemys scripta elegans*, red-eared slider, turtle, Baquacil®, *Salmonella*, Salmonellosis.

INTRODUCTION

Salmonella Newport was first isolated from a chelonian, *Geochelone giganteas*, by McNeil and Hinshaw in 1946. It wasn't until almost seven years later that the first case of turtle-associated salmonellosis (TAS) in a human was reported (Boycott, *et al*, 1953). The apparent incidence of TAS continued to increase over the next two decades (Williams and Heldson, 1965). The public health concern for TAS during this period was limited, but would change with the increased incidence of cases reported in young children. The first case of TAS in a child was reported in 1963 (Hersey and Mason, 1963). *Salmonella* Hartford was recovered from a 7-month old infant with diarrhea, vomiting, and fever. An investigation of the infant's environment resulted in the isolation of the same serotype from the family's pet turtle.

The increased incidence of TAS in children during the late 1960s and early 1970s became an important concern to both state and federal health officials. In 1972, the Food and Drug Administration required certification verifying *Salmonella*-free status for the interstate transport of pet turtles. This program was found to be ineffective. A study conducted by the Center for Disease Control concluded that 38% of the animals certified to be *Salmonella*-free were actually carriers (CDC, 1974).

Therefore, in 1975, the Food and Drug Administration restricted the intra- and interstate sales of all turtle eggs and live turtles with a carapace length less than 10.2 cm. The decision to restrict the sale of turtles with a total length less than 10.2 cm (4 in) was based on the assumption that these animals would fit into the mouth of young children. Enforcement of this policy resulted in a 77% reduction in the incidence of cases in those states not producing turtles (Cohen, *et al*, 1980).

Exceptions to this 1975 law were made for marine turtles and educational and scientific institutions. Violators of this law are provided a written demand to destroy the animals under FDA supervision within 10 days. Violators are also subject to a fine not more than $1000 and/or imprisonment of not more than one year for each violation. Herpetoculturists that breed exotic chelonians have avoid-

ed prosecution by selling animals under the guise of educational animals. Currently, there are several hundred reptile swap meets a year in the United States where chelonians with a carapace length less than 10.2 cm can be purchased. In addition, chelonians less than 10.2 cm in length are routinely sold via the internet. The availability of these chelonians, in combination with the apparent public health concern for TAS, suggest that there is a need to develop methods to minimize *Salmonella* spp. in the chelonians or their environment to minimize the health risks associated with pet reptile ownership.

Attempts to reduce or eliminate *Salmonella* spp. in turtles with antimicrobials were initiated after the FDA regulation was implemented in 1975. Treatment of hatchlings with oxytetracycline in their tank water for up to 14 d reduced shedding in treated turtles, but did not affect enteric colonization (Siebling, *et al*, 1975). Treatment of the freshly laid eggs with oxytetracycline or chloramphenicol with a temperature differential egg dip method was also successful at eliminating *Salmonella* spp. in eggs less than one day old, but did not clear eggs greater than two days old (Siebling, *et al*, 1975). Large-scale experimentation on commercial turtle farms with surface decontamination and pressure or temperature differential treatment of eggs with gentamicin dip solutions for eggs greater than two days old, followed by hatching the eggs on *Salmonella*-free bedding, substantially reduced *Salmonella* sp. infections and shedding rates in hatchling turtles (Siebling, *et al*, 1984). Forty percent of the eggs not treated with the gentamicin were found to harbor *Salmonella* sp., whereas only 0.15% of the treated eggs were positive. Legislative implementation of this concurrent method of surface decontamination and gentamicin treatment by the Louisiana Department of Agriculture in 1985 was hailed as victory by turtle farmers.

Unfortunately, the use of gentamicin and the other antimicrobials has led to an even greater concern due to the development and persistence of antimicrobial resistant strains of *Salmonella* sp. In a 1988 study of red eared sliders, *Trachemys scripta elegans*, eggs exported to Canada from four Louisiana turtle farms, six of 28 lots tested (21%) from three of four exporters were positive for *Salmonella* spp. (D'Aoust, *et al*, 1990). Of the 37 *Salmonella* strains isolated, 30 (81%) were gentamicin resistant (D'Aoust, *et al*, 1990). Similar results have been reported from samples collected directly from the farms in Louisiana. Shane, *et al*, 1990, collected environmental samples and live hatchlings directly from two Louisiana turtle farms. Isolates of *S. arizonae* and *S. poona* collected from turtles at one of the farms were resistant to erythromycin, gentamicin, tetracycline, and sulfonamide. Pond water samples from both farms showed similar antimicrobial resistant patterns to erythromycin. In 1988, 115 batches of turtle hatchlings were submitted from 28 farms to the Louisiana Department of Agriculture and Forestry for analysis (Shane, *et al*, 1990). Five (4.3%) *Salmonella* isolates were obtained. Four of the organisms were submitted for serotyping; three were *S. arizona* and one was *S. poona*. All four isolates were resistant to erythromycin, gentamicin, tetracycline, and sulfonamide. The findings of these studies suggest that the application of a single intervention, such as egg washing, will not be sufficient to suppress or eliminate *Salmonella* sp. from captive bred chelonians.

To successfully manage *Salmonella* sp. in chelonians, a series of interventions will be necessary. A methodical approach comprised of treatment interventions that reduce *Salmonella* sp. in adult breeding chelonians, reduce *Salmonella* sp. contamination of the egg, reduce *Salmonella* sp. colonization of the hatchling, and reduce *Salmonella* sp. dissemination in the environment are required. Each of these management schemes will require extensive research. The purpose of this research was to evaluate a specific method of reducing *Salmonella* sp. in the environment of hatchling chelonians.

Polyhexamethylene biguanide is a sanitizing agent that is considered safe for human and animal use. This compound has been used as a mouth rinse for humans (Rosin, *et al*, 2001), a microbicide for chicken eggs (Cox, *et al*, 1994), and a treatment for protozoal fungal keratitis (Panda, *et al*, 2003). The antimicrobial effect of this compound varies with concentration, being bacteriostatic at low concentrations and bacteriocidal at higher concentrations. There are many different derivatives of polyhexamethylene biguanide. One such derivative, Baquacil® (poly-iminoimidocarbonylimino- hexamethylene hydro chloride) (Avecia Inc., Wilmington, DE 19850 USA), is a commercial swimming pool sanitizer and algistat. This product is used as a safe alternative to chlorine for swimming pools. Because Baquacil® is considered safe for humans, its application as a microbicide may prove useful for captive chelonians.

The primary objective of this study was to determine if Baquacil® would suppress or eliminate *Salmonella* sp. in the aquatic habitat of the slider.

Materials and Methods

This study was conducted in accordance with the regulations specified by the Louisiana State University Institutional Animal Care and Use Committee. Fifty-five hatchling *Salmonella*-positive sliders were used for this study. The sliders were housed in individual plastic containers with approximately 1 liter of chlorinated water or a dechlorinated water and Baquacil® (25 or 50 ppm) solution. The sliders were randomly assigned to three different treatment groups using a random generator: Group 1) 25 ppm Baquacil® (n=20), Group 2) 50 ppm Baquacil® (n=20), and Group 3) chlorinated tap water (n=15). Baquacil® concentration, water pH, and alkalinity were determined using Aquachek pool and spa test strips (Environmental Test Systems, Inc., Elkhart, IN). The ambient air temperature was maintained between 24 – 27°C (76 – 80°F), and the water temperature maintained between 21 – 23°C (70 – 73°F). The sliders were provided a photoperiod comprised of 12 hr of light and 12 hr of darkness. The pH of the water was adjusted to 7.5 and the alkalinity maintained between 80 – 120 ppm. The water from the enclosures was changed weekly, and fresh Baquacil® solution was made weekly. The sliders were offered a *Salmonella*-free commercial turtle pellet food (Fluker Farms, Port Allen, LA) daily.

Water samples were collected three times a week for four

Figure 1. Prevalence of *Salmonella* sp. in the aquatic habitat of RES. Days of the week are recorded as M, W, and F for Monday, Wednesday and Friday, respectively. The number following each day represents the week of the study (1 – 4).

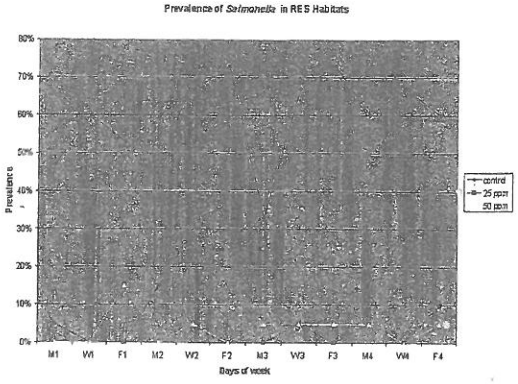

Table 1. Prevalence and 95% confidence intervals (in parentheses) of *Salmonella* sp. in the water samples collected from RES habitats during the study.

Week 1	Day 2	Day 4	Day 6
Control	47% (22-73)	60% (35-85)	53% (28-88)
25 ppm	5% (0-14)	0 (0-15)	0 (0-15)
50 ppm	0 (0-15)	0 (0-15)	15% (0-15)
Week 2	**Day 2**	**Day 4**	**Day 6**
Control	60% (35-85)	40% (15-65)	73% (50-96)
25 ppm	15% (0-30)	10% (0-23)	0 (0-15)
50 ppm	0 (0-15)	5% (0-14)	0 (0-15)
Week 3	**Day 2**	**Day 4**	**Day 6**
Control	47% (22-72)	67% (43-91)	67% (43-91)
25 ppm	5% (0-14)	5% (0-14)	0 (0-15)
50 ppm	5% (0-14)	5% (0-14)	5% (0-14)
Week 4	**Day 2**	**Day 4**	**Day 6**
Control	60% (35-85)	67% (43-91)	53% (28-88)
25 ppm	5% (0-14)	0 (0-15)	10% (0-23)
50 ppm	5% (0-14)	0 (0-15)	5% (0-14)

ference (p=0.001) in the prevalence of *Salmonella* sp. between the original (19%, 113/594) and DSE (14%, 83/594) cultures. Overall, there was an 11.7% (70/594) disagreement between the two groups. Interestingly, the frequency of DSE culture-positive only samples was greatest in the 25 and 50 ppm treatment groups, suggesting that the original numbers of organisms collected from the treated samples were low.

Histologically, the eyes, corneas, eyelids, tongue, oral mucosa, skin, and carapace of the 50 ppm Baquacil® treated sliders were no different than those of the control group. In both groups, the eyelids and corneas were uniformly thick with normal epithelial cells and no evidence of degeneration, erosion or inflammation. Internal ocular structures were within normal limits. Likewise, the oral mucosa, tongue, skin, and carapace and underlying structures were normal in both groups.

Discussion

Chelonian carriers of *Salmonella* spp. are a concern of public health officials, and the propagation of *Salmonella*-free chelonians is a primary objective for chelonian producers. Currently, chelonian eggs are sanitized using a combination of washing with water or sodium hypochlorite solution and temperature or pressure differential treatment using gentamicin. Although the apparent prevalence of *Salmonella* sp. in these eggs is very low, occasional cases of antimicrobial resistant *Salmonella* sp. are reported (D'Aoust, *et al*, 1990, Shane, *et al*, 1990). In addition, once the chelonians are removed from a "clean environment" and disseminated into the pet retail trade, the industry has no control method in place to prevent the re-colonization of a chelonian or to suppress or eliminate shedding in *Salmonella*-positive chelonians.

Polyhexamethylene biguanide has been found to be effective at eliminating experimentally inoculated *Salmonella* Typhimurium from the surface of chicken eggs. In a study to evaluate the efficacy of several different microbicides, including quaternary ammonium, peroxygen compounds, hydrogen peroxide, ethylene oxide, phenols, and sodium and potassium hydroxide, only the polyhexamethylene biguanide (0.035%, Cosmocil CQ, ICI Americas, Inc., Wilmington, DE) product was 100% effective at eliminating the salmonellae (Cox, *et al*, 1994). The results of this study also indicate that polyhexamethylene biguanide products have some effect against reptile serotypes.

Because hatchling chelonians serve as the primary source of infection for pet owners, neonates are a logical starting point for programs focused on suppressing or eliminating *Salmonella* sp. The primary objective of this study was to determine if Baquacil® could be used in a bath to suppress or eliminate *Salmonella* sp. in the water column of sliders. The findings of this study suggest that Baquacil® can suppress *Salmonella* sp. in the water column of sliders. Although this product was not 100% effective, it did significantly reduce the presence of *Salmonella* sp. in the water column.

The variable prevalence of *Salmonella* sp. in these slid-

ers, especially the control group, was not unexpected. Transient shedding of *Salmonella* sp. has been documented previously in reptiles (Mitchell, 2001). Inappropriate environmental conditions and other causes of physiologic stress may increase the rate of shedding. Historically, the management of sliders in captivity was unacceptable. The public health concern identified in the 1960's and 1970's was likely the result of a limited understanding of the husbandry requirements of these animals. In this study, even though the RES were housed in simple enclosures, the prevalence of *Salmonella* sp. in the water column never approached 100%. These finding suggest that, even in the control group, the risk of contracting TAS is not constant. Providing an appropriate environment and diet, in addition to using a sanitizing agent such as Baquacil®, may reduce the zoonotic health risk associated with these reptiles.

Because the purpose of this study was to determine the prevalence of *Salmonella* sp. in the habitats of RES at given time points, an enrichment broth and DSE were used to increase the likelihood of isolating the organism. Attempts to isolate *Salmonella* sp. without enrichment may result in misclassification (false-negatives). However, enrichment broth may also mask the true risk associated with this organism. In general, approximately $10^3 - 10^6$ *Salmonella* organisms are required to infect a human. There are a number of factors that can affect a human's susceptibility to contracting TAS, including age, previous exposure and immune status. Based on the findings of this study, a large number of the positive isolates identified in the treatment groups were only characterized after DSE, suggesting the actual number of organisms in the water column may be small. Additional research evaluating the numbers of organisms in the treated water samples without enrichment confirms that they are $< 10^3$ (Mitchell, unpublished research).

There was no significant difference among treatment groups in the *Salmonella* status of the sliders at necropsy. The similar prevalence of *Salmonella* between the three groups suggests that Baquacil® has no effect on the colonization of *Salmonella* in these animals. However, it was interesting to note that a percentage (control: 20%, 25 ppm: 30%, 50 ppm: 25%) of these turtles were *Salmonella*-negative at the time of the necropsy. This finding may have been the result of using microbiological culture as the method of detection. Mitchell (2001) estimated the sensitivity of microbiological culture as a method of detecting *Salmonella* sp. in green iguanas, *Iguana iguana*, to be approximately 70%. This would suggest that approximately 3/10 samples could be misclassified as false negative samples. In addition, if these animals were harboring low numbers of salmonellae, the assay could have been insufficiently sensitive to detect them. However, DSE was used to increase the likelihood of detecting low numbers of *Salmonella* sp. Another possible explanation for this finding is that the sliders could have self-cleared the *Salmonella* sp. *Salmonella* sp. infections and carrier states are generally considered to be self-limiting in mammals, and persistent in reptiles. Based on these findings, this may not be the case, and some reptiles may also self-clear or not remain colonized.

There were no significant histologic lesions in the RES treated with 50 ppm Baquacil®. The high dose (50 ppm) used for this study is similar to that recommended for the treatment of swimming pools and is lower than that used to treat experimental fungal keratitis in rabbits (Panda, *et al*, 2003). Because the gastrointestinal tracts were removed from these animals for culture, we were unable to evaluate them for pathologic changes. However, the eyes, palpebrae, oral cavity, skin and shell were evaluated and lacked microscopic evidence of damage. Future studies should evaluate longer-term exposure to Baquacil® and complete necropsies should be performed to determine if there are any pathologic lesions associated with the ingestion of Baquacil® treated water.

Conclusions

The results of this study are promising as an initial step in the development of an intervention plan for the management of *Salmonella*-positive chelonians. Additional long-term studies to evaluate the effects of this compound in different species of chelonians and on specific reptile *Salmonella* serotypes should be pursued. In addition, studies to evaluate the potential for the development of resistance to Baquacil® need to be pursued.

Acknowledgements

The authors wish to thank the Louisiana Department of Agriculture, the Louisiana State University Agriculture Center and Extensions Offices, Fluker Farms, and all of the aquatic chelonian farmers from Louisiana for funding and supporting this project.

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