Evaluating the Effect of Baquacil® and Sanosil® on Salmonella spp., in the Aquatic Habitat of the Red-Eared Slider Turtle, Trachemys scripta elegans

Trevor T. Zachariah1, DVM, MS, Mark A. Mitchell2, DVM, MS, PhD, Verna F. Serra1, BA, Michael Walden3, MS, DVM, Rudy W. Bauer3, DVM, PhD, DACVP

1. Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA
2. Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802, USA
3. Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

Abstract: Turtle-associated salmonellosis was first recognized as a public health concern in the 1960's, particularly due to an increase in the incidence of disease among children. In response to the public health threat, the United States Food and Drug Administration (FDA) implemented regulations in 1975 restricting the sale of turtles with a carapace length <10.2 cm. Since that time, the turtle industry has pursued research to eliminate Salmonella spp. Recent work has focused on identifying non-antibiotic products to reduce or eliminate Salmonella and reverse the FDA regulations. Baquacil® and Sanosil® are commercial non-antibiotic, antimicrobial products. Eighty-four red-eared slider turtle hatchlings, Trachemys scripta elegans, were used to evaluate the efficacy of these products as a method to suppress Salmonella spp., in the turtles’ habitats. The turtles were maintained individually in plastic containers that contained chlorinated tap water, chlorinated tap water and 10, 50, or 100 ppm Sanosil®, or dechlorinated tap water and 5, 10, or 50 ppm Baquacil®. Water samples from each container were collected twice weekly for two months, and the Salmonella status of the samples determined by standard microbiological culture, including delayed secondary enrichment. Water samples from containers with 50 ppm Baquacil® were less likely to be positive for Salmonella than those from the control group (p < 0.0001). The use of delayed secondary enrichment significantly increased the recovery of Salmonella spp., from the water samples (p < 0.0001). The intestinal tracts of the turtles were cultured for Salmonella spp., at the conclusion of the study. There was no significant difference in the Salmonella spp., status of the intestinal cultures from any of the turtles (p = 0.08). No gross or histopathologic lesions in the turtles were found to be associated with any of the Baquacil® or Sanosil® concentrations. A concentration of 50 ppm Baquacil® may be used to decrease the prevalence of Salmonella in the aquatic habitat of the red-eared slider turtle.

Key Words: red-eared slider turtle, Trachemys scripta elegans, Salmonella, salmonellosis, Baquacil®, Sanosil®.

INTRODUCTION

Reptiles represent an important segment of the pet market in the United States. According to a 2005 - 2006 National Pet Owners Survey, more than 69 million households own a pet, and approximately 4.4% of these households have reptiles (APPMA, 2006). The same survey reported that there is an average of 3.7 reptiles per household, or a total of 11 million reptiles in the United States. The number of reptiles being imported into the United States each year is also on the rise, with over one million animals being imported annually (Chomel, et al, 2007). This close association between reptiles and humans has been associated with an increased risk of zoonotic disease transmission, particularly salmonellosis.

Salmonella was first identified in snakes in 1943 (Hinshaw and McNeil, 1944), and was found soon thereafter in chelonians and lizards (McNeil and Hinshaw, 1946). The first reported case of turtle-associated salmonellosis occurred in 1953 (Boycott, et al, 1953), while the first reported case of turtle-associated salmonellosis in a child was reported in 1963 (Hersey and Mason, 1963). During the 1960s and early 1970s, the incidence of turtle-associated salmonellosis grew, particularly in children. It was estimated that by 1971, 15 million pet turtles were being sold annually, 4.2% of United States households owned a pet turtle, and that these animals accounted for 14% (280,000) of human salmonellosis cases annually (Lamm, et al, 1972).

The Washington State Health Department investigated a series of 21 index cases of turtle-associated salmonellosis in the Seattle area in 1965, all but one of which involved a child (Baker, et al, 1972). This helped in part to prompt a ban on the sale of pet turtles by the Washington State Board of Health in 1968. In 1972, the United States Food and Drug Administration imposed regulations that required Salmonella-
free certification of all interstate turtle shipments. Subsequently, a study conducted by the Centers for Disease Control found that 38% of the certified turtles harbored *Salmonella* (CDC, 1974). In 1975, the FDA implemented more stringent regulations that restricted the intra- and interstate sale of turtle eggs and live turtles less than 10.2 cm (4 in) in carapace length. These regulations effectively halted the sale and ownership of turtles in the United States. After the enforcement of the regulations, there was a marked reduction of approximately 77% in the number of cases of turtle-associated salmonellosis in children by 1976, which equated to approximately 200 cases (Cohen, et al., 1980).

The 1975 FDA regulations limiting pet turtle sales did contain exceptions for marine turtles and turtles sold for educational and scientific purposes. Herpetoculturists have circumvented the ban by selling chelonians under the guise of the latter two purposes, and chelonians with a carapace length of less than 10.2 cm are readily available for purchase on the internet and at hundreds of reptile expositions and swap meets in the United States. Due to the public health concern for turtle-associated salmonellosis, and the continued availability of pet turtles, a need to develop intervention methods that minimize the likelihood of disseminating *Salmonella* spp., between chelonians and their environment exists.

Various procedures for reducing or eliminating *Salmonella* spp., from turtles or turtle eggs have been investigated since the 1975 FDA regulations. Siebeling, et al., (1975) found that hatchling red-eared slider turtles, *Trachemys scripta elegans*, treated with terramycin or tylosin in their tank water for up to 14 d had reduced shedding of *Salmonella*. However, the antibiotic treatment did not affect enteric colonization. Gentamicin, an aminoglycoside, was evaluated as a potential treatment using techniques described in poultry production (Siebeling, et al., 1984). Exposing red-eared sliders eggs to gentamicin via temperature- or pressure-differential methods was found to decrease the prevalence in the eggs to <2% (Siebeling, et al., 1984). In 1985, this treatment method was adopted by the Louisiana Department of Agriculture and became a mandatory requirement.

The use of antibiotics to treat hatchling turtles and turtle eggs has led to multiple findings of resistance in strains of *Salmonella*. D’Aoust, et al., (1990) tested 28 lots of red-eared sliders eggs that were exported from Louisiana to Canada and found that six lots (21%), representing three of four exporters, yielded *Salmonella* from the eggs or the packing moss in which they were shipped. Gentamicin resistance was found in 81% (30/37) of the *Salmonella* strains recovered. In a study of two turtle farms in southern Louisiana, hatchlings from one farm yielded *Salmonella* strains resistant to erythromycin, gentamicin, tetracycline, and sulfonamides. *Salmonella* isolates from pond water on both farms showed similar resistance patterns (Shane, et al., 1990). An examination of the records of 115 batches of hatchlings from 28 turtle farms delivered to the Louisiana Department of Agriculture and Forestry in 1988 revealed an additional four *Salmonella* isolates that were resistant to the same antibiotics (Shane, et al., 1990).

Due to the emergence of antibiotic-resistant *Salmonella* strains from turtles and eggs, more recent work has focused on the use of non-antibiotic antimicrobials for treatment. Mitchell, et al., (2005) showed that the application of the sanitizing agent polyhexamethylene biguanide in the water of red-eared sliders at concentrations of 25 and 50 ppm significantly reduced the prevalence of *Salmonella* in the aquatic medium, but did not eliminate it from the turtles. The compound was found to be safe for the turtles at both the 25 and 50 ppm concentrations. In a red-eared sliders egg treatment study, a combination of sodium hypochlorite and PHMB was used as a bath or pressure-differential dip. Both methods were found to significantly reduce *Salmonella* spp., from the egg or the resulting hatchlings compared to controls (bath odds ratio = 0.2, dip odds ratio = 0.01) (Mitchell, et al., 2007). It was also found that polyhexamethylene biguanide used in a pressure-differential dip was more effective than when it was used in a bath.

The application of any one method to suppress or eliminate *Salmonella* from captive chelonians is not likely to be successful. Instead, a serial, multimodal approach is necessary. Mitchell, et al., (2005) have suggested that to minimize the zoonotic risk associated with these animals, a program focused on minimizing the amount of *Salmonella* spp., at each life stage of the turtles in captivity would be needed. These different life stages include the adult breeding animals, eggs, and hatchlings. Minimizing *Salmonella* spp., in the environment of these animals would also be considered important. Because the hatchlings serve as the primary source of infection for humans, this would appear as the most appropriate point to start a control program. The purpose of this study was to evaluate *Salmonella* spp., control methods for captive hatchling turtles.

Two non-antibiotic, antimicrobial products have been identified for this purpose. Baquacil® (Arch Chemicals, Inc., Norwalk, CT) contains the polyhexamethylene biguanide derivative poly-iminoimidocarbonylimino-hexamethylene hydrochloride. It is a chlorine alternative for use as a pool sanitizer and algistat. Sanosil® (Sanosil Ltd., Hombrechtikon, Switzerland) contains a combination of 7.5% hydrogen peroxide and 0.0075% silver. It is a disinfectant that is labeled for use in industrial, agricultural, medical, and recreational fields, among others.

The primary objective of this study was to evaluate the efficacy of Baquacil® and Sanosil® against *Salmonella* spp., in the aquatic habitat of red-eared sliders. A secondary objective was to determine the safety of Baquacil® and Sanosil® for red-eared sliders. A final objective was to determine if delayed secondary enrichment increases the likelihood of isolating *Salmonella* spp., from the aquatic habitat of red-eared sliders. There were four specific hypotheses evaluated in this study:

1. Water samples from turtle habitats treated with Baquacil® or Sanosil® would be less likely to be *Salmonella*-positive than samples from the control group (H0: μT = μC; H1: μT < μC).
2. There would be no difference in the prevalence of *Salmonella* in gastrointestinal samples from the turtles in the Baquacil® or Sanosil® groups and the control group (H0: μT = μC; H1: μT ≠ μC).
3. There would be no difference in the frequency of *Salmonella* isolation between the initial and the delayed secondary enrichment cultures (H0: μI = μDSE; H1: μI ≠ μDSE).
4. There would be no difference in the pathological findings between the Baquacil® or Sanosil® groups and the control group (H0: μT = μC; H1: μT ≠ μC).
MATERIALS AND METHODS

This study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Eighty-four hatchling red-eared sliders were obtained from an aquatic chelonian farm in Ponchatoula, LA. The turtles were collected from a group of animals that were not treated as eggs for Salmonella using the techniques outlined in Mitchell, et al., (2007). The turtles were housed in individual plastic containers and provided a mortar brick for basking and approximately two liters of chlorinated tap water. The turtles were examined for any obvious physical abnormalities or behaviors. Each turtle was weighed to the nearest 0.1 g.

Before the treatments were applied, all the turtles were kept in chlorinated tap water for a two-month acclimation period and confirmed to be shedding Salmonella using standard microbiological techniques. A total of 3 ml of water was collected from around the base of the brick in each enclosure using a new, sterile 3 ml syringe. The samples were added to 7 ml of selenite enrichment broth and incubated at 37°C (98.6°F) for 48 hr under aerobic conditions. After incubation, the enriched selenite cultures were mixed on a Vortex agitator for five seconds. A heat-sterilized bacterial loop was used to transfer an aliquot of enriched broth to the surface of a Petri dish containing xylose-lysine-tergitol agar (Hardy Diagnostics, Santa Maria, CA). Streaked plates were incubated at 37°C (98.6°F) for 48 hr under aerobic conditions. Presumptive Salmonella colonies were identified based on colony characteristics, including circular to irregular shape, clear growth, hydrogen sulfide (H₂S) production, and lack of lactose fermentation. Selected colonies were evaluated on indicator media including urea agar, lysine iron agar (LIA), and triple sugar iron agar (TSI). A heat-sterilized bacterial loop was used to streak a portion of a suspect colony onto slants of urea, LIA, and TSI agar. A heat-sterilized inoculation needle was used to stab a portion of a suspect colony twice into the LIA and once into the TSI. The preparations were incubated aerobically at 37°C (98.6°F) for 24 hr. The presence of Salmonella was denoted by a negative urea test, positive LIA with H₂S production, and alkaline over acid with H₂S production in the TSI.

A random number generator was used to assign the animals to seven different groups: Group 1) 10 ppm Baquacil®, Group 2) 5 ppm Baquacil®, Group 3) 50 ppm Sanosil®, Group 4) 50 ppm Baquacil®, Group 5) 10 ppm Sanosil®, Group 6) chlorinated tap water, Group 7) 100 ppm Sanosil®. Each treatment group was comprised of 12 turtles. The water from the enclosures was changed on the same day each week, sanitized plastic containers were exchanged for the old ones, and fresh treatment solutions were made weekly. The solutions of Baquacil® were made by dechlorinating (Aqua-Plus, Rolf C. Hagen Corp., Mansfield, MA) and adjusting the pH of the tap water to 7.5 (Proper pH® 7.5, Aquarium Pharmaceuticals, Inc., Chalfont, PA). No special treatments were applied to the Sanosil® solutions. The ambient air temperature of the room the animals were housed in was maintained between 24 - 27°C (76 – 80°F), and the water temperature was maintained between 21 - 23°C (70 – 73°F). The turtles were provided a 12 hr photoperiod. A Salmonella-free commercial turtle pellet food (Fluker’s Aquatic Turtle Diet, Fluker Farms, Port Allen, LA) was offered to the animals daily.

After the initial treatment water change, water samples were collected twice a week for eight weeks. Sample collection was always performed on days three and six for a given week. Every sample was evaluated for Salmonella using the techniques described previously. A delayed secondary enrichment was performed on each negative sample to increase the likelihood of identifying low concentrations of Salmonella. For the procedure, the original selenite samples were placed at room temperature (24°C, 75°F) for 96 hr. A 3 ml aliquot of the original selenite sample was added to 7 ml of selenite enrichment broth and incubated aerobically at 37°C (98.6°F) for 48 hr. The samples were processed using the techniques described previously.

At the conclusion of the eight-week trial, all of the turtles were weighed and anesthetized with ketamine hydrochloride (100 mg/kg/turtle, intramuscular) (Ketaset®, Fort Dodge Animal Health, Overland Park, KS). The turtles were then humanely euthanized using an overdose of pentobarbital sodium and phenytoin sodium (0.1 ml/turtle, intracereomg) (Beuthanasia®-D Special, Schering-Plough Animal Health Corp., Union, NJ). The firmness of the turtles' shells was measured using an ordinal scale: 0 = firm shell, 1 = mildly distensible shell, 2 = easily distensible shell. To determine the shell score, the bridge of the shell was gripped between the thumb and index finger of one hand and squeezed. All of the turtles were scored by one evaluator.

A random number generator was used to select nine turtles from each treatment group for evaluation of the gastrointestinal tract for presence of Salmonella. Sterile techniques were used to remove the gastrointestinal tract (Mitchell, et al., 2005). Scissors were used to cut the bridge of the shell and facilitate removal of the plastron. The gastrointestinal tract was excised, placed into 7 ml of enriched selenite broth, and a sterile cotton-tipped applicator used to macerate the tissues. The samples were further macerated and mixed using a Vortex agitator for five seconds. The samples were incubated aerobically at 37°C (98.6°F) for 48 hr and processed using the techniques described. A 96 hr delayed secondary enrichment was performed on all the samples that failed to yield Salmonella from the initial culture.

The three remaining turtle carcasses from each treatment group were submitted for histopathologic evaluation. Scissors were used to cut the bridge of the shell and remove the plastron. The carcass was then placed into 100 ml of 10% neutral buffered formalin and fixed for seven days. A complete histologic review of all the tissues was performed. The tissues were processed routinely, sectioned (4 μm thicknesses), stained with hematoxylin and eosin, and examined microscopically. The amount of ossification at the midline and the bridge of the carapace of each turtle was measured on an ordinal scale, from 0 = no ossification to 4 = nearly complete ossification.

The sample size required for this study was calculated under the following assumptions and criteria: that the proportion of Salmonella in the water column of the turtles in the control group would be >0.9, the prevalence of Salmonella in the water column of the turtles in the treatment groups would be <0.1, that the α = 0.5, and that the power = 0.8. The 95% binomial confidence intervals were calculated for each of the proportion estimates and odds ratios. The first hypothesis tested in this study was that the water samples from turtle habitats treated with Baquacil® or Sanosil®...
would be less likely to be *Salmonella*-positive than samples from the control group (H0: \( \mu_T = \mu_C \); H1: \( \mu_T < \mu_C \)). A Cochran’s Q test was used to determine if there was a significant difference in the *Salmonella* status of the water samples over time. A multinomial logistic regression model was developed to predict the *Salmonella* status of a sample using the variables treatment group (Group), day of sample collection (Day), and week of study (Week). The second hypothesis was that there would be no difference in the prevalence of *Salmonella* in gastrointestinal samples from the turtles in the Baquacil® or Sanosil® groups and the control group (H0: \( \mu_T = \mu_C \); H1: \( \mu_T \neq \mu_C \)). A chi-square test was used to determine if there was a significant difference between treatment groups for prevalence of *Salmonella*. The third hypothesis was that there would be no difference in the frequency of *Salmonella* isolation between the initial and the delayed secondary enrichment cultures (H0: \( \mu_I = \mu_{DSE} \); H1: \( \mu_I \neq \mu_{DSE} \)). All of the paired initial and delayed secondary enrichment cultures throughout the study were tabulated. A McNemar’s change test was used to determine if there was an overall significant difference in the frequency of *Salmonella* isolation between the initial and delayed secondary enrichment cultures. Each treatment group was then evaluated with a McNemar’s change test using a binomial distribution. The final hypothesis tested was that there would be no difference in the pathological findings between the Baquacil® or Sanosil® groups and the control group (H0: \( \mu_T = \mu_C \); H1: \( \mu_T \neq \mu_C \)). A Kruskal-Wallis one-way analysis of variance (ANOVA) was used to determine if there was a significant difference in shell score, carapace midline ossification, or bridge ossification between treatment groups. The difference in pre- and post-treatment weights was calculated for each turtle. A Shapiro-Wilk test was used to determine the normality of the weight difference data. A Kruskal-Wallis ANOVA was used to determine if there was a significant difference in weight change between treatment groups. To determine differences in the prevalence of histopathologic abnormalities between treatment groups, the 95% CI were compared visually. Values of \( p < 0.05 \) were considered statistically significant. Statistical analysis was performed using SPSS 11.0 (SPSS, Inc., Chicago, IL).

**Results**

All of the turtles remained clinically normal throughout the study period. There was a significant within-groups difference in the *Salmonella* status of the water samples over time (Cochran’s Q = 103.3, \( p < 0.0001 \)). The multinomial logistic regression model was found to be significantly different from a null model at predicting the outcome (-2 log likelihood \([2LL] = 406.86, X^2 = 263.58, p < 0.0001\), and demonstrated adequate goodness-of-fit (Pearson chi-square = 108.76, \( p = 0.19 \)). No significant interactions among the variables were found. Each of the variables was found to have a significant effect in the model (Group: \(-2LL = 591.26, X^2 = 184.4, p < 0.0001\); Day: \(-2LL = 427.72, +^2 = 20.86, p < 0.0001\); Week: \(-2LL = 477.81, X^2 = 70.95, p < 0.0001\)). Samples collected from Group 4 (50 ppm Baquacil®) were significantly more likely, and those collected from Group 5 (10 ppm Sanosil®) were significantly less likely to be *Salmonella*-negative than those from the control group (Table 1 and Figure 1). Samples collected on Day 6 were significantly less likely to be *Salmonella*-negative than those from Day 3 (Table 1). Samples collected during weeks five through eight were significantly more likely to be *Salmonella*-negative than those collected during the first week (Table 1).

There was no significant difference in the prevalence of *Salmonella* in the gastrointestinal samples between any of the treatment groups (\(X^2 = 11.3, p = 0.08\)). There was an overall significant difference in the frequency of *Salmonella* isolation between the initial and delayed secondary enrichment cultures (\(X^2 = 49.0, p < 0.0001\)). There was disagreement between the two groups in 51 of the 775 times (6.6%) that delayed secondary enrichment was employed. Interestingly, all the treatment groups but Group 7 (100 ppm Sanosil®) also showed a significant difference in the frequency of *Salmonella* spp., isolation between the initial and delayed secondary enrichment cultures. There was no significant difference in the shell scores (\(X^2 = 3.29, p = 0.77\)), carapace midline ossification scores (\(X^2 = 10.23, p = 0.12\)), or bridge ossification scores (\(X^2 = 2.56, p = 0.86\)) between any of the treatment groups. The pre- and post-treatment differences in weight for the turtles were not normally distributed (\(p < 0.0001\)). There was no significant difference in the weight differences between any of the treatment groups (\(X^2 = 6.1, p = 0.41\)). All but one of the turtles gained weight over the course of the study. The median weight gain was 2.2 g (min/max: -1.1/10.7; 10 - 90% quantiles: 0.8 - 5.1).

All of the turtles had mild granulocytic accumulations in the liver and kidney consistent with extramedullary hematopoiesis. Two turtles had noteworthy histopathologic findings. One turtle from Group 7 (100 ppm Sanosil®) had mild granulomatous omphalophlebitis with fibrous and granulomatous coelomitis associated with a ruptured yolk sac. One turtle in Group 4 (50 ppm Baquacil®) had severe heterophilic cuffing of the hepatic and renal vasculature with perivascular heterophils observed in the vasculature of the carapace. The heterophilic cuffing was often severe enough to displace the surrounding tissue, but no significant tissue dam-

**Figure 1.** Proportion of *Salmonella*-positive samples for Group 4 (50 ppm Baquacil®) and Group 5 (10 ppm Sanosil®) compared to the control group during the course of the study. — 50 ppm Baquacil®, — 10 ppm Sanosil®, — Control
Table 1. Statistics for significant variables in the multinomial logistic regression model: p-value, odds ratio (OR), and 95% confidence interval (CI) of the odds ratio.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 4</td>
<td>&lt; 0.0001</td>
<td>3.85</td>
<td>2.4 - 6.17</td>
</tr>
<tr>
<td>Group 5</td>
<td>&lt; 0.0001</td>
<td>0.17</td>
<td>0.1 - 0.27</td>
</tr>
<tr>
<td>Day 6</td>
<td>&lt; 0.0001</td>
<td>0.58</td>
<td>0.45 - 0.73</td>
</tr>
<tr>
<td>Week 5</td>
<td>0.04</td>
<td>1.6</td>
<td>1.01 - 2.55</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.002</td>
<td>2.08</td>
<td>1.3 - 3.32</td>
</tr>
<tr>
<td>Week 7</td>
<td>&lt; 0.0001</td>
<td>3.54</td>
<td>2.17 - 5.78</td>
</tr>
<tr>
<td>Week 8</td>
<td>&lt; 0.0001</td>
<td>2.91</td>
<td>1.8 - 4.7</td>
</tr>
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age was observed to be associated with the inflammatory cells. Differential diagnoses for this condition included septicemia, aberrant/extreme extramedullary hematopoiesis, and myeloproliferative disease.

**Discussion**

Since the implementation of the 1975 FDA regulation restricting the sale of turtles < 10.2 cm in the United States, the aquatic chelonian industry has been focused on identifying non-antibiotic methods to eliminate/suppress Salmonella spp., in turtle hatchlings and their environment. Because it is financially and logistically difficult to treat the adult breeding turtles on a farm, the logical point of intervention is with the hatchling turtles. This also makes sense because the young turtles are the animals that will be distributed among the public and serve as the primary source of infection for humans.

Since it has been shown that the primary route of Salmonella infection in red-eared sliders is via a horizontal route through egg contamination during deposition (Izadjoo, et al., 1987), there has been a focus on eliminating Salmonella from red-eared sliders eggs and the hatching environment. Current methods for treating eggs include a combination of washing with water or sodium hypochlorite solution and temperature- or pressure-differential treatment using gentamicin (Mitchell, et al., 2005) or Baquacil®. The eggs are then incubated in sanitized plastic containers without substrate until hatching. These methods have led to an apparent low prevalence of Salmonella among red-eared sliders eggs. However, the use of antibiotics for this purpose has led to reports of Salmonella isolates that are resistant (D’Aoust, et al., 1990, Shane, et al., 1990), further increasing the public health risk of these animals.

Another concern for the aquatic chelonian industry is that once the hatchlings are shipped from the farms and disseminated into the pet trade, there are no methods in place to control re-colonization by or shedding in animals that are positive for Salmonella. This concern, along with the problems of turtle-associated salmonellosis and antibiotic-resistant Salmonella, has lead to research into non-antibiotic antimicrobials that are effective against Salmonella in turtle eggs and hatchlings.

Polyhexamethylene biguanide is an antimicrobial that has been found to be safe for use as a mouth rinse in humans (Rosin, et al., 2001) and as a treatment for keratitis in animals (Panda, et al., 2003). Cox, et al., (1994, 1998, 1999) evaluated the effect of different antimicrobial compounds against Salmonella organisms on experimentally inoculated chicken eggs. The compounds tested included quaternary ammonium, peroxoxyen compounds, hydrogen peroxide, ethylene oxide, phenols, and sodium and potassium hydroxide, and polyhexamethylene biguanide. The polyhexamethylene biguanide was the only compound that had a consistently high efficacy against Salmonella. In a recent study of red eared sliders egg treatment methods, it was found that 50 ppm Baquacil® combined with sodium hypochlorite used as a bath or pressure-differential treatment significantly reduced the prevalence of Salmonella on egg surfaces and the resulting hatchlings (Mitchell, et al., 2007). It has also been shown that Baquacil® significantly reduces the prevalence of Salmonella sp. in the aquatic habitat of red-eared sliders when added to the water of captive hatchlings at concentrations of 25 and 50 ppm (Mitchell, et al., 2005). Anecdotally, aquatic chelonian farmers report that 5 – 10 ppm concentrations of Baquacil® are effective as red-eared sliders egg treatments based on polymerase chain reaction testing of treatment solutions.

The findings of this study suggest that Baquacil® can significantly reduce the prevalence of Salmonella in the habitat of red-eared sliders, but only at a concentration of 50 ppm. These results are consistent with those obtained by Mitchell, et al., (2005). The results are also consistent with the fact that polyhexamethylene biguanide is variable in its antimicrobial effect, with bacteriostatic action at low concentrations and bactericidal action at higher concentrations (Mitchell, et al., 2005). The fact that the present study did not find significance when Baquacil® was used at 5 ppm or 10 ppm suggests that aquatic chelonian farmers may need to modify their current treatment protocols.

The results of this study suggest that Sanosil® at concentrations of 10, 50 and 100 ppm cannot significantly decrease the prevalence of Salmonella in the aquatic habitat of red-eared sliders. The fact that the 10 ppm Sanosil® treatment (Group 5) was found to be significantly less protective against Salmonella than the control (Group 6) is likely a cause of variation in Salmonella excretion by red-eared sliders and the number of organisms collected in a given sample. Hydrogen peroxide or silver would not promote the growth of bacteria despite their apparent lack of antimicrobial efficacy in this study. The antimicrobial effects of hydrogen peroxide (Juvénal et al., 1999, Aarestrup and Hasman, 2004) and silver particles (Feng, et al., 2000, Morones, et al., 2005) are well known. However, hydrogen peroxide is rapidly degraded when exposed to light and the environment, and its antimicrobial effect may therefore be quite transient and insubstantial.

Silver particles exert their antibacterial effect principally through interaction with cell membranes. This interaction can disrupt the permeability of membranes (Sondi and Salopek-Sondi, 2004), leading to cell lysis (Gogoi, et al., 2006). It can also interfere with cell respiration by disrupting the proton motive force involved in oxidative phosphorylation (Dibrov, et al., 2002). It has also been shown that the antibacterial effect of silver particles is dependent on size (Morones, et al., 2005, Panacek, et al., 2006), with small particles being more effective due to their large surface area to volume ratio. This allows more surface area for interaction with cell membranes. The silver particles in Sanosil® may have been too large to have a significant effect on the Salmonella in the red-eared sliders water. It is also possible that they settled to the bottom
of the water column, limiting their interaction with *Salmonella* organisms in suspension. Another possibility is that silver particles were inactivated by binding to organic material from red-eared sliders excretions or the daily food ration. Silver ions are known to bind to proteins and nucleic acids (Brett, 2006).

The effect of time was significant in this study, as expressed by the variables day and week. Samples collected on Day 6 were significantly more likely to be positive for *Salmonella* than those collected on Day 3. This is likely due to the degradation of the treatment chemicals between water changes, and therefore the decrease in their antimicrobial efficacy. During this same period, the red-eared sliders would have continued to shed *Salmonella*, increasing the number of organisms in the water column. Based on these findings, the authors recommend changing the water of a red-eared sliders habitat frequently (every 24 - 72 hr) to reduce the likelihood of exposure to *Salmonella* spp.

Water samples collected during weeks five through eight were significantly more likely to be free of *Salmonella* compared to week 1. Because there was no significant interaction between the variables Group and Week in the regression model, it is unlikely that this effect was due to the Baquacil® or Sanosil® treatments. It is possible that the amount of *Salmonella* spp., shed by the red-eared sliders decreased during the course of the study. These animals were hatched in sanitized incubators and did not feed for the first few weeks of life while absorbing their yolk sacs, which limited their exposure to microorganisms that could colonize their intestinal tract. Once enrolled in this study, they were exposed to water, bricks, and food rations. These could have acted as sources of colonizing microflora that then had a competitive exclusion effect on the *Salmonella* spp.

In this study, Baquacil® was not 100% effective at eliminating *Salmonella* spp., from the water of red-eared sliders. Overall, only five of the 84 turtles had no *Salmonella* spp., cultured from any of the samples taken during the study, although four of these were in Group 4 (50 ppm Baquacil®). Only two of the 84 turtles were positive for *Salmonella* spp., on every sample, and these were in Group 5 (10 ppm Sanosil®). The rest of the turtles had variable shedding over the course of the study. This is not surprising, since it has been shown that reptiles can shed *Salmonella* sp. intermittently (Burnham, et al, 1998, Mitchell, 2001). This also suggests that the risk of turtle-associated salmonellosis is not constant. An additional consideration is the amount of physiologic stress with which an animal deals. Inappropriate husbandry and other causes of stress can increase the rate of *Salmonella* shedding, as has been demonstrated with the ability of dehydration to activate latent *Salmonella* infections in red-eared sliders (DuPonte, et al, 1978). This may partially help to explain the public health concern of turtle-associated salmonellosis identified in the 1960’s and early 1970’s. At that time, the captive management of aquatic chelonians was inadequately understood and executed, leading to an average captive life span of just two months for red-eared slider hatchlings (Lamm, et al, 1972).

The purpose of this study was to determine the prevalence of *Salmonella* in the habitats of red-eared sliders, and in order to increase the likelihood of isolating the organism, an enrichment broth and delayed secondary enrichment were used. Attempts to isolate *Salmonella* without enrichment may result in sample misclassification (false-negative), since the number of organisms present is likely to be small. The overall significant difference found between the initial and delayed secondary enrichment cultures in this study also suggests that the number of *Salmonella* organisms in the samples was small. Mitchell, et al, (2005) reported that samples of water treated with Baquacil® resulted in a reduction in the numbers of *Salmonella* to <10^3. Thus, enrichment of samples may inflate the true risk associated with *Salmonella* in the red-eared sliders habitat, since approximately 10^3 - 10^6 organisms are needed to infect a human. However, factors such as age, previous exposure, and immune status can affect a human’s susceptibility to turtle-associated salmonellosis.

The majority of gastrointestinal samples (58/63, 92%) were positive for *Salmonella*, and there was no significant difference in the prevalence of positive samples between the treatment groups. These findings are similar to those found by Mitchell, et al, (2005), and suggest that neither Baquacil® nor Sanosil® has an effect on the colonization of *Salmonella* in the intestinal tract of red-eared sliders. The fact that approximately 8% of the samples failed to yield *Salmonella* could be a result of the use of microbiological methods to detect the organisms or self-clearance by the red-eared sliders. It has been estimated that the sensitivity of culture without delayed secondary enrichment as a method to detect *Salmonella* spp., in organ samples from green iguanas, *Iguana iguana*, is approximately 70% (Mitchell, 2001).

Therefore, the possibility exists that up to 30% of samples could have been misclassified as false negatives. However, the real estimate is likely much smaller due to the use of delayed secondary enrichment to increase the chance of isolating *Salmonella*. It is also possible that the red-eared sliders cleared themselves of *Salmonella*, though this is not a likely occurrence. In mammals, including humans, *Salmonella* infections are generally self-limiting (Acha and Szyfres, 2001), but infections in reptiles are considered to be persistent. *Salmonella* spp., are considered to be part of the indigenous gastrointestinal flora of reptiles, making them carriers of the organism. It is also unlikely that the treatment chemicals had an effect on *Salmonella* colonization of the red-eared sliders. Though the animals imbibed the treated water, the chemicals would likely have been degraded by the acidic environment of the stomach. Reports of gastric pH in feeding chelonian species have ranged from 2.0 to 6.0 (Skoczylas, 1978).

Though there was no significant difference in the prevalence of *Salmonella*-positive gastrointestinal samples between the treatment groups, the p-value of the chi-square test (p = 0.08) approached the significance level of p <0.05 for this study. Because of this, a power analysis was performed, and the power of the chi-square test was found to be 0.6. This value is lower than the accepted robust power level of 0.8 or greater, suggesting the potential of a type II error. This is not surprising, considering the small sample size of nine red-eared sliders from each treatment group. However, when examining the data, only Group 1 (10 ppm Baquacil®) appeared different from the other groups (Group 1: 66.7%; other groups: 88.9 – 100%). The authors have no biological explanation for this finding.

Only two of the red-eared sliders submitted for necropsy and histopathologic examination had significant lesions identified. The lesions in both of these animals were internal processes and were unlikely to have been induced by the treatments used in this study. Both Baquacil® and Sanosil® exert their effects in the environment, and lesions associated with either product would be predicted to occur in the integu-
mentary system or external organs (e.g., conjunctiva and cornea of the eyes). This was not seen in any of the animals examined. Shell and ossification scores were used in this study because it has been noted that concentrations of Baquacil® greater than 50 ppm may cause osteopenia in hatchling red-eared sliders (Mitchell, et al, unpublished data). The differences seen in the shell and ossification scores in this study were not significant and were most likely due to natural variation.

CONCLUSIONS

The findings of this study confirm what has been previously shown by Mitchell, et al, (2005), that the polyhexamethylene biguanide product Baquacil® can effectively decrease the prevalence of Salmonella in the water column of hatchling red-eared slider turtles. Unlike the previous study, it was found that only a concentration of 50 ppm in the red-eared slider turtle’s habitat was significant. This is an indication that the efficacy of lower doses of polyhexamethylene biguanide against Salmonella is not adequate and use of such should be avoided. While a concentration of 50 ppm Baquacil® reduced the prevalence of Salmonella, there was no expectation that this concentration would eliminate the organism from the red-eared slider turtle’s habitat. Indeed, the treatment had no effect on the colonization of Salmonella in the gastrointestinal tract of the hatchlings, so they would continue to be a source of the organisms. The Baquacil® also appeared to lose its efficacy over the time between water changes. However, there was a general trend over the course of the study in which the prevalence of Salmonella decreased. Investigations into whether this trend would continue with prolonged use of polyhexamethylene biguanide treatment should be conducted. It also remains to be seen if Baquacil® use at greater concentrations may be more effective at reducing the prevalence of Salmonella in the habitat of red-eared slider turtles. Higher concentrations of polyhexamethylene biguanide would also have to be investigated for safety. In this study, no significant lesions were caused by either the Baquacil® or the Sanosil® products.

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