

Eradication of *Arizona hinshawii* from Artificially Infected Turtle Eggs

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Turtle eggs, 24 h old, were infected with *Arizona hinshawii* and treated 48 h later with gentamicin sulfate (Garasol; Shering Corp., Allantown, N.J.) by pressure differential egg dip treatment to ascertain the concentration of this reagent required to eradicate this pathogen from eggs. Infected eggs treated with 1,000 or 1,500 µg of gentamicin per ml of dip solution eliminated detectable *A. hinshawii* from eggs as determined by testing shells and embryo-yolk homogenates of 12-day-old eggs and the gastrointestinal tracts, kidneys, livers and gall bladders, and yolks of 50-day-old embryos. Treated eggs produced hatchlings which did not excrete detectable *A. hinshawii* at 72 h or 30 days after hatching, nor was this organism recovered from the visceral organs of these hatchlings when necropsied 30 days after hatching. Bacteriological assays on infected nontreated eggs showed that greater than 70% of the eggs harbored *A. hinshawii*, and eggs in this group produced hatchlings which actively excreted and harbored *A. hinshawii*. Eggs not infected or treated also produced turtles which excreted and systemically carried *A. hinshawii* and *Salmonella* spp. though not at the same level as did the turtles produced from infected, nontreated eggs.

The ban on interstate shipment, public sale, and importation of viable turtle eggs and live turtles with a carapace length of less than 4 in. (ca. 10.16 cm) did not bring about the immediate demise of the Louisiana turtle farming industry. Over the past 20 years the industry, in addition to domestic sales, attracted a sizable foreign market which presently sustains an annual export trade of an estimated 3×10^6 to 4×10^6 turtles. To date, countries which constitute the European and Far Eastern markets have not imposed restrictions or bans on the importation of turtles from the United States. It must be anticipated that foreign markets may be closed in the future for the reason that the ban was imposed in the United States; turtle hatchlings carry and excrete *Salmonella* and *Arizona* spp. (3, 4, 6, 15). Louisiana turtle raisers and shippers, when faced with the domestic ban, implemented measures at a commercial level to reduce the bacterial numbers in the 24-h egg through a sanitization procedure, eliminate *Salmonella* and *Arizona* spp. from the sanitized egg by treating eggs with antimicrobial agents, and modernize the egg-hatching procedure to prevent recontamination of sanitized and treated eggs. The impetus for these efforts are twofold: first, to export a clean, safe pet to maintain a lucrative foreign market, and second, to convince domestic regulatory agencies that a *Sal-*

monella-free turtle can be hatched and maintained.

It has been reported that *Salmonella* spp. can readily penetrate the shell of intact hen eggs from an external position (11, 14, 16), an event which may also be true for turtle eggs (2). The dip treatment of chicken and turkey eggs in solutions of antibiotic and other antimicrobial agents is widely used in the poultry industry to eradicate *Salmonella*, *Arizona*, and *Mycoplasma* spp. (1, 5, 7, 8). Saif et al. (7, 8) reported that gentamicin eradicated *Salmonella* spp. from both experimentally and naturally infected turkey eggs. In a previous report (10), it was found that naturally infected turtle eggs treated in dip solutions of either Terramycin (Te) or Chloromycetin produced turtles that did not excrete *Salmonella* spp. or *Arizona hinshawii* for the duration of the experiment (240 days), nor were these organisms recovered from whole-turtle homogenate. In 1974, the Food and Drug Administration approved gentamicin sulfate (Garasol; Shering Corp., Bloomfield, N.J.) for the dip treatment of turkey eggs for the eradication of *Salmonella* and *Arizona* spp. Gentamicin appeared to be better suited to treat turtle eggs at a commercial level than either Te or Chloromycetin for a variety of reasons. Antimicrobial activity is not lost from gentamicin solutions when exposed to freezing, pasteurization, or auto-

TABLE 1. Numbers of mesophilic aerobic bacteria per gram of turtle egg homogenate at three points during the egg sanitization procedure

Date and treatment	No. of eggs tested	Bacterial no. per g of egg ^a
April 28		
Before sanitization	10 ^b	5×10^5 – 7.5×10^5
After 5-min wash ^c	10	2.6×10^3 – 4×10^3
After Clorox dip ^d	10	80–5,600
May 29		
Before sanitization	10	2.1×10^6 – 3.9×10^6
After 5 min wash	10	3.5×10^3 – 16.0×10^3
After Clorox dip	10	100–500

^a Plate count agar, 35°C for 48 h.

^b Eggs ranged in weight from 7 to 13 g.

^c Spray delivery, ca. 30°C.

^d Five-minute immersion in Clorox bath, 230 ppm (230 µg/ml).

clave temperatures, a feature which would permit the farmers to sterilize and filter egg dip solutions between treatments and store the solutions in freezers between seasons.

Before embarking on a large scale egg dip treatment venture at a commercial farm, a pilot study, the subject of this paper, was done in the laboratory to determine the efficiency of various gentamicin concentrations in eliminating *A. hinshawii* from artificially infected eggs and the resultant hatchlings.

MATERIALS AND METHODS

Turtle eggs. Fertile turtle eggs (*Pseudemys scripta-elegans*) less than 24 h old were sanitized at the farm in the following way. The eggs were collected from the dirt nest and placed in single layers in vinyl-coated wire trays. Each tray, which held 75 to 80 eggs, was washed for 5 min under warm running water, immersed for 5 min in a 40°C Clorox-water bath (4 ml of Clorox per liter of water) and dried in a 40°C oven for 15 min.

Egg infection by the PD method. For infecting eggs by the pressure differential (PD) method, an *A. hinshawii* isolate recovered from a turtle egg (9) was used as the infecting agent. The organism was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 18 h at 35°C, and 1 ml was transferred to 1,000 ml of sterile 0.15 M NaCl and stirred for 5 min at room temperature. The infecting *A. hinshawii* suspension on 10 test dates contained between 8×10^5 and 8×10^6 *A. hinshawii* organisms per ml of suspension.

Each experiment, in which 150 to 300 eggs were used, was subdivided into three experimental groups. The infected, treated (IT) eggs were artificially infected with *A. hinshawii* and subsequently treated with gentamicin. The infected, nontreated (I-NT) control group eggs were infected only, and the third group contained noninfected and nontreated (NI-NT) eggs. The IT and I-NT eggs were infected with *A. hinshawii*

in the following manner. The eggs were immersed in the infecting *A. hinshawii* suspension in a plastic container which was inserted into a modified pressure cooker pan. The pressure cooker lid was fitted with a nozzle so that pressure tubing could be secured and connected to a portable vacuum pump. A vacuum was drawn to 27 in. (ca. 68.6 cm) for 5 min and then released over a 45- to 60-s interval. The eggs were soaked for an additional 10 min and placed in sterile, vinyl-coated wire trays which were inserted into plastic hatching containers. The infected eggs were incubated at 30 to 33°C for 48 h before further manipulation.

Treatment of infected eggs with gentamicin by the PD method. The eggs in the IT group were divided into groups of 30 to 50 eggs, and each group was treated with a different concentration of gentamicin by the PD egg dip method. The protocol used for egg infection, described above, was used for egg treatment. Stock gentamicin (Garasol; 50 mg/ml) was added to sterile distilled water to give 62, 125, 250, 500, 1,000, and 1,500 µg/ml of treatment solution. After the 15-min treatment process, the IT eggs were placed in sterile trays that were fitted into sanitized hatching chambers and incubated. The eggs were hatched in approximately 60 days in a closed system with outside contamination kept to a minimum. The eggs were inspected weekly, and nonfertile and fungus-contaminated eggs were removed.

Egg assays for *A. hinshawii*. Five eggs from each of the treatment subgroups and the I-NT and NI-NT control groups were removed for bacteriological assay on days 10 and 50 after antibiotic treatment. They were tested for the presence of the test organism *A. hinshawii* and for *Salmonella* spp. which might have been introduced naturally into the egg before or shortly after they were laid. The day 10 assay was done in the following manner. The entire shell surface of each egg was swabbed, the shell was opened and the egg contents (12-day-old embryo and yolk) were decanted and minced, and then the exposed inner aspects of the empty shell were swabbed. Each sample was inoculated into tetrathionate broth (9). On day 50, five eggs from each group were removed and their outer shells were swabbed, and then the viable hatchling was lifted through an opening made in the iodine-sterilized shell, asphyxiated in chloroform, and surface sterilized in a 2% iodine bath. The plastron was removed, and the yolk sac, both liver lobes (with intact gall bladder), the kidneys, and the entire length of the gastrointestinal tract, excluding the stomach, were removed. Each tissue was minced with a scissor and inoculated into 10 volumes of tetrathionate broth.

The remaining eggs in each experimental group were hatched, and the hatchlings were placed in 1,000-ml covered beakers containing 50 ml of sterile water, five hatchlings per beaker. The water from each beaker was assayed at 72 h and 30 days after hatching for the presence of *A. hinshawii* or *Salmonella* spp. (excretion assay) by methods previously reported (9).

The eggshell debris from each hatching chamber was preenriched in 1,000 ml of lactose broth for 24 h at 37°C, and 10 ml was subcultured into 90 ml of tetrathionate broth.

Egg homogenate was prepared to determine levels of mesophilic aerobic bacteria per gram and also to determine the number of *A. hinshawii* organisms in or

TABLE 2. Bacteriological assay of artificially infected turtle eggs for the presence of *A. hinshawii* 10 and 50 days after treatment with various concentrations of gentamicin by the PD egg dip method

Treatment group (μg of gentamicin per ml) ^a	Expt no.	No. of eggs positive for <i>Arizona/Salmonella</i> spp. on day:										
		10				50						
		No. of eggs tested	Shell		Yolk-embryo	No. of eggs tested	Shell		Embryo			
Outer	Inner		Outer	Inner			Yolk	Liver	Kidney	GI ^b		
I-NT	7	35	27	6	5	29	20	9	5	6	7	7
NI-NT	7	35	0	0	0	25	0	0	0	0	2 ^c	0
PD (1,500)	5	24	0	0	0	20 ^d	0	0	0	0	0	0
PD (1,000)	7	35	0	0	0	30 ^d	0	0	0	0	0	0
PD (500)	6	30	2 ^c	0	0	30	0	0	0	0	0	0
PD (250)	5	25	0	0	0	20 ^d	0	0	0	0	0	0
PD (125)	4	20	1 ^e	1 ^c	0	20	0	0	0	0	0	0
PD (62)	1	5	0	0	0	5	0	0	0	0	2 ^c	0

^a PD, Artificially infected eggs treated by PD dip treatment with indicated gentamicin dose.

^b GI, Gastrointestinal tract.

^c *Arizona hinshawii*.

^d 1 Experimental group did not hatch, owing to a fungal bloom.

^e *Salmonella* C₁.

on eggs at 48 h after artificial infection by methods previously reported (9, 10).

Bacteriology. The tetrathionate broth enrichment cultures were incubated at 37°C for 48 h and then streaked onto brilliant green agar (Difco Laboratories, Detroit, Mich.) containing 80 mg of sulfadiazine per liter and onto bismuth sulfite agar (BBL). Three isolates were picked from each plate and identified by methods previously reported (9).

MIC determination. Twofold dilutions of gentamicin in 0.5-ml volumes of antibiotic assay medium no. 3 (Difco) were made. The inoculum, 0.5 ml (10^5) cells of the test *A. hinshawii* organism or isolates recovered from eggs treated with gentamicin or from hatchlings produced from these eggs, was added to each tube. The minimal inhibitory concentration (MIC) was recorded as the tube containing the lowest dilution of gentamicin inhibiting visible turbidity after incubation at 37°C for 24 h.

RESULTS

Freshly dug turtle eggs are coated with a mucous layer, deposited on the shells as they pass through the cloacal opening, and dirt acquired from the nest. The mucous and dirt present a barrier to antimicrobial agents in treatment baths, in addition to contributing a sizable bacterial population to the egg surface. To combat this obstacle, a sanitization regimen was designed to both clean the eggshell surface and reduce the bacterial load on the shell. Table 1 shows the levels of aerobic mesophilic bacteria per gram of egg at three points in the sanitization process. Ten eggs were randomly selected from 24-h nests during the last week of April (the first week of the egg-laying season). These eggs showed 5×10^5 to 7.5×10^5 organisms per g of egg homogenate. The level of bacterial contamination on the egg was reduced after the 5-min warm-water washing step. The Clorox treatment

bath (ca. 230 ppm [230 $\mu\text{g}/\text{ml}$]) reduced the bacterial load to 80 to 5,600 organisms per g of egg homogenate. The experiment was repeated in late May during the height of the egg-laying season, and the results (Table 1) showed the same reduction in bacterial numbers seen in the first experiment.

An attempt was made to determine the level of infection in eggs artificially infected with *A. hinshawii*. From a group of 150 eggs that had been infected with *A. hinshawii* 48 h previously, 10 eggs were selected. They were homogenized individually and plated in bismuth sulfite pour plates. Artificial infection by the PD egg dip method resulted in 1.3×10^4 to 360×10^4 H₂S-producing organisms per g of egg. *Salmonella* and *Arizona* spp. were not detected in the homogenate of 10 sanitized eggs, which were not infected with *A. hinshawii*.

On seven occasions from May 13 through July 9, sanitized eggs were obtained from three commercial turtle farms, infected with *A. hinshawii*, and treated with various concentrations of gentamicin. Table 2 shows the results of the 10- and 50-day egg assays on control and treatment group eggs. It is apparent that I-NT eggs were successfully infected with *A. hinshawii*, for 27 of 35 eggs tested on day 10 and 20 of 29 eggs tested on day 50, approximately 1 week before hatching, were positive for *A. hinshawii*. From the NI-NT group, 60 eggs were negative on the day 10 assay, and the kidneys from two turtles were positive for *A. hinshawii* on the day 50 assay.

A. hinshawii-infected eggs treated with 1,500 or 1,000 μg of gentamicin per ml of dip solution were uniformly negative for *A. hinshawii*. Eggs treated with 500, 250, 125, or 62 μg of gentamicin per ml of dip solution showed at least two

TABLE 3. Bacteriological assay of egg shell residue and container water (excretion assay) holding hatchlings from control and treatment groups

Treatment group, (μg of gentamicin per ml) ^a	Shell and residue		Excretion assay	
	Expt no.	No. of positive eggs	No. of containers positive for <i>Salmonella</i> and <i>Arizona</i> spp./total no. of containers ^b	
			72 h	30 d
I-NT	6	6	12/12	11/12
NI-NT	5	3	12/21 ^c	12/21 ^c
PD (1,500)	3	0	0/7	0/7
PD (1,000)	6	0	0/14	0/14
PD (500)	6	0	1/13 ^c	2/13 ^{c,d}
PD (250)	4	0	0/11	1/11 ^c
PD (125)	4	1	0/10	2/10 ^c
PD (62)	1	0	0/3	0/3

^a PD, Artificially infected eggs treated by PD dip method with indicated gentamicin dose.

^b Five hatchlings per container.

^c *A. hinshawii*.

^d *Salmonella* serogroup C₁.

eggs positive for *A. hinshawii* in each of these treatment groups on day 10 or 50. In one instance, *Salmonella* serogroup C₁ was recovered from an outer shell swab from one egg 10 days after treatment with 125 μg of gentamicin.

The hatchlings began to peck and emerge from the eggs on days 55 through 60 and were immersed for 30 s in a Clorox solution to remove debris and distributed to 1,000-ml beakers. The water from each beaker was tested for the presence of *A. hinshawii* or *Salmonella* spp. or both 72 h and 30 days after hatching. Hatchlings from two of the five NI-NT groups, which occupied 12 of the 21 beakers, excreted detectable *A. hinshawii* into their container water. The hatchlings from one experiment did not excrete pathogens even though the eggshells were positive. IT eggs treated with 1,000 or 1,500 μg of gentamicin per ml of dip solution did not produce hatchlings which excreted detectable levels of *A. hinshawii*, nor was the organism recovered from the eggshell residue from the nine hatching chambers which constituted these two experimental treatment groups. Of the IT eggs treated with 125, 250, or 500 μg of gentamicin per ml of dip solution, 2 of 13, 1 of 11, and 2 of 10, respectively, produced hatchlings which excreted *A. hinshawii* (Table 3).

After the day 30 excretion assay was completed, two or three hatchlings from each container that had been negative for *A. hinshawii* and *Salmonella* spp. on both excretion assay dates were necropsied, and their organs were bacteriologically tested for the presence of *A. hinshawii* and *Salmonella* sp. (Table 4). Since all 12 containers from the I-NT experiments were positive, 10 hatchlings were selected and necropsied, and all 10 were positive for *A. hinshawii*.

Fifteen hatchlings from nine containers which

housed NI-NT turtles that had been negative for *A. hinshawii* and *Salmonella* spp. in the excretion assays were necropsied, and four of these were positive (Table 4). These turtles were hatched from NI-NT eggs in experiments which had yielded *A. hinshawii* isolates in either the shell assay (Table 3) or the day 50 embryo assay (Table 2).

Seventy hatchlings produced from IT eggs treated with 500, 1,000, or 1,500 μg of gentamicin per ml of dip solution were necropsied, and the tissues tested were negative for *A. hinshawii*. Of 45 hatchlings tested from eggs treated with 62, 125, or 250 μg of gentamicin, 5 were positive.

It is probable that, in some instances, turtle eggs are not recovered from nests until 24 to 48 h after deposition. For this reason, a 48-h time interval between artificial infection and treatment was used. In the last experiment (Table 5), fresh eggs were infected with *A. hinshawii*; one group was treated 7 days later and a second group was treated 14 days later with 1,000 μg of gentamicin. Eggs from each of these two treatment groups were tested for *A. hinshawii* immediately before treatment (days 7 and 14 after infection) and 10 days after treatment. Eggs artificially infected with *A. hinshawii* were positive when tested after infection and negative when tested 10 days posttreatment (Table 5).

MIC tests. On six occasions, the infecting organism, *A. hinshawii*, was tested by the tube dilution method to determine the MIC of gentamicin for this isolate. On each testing date, the MIC for the infecting *A. hinshawii* culture was 0.78 μg of gentamicin per ml of broth. Seventeen *A. hinshawii* and two *Salmonella* C₁ isolates recovered from eggs, shells, container water, or hatchlings produced from eggs treated with gen-

TABLE 4. Bacteriological assay of visceral organs from turtle hatchlings produced by Garasol and nontreated eggs 30 days after hatching

Treatment group (μg of gentamicin per ml) ^a	No. of hatchlings necropsied	No. of organs positive for <i>Salmonella</i> and <i>Arizona</i> spp.			
		Yolk	Liver	Kidney	Gastrointestinal tract
I-NT	10	4	8	9	10
NI-NT	20	1 ^b	1 ^b	4 ^b	3 ^b
PD 1500	15 ^c	0	0	0	0
PD 1000	25 ^c	0	0	0	0
PD 500	30	0	0	0	0
PD 250	20	0	0	2	0
PD 125	20	0	0	0	2
PD 62	5	0	0	0	1

^a PD, Artificially infected eggs treated by PD dip treatment with indicated gentamicin dose.

^b *Arizona* spp. were isolated from tissues of turtles produced by eggs not purposely infected.

^c One experimental group did not hatch, owing to a fungal bloom.

tamicin were also tested. The MIC ranged from 0.39 to 1.56 μg of gentamicin per ml for these 17 isolates, indicating no apparent increase in resistance of *A. hinshawii*.

DISCUSSION

In order for the Louisiana turtle farming industry to survive when faced with the 1975 ban on interstate shipment and sale of turtle hatchlings, it had to revolutionize the time-honored methods used to hatch turtle eggs, greater than 50% of which were actively excreting *Salmonella* spp. or *A. hinshawii* or both (9, 10). The egg-hatching methods were modified to include an egg sanitization procedure to reduce the numbers of bacteria on the shell surface, and after sanitization, the detectable numbers of aerobic mesophilic bacteria were reduced from 10^5 to 10^2 organisms per g of egg homogenate. An egg treatment regimen was designed to eradicate *Salmonella* and *Arizona* spp. from the turtle egg at a time when these pathogens were present in low numbers. Previously, it was reported (10) that 24-h turtle eggs treated by temperature differential with Te or Chloromycetin at concentrations of 1,000 μg per ml of dip solution produced hatchlings which did not excrete *Salmonella* spp. or *A. hinshawii*. Subsequent to this report, the Food and Drug Administration approved the commercial use of gentamicin sulfate (Garasol) for use in dip solutions for the eradication of *Salmonella* and *Arizona* spp. from turkey eggs. Also, a chamber was designed and used to hatch turtle eggs which would diminish the chances of recontaminating eggs or subsequent hatchlings with the pathogens.

The primary objective was to determine the concentration of gentamicin required to eradicate *A. hinshawii* from artificially infected eggs and subsequent hatchlings. *A. hinshawii* was selected as the infecting organism because great-

er than 50% of the isolates recovered from infected turtle hatchlings in previous studies were *A. hinshawii* (9, 10), and Wells et al. (13) reported that arizonae exhibit a greater tendency toward systemic infection in turtles than do salmonellae. There has been one previously reported attempt to artificially infect turtle eggs with *Salmonella* spp. Feeley and Tregar (2) exposed one group of turtle eggs to 10^4 *Salmonella braenderup* organisms for 1 h at 37°C and a second group for 24 h. The eggs were exposed to 0.1 ml of the infecting organism in U-shaped receptacles. Of 41 eggs exposed for 1 h, 26% were penetrated by *S. braenderup*, and 54% of 46 eggs exposed for 24 h were penetrated. Unfortunately, only 6 eggs hatched from the remaining 147 eggs; however, each of these hatchlings actively excreted *S. braenderup*. In our study, utilization of the PD method to infect fresh turtle eggs was successful. The level of infection 48 h after exposure to *A. hinshawii* showed a minimum of 1.3×10^4 organisms in 1 ml of whole-egg homogenate, and these numbers are considerably higher than those which can be detected in naturally infected eggs (10). The I-NT control group showed that *A. hinshawii* was recovered from 73% of the eggs tested (Table 2). Sixty hatchlings produced from the remaining I-NT eggs excreted *A. hinshawii* (Table 3), and when 10 of these hatchlings were necropsied, *A. hinshawii* was isolated from the gastrointestinal tract from each animal and from the kidneys and livers of 9 and 8 animals, respectively. These findings show clearly that the PD method efficiently delivered *A. hinshawii* into the turtle egg.

On seven and five occasions, IT eggs were treated with 1,000 and 1,500 μg of gentamicin per ml of dip solution, respectively (Table 2). Of the 109 eggs selected from these two treatment groups, neither *A. hinshawii* (introduced artificially) nor *Salmonella* spp. (contracted naturally) was recovered from these eggs on the day 10

TABLE 5. Bacteriological assay of artificially infected turtle eggs treated with 1,000 µg of gentamicin per ml of dip solution on days 7 and 14 postinfection

Expt group	Pretreatment assay ^a		Posttreatment assay ^a	
	Day ^b	No. positive/ no. tested	Day ^b	No. positive/ no. tested
Noninfected	2	1/5	21	0/5
I-NT	2	4/5	21	4/5
IT (7 days later)	7	5/5	14	0/5
IT (14 days later)	14	4/5	21	0/5

^a Outer and inner shell swabs and yolk-embryo were tested.

^b Day into the 55- to 60-day egg incubation period.

or the day 50 assay. Eggshells and residue collected from the hatching chambers holding the treated eggs were negative. A total of 105 hatchlings produced by eggs in the two treatment groups did not excrete detectable levels of *A. hinshawii* or *Salmonella* spp., and when 40 hatchlings from these two treatment groups were necropsied, *A. hinshawii* was not isolated from the visceral organs tested or the yolk sac (Table 4). Infected eggs treated with 500, 250, 125, or 62 µg of gentamicin per ml of dip solution yielded at least one *A. hinshawii* isolate during the follow-up egg, excretion, and necropsy assays (Tables 2, 4, and 5).

The finding that eggs in the NI-NT group yielded only 2 eggs positive for *A. hinshawii* of 60 tested may reflect low natural infection levels or indicate that the egg sanitization regimen dramatically reduced the numbers of naturally acquired *Arizona* and *Salmonella* spp. Hatchlings produced by NI-NT eggs in 12 of the 21 vessels excreted both *A. hinshawii* and *Salmonella* serogroup C₁ (Table 3) on the two assay dates. Ten animals each from the excretion-negative and the excretion-positive vessels were necropsied, and four animals from the latter vessels were positive for *A. hinshawii* and *Salmonella* spp. Unfortunately, an additional control group was not included in this study (non-sanitized, noninfected, nontreated eggs) to determine whether the natural infection rates in the NI-NT groups were influenced by the egg sanitization regimen.

Trust and Bartlett (12) questioned the wisdom of aquaria fanciers who, in their attempts to minimize bacterial disease during shipment and maintenance of frogs, snails, and other aquaria animals, treat these pets with various antibiotics. They reported the isolation of 13 *Salmonella* serotypes from snails, frogs, and aquaria water which exhibited multidrug resistance to 9 to 16 antibiotics, including gentamicin. The MIC of gentamicin among the salmonellae isolates ranged from 12.5 to 16 µg/ml (12). We tested 17

A. hinshawii isolates recovered from eggs and hatchlings produced from eggs treated with gentamicin (500 µg/ml or less), and none of these isolates displayed a gentamicin MIC significantly greater than that for the *A. hinshawii* isolate used to infect the eggs.

Relative to the findings of Trust and Bartlett (12) was a study conducted in our laboratory. In 1974, it was reported (9) that turtle hatchlings placed in treatment baths of Te for 7 to 14 days failed to eliminate systemic *Salmonella* and *Arizona* infections, whereas treatment of naturally infected turtle eggs in Te baths (1,000 to 2,000 µg/ml) by the temperature differential method eradicated detectable *Salmonella* and *Arizona* spp. from the eggs and the subsequent hatchlings (10). These two studies yielded 310 *Salmonella* and *Arizona* isolates, 190 from hatchlings treated in Te baths (9), 67 from eggs treated with 500 µg of Te or less, and 53 isolates from nontreated eggs and turtles. These isolates were tested for Te resistance and the capacity to transfer Te resistance to a recipient R-negative strain of *Escherichia coli* (H. D. Braymer, Louisiana State University, Baton Rouge, personal communication). Of the 190 isolates recovered from hatchlings treated with Te, 97 were resistant to 20 µg of Te, and 52 of these isolates were capable of transferring Te resistance to the recipient *E. coli* strain, whereas the level of Te resistance in *Salmonella* and *Arizona* isolates from Te-treated eggs (3.0%) was identical to that in isolates recovered from nontreated eggs or hatchlings.

Kaufmann and Morrison (3) suggested that the turtle egg may become contaminated with *A. hinshawii* or *Salmonella* spp. through ovarian transmission, whereas we feel (10) that they become infected through surface contamination shortly after deposition in the nest. Regardless of which infection mechanism is operative, eggs treated with 1,000 or 1,500 µg of gentamicin by the PD method produced *Salmonella*- and *Arizona*-free turtles.

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