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Treatment of *Salmonella-Arizona*-Infected Turtle Eggs with Terramycin and Chloromycetin by the Temperature-Differential Egg Dip Method

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Attempts to eliminate *Salmonella* and *Arizona* infection from newly hatched turtles were made by dipping fresh eggs in cold solutions of Terramycin and Chloromycetin at 1,000, 1,200, 1,500 and 2,000 μg per ml for either 10, 20, or 30 min. Control groups consisted of hatchlings produced from nondipped eggs or eggs dipped in chilled water. In two of the four experiments 5 to 10 eggs were blended on days 15, 30, and 45 post antibiotic dip treatment. Twenty-five to 60 hatchlings from each control or experimental dip group were held in containers and the water was tested (excretion method) for *Salmonella* and *Arizona* every 15 or 30 days for 180 to 210 days after hatching. Representative turtles were homogenized (blending method) to determine if systemic infections were present. All specimens tested were enriched in tetrathionate and selenite cystine broth. Nondipped eggs and water-dipped eggs routinely showed *Salmonella* and *Arizona* present in egg homogenate and hatchlings emerging from these eggs excreted these pathogens. Terramycin- and Chloromycetin-dipped eggs were uniformly negative for these pathogens, only if fresh eggs were dipped. Bacteriological assay of container water and whole turtle homogenate from hatchlings were negative for *Salmonella* and *Arizona* if eggs were dipped in 1,000 μg of Terramycin early in the egg laying season or if eggs were dipped in 1,500 or 2,000 μg of Terramycin per ml late in the egg laying season. The results of temperature-differential egg dip studies suggest that this is a feasible and promising method by which to eradicate *Salmonella* and *Arizona* from the turtle.

The rearing of baby turtles for commercial sale as pets, primarily a Louisiana-based industry, is currently under legislative prohibition by both federal and state agencies because these animals carry and excrete *Salmonella-Arizona* organisms (3, 8, 17). Before 1970 the turtle industry brought 2.5 million dollars into the state annually and because of its economic value numerous attempts have been made to eliminate these pathogens from the hatchling and/or its environment. On 23 June 1975 the Food and Drug Administration banned the public sale, interstate shipment, and importation of viable turtle eggs and live turtles with a carapace length of less than 4 inches (ca. 10.16 cm).

Kaufmann et al. (4) treated a commercial turtle breeding pond with copper sulfate and maintained this chemical at 2 to 5 μg/ml of pond water from April through September 1970. Although *Salmonella-Arizona* numbers in these waters were significantly suppressed, the *Salmonella* excretion rate for hatchlings from eggs laid by adult females in the treated pond was not reduced when compared to *Salmonella* excretion rates for turtles hatched from eggs gathered from a nontreated pond.

Attempts were made to treat infected hatchlings with Terramycin (Te), dissolved in treatment baths (11, 12). Although treatment with 200 to 1,200 μg of Te per ml of container water for 7 or 14 days did eliminate excretion of detectable *Salmonella-Arizona* by the treated turtle for several weeks post-treatment, it did not eradicate the systemic presence of these organisms. This approach was fraught with problems in that it was most difficult to treat thousands of turtles at one time on an industrial scale and also the fear of generating antibiotic-resistant strains of *Salmonella* existed.

It was the intent of this investigation to explore the feasibility of dipping fresh turtle eggs in solutions of antibiotic in an attempt to eliminate *Salmonella-Arizona* from the egg and thus hatch a "*Salmonella*-free" turtle. The poultry industry has reported considerable success in the elimination of enteric pathogens and *Mycoplasma* from turkey and chicken eggs when they are dipped in antibiotic solutions by the temperature or pressure differential egg dip

method (1, 6, 7, 9, 10, 14). Turtle eggs were gathered and dipped by the temperature-differential method in chilled solutions of Te or Chloromycetin (Cl). Hatchlings from dipped eggs were assayed by both the excretion and blending methods for 5 to 9 months post-hatching to determine if they were either excreting or harboring *Salmonella* systemically. The findings tentatively suggest that the antibiotic egg dip treatment is a feasible and promising approach toward the elimination of *Salmonella-Arizona* from the baby turtle.

MATERIALS AND METHODS

Eggs and turtles. Four egg dip experiments were performed at two commercial turtle breeding farms in southern Louisiana (Pontchatoula and Pierre Part, La.). The eggs were treated and incubated at the farm. Hatched baby turtles were taken to the laboratory for follow-up studies. The turtle under study was the red-eared slider (*Pseudemys scripta-elegans*).

Egg dip protocols. Turtle eggs were gathered and treated on the same day unless otherwise stated. Immediately after eggs were dug they were washed in running water, placed in wire baskets (two layers) and incubated at 30 C for 3 to 4 h. Antibiotic dip baths were prepared by dissolving Te (Charles Pfizer, New York) or Cl (Parke-Davis Co., Detroit, Mich.) in water to give a final concentration of 1,000, 1,200, 1,500, or 2,000 μg per ml in a final volume of 4 to 8 liters. Both plastic and stainless-steel dip tanks were used. The dip baths were refrigerated and used when the antibiotic solution reached 6 to 12 C. The egg trays, which contained 60, 170, or 220 prewarmed eggs, were completely immersed in the cold antibiotic solution for 10, 20, or 30 min. At the same time washed and prewarmed eggs were dipped in cold water (6 to 12 C) for the same time intervals (water-dipped control group). The egg trays were removed from the dip solution and inserted into plastic bags which were sealed and incubated at 30 C for 55 to 65 days. In the first egg dip experiment five eggs were taken before dip treatment and again from each control and experimental group on days 15, 30, and 45 post-dip. These eggs were blended and the egg homogenate was enriched and cultured for *Salmonella* and *Arizona*. In the second egg dip experiment 10 eggs were removed from each control and experimental group and blended.

Egg blending assay. Five or 10 viable turtle eggs were placed in a sterile 200-ml capacity stainless-steel cup. The eggs were blended at the lowest setting on the Sorvall Omnimixer for 2 min at 4 C. Twenty-five milliliters of egg homogenate was inoculated into 225 ml of both tetrathionate broth (Difco) containing 10 mg of brilliant green dye per liter (TBG) and into 225 ml of selenite cystine broth (Difco). After incubation at 37 C for 48 h, a 5-mm loopful of broth was streaked onto brilliant green agar (Difco) containing 80 mg of sulfadiazine per liter and onto bismuth sulfite agar (Difco). The brilliant green and bismuth sulfite plates were incubated at 37 C for 24 h; three lactose-negative colonies were picked from brilliant green agar and three black colonies were picked from bismuth sulfite agar and inoculated into triple sugar iron agar and lysine iron agar (Difco). Subsequent inoculations were made from triple sugar iron agar to urea agar slants, motility-indole-ornithine deeps, malonate broth and KCN broth (Difco). In addition to biochemical characterization, *Salmonella* were screened serologically in commercially available polyvalent and group-specific O antiserum (Difco). Suspected *Arizona* isolates (malonate positive) were inoculated into Trypticase soy-tryptose broth (Trypticase soy [BBL] and 13 g of tryptose [Difco] per liter) and then screened serologically in phase 1 and phase 2 H antiserum (Difco). All TBG broth cultures which were negative for *Salmonella* and *Arizona* were subcultured to a second TBG (secondary enrichment) when the primary enrichment was 1 week old. These were processed as discussed above.

Hatchling follow-up excretion and blending studies. Newly hatched baby turtles from each of the four egg dip experiments were brought to the laboratory within 2 days after hatching. During follow-up studies turtles in each experimental group were housed in groups of five at room temperature in 1,000-ml beakers covered with aluminum foil which contained 50 ml of sterile water. These turtles were not fed throughout the testing period since for several months post-hatching these animals receive nourishment from a yolk sac which involutes through the plastron before hatching. Regardless of whether assays were to be performed the turtles were transferred to sterile containers every week.

There were 25 to 60 turtles in each experimental and control group, dependent upon the number of eggs dipped and removed for blending studies and on the hatch rate.

Water was removed from each beaker in the experimental and control groups in each of the four egg dip experiments and was assayed for *Salmonella* and *Arizona* on a weekly, bimonthly, or monthly basis depending upon the experiment (excretion assay). In all instances 20 ml of water was inoculated into 180 ml of TBG and into 180 ml of selenite cystine broth. These were then processed as discussed above.

Unless the experimental or control group consisted of 30 turtles or less, in which case five turtles were used, 10 turtles were removed from each group every 30 days and blended as described elsewhere (blending method) (11). Twenty milliliters of turtle homogenate was enriched in 180 ml of both TBG and selenite cystine broth and processed as described above. In some cases turtles were blended every 15 days post-hatching.

RESULTS

The first egg dip experiment was performed in early April 1974. Two- to 7-day-old eggs were separated from fresh eggs (2- to 12-h-old), and then each group was dipped separately into either Te or Cl treatment baths, both of which contained 1,000 μg of antibiotic per ml. A second group of fresh eggs was dipped into cold

water and served as a control group. Table 1 shows the results of blending studies done on representative eggs taken from each experimental and control group 15, 30, and 45 days after dip treatment. *Salmonella* serogroups C_1, C_2, E_1, and *Arizona* were isolated from turtle egg homogenate prepared from fresh nondipped eggs. *Salmonella* E_1 was also recovered from blended nondipped eggs on days 30 and 45 into the incubation period and *Arizona* was isolated from these eggs on day 45. Egg homogenates prepared from fresh eggs, dipped in either Cl (Cl-dip) or Te (Te-dip), were negative for these pathogens on each assay date, whereas egg homogenates prepared from old eggs dipped in Cl or Te were positive for *Salmonella* group B on days 30 and 45 post-dip.

Hatchlings from each experimental group were brought to the laboratory and maintained in groups of 5, in 50 ml of water, and assayed bimonthly by the excretion method through 128 days and then on day 180 and 220 after hatching (Table 2). Nondipped and water-dipped eggs produced turtles which excreted *Salmonella* group G and *Arizona*, respectively, on each assay date. Fresh eggs dipped for 10 min in Te produced turtles which did not excrete detectable *Salmonella* or *Arizona* through 220 days after hatching. *Salmonella* C_1 was isolated from 72-h container water from one of the four beakers which housed hatchlings produced by fresh eggs dipped in Cl. This organism was not recovered again from this beaker during subsequent bacteriological assays.

On days 7, 58, 114, and 220 after hatching, five turtles from each of the five experimental groups were blended and the resulting whole turtle homogenate was assayed for *Salmonella*-

TABLE 1. *Bacteriological examination of turtle egg homogenate for Salmonella-Arizona after treatment for 10 min in cold solutions of Te or Cl at 1,000 μg/ml*

Experimental group[b]	Before treatment	Days post dip treatment[a]		
		15	30	45
Nondipped	C_1, C_2, E_1,[c] *Arizona*	0	E_1	E_1, *Arizona*
Water dipped		0	B, *Arizona*	B, *Arizona*
Cl-dip		0	0	0
Cl-dip (old)[d]		0	B	B
Te-dip		0	0	0
Te-dip (old)[d]		0	B	B

[a] Five eggs from each experimental group were blended at 4 C at 10 speed in a Sorvall Omnimixer for 2 min.
[b] Forty to 60 eggs in each experimental group.
[c] *Salmonella* serogroup isolated.
[d] Eggs were 2 to 7 days old.

TABLE 2. *Bacteriological examination of water for Salmonella-Arizona from containers holding turtles produced from nondipped, water-dipped, and Te- or Cl-dipped eggs, at 1,000 μg/ml*

Experimental group[b]	Water tested on days post-hatching[a]												
	3[c]	16	23	30	44	58[c]	72	86	100	114[c]	128	180	220[c]
Nondipped	+[d]	+	+	ND[e]	ND	+	+	ND	ND	+	+	ND	+
Water-dipped	+[f]	+	+	+	+	+	+	+	+	+	+	ND	+
Cl-dip	+[g]	0	0	0	0	0	0	0	0	0	0	0	0
Cl-dip (old)[h]	+[i]	+	+	ND	ND	+	+	ND	ND	ND	ND	+	+
Te-dip	0	0	0	0	0	0	0	0	0	0	0	0	0
Te-dip (old)[h]	+[i]	+	+	ND	ND	+	+	ND	ND	ND	+	+	+

[a] Container water was changed weekly.
[b] Twenty-five to 30 turtles in each group, housed five per 1,000-ml beaker.
[c] Five turtles were removed from each group and blended.
[d] *Salmonella* group G was isolated on each test date for the nondipped group.
[e] ND, Not done.
[f] *Arizona* was isolated on each test date for the water-dipped group.
[g] *Salmonella* group C_1 was isolated from one of four beakers.
[h] Eggs 2 to 7 days old.
[i] *Salmonella* group B was isolated on each test date for old eggs dipped in either Te or Cl.

Arizona. The results of the blending study are shown in Table 3. Again, as was the case in the excretion studies, *Salmonella* G and *Arizona* were isolated from whole turtle homogenate prepared from turtles which hatched from nondipped and water-dipped eggs, respectively. *Salmonella* group B was recovered from homogenates prepared from hatchlings produced by old eggs dipped in either Te or Cl. These pathogens were not isolated from turtle homogenate when hatchlings from fresh egg dip groups were blended.

In late May 1974, during the height of the egg laying season, the second egg dip experiment was performed. Three groups of 220 eggs each (Te-dip 1, Te-dip 2, and Te-dip 3) were sequentially dipped into the same Te bath, which contained 1,000 μg of Te per ml, for 20 min each at 12 C. Sufficient time was allowed to elapse between each dip so that the dip solution returned to 12 C. Table 4 shows the results of egg homogenization studies done on eggs from dip and control groups before and subsequent to treatment. *Arizona* was isolated from nondipped whole egg homogenate and also from shell surface swabs taken from 10 nontreated eggs; however, when the yolk, which was aseptically collected and pooled from these same 10 eggs, was enriched and cultured, neither *Salmonella* nor *Arizona* was isolated. *Arizona* was isolated from eggs before and after the cold water dip treatment and also on days 15, 30, and 45 post-Te dip. *Arizona* was also recovered from the Te-dip 1 group eggs before treatment, but not from Te-dip 3 group eggs after this lot had been dipped. Sixty turtles from Te-dip 1, 35 turtles from Te-dip 2, and 50 turtles from Te-dip 3 were taken to the laboratory and maintained in groups of 5 in beakers containing 50 ml of water, and the container water was assayed bimonthly for *Salmonella* and *Arizona* by the excretion method through 90 days and then monthly through 210 days post-hatching (Table 5). *Salmonella* group B was isolated from the water of one of the six remaining beakers on day 120 post-hatching in Te-dip 1, and the turtles in this same container were also positive for serogroup B when blended and assayed on this date. None of the other 11 beakers were positive during the 210-day assay period. Two beakers from group Te-dip 2 were positive, *Arizona* was isolated by the excretion assay on day 90 from one, and *Salmonella* group B was isolated from homogenate prepared from turtles in

TABLE 3. *Bacteriological examination of turtle homogenate for Salmonella-Arizona from hatchlings 7, 58, 114, and 220 days after hatching from nondipped, water-dipped, and Te- and Cl-dipped eggs, at 1,000 μg/ml*

Expt group[a]	Blending assays on days post-hatching[b]			
	7	58	114	220
Nondipped	G[c]	G	ND[d]	G
Water-dipped	*Arizona*	*Arizona*	*Arizona*	*Arizona*
Cl-dip	0	0	0	0
Cl-dip (old)[e]	B	B	B	B
Te-dip	0	0	0	0
Te-dip (old)[e]	B	B	B	B

[a] Twenty-five to thirty turtles in each group at outset.
[b] Five turtles were removed from each experimental group and blended for 2 min at 16,000 rpm in a Sorvall Omnimixer at 4 C.
[c] *Salmonella* serogroup isolated.
[d] ND, Not done.
[e] Eggs were 2 to 7 days old when dipped.

TABLE 4. *Bacteriological examination of turtle egg homogenate for Salmonella-Arizona after three groups of fresh turtle eggs were sequentially dipped for 20 min each in cold Te at 1,000 μg/ml*

Expt group	No. of eggs per group	Days post dip treatment[a]				
		0		15	30	45
		Before dip	After dip			
Nondipped	100	*Arizona*	ND[b]	ND	ND	ND
Shell swabs	10	*Arizona*, B[c]	ND			
Pooled yolk[d]	10	0	ND			
Water dip	100	*Arizona*	*Arizona*	*Arizona*	*Arizona*	*Arizona*
Te-dip 1[e]	220	*Arizona*	ND	0	0	0
Te-dip 2[e]	220	ND	ND	0	0	0
Te-dip 3[e]	220	ND	0	0	0	0

[a] Ten eggs from each treatment and control group were blended for 2 min at low speed in a Sorvall Omnimixer at 4 C.
[b] ND, Not done.
[c] *Salmonella* serogroup isolated.
[d] Yolk was pooled from the 10 eggs which had been swabbed, enriched in TBG, and cultured.
[e] Groups were sequentially dipped in same Te treatment bath at 12 C for 20 min.

TABLE 5. *Bacteriological examination of container water and turtle homogenate for Salmonella and Arizona from hatchlings produced by three lots of eggs which had been sequentially dipped in Te (1,000 µg/ml) for 20 min*

Expt group[a]	Specimen tested	Excretion and blending studies, days post-hatching										
		10	15	30	45	60	75	90	120	150	180	210
Te-dip 1	Water	0	ND[c]	0	0	0	0	0	B 1/6	0	0	0
	Homogenate[b]			0[d]		0		0	B 1/2	0		0
Te-dip 2	Water	0	0	0	0	0	0	Ariz 1/4	0	0	0	0
	Homogenate[b]			0		0		0	0	B 1/2		0
Te-dip 3	Water	ND[c]	ND[c]	B 6/10[e]	0	Ariz 2/10	B 6/8	B 5/6	ND	B, Ariz 2/6	B 1/4	B 1/2
	Homogenate[b]		ND[c]	ND[c]		B 2/2		B 1/2	ND	B 1/2	B 2/2	B 1/2

[a] Sixty turtles in group Te-dip 1, 35 turtles in group Te-dip 2, and 50 turtles in Te-dip 3, maintained five turtles per container in which water was changed weekly.

[b] At 30-day intervals 5 or 10 turtles in each dip lot were blended in a Sorvall Omnimixer and the homogenate was assayed for *Salmonella-Arizona*.

[c] Early studies could not be done; campus steam supply shut down for 18 days.

[d] Turtles blended came from containers which had previously been negative on excretion assays.

[e] Represents number of containers positive for *Salmonella-Arizona* (numerator) over the number of containers tested (denominator).

a second container on day 150. Six of the 10 beakers, when tested for the first time 30 days after the turtles had hatched, revealed that hatchlings in the Te-dip 3 group were excreting *Arizona* and/or *Salmonella* group B. This same organism was also recovered from homogenate prepared from the turtles in each of these same six beakers when they were blended on subsequent assay dates. In addition, the turtles in a seventh beaker were positive when blended and these animals had been negative on all previous excretion assays (*Salmonella* group B, day 210). It is of interest to note that *Arizona* was isolated from the water on days 60 and 150, but not from turtle homogenate on any assay date.

The third egg dip study was performed in late June, toward the end of egg laying season. Egg lots consisting of 1,000 eggs each were dipped for 30 min in Te (1,200 µg/ml). Eggs were dipped on 4 different days (dip 18, dip 22, dip 23, and dips 26-1 and 26-5). Dip 26 included 5 lots of 220 eggs each dipped sequentially in the same Te bath; however only the first (26-1) and fifth (26-5) lots were included in this study. Egg homogenate studies were not done in this experiment. Table 6 shows the results of water (excretion) and whole turtle homogenization (blending) studies done at 30-day intervals on 60 hatchlings from each dip group through 210 days post-hatching. The turtles in one of the 12 beakers, in dip group 18, excreted *Salmonella* serogroup D during one assay period (day 30); however, these same turtles when subsequently blended on day 210 were negative for *Salmonella* and *Arizona*. In this same experimental group turtles blended on days 60 and 120 were positive for *Salmonella* C_2 and B, respectively, and neither of these organisms had been detected in the container water holding these turtles on previous testing dates. Except for water assays done on day 7 post-hatching, when dip groups 22, 23, 26-1, and 26-5 showed 1, 5, 3, and 1 of 12 beakers positive for *Arizona*, all subsequent excretion assays done on water samples from these beakers were negative. Group 26-1 was negative for *Salmonella* and *Arizona* on all excretion and blending assays after day 7. On day 90 after dip treatment, blending studies on five turtles from dip group 23 and 5 from group 26-5 revealed the presence of *Salmonella* serogroup B in the homogenate. *Salmonella* serogroup B was also isolated from whole turtle homogenate prepared from five turtles in dip group 22, 120 days after dip treatment. Neither *Salmonella* nor *Arizona* had been isolated from the water on previous excretion assay dates from the containers holding these three subgroups. Again, as was observed in the second experiment, *Arizona* was recovered from water and *Salmonella* from turtle homogenate.

At the time the hatchlings were brought to the laboratory, the contents of the hatching containers, which included broken shells, water of condensation, and feces, were collected. The residue was enriched in TBG and selenite cystine broth and cultured. The contents of the hatching containers for groups 18, 22, 23, 26-1, and 26-5 were negative for *Salmonella* and *Arizona* (not shown in table).

Since both *Arizona* and *Salmonella* could be isolated from hatchlings produced by eggs dipped in 1,000 or 1,200 µg of Te per ml of the treatment bath, it appeared that eggs laid and gathered in late May and early June might be more heavily contaminated than the April eggs

used in experiment 1. Therefore, the fourth egg dip experiment was carried out by dipping eggs in 1,000, 1,500 or 2,000 μg of Te per ml.

Table 7 shows the results of excretion and blending studies done on turtles which hatched from eggs dipped for 30 min in 1,000, 1,500 or 2,000 μg of Te per ml. Approximately 100 fresh and 100 old eggs were dipped into 1,000 μg of Te per ml. The turtles in one of the eight containers holding hatchlings from fresh eggs dipped in 1,000 μg of Te excreted detectable *Arizona* on each assay date, and when these turtles were blended 180 days post-hatching *Arizona* was isolated from the whole turtle homogenate. Old eggs (2 to 10 days old) produced fewer hatchlings; however, water in two of the five containers when assayed after 72 h showed *Arizona* to be present. All subsequent excretion assays done on these two containers and blending studies done on the turtles in these containers (day 30 and day 60) were negative for *Arizona*. Excretion and blending studies done on the water and turtles produced by eggs dipped in either 1,500 or 2,000 μg of Te per ml were uniformly negative through 150 days post-hatching. There were 25 turtles in each of these last two groups, which represented a poor hatch rate (25%).

DISCUSSION

This investigation revealed that turtle eggs can be immersed in chilled solutions of Te containing 1,000, 1,200, 1,500, or 2,000 μg per ml for as long as 30 min with little or no discernible loss in viability or hatchability. A poor hatch rate was observed in the fourth egg dip

TABLE 6. *Bacteriological examination of container water and turtle homogenate for Salmonella-Arizona produced by eggs dipped for 30 min in Te at 1,200 μg/ml on 4 different days*

Expt group[a]	Specimen tested	Excretion and blending studies, days post-hatching										
		7	15[b]	30[b]	45	60[b]	75	90[b]	120[b]	150	180	210[b]
Te-dip 18	Water	0	0	D, 1/12[c]	0	0	0	0	0	0	0	0
	Homogenate		0	0		C_2, 1/2		0	B, 1/2			0
Te-dip 22	Water	Ariz., 1/2	0	0	0	0	0	0	0	0	0	—[d]
	Homogenate		0	0		0		0	B, 1/2			
Te-dip 23	Water	Ariz., 5/12	0	0	0	0	0	0	0	0	0	0
	Homogenate		0	0		0		B, 1/2	0			0
Te-dip 26-1	Water	Ariz., 3/12	0	0	0	0	0	0	0	0	0	—[d]
	Homogenate		0	0		0		0	0			
Te-dip 26-5	Water	Ariz., 1/2	0	0	0	0	0	0	0	0	0	—[d]
	Homogenate		0	0		0		B, 1/2	0			

[a] Twelve containers in each group, five turtles per container in 50 ml of water changed each week.
[b] Two containers removed from group and turtles blended in a Sorvall Omnimixer for 2 min at 16,000 rpm.
[c] *Salmonella* serogroup isolated; number of containers positive for *Salmonella-Arizona* (numerator) over number of containers tested (denominator).
[d] Remaining animals died before assay date.

TABLE 7. *Bacteriological examination of container water and turtle homogenate from turtles produced by eggs which were dipped for 30 min in treatment baths containing 1,000, 1,500, or 2,000 μg of Te per ml*

Expt group[a]	Specimen tested	Excretion and blending studies, days post-hatching									
		3	15	30	45	60	75	90	120	150	180
Te-1000 (fresh)	Water	0	Ariz 1/8[c]	Ariz 1/7	Ariz 1/6	Ariz 1/5	Ariz 1/4	Ariz 1/4	Ariz 1/3	Ariz 1/2	Ariz 1/2
	Homogenate[b]		0[d]	0	0	0		0	0		Ariz 1/2
Te-1000 (old)	Water	Ariz 2/5	0	0	0	0	0	0	0	0	0
	Homogenate[b]		0	0	0	0					0
Te-1500	Water	0	0	0	0	0	0	0	0	0	—[e]
	Homogenate[b]			0		0		0		0	
Te-2000	Water	0	0	0	0	0	0	0	0	0	—[e]
	Homogenate[b]			0		0		0		0	

[a] Twenty-five to 40 turtles per group, maintained five animals per container in 50 ml of water changed each week.
[b] Turtles in one container were removed from experimental group and blended at 15- and/or 30-day intervals.
[c] Number of samples positive for *Salmonella* or *Arizona* (numerator) over number of samples tested (denominator).
[d] Turtles blended came from containers which had previously been negative for excretion assays.
[e] Remaining animals died before assay date.

experiment, but this could be attributed to high egg infertility rates often observed at the end of the 4-month egg laying season. Turtle eggs were hatched in sealed plastic containers and the hatch rates approached 85%. The containers were not packed with any bedding material, which represented a major departure from the time-honored egg hatching procedure which was previously performed by burying eggs in peat moss, saw dust, or dirt in hot-bed frames. The bedding material constituted a potential continued source for *Salmonella-Arizona* egg contamination since in some instances the material was reused without proper sanitization.

Turtle eggs which had been laid and gathered on the same day and washed under running water showed that *Salmonella* and/or *Arizona* was present in blended egg homogenate (Tables 1 and 4). The eggs may become contaminated with these pathogens as they develop in infected ovarian tissue. Kaufmann and Morrison (5) isolated *Salmonella rubislaw* from the ovarian tissue of eight adult breeders, out of 10 tested, and they also isolated this same serotype from the internal contents tested from 2 of 20 fresh turtle eggs. It is also possible that clutches of eggs laid by noninfected breeders in the dirt on a confined pond levee could become contaminated by an alternate pathway. The bank soil has heavy turtle traffic during the months of April through July when each of 6,000 to 8,000 females in the average breeding pond lay two to three clutches of eggs. The female before excavating a nest urinates on the selected nest site to soften the sunbaked soil. The female takes into her bladder 40 to 60 ml of pond water just before searching for a nest site. Kaufmann and Morrison (5) and Kaufmann et al. (4) reported that *Salmonella-Arizona* most-probable-number (MPN) determinations done on water from commercial breeding ponds during the egg laying season may reach 240 organisms per 1,000 ml of water. We have also made similar studies and found elevated *Salmonella-Arizona* MPN values of 120 to 720 organisms per 100 ml of pond water during the egg laying months (unpublished data). A female may then take in "infected" pond water and contaminate the soil in her selected nest site. It has been documented that *Salmonella* organisms readily penetrate the chicken egg (13, 16, 18). Feeley and Tregar (2) reported that of 41 turtle eggs exposed to *Salmonella braenderup* for 1 h 26.8% were penetrated. Fifty-four percent of 46 eggs were penetrated when eggs were exposed for 24 h. Only six of 147 exposed eggs hatched; however, each hatchling was shown to be excreting *S. braenderup*.

With these concerns in mind we felt that the best time to eradicate *Salmonella-Arizona* from the turtle might be at the egg stage, and more specifically the freshly laid egg which might harbor the fewest number of *Salmonella-Arizona*. In the first experiment prewarmed fresh eggs dipped in cold treatment baths containing 1,000 μg of Te or Cl per ml produced turtles which did not excrete *Salmonella* or *Arizona* for 220 days post-hatching. Nondipped eggs, water-dipped eggs, and 2- to 7-day-old eggs dipped in Te or Cl produced turtles which excreted detectable *Salmonella* or *Arizona* on each assay date throughout the test period. When representative turtles from each of these experimental groups were blended, the findings were similar to the excretion studies.

In the second egg dip experiment three lots of fresh eggs were dipped sequentially into the same dip bath, for 20 min each, which contained 1,200 μg of Te per ml. Egg blending studies showed no detectable *Salmonella* or *Arizona* in the eggs of the three dip groups, whereas *Arizona* was recovered from water-dipped eggs. *Arizona* was isolated from shell surface swabs of nondipped eggs but not from the internal egg content. Turtles produced from eggs in the third dip group (Te-dip 3) excreted *Arizona* and *Salmonella* serogroup B and the infection rate in this group, determined by both excretion and blending studies, indicated a greater level of infection in these turtles (seven of ten containers positive) than the first or second dip groups (one of twelve and two of seven positive, respectively). There are two possible explanations for these observations: first, the eggs were not adequately sanitized before dip treatment, and the accumulation of considerable organic material and dirt leached off the eggs during the first and second dip treatments might have hindered or tied up Te activity; secondly, it is conceivable that significant amounts of Te activity were removed by the eggs during the first and second dips so that suboptimal Te activity remained. Unfortunately, samples from the antibiotic baths were not assayed for Te concentration before and after dipping to determine what Te activity was removed by the eggs during treatment.

The results of the third experiment showed that hatchlings produced by eggs in four independent egg lots dipped in 1,200 μg of Te per ml for 30 min on 4 different days excreted detectable levels of *Arizona* 7 days after hatching; however, all subsequent water assays performed on these containers were negative. No reasonable explanation can be offered at this time as to why *Arizona* was not recovered again by either blending or excretion assay.

Nor was this organism isolated from the container residue (shells and water) which had been enriched and cultured when the turtles hatched. One dip group, Te-dip 26-1, was negative on all excretion and blending assays done after day 7, while on three occasions *Salmonella* D, C_2 and B were recovered from turtle container water (day 30) and whole turtle homogenate (days 60 and 120) from dip group Te-dip 18, and whereas turtles in one container in each of the other three dip groups in this experiment were positive for *Salmonella* serogroup B when they were blended and tested. In each of these instances (except day 30, Te-dip 18) these organisms were not detected in the container water on previous excretion assays or in the water sample tested concurrently with the blending study. This could reflect a systemic infection in tissue such as the spleen or liver which does not necessarily contribute these organisms to the excretion products (urine or feces), and they would not be detected unless the animals were blended or the respective tissues were tested (12). Wells et al. (15) reported the isolation of *Arizona* from baby turtle kidney, liver and ovarian tissue, but not from the container water holding these animals.

The last experiment showed that hatchlings from eggs dipped in 1,500 or 2,000 μg of Te per ml of treatment bath did not excrete *Salmonella* or *Arizona* nor were these pathogens isolated from whole turtle homogenate prepared periodically from these turtles over a 180-day period.

Stuart and Keenum (14) reported that dipping chicken eggs in solutions containing neomycin sulfate at 4,800 μg/ml was of value for controlling *Salmonella pullorum* in naturally infected eggs. Lucas et al. (7) dipped turkey eggs that were infected experimentally with *S. saint paul*, *S. typhimurium*, and *Arizona* organisms. They reported that kanamycin, spectinomycin, and neomycin reduced the rate of recovery of organisms. Saif et al. (10) dipped naturally and experimentally infected turkey eggs in solutions of gentamicin sulfate and reported the elimination of *Arizona*. In a second study Saif and Shelly (9) exposed 445 turkey eggs in groups of 10 to 25 different *Salmonella* serotypes by the pressure-differential procedure. These eggs were then dipped in 1,000 μg of gentamicin sulfate and 6 to 8 days later 2% were infected, whereas 80.1% of infected non-dipped control eggs were infected.

If anything, the turtle egg is more permeable than the chicken or turkey egg and depending upon the relative humidity in the hatching room or container the turtle egg will swell or shrink within the boundary of an extremely flexible shell. The preliminary findings of our investigation suggest that the treatment of infected turtle eggs by the temperature-differential method is a feasible approach toward eliminating *Salmonella* and *Arizona*. The turtle once hatched can be kept isolated in the hatching tray and as a result contamination can be avoided at the farm since these animals need not be fed for 6 to 9 months post-hatching.

The antibiotic dip treatment coupled with an improved, possibly mechanized, egg washing process and pre-dip sanitization procedures certainly would improve the effectiveness of antibiotic penetration and possibly stability. Efforts must be made to assay the amount of antibiotic taken into the egg when dipped either by temperature- or pressure-differential methods. Dip solutions must also be monitored to determine levels of antibiotic in the bath during usage. All of these factors are being examined during the present egg laying season. We are also currently testing Garasol (gentamicin sulfate) by both the temperature- and pressure-differential egg dip methods, which recently received Food and Drug Administration approval for turkey egg dip treatment.

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LITERATURE CITED

1. Barnes, L. E., J. I. Lay, and S. M. Bickford. 1973. Gentamicin levels in chicken eggs and tissues of progeny following temperature differential dipping. Avian Dis. 17:594-597.
2. Feeley, B. S., and M. D. Tregar. 1969. Penetration of turtle eggs by *Salmonella braenderup*. Public Health Rep. 84:156-158.
3. Kaufmann, A. F. 1966. Pets and *Salmonella* infections. J. Am. Vet. Med. Assoc. 149:1655-1661.
4. Kaufmann, A. F., M. D. Fox, G. K. Morris, B. T. Wood, J. C. Feeley, and M. K. Frix. 1972. Turtle-associated Salmonellosis. III. The effects of environmental *Salmonellae* in commercial turtle breeding ponds. Am. J. Epidemiol. 95:521-528.
5. Kaufmann, A. F., and Z. L. Morrison. 1966. An epidemiologic study of salmonellosis in turtles. Am. J. Epidemiol. 84:364-370.
6. Levine, P. D., and J. Fabricant. 1962. Effects of dipping eggs in antibiotic solutions on PPLO transmission in chickens. Avian Dis. 6:72-85.
7. Lucas, T. E., M. C. Kumar, S. H. Kleven, and B. S. Pomeroy. 1970. Antibiotic treatment of turkey hatching eggs preinfected with *Salmonella*. Avian Dis. 14:455-462.
8. Rosenstein, B. J., P. Russo, and M. C. Hinchliff. 1965. A family outbreak of Salmonellosis traced to a pet turtle. N. Engl. J. Med. 272:960-961.

9. Saif, Y. M., and S. M. Shelly. 1973. Effect of gentamicin sulfate dip on *Salmonella* organisms in experimentally infected turkey eggs. Avian Dis. 17:574-581.
10. Saif, Y. M., L. C. Ferguson, and K. E. Nestor. 1971. Treatment of turkey hatchling eggs for control of *Arizona* infection. Avian Dis. 15:448-461.
11. Siebeling, R. J. 1975. Preliminary observations on the treatment of baby turtles (*Pseudemys scripta-elegans*) for the elimination of *Salmonella-Arizona*, p. 243-260. *In* R. L. Amborski, M. A. Hood, and R. R. Miller (ed.), Proc. Gulf Coast Regional Symp. on Diseases of Aquatic Animals. Louisiana State University Press, Baton Rouge.
12. Siebeling, R. J., P. M. Neal, and W. D. Granberry. 1974. Evaluation of methods for the isolation of *Salmonella* and *Arizona* organisms from pet turtles treated with antimicrobial agents. Appl. Microbiol. 29:240-245.
13. Stokes, J. L., W. W. Osborne, and H. G. Bayne. 1956. Penetration and growth of *Salmonella* in shell eggs. Food Res. 21:510-518.
14. Stuart, E. E., and R. D. Keenum. 1970. Preincubation treatment of chicken hatching eggs infected with *Salmonella pullorum*. Avian Dis. 14:87-95.
15. Wells, J. G., G. McC. Clark, and G. K. Morris. 1974. Evaluation of methods for isolating *Salmonella* and *Arizona* organisms from pet turtles. Appl. Microbiol. 27:8-10.
16. Williams, J. E. 1968. The penetration patterns of *Salmonella typhimurium* through the outer structures of chicken eggs. Avian Dis. 12:445-446.
17. Williams, L. P., and H. L. Helsdon. 1965. Pet turtles as a cause of human salmonellosis. J. Am. Med. Assoc. 197:347-351.
18. Wright, G. W., and J. F. Frank. 1956. Penetration of eggs by *Salmonela typhimurium*. Can. J. Comp. Med. 20:453-457.