PENETRATION OF TURTLE EGGS BY *SALMONELLA BRAENDERUP*

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THE FACT that *Salmonella*-infected pet turtles can cause salmonellosis in persons has been well documented (*1–3*). Prevention of such cases of salmonellosis would require pet turtles to be free of salmonellae. One method of obtaining *Salmonella*-free pet turtles might be to put antibiotic drugs in their food or water, but experiments along this line have been unsuccessful (personal communication, V. D. Foltz, Kansas State University, July 1967).

Another solution would be to prevent the turtles from becoming infected in the first place. Investigations into where and how turtles become contaminated show that pet turtles excrete salmonellae in homes, at pet shops, in holding tanks of wholesalers, and at the breeding farms (*4–6*).

The purpose of this paper is twofold. The first is to test the hypothesis that the eggs from which pet turtles hatch can be penetrated by salmonellae. The second is to discuss the significance to public health workers of penetration if it occurs.

Materials and Methods

Turtle eggs (*Pseudeyms scripta-elegans*) were collected at a large commercial turtle farm in the Southeast. Six eggs were gathered from each of 49 randomly selected nests that contained 8 to 10 eggs each. The 294 eggs were divided into three groups of 98 eggs per group. Two groups of eggs were exposed to salmonellae; one group of 98 for approximately 1 hour; the other group of 98 for approximately 24 hours. The third group of 98 eggs was not exposed to salmonellae and served as the control group. Any eggs that remained in the nests were collected and retained by the breeder for hatching and sale. At the time of collection, each egg was marked so that we would know from which nest it originated. This method allowed the distribution of two eggs from every nest into each of the three experimental groups.

The eggs that were exposed for 1 hour were placed in U-shaped receptacles (7/8-inch diameter) of plastic agglutination trays containing 0.1 ml. of a 10^5 *Salmonella braenderup* per ml. suspension. This inoculum was freshly prepared in an improvised laboratory at the turtle farm. A loopful of an actively growing broth culture of *S. braenderup* grown at 37° C. was inoculated into 10 ml. of a medium consisting of equal parts of trypticase soy broth (Baltimore Biological Laboratories) and tryptose broth (Difco Laboratories) and incubated overnight at 37° C. An 0.1 ml. amount of this overnight culture was transferred into 100 ml. of sterile distilled water that was of the same approximate temperature as the eggs. After an hour in the inoculum, the eggs were wiped off with 70 percent alcohol, placed in plastic bags, and transported to the National Communicable Disease Center.

The eggs that were exposed for 24 hours were placed in 7-dram plastic snap-cap vials containing 0.2 ml. of the *S. braenderup* inoculum and transported to the Center. After approximately 24 hours, the plastic vials were opened, and the eggs were removed and washed with 70 percent alcohol. The control group of eggs was not exposed to the *S. braenderup* inoculum. They were washed off with 70 percent alcohol and transported to the Center in plastic bags. It should be noted that seven eggs of the first group were exposed for 2 hours.

All eggs, inoculated and control, were then

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placed in sterile refrigerator jars containing sterile gauze moistened with sterile water. Each jar contained two eggs that had received the same treatment and had originated from the same clutch. The jars were covered with perforated wax paper to allow air exchange, then incubated at 28° C. The jars were inspected daily for moisture content and sterile water was added when needed.

After a week, one egg was removed from each refrigerator jar for culturing. The eggs were washed in a strong tincture of iodine solution and cut open at the end not exposed to *S. braenderup*. The egg contents were dropped into refrigerator jars containing 50 cc. of lactose broth and incubated at 37° C. for 24 hours. Transfers were then made both to tetrathionate broth and brilliant green agar with 80 mg. of sulfadiazine per 100 ml. agar. The tetrathionate broth was incubated for 24 hours and streaked on brilliant green sulfadiazine media, suspect colonies were picked to triple sugar iron media, and the salmonellae were identified by standard methods (*7*). The second egg in each jar was retained for hatching.

Results

Table 1 shows that 26.8 percent of the 41 eggs exposed to *S. braenderup* for an hour were penetrated. The percentage of penetration increased to 42.9 percent of 7 eggs that were exposed for an additional hour. Highest penetration in the experiment, 54.4 percent of 46 eggs, occurred when the eggs were exposed for 24 hours. No salmonellae were isolated from the eggs serving as controls. The difference in penetration between the eggs exposed for 1 hour and the eggs exposed for 24 hours was significant ($P=0.01$); but the difference between the eggs exposed 1 hour and the eggs exposed for 2 hours was not significant ($P=0.39$), according to probability tests (*8*).

Only six turtles hatched from the 147 eggs retained for hatching. Table 2 shows that 100 percent of the turtles excreted *S. braenderup* on hatching from exposed eggs, but no salmonellae were isolated from the one turtle that hatched from the unexposed egg. The poor hatch was caused by our inability to maintain proper humidity. The eggs that failed to hatch either became waterlogged or dried up. Since the purpose of this part of the study was to obtain living baby turtles and determine whether or not they excreted salmonellae, no attempt was made to culture nonviable eggs.

Discussion

Turtle egg shells are permeable and readily permit passage of water and gas (*9*). It has been demonstrated that *Salmonella* penetrate chicken eggs (*10*, *11*). These facts imply *Salmonella* penetration of turtle eggs could occur. Consequently, the speed of penetration, the direct relationship between length of exposure and percentage of eggs penetrated, the overall high percentage of eggs penetrated, and the hatching of infected turtles from eggs artificially exposed to salmonellae was not surprising.

If penetration were possible, it could occur under the artificial conditions of this experiment.

The significance to workers in public health is that the conditions of the experiment may be duplicated at commercial turtle farms where rendered meat and poultry, often heavily contaminated with salmonellae, are thrown into the turtle ponds to serve as food for breeding turtles. Both the water in the ponds and the ground surrounding them thus may become

Table 1. Results of culture of contents of turtle eggs exposed to *Salmonella braenderup*

Period of exposure	Number exposed [1]	Number positive	Percent positive
1 hour	41	11	26. 8
2 hours	7	3	42. 9
24 hours	46	25	54. 4

[1] Less 1 egg from 1-hour group and 3 eggs from 24-hour group that were cracked in transit to the center.

Table 2. Number and percent of newly hatched turtles positive to *Salmonella braenderup*

Period of exposure	Number of eggs exposed	Number of hatched turtles	Number positive	Percent positive
1 hour	42	2	2	100
2 hours	7	0	0	0
24 hours	49	3	3	100

heavily seeded with salmonellae. (Unpublished paper, by J. C. Feeley, M. D. Treger, and A. F. Kaufmann, "*Salmonella* culture survey of a turtle farm.") Consequently eggs could be exposed to salmonellae at the pond, during passage through their mothers' contaminated cloacas, or during contact with contaminated soil in the nests.

Also, the warm moist environment during the 2-month period of incubation would allow salmonellae to survive on the egg surfaces and favor penetration.

Penetration of turtle eggs by salmonellae does not appear to be limited to freshly laid eggs. Four eggs approximately 3 weeks old were tested by the methods of Williams and Whittemore (*12*). One out of three of these eggs artificially exposed to *Salmonella typhimurium* was penetrated (personal communication, Dr. J. E. Williams, U.S. Department of Agriculture, Agricultural Research Services, Athens, Ga., July 1967). The fourth egg was not exposed and served as a control.

Summary

Turtle eggs were collected from a large commercial turtle farm in the Southeast. The 294 eggs collected were divided into three groups of 98 eggs per group. Two groups of eggs were exposed to *Salmonella braenderup*; one group for approximately 1 hour; the other group for approximately 24 hours. The third group of 98 eggs was not exposed to *S. braenderup* and served as controls. Half the eggs were cultured for salmonellae after a week's incubation at 28° C. The remaining eggs were incubated until either baby turtles hatched from them or until the eggs obviously were no longer viable. The hatched turtles were cultured, and the nonviable eggs were discarded. Penetration occurred in 26.8 percent of the eggs exposed for 1 hour and 54.4 percent of the eggs exposed for 24 hours. The difference between the two percentages was statistically significant.

Only six turtles hatched from the 147 eggs that were retained for hatching. Five of the turtles came from exposed eggs and proved to be infected with *S. braenderup*. The one turtle that hatched from an unexposed egg was negative for salmonellae.

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