Evaluation of Methods for Isolating *Salmonella* and *Arizona* Organisms from Pet Turtles

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Two methods for isolating *Salmonella* and *Arizona* organisms from turtles, blending and excretion, were evaluated, and the percentage of isolates obtained by each method was compared with the percentage of isolates obtained by culture of turtle organs. The blending and excretion methods were equally effective in detecting the overall incidence of *Salmonella* and *Arizona* infections in turtles. The percentage of isolates obtained by specific organ culture, however, was less than the percentage obtained by the other two methods. The blending method detected a greater number of turtles with *Arizona* infections than did the excretion method, but there was no difference in the number of *Salmonella* infections detected by the two methods. The frequency of isolation of *Arizona* organisms from organs other than the small intestine and colon was higher than that of *Salmonella*.

Pet turtles have been shown to be carriers of *Salmonella* which cause human infections (5, 7, 8). Recent data have shown that, of the estimated $2 \times 10^4$ annual cases of human salmonellosis in the United States, approximately $3 \times 10^3$ are associated with pet turtles (6). Because of the magnitude of the problem, federal regulations prohibiting the importation of pet turtles and interstate shipment of turtles infected with *Salmonella* or *Arizona* have been implemented.

Federal and some state regulations require that lots of turtles must be proven free of *Salmonella* and *Arizona* bacteriologically before interstate shipment. The purpose of this study was to evaluate two methods of isolating *Salmonella* and *Arizona* from turtles and to compare these methods with the culture of specific organs.

**MATERIALS AND METHODS**

One hundred baby red-eared turtles (*Pseudemys scripta elegans*) were purchased from a commercial turtle breeder in southeastern Louisiana. The turtles were shipped together in a single container.

Fifty turtles were used to evaluate the excretion procedure. Each turtle was placed in a sterile 400-ml beaker containing 10 ml of sterile water. All beakers were covered with foil and held at room temperature. The turtles were not given food because young turtles do not eat for several months after hatching. After 1 week, during which the water was not changed, 1 ml of the water was pipetted from each beaker into 10 ml of tetrathionate broth (Difco) containing 10 mg of brilliant green dye per liter (TET). After the tubes of broth were incubated at 37 °C for 48 h, a 5-mm loopful of the broth was streaked on brilliant green agar (Difco) containing 80 mg of sulfadiazine per liter (BGS). The BGS plates were incubated at 37 °C for 24 h, and three lactose-negative colonies were picked to triple sugar iron agar, lysine iron agar, and Trypticase soy tryptose broth (15 g of Trypticase soy [BBL] and 13 g of tryptose broth [Difco] per liter). *Salmonella* and *Arizona* isolates were identified by methods described by Galton, Morris, and Martin (4). *Salmonella* organisms were first screened serologically in Spicer-Edwards pooled antisera (1) and then definitively serotyped (2).

The other 50 turtles were used to evaluate the blending method. Each turtle was blended in 50 ml of TET in an Oster disintegrator. The turtle was inserted through the center cap of the cover while the blender was operating at high speed (15,000 to 20,000 rpm). Although disintegration of the turtle was immediate, blending was continued for 1 min. After blending, the contents were transferred to a 300-ml jar and incubated at 37 °C for 48 h. Subsequent culturing was performed by the procedures described above.

Isolation of *Salmonella* and *Arizona* organisms from specific organs was attempted on the 50 turtles used in the excretion procedure immediately after culturing. Each turtle was killed with an intraperitoneal injection of 0.5 ml of pentobarbital sodium and was washed in a 2% iodine solution. After removing the plastron, samples of bile, liver, spleen, egg yolk, kidney, small intestine, and colon were obtained; bile was aspirated with a sterile hypodermic syringe, and the organ samples were collected in the order listed to prevent contamination from intestinal content leakage. All samples were placed in tubes containing 10 ml of TET. Dissection instruments were dipped in 70% alcohol and flamed between the collection of each sample. Cultures were processed in the manner already described.
RESULTS

Blending and excretion methods were equally effective in detecting the overall incidence of Salmonella and Arizona infection in turtles. Forty-nine turtles were positive for at least one organism by the blending method, and 48 were positive by the excretion method (Table 1). Only 36 turtles were positive when specific organs were cultured, which was a significantly lower number than by the other two methods ($P < 0.005$).

Salmonella infections were detected in 17 turtles by the blending method and 24 by the excretion method. There is no significant difference between these values. The blending method did detect a significantly greater number of turtles with Arizona infections (45 turtles) than did the excretion method (32 turtles, $P < 0.005$).

The number of Salmonella and Arizona isolations from individual organs is shown in Table 2. Salmonella and Arizona species were recovered most frequently from the small intestine and colon. One of these two organs was positive in 35 of the 36 infected turtles. Salmonella and Arizona were found with equal frequency in the small intestine and colon, but in other organs the frequency of Arizona infections was significantly higher than Salmonella ($P < 0.005$).

By specific organ culture, 19 of the turtles showed mixed infections with Salmonella and Arizona. In 10 of these 19 turtles, Salmonella and Arizona isolates were from different organs. In the remaining turtles, both organisms were recovered from the same organ, the colon.

A comparison of Salmonella serotypes isolated by the excretion and blending procedures indicated no difference in serotype selectivity by either procedure. Salmonella java and Salmonella branderup were the only serotypes isolated by both procedures (Table 3). Although Salmonella isolates from the organ cultures were not definitively serotyped, they fell into the same groups as Salmonella java and Salmonella branderup (B and C).

DISCUSSION

Although the excretion and blending procedures were equally effective in detecting Salmonella and/or Arizona infections in turtles, fewer positive results were obtained by culturing individual organs than by either the blending or excretion method. However, only a small

| Procedures | No. | Serogroup |
|------------|-----|-----------|
| Excretion  | 21  | Group C<sup>a</sup><sup>b</sup> |
|            |     | Group B<sup>c</sup> |
| Blending   | 16  | Group C<sup>i</sup><sup>a</sup> |
|            |     | Group B<sup>c</sup> |
| Organ isolations | 31 | Group C<sup>i</sup> |
|            |     | Group B<sup>c</sup> |

<sup>a</sup> All identified as Salmonella branderup.
<sup>b</sup> One sample contained both Salmonella branderup and Salmonella java.
<sup>c</sup> All identified as Salmonella java.

| Selected turtle organs | No. of organs cultured | Salmonella isolates | Arizona isolates | Mixed isolations | Total isolations |
|------------------------|------------------------|---------------------|-----------------|-----------------|-----------------|
|                        |                        | No. | %    | No. | %    | No. | %    | No. | %    |
| Yolk                   | 50                     | 0   | 0    | 3   | 6    | 0   | 0    | 3   | 6    |
| Bile                   | 50                     | 1   | 2    | 2   | 4    | 0   | 0    | 3   | 6    |
| Liver                  | 50                     | 1   | 2    | 4   | 8    | 0   | 0    | 5   | 10   |
| Spleen                 | 50                     | 0   | 0    | 4   | 8    | 0   | 0    | 4   | 8    |
| Small intestine        | 50                     | 10  | 20   | 7   | 14   | 0   | 0    | 17  | 34   |
| Colon                  | 50                     | 10  | 20   | 15  | 30   | 9   | 18   | 25  | 50   |
| Kidney                 | 50                     | 1   | 2    | 5   | 10   | 0   | 0    | 6   | 12   |

* Numbers in parentheses are percentages.

TABLE 1. Comparison of the blending, excretion, and individual organ procedures for the isolation of Salmonella and Arizona organisms from 50 turtles

TABLE 2. Number of Salmonella and Arizona isolations from selected turtle organs

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portion of each organ was cultured, thereby reducing the likelihood of detecting mild infections.

The difference in the frequency of recovery of Arizona organisms by the blending and excretion methods is probably due to pathogenic and cultural characteristics of that organism. From the organ culture data, it appears that arizonae have a greater tendency toward systemic involvement than do salmonellae. As a result, Arizona infection limited to kidney, ovary, and/or liver would be missed by the excretion technique but would be detected by the blending procedure, thus accounting for the higher rate of recovery of arizonae by the blending procedure.

Edwards and co-workers (3) noted that a high percentage of Arizona isolates from humans were from blood and localized infections in contrast to non-host-adapted salmonellae. This suggests that Arizona may be more invasive in man than non-host-adapted Salmonella, and our data indicate that this is also true in turtles.

The blending procedure detected more Arizona infections, but the blending and excretion methods appeared to be equally sensitive in detecting overall infections with Salmonella or Arizona, or both. Because current regulations condemn lots of turtles found contaminated with either Salmonella or Arizona, either of these procedures would be satisfactory. Although some systemic Arizona infections may be missed, the number of turtles having such infections without concomitant intestinal involvement would be small, and this would not allow for a significant number of infected lots of turtles to be overlooked.

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