The Moroccan Food Snail, Helix aspersa, as a Source of Salmonella

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A total of 270 samples, nine lots of 30 samples each, of imported Moroccan food snails was examined for the presence of Salmonella. Eighty-four samples (an overall incidence of 31.11%) and all nine lots contained Salmonella. No significant difference \( P > 0.25 \) in the number of positive samples was observed by using either selenite cystine broth or tetrathionate broth when the samples had been pre-enriched in lactose broth. When used as direct selective enrichments with samples not pre-enriched in lactose broth, tetrathionate broth was significantly \( P < 0.05 \) more productive than selenite cystine broth. The overall detection of Salmonella-positive samples by direct enrichment was significantly greater \( P < 0.001 \) than by pre-enrichment. A variety of uncommon serotypes representative of several somatic groups was isolated. This study reports the occurrence and incidence, and the concomitant human health potential, of Salmonella in one species of live, imported food snails.

In the past, processed foods have been the source of the majority of samples examined by the Food and Drug Administration (FDA) for the presence of Salmonella. During the past year, however, live turtles and imported frog legs have posed the largest regulatory problems with respect to Salmonella contamination. Recently, imported live food snails have been added to this list.

In this country, snails are becoming increasingly popular as a food delicacy, and in France more than 600 million land snails are consumed each year (8). Of the several varieties of edible snails available, Helix aspersa is especially favored, escargots à la bourgogne being a well-known dish in Paris. Since these snails are usually obtained from swamps and marshes, they may contain parasites and pathogenic microorganisms of which they cannot purge themselves. This study was undertaken to determine if Salmonella is a serious problem in snails, and, if so, to determine the efficiency of various analytical methods for recovering this pathogen.

MATERIALS AND METHODS

During a 6-month period, nine lots of the Moroccan food snail, Helix aspersa, imported into the port of New York, were sampled and analyzed for the presence of Salmonella. All snails were shipped from Morocco in straw hampers or baskets, and upon arrival, FDA import inspectors collected samples from 30 hampers per lot. Each sample consisted of 35 snails and was collected in a cardboard carton or plastic bag with perforations and maintained at room temperature during shipment to the laboratories in Washington, D.C. for analysis.

The live individual snails ranged from 7 to 10 g. Only whole, live snails with shells intact, usually 10 to 15 snails, were weighed into a sterile, foil-covered beaker to approximate a 100-g sample. A volume of sterile, distilled water equal in weight to the snail sample was added to a sterile high-speed blender jar containing the snail sample. This mixture was blended for 90 s at high speed (approximately 14,000 rpm). Total disintegration of the snails was usually complete in 15 to 20 s, but blending was continued to assure complete homogeneity of the mixture.

The relative efficiency of the lactose pre-enrichment and direct enrichment methods of the Association of Official Analytical Chemists (1) and the Bacteriological Analytical Manual (6) for the isolation of Salmonella from snails was determined in the following manner. Fifty-milliliter aliquots of snail homogenate were pipetted into three sterile, 500-ml Erlenmeyer flasks containing 200 ml of either 35 C.-tempered lactose broth (Difco), selenite cystine (SC) broth (Difco), or tetrathionate (TT) broth (Difco) with 10 mg of brilliant green dye per liter. The broths were made in a greater than normal concentration to compensate for the distilled water added to the sample. The final concentrations of the various ingredients of the respective broths were those prescribed by the manufacturer (Difco). The flasks were swirled to completely mix the contents, the pH was adjusted to 6.8 ± 0.2, and the flasks were placed in a walk-in incubator (35 C) for 24 h. A 1-ml portion of the lactose
pre-enrichment mixture was then subcultured to sterile tubes containing 9 ml of fresh, 35 C-tempered SC or TT broth. After an additional 24 h of incubation, the selective enrichment broths, from both the pre-enriched and directly enriched samples, were streaked with a 3-mm loop to plates of brilliant green, bismuth sulfite (Difco), and Salmonella-Shigella agars. The bismuth sulfite agar plates were refrigerated for 24 h before being used. All agar plates were incubated at 35 C and examined after 24-h incubation period. The bismuth sulfite agar plates were examined after 24 h, but were incubated an additional 24 h and re-examined. When present, at least two colonies suspicious for *Salmonella* were picked per plate in tandem to triple sugar iron agar (Difco) and lysine iron agar (Difco). Cultures giving reactions typical for *Salmonella* were screened biochemically (4). The somatic and flagellar serological grouping, as described by Edwards (3), was followed by definitive serotyping (4). Because many of the isolates in this study have not been commonly isolated from foods by FDA, confirmation of selected isolates was performed by the Enteric Bacteriology Laboratory, National Center for Disease Control, Atlanta, Ga.

RESULTS AND DISCUSSION

Of nine lots of Moroccan food snails imported into this country, all were positive for *Salmonella*, with positive samples per lot ranging from 10 to 50% (Table 1). Of a total of 270 samples, 84 were positive for *Salmonella* (31.11%).

When SC and TT were used with samples that had been pre-enriched in lactose broth, 24 *Salmonella*-positive samples were obtained with SC broth and 23 with TT broth (Table 2), which is not a significant difference (*P > 0.25*). When these selective broths were used with samples that had not been pre-enriched in lactose broth, 28 positive samples were obtained with SC broth and 65 with TT broth, this difference being significant (*P < 0.05*).

Most of the procedures in the *Bacteriological Manual* (6) for the examination of foods for the presence of *Salmonella* require the pre-enrichment of the sample in 0.5% lactose broth, a practice based on the findings of North (7). *H. aspersa* is commonly found living in marshes, swamps, and certain terrestrial habitats having deposits of limestone rock (2, 5, 9); consequently, the presence of microflora and microfauna competitive to *Salmonella* can be expected. The possibility exists that samples containing large numbers of such competitive organisms could conceivably overgrow the *Salmonella* in a nonselective medium such as lactose broth. Supporting data for this possibility are seen in Table 3. Of the 84 *Salmonella*-positive samples, nine were exclusively positive by lactose pre-enrichment followed by selective enrichment either in SC or TT broth; 48 were exclusively positive for *Salmonella* when either SC or TT broth was used without the lactose pre-enrichment step. Even though this difference is significant (*P < 0.001*), the use of both the pre-enrichment and direct selective enrichment procedures is recommended for recovery of the maximum number of *Salmonella*-positive samples. Had the examination of snails for *Salmonella* been limited to the direct enrichment procedure, nine *Salmonella*-positive samples would have gone undetected; 27 were positive for *Salmonella* by both procedures.

Table 4 shows the *Salmonella* serotypes isolated from snails. Except for *S. montevideo*, *S. bredeney*, and *S. newport*, these serotypes have not been frequently isolated from foods by FDA. One sample contained a *Salmonella* culture with the antigenic formula of 16:g,m,t:-. The decision as to whether it is a new serotype or a variant of *S. mobeni* (16:g,m,s,t:-) has not yet

### Table 1. The relative occurrence of *Salmonella* in nine lots of *H. aspersa*

| Lot no. | No. of samples analyzed | No. of samples positive for *Salmonella* | Samples positive for *Salmonella* (%) *a* |
|---------|------------------------|----------------------------------------|----------------------------------------|
| 1       | 30                     | 3                                      | 10.00                                  |
| 2       | 30                     | 15                                     | 50.00                                  |
| 3       | 30                     | 15                                     | 50.00                                  |
| 4       | 30                     | 8                                      | 26.67                                  |
| 5       | 30                     | 8                                      | 26.67                                  |
| 6       | 30                     | 11                                     | 38.67                                  |
| 7       | 30                     | 11                                     | 38.67                                  |
| 8       | 30                     | 8                                      | 26.67                                  |
| 9       | 30                     | 5                                      | 16.67                                  |

*The average is 31.11%.*

### Table 2. Comparison of various analytical methods for the recovery of *Salmonella* from *H. aspersa*

| Lot no. | No. of samples positive for *Salmonella* | SC pre-enriched | TT pre-enriched | Direct |
|---------|----------------------------------------|-----------------|-----------------|--------|
| 1       | 0                                      | 1               | 0               | 2      |
| 2       | 4                                      | 4               | 3               | 15     |
| 3       | 8                                      | 4               | 3               | 14     |
| 4       | 0                                      | 0               | 3               | 8      |
| 5       | 3                                      | 5               | 4               | 6      |
| 6       | 5                                      | 6               | 4               | 5      |
| 7       | 0                                      | 0               | 9               | 5      |
| 8       | 3                                      | 1               | 1               | 6      |
| 9       | 1                                      | 2               | 1               | 4      |

* *Brilliant green dye (10 mg of dye per liter of broth base) was added.*

Analytical
Snails have long been recognized as an integral part of several food chains (9). Surviving mainly on decaying vegetation, the snails in turn become food for fish and a large variety of wild animals. Waste material from snails plays a significant role in the organic content of the soils and waters. It is not known if Salmonella contamination is specific for H. aspersa or whether other snail species may also be affected. Due to rapidly increasing cost of importing snails, several suppliers are turning to commercial production. More studies are planned to determine if these domesticated snails, as well as the common household aquaria snails, pose a significant health hazard to humans.

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