Isolation of Salmonellae and Other Potential Pathogens from the Freshwater Aquarium Snail *Ampullaria*

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The freshwater aquarium snail (*Ampullaria* spp.) was demonstrated to carry as many as 10⁸ viable mesophilic bacteria per g of meat plus shell. Some 16 genera of bacteria were identified, with gram negatives predominating. Enrichment culture techniques enabled the isolation of salmonellae from 24 of 42 lots of 200 g each. The salmonellae comprised eight different serotypes, including *Salmonella newport*, *Salmonella saint-paul*, and *Salmonella infantis*. This association of salmonellae with snails may contribute to cases of human salmonellosis, since other aquarium species have already been shown to contribute to many such cases. The snails were also found to commonly harbor *Pseudomonas aeruginosa* and, occasionally, *Edwardsiella tarda*.

Organisms of the genus *Salmonella* are among the most widely distributed pathogens in nature, and *Salmonella* infection is widespread in humans and other animals throughout the world (4, 18). Because of its universal incidence, its diverse manifestations, and its complex epidemiology, salmonellosis now constitutes a serious threat to the health of humans and other animals (18, 25). The problem is of such magnitude that, in spite of numerous publications on the salmonellae and their effects on various hosts, few countries can assess accurately the annual toll of human illness or economic loss of livestock as a result of these infections (18). To eradicate such an infection, it is necessary to identify the main reservoirs and vehicles of *Salmonella* and to break the links in the chain of transmission of the organism to humans.

Recently, there has been considerable publicity about the public health risks associated with the sale of small pet aquarium turtles and tortoises. One estimate puts the number of cases of human salmonellosis associated with these pets at 300,000 per annum in the United States (27). In Canada, the association of salmonellae with these species has resulted in laws banning the importation of aquarium turtles and tortoises. In the United States, the Food and Drug Administration has also banned the interstate sale of these animals.

For the past 2 years, this laboratory has been examining the bacterial population of other aquarium species (23) and aquarium diets (24). Recently, we turned our attention to the freshwater aquarium snail (*Ampullaria* spp.), since it has been observed that edible snails obtained from swamps in Morocco for consumption in North America carry with them *Salmonella* species (2). Significant numbers of aquarium snails are sold to the public, and, if carrying salmonellae, these snails may present a public health risk similar to that presented by the aquarium turtles. The present study therefore was designed to determine the microflora associated with the common aquarium snail, to quantitate their bacterial load, and to demonstrate the presence or absence of salmonellae in this microflora.

**MATERIALS AND METHODS**

Snail samples. A total of 49 samples of snails were purchased from 33 retail outlets in Duncan, Nanaimo, Port Alberni, Vancouver, and Victoria, British Columbia. Snails were supplied in plastic containers containing water taken from the aquarium in which the snails had been housed. Samples were transported to the laboratory and assayed immediately. Snail samples weighed from 3.2 to 50.6 g, with a mean weight of 8.2 g. An additional 42 lots of 200 g each were tested. These snails were obtained directly from a supplier in Florida.

Quantitative bacteriological examination. Samples were diluted with iced 0.1% (wt/vol) peptone water (pH 7.2) and homogenized in a sterile blending jar at high speed six times, for 30 s each time. To ensure that overheating did not occur, the diluted sample was cooled in an ice bath between homogenizations. Duplicate dilutions were prepared in 0.1% peptone water, and the viable mesophilic bacteria present were enumerated on Trypticase soy agar (Baltimore Biological Laboratories [BBL]), MacConkey agar (BBL), and pseudocel agar (BBL) by the drop-plate method of Miles and Misra (16).
Inoculated Trypticase soy agar was incubated aerobically and anaerobically at 20, 30, or 37 C for 48 h, whereas inoculated MacConkey agar and pseudocel agar were incubated aerobically at 37 C for 48 h. Anaerobiosis was obtained by using anaerobic jars with disposable hydrogen-carbon dioxide generator envelopes (BBL).

The procedures of the American Public Health Association, Inc. (1) were followed for most-probable-number estimates. Presumptive coliforms were estimated by the 10:1:0.1 multitube method by using MacConkey broth (BBL) (8, 27) and were confirmed by separate loop transfer from positive presumptive coliform tubes to MacConkey broth and incubated at 44.5 ± 0.5 C. The most-probable-number estimation of enterococci was carried out in ethyl violet azide broth (BBL) (14) and was confirmed by separate loop transfers to fresh ethyl violet azide broth.

Qualitative bacteriological enumeration. A representative of all the colony types appearing on plates containing 30 to 50 colonies was selected. Three different persons were used to pick colonies to further reduce selection bias. The isolates were then purified and maintained by weekly transfer on Trypticase soy agar and storage at 4 C. Selenite-F broth (BBL) and tetradionate-brilliant green broth (27) were also inoculated with samples and incubated at 37 and 41.5 C. After 48 h the Selenite-F and tetradionate-brilliant green broths were streaked onto Salmonella-Shigella agar (BBL), brilliant green agar (BBL), and bismuth sulfite agar (BBL). Similarly, cetrimide broth was inoculated and incubated at 42.5 C and then streaked onto pseudocel agar (BBL). The tests used to characterize the isolates were those previously described (23) and were obtained from Edwards and Ewing (9), Skerman (21), and Smith et al. (22). Final identification of the isolates was based on the schemes of Buchanan and Gibbons (5), Shewan et al. (20), Bain and Shewan (3), Cowan and Steele (7), Hugh (11), Edwards and Ewing (9), Weaver et al. (26), and Lewis (13). Separation of Vibrio species from the otherwise Vibrio-like anaerogenic aeromonads and plesiomonads was facilitated by testing for acetoin production, sensitivity to compound 0/129, fermentation of inositol, liquefaction of gelatin, and decarboxylation of lysine (10). Identification within the Enterobacteriaceae was confirmed by using the API system (Analytab Products, Inc., New York). The serotypes of isolates biochemically identical to salmonellae were determined with "O" and "H" antisera (BBL) and confirmed serologically by the National Enteric Reference Laboratory.

RESULTS

Quantitative bacteriological examination. The results in Table 1 show that snails are populated by as many as 10^6 viable mesophilic bacteria per g of meat plus shell. The mean counts obtained when plated dilutions were incubated at 20, 30, or 37 C were similar, as were the numbers of bacteria recovered when plates were incubated anaerobically. As many as 10^6 of these organisms were capable of growth in the presence of cetrimide at 37 C (pseudocel agar), although the majority of these organisms did not produce pyocyanin, suggesting that they were not Pseudomonas aeruginosa. Typical pigment-producing strains of this organism were, however, isolated on occasion from dilutions as high as 10^4. Some 10^5 of the organisms present were capable of growth in the presence of bile salts (on MacConkey agar), suggesting a likelihood of fecal origin for many of the organisms. Of these, as many as 10^6 were lactose fermenters.

The results of the most-probable-number assays performed on the snails are shown in Table 2. Using this technique, as many as 10^6 completed coliforms per 100 ml were demonstrated, and countable numbers of coliforms were present in all samples tested. As many as 10^9 of these organisms were fecal coliforms, which were recovered from 6 of the 23 samples tested. All samples tested contained countable enterococci with numbers as high as 10^9/100 ml.

Qualitative bacteriological examination. Some 727 of the most numerous species were characterized, and the results are shown in Table 3. One of the most common genera isolated was Pseudomonas. The majority of the

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<th>Incubation conditions</th>
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<td>Mean</td>
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<td>20 C, TSA</td>
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<td>37 C, PA</td>
<td>26</td>
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<td>37 C, Mc (total)</td>
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<td>37 C, Mc (lactose +)</td>
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<td>Anaerobic incubation</td>
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a TSA, Trypticase soy agar; PA, pseudocel agar; Mc, MacConkey agar.
b TFC(n), Too few organisms to count in (n) samples.
pseudomonads were nonpigmented species; however, typical pyocyanin-producing *P. aeruginosa* was isolated from 28 samples, whereas fluorescent strains were isolated on 14 occasions. The most common aeromonad isolated was *Aeromonas hydrophila*, recognized in 19 samples.

The *Enterobacteriaceae* were well represented and comprised 69% of the isolates. A large number of *Citrobacter* were characterized, *C. freundii* on 244 occasions and *C. diversus* on 76 occasions, in part due to the fact that H₂S-producing colonies on *Salmonella-Shigella* agar plates were closely examined to determine the presence of *Salmonella*. *Proteus* species were identified in 62 samples and included *P. mirabilis*, *P. morganii*, *P. rettgeri*, and *P. vulgaris*. *Escherichia coli* was observed in 20 samples, and in 21 samples containing *Enterobacter* 68% were *E. cloacae* and 32% were *E. hafniae*.

No *Salmonella* were isolated from the first 49 samples of snails (average weight, 8.2 g). However, when the sample size was increased *Salmonella* was readily isolated. When an additional 42 lots of 200 g each were processed by the regular procedures, *salmonellae* were isolated from 24 lots. These *salmonellae* were shown to belong to eight different serotypes (Table 4). It should be noted that more than one serotype per sample was encountered in 10 of the samples tested. In addition to these *salmonellae*, *Edwardsiella tarda* was identified on 10 occasions.

Gram-positive identifications include 30 isolations of enterococci and 29 isolations of clostridia, which included *Clostridium bifermens*, *C. botulinum*, *C. butyricum*, *C. fallax*, *C. glycolicum*, *C. perfringens*, *C. sordellii*, and *C. subterminale*.

**DISCUSSION**

This study has revealed a previously unreported zoonotic reservoir of *salmonellae*. This disquieting finding has obvious public health implications since it seems likely that the snails contaminate other aquarium species, as well as the water and hardware of aquaria, with these pathogenic bacteria. There is reason to believe that this association of *salmonellae* with snails will contribute to cases of human salmonellosis, since other aquarium species, namely, the small terrapins, have already been shown to contribute to many cases of human salmonellosis. It would seem desirable that regulatory action be taken to control the international and intranational shipment and subsequent sale of aquarium snails unless they can be certified *Salmonella*-free. At the very least, those persons handling large shipments of these invertebrates must be made aware of the possible risk of infection.

Various agencies, including the World Health Organization, are becoming concerned with the increasing incidence of *Salmonella* infections worldwide. Of the *salmonellae* detected in the current investigation, *Salmonella newport*, *S. saint-paul*, and *S. infantis* are among the 10 most frequently isolated serotypes from human sources (6); however, increasing numbers of new serotypes are also being associated with human infections. It is
worth noting that the biochemical characteristics and serological properties of a number of these isolates were demonstrated to be unique, and a complete description of these and other unique salmonellae isolated from aquarium sources will appear in a subsequent report.

The present investigation has revealed that aquarium snails are also carriers of *E. tarda*. This species is capable of producing disease patterns similar to those caused by *Salmonella* (12) as well as other diseases of humans and other animals (19). The association of *P. aeruginosa* with aquarium snails may also have public health significance. This is the first report of such an association, and the findings point to the aquarium as being another source of nosocomial infections with this species. Although it is hard to quantify the numbers of aquaria present in hospitals and rest homes, it is certainly not uncommon to find aquaria in pediatric wards, and in less careful hospitals aquaria are even found in burn wards. These aquaria are maintained by hospital staff, presenting the opportunity for these workers to come into direct contact with *P. aeruginosa*. If adequate disinfection procedures are not followed after contact with aquaria, a chance certainly exists for the spread of this potential pathogen to patients. The same can be said for doctors’ and dentists’ offices. It is important to note that since the snails will contaminate the aquarium water direct contact with the snails is not necessary, and simple contact with the water or aquarium hardware will suffice. In fact, the potential often exists for a more widespread distribution of the organism from the aquarium by aerosol formed by the action of the aquarium aerator. Because of this potential public health risk posed by *P. aeruginosa*, studies have been initiated to type isolates of this organism from aquarium sources to determine whether they include types known to be associated with human infections.

The other bacterial species found to be associated with the snails were similar to those found in water containing ornamental fish and small pet green turtles, although the total numbers of these bacteria on the snails were generally higher than those present in the water in these other studies. In terms of human infection, most of these organisms can be regarded as nonpathogens. However McCoy and Seidler (15) have suggested that the possibility of human infections from some of these species should not be overlooked. For example, there are increasing numbers of reports of human infections in which fluorescent pseudomonads, aeromonads, and *Citrobacter* and *Enterobacter* species are implicated. These infections include genito-urinary tract infections, enterocolitis, and diarrhea in children and bacteremia and septicemia in debilitated persons. All these species are present in significant numbers on the aquarium snails examined.

The methods used for commercial production of aquarium snails facilitate their accumulation of large numbers of microorganisms, since these invertebrates breed and feed on mud flats. If these snails are to continue to be offered for sale to the public, studies must be initiated to determine the procedures necessary to rid them of pathogenic microorganisms.

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**LITERATURE CITED**


