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Small Pet Aquarium Frogs as a Source of *Salmonella*

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Salmonellae were isolated from 21% of the samples of freshwater aquarium frogs tested and from 25% of the samples of aquarium water containing these frogs. The salmonellae were *Salmonella arizonae*, *S. bovis-morbificans*, *S. hadar*, *S. saint-paul*, *S. typhimurium*, and *S. worthington*. These isolations were made over a period of 9 months and from three different cities. This association of salmonellae with frogs may contribute to cases of human salmonellosis since other aquarium species have already been shown to contribute to such cases.

Recently, considerable attention has been focused on the carriage of salmonellae by small pet turtles and tortoises (12). It was demonstrated (12) that cases of human salmonellosis were caused by these associated salmonellae, which suggests that the association of salmonellae with other ornamental aquarium species could constitute a hazard to those who transport, sell, or purchase such animals. More recently, it was found that *Salmonella* carriage by ornamental aquarium species was not restricted to turtles, but that aquarium snails are also reservoirs of salmonellae (1).

Other studies have established that feral frogs, as well as frogs legs destined for human consumption, can carry salmonellae (7). Small frogs are also imported into North America from Africa and South America to be sold to the public as pets for ornamental aquaria. The present study was undertaken to determine whether these aquarium frogs also represent a source of salmonellae and so pose a public health risk.

MATERIALS AND METHODS

The frogs studied were all small frogs sold as aquarium-housed pets (including *Xenopus* spp., *Hynochirus* spp., and *Pipa pipa*) and were purchased from 16 retail outlets in Victoria, Sidney, and Vancouver, British Columbia, Canada. The survey included all of the stores stocking these animals in each of the above-mentioned cities. The frogs were packaged by the retailer in clean, new, plastic bags marketed for the transportation of aquatic pets. Water taken from the aquaria in which the frogs had been housed was included to provide an aqueous environment during the transfer. The frogs taken for sampling were the only species contained within single aquaria in each retail outlet sampled. Samples reached the laboratory within 18 h of purchase. Immediately upon arrival at the laboratory, frogs and water were subjected to the assay procedures outlined.

Quantitative examination. When the samples arrived at the laboratory, 250-ml volumes of water were transferred to an iced, sterile container to wait subsequent testing. The frogs constituting each sample were weighed in petri dishes. Each water sample reported represents water from one aquarium, and each frog sample represents an average of five frogs, with an average sample weight of 3.9 g (range, 17.8 to 1.2 g), from the same aquarium.

Duplicate dilutions of the water samples were prepared in 0.1% (wt/vol) peptone-water (pH 7.2), and the viable mesophilic bacteria present were enumerated on Trypticase soy agar (TSA, Baltimore Biological Laboratories [BBL]) by the drop plate method of Miles and Misra (5). Inoculated media were incubated aerobically and anaerobically at 37°C for 48 h. Anaerobiosis was obtained by using catalytic anaerobic jars with disposable H_2-CO_2 generator envelopes (BBL). Dilutions were also enumerated on MacConkey agar (BBL) after incubation at 37°C for 24 h. Numbers of lactose-fermenting organisms were noted.

Qualitative examination. Frog and water samples were separately examined for the presence of salmonellae. Portions (10 to 20% by volume) of the water were inoculated into freshly prepared selenite-F broth (BBL) and tetrathionate broth (BBL) to which 10 mg of brilliant green per liter had been added (12). Duplicate sets of inoculated media were incubated, one at 37°C and the other at 41.5°C. The broths were plated at 18 and 48 h and subcultured to fresh selenite-F broth or tetrathionate broth at 18 h. Plates of salmonella-shigella agar and of bismuth sulfite agar (BBL) were streaked at each sampling and incubated at 37 and 41.5°C for 48 h. Representative colonies from plates displaying growth were transferred to TSA for purification prior to identification and were stored on TSA slants at 4°C.

Frogs were anaesthetized by exposure to cold and then placed in a sterile blender jar with sufficient

iced peptone-water to produce a 1:10 (wt/vol) dilution. Homogenization was obtained in 3 min, blending for 30-intervals. To ensure that overheating did not occur, the homogenate was cooled in an ice bath between mascerations. Portions (10 to 20% [wt/vol]) of the homogenate were inoculated into selenite-F broth and tetrathionate-brilliant green broth and examined as described for the water samples.

Colonies capable of growth on the selective agars used were screened on the basis of lactose utilization and the ability to decarboxylate lysine to separate *Salmonella* and *Edwardsiella* from late-lactose-positive and late-lactose-negative *Citrobacter*. Isolates were further characterized by their colonial morphology and pigmentation, as well as by the shape, arrangement, staining characteristics, flagellar arrangement, and motility of cells. Biochemical tests used to characterize the isolates were those described by Edwards and Ewing (3), Skerman (8), Smith et al. (9), and Weaver et al. (11). Isolates conforming to the *Salmonella-Arizona* group were serotyped in agglutinating O and H sera (BBL) and subsequently serologically characterized more fully at the Laboratory Centre for Disease Control, Ottawa.

RESULTS

A total of 493 colonies were isolated and identified. The normal mesophilic flora was evaluated initially and found to be similar to the microflora found in previous aquatic samples examined (1, 10). In addition to the aerobic and facultative isolations of *Alcaligenes* sp. (1), *Acinetobacter* sp. (8), *Aeromonas hydrophila* (10), *Chromobacterium violaceum* (1), *Flavobacterium* (3), *Pseudomonas aeruginosa* (11), *P. fluorescens* (3), *Pseudomonas* sp. (8), and *Vibrio* sp. (1), the anaerobic isolations included *Clostridium* sp. (3), *Bacteroides* sp. (8), and *Fusobacterium* sp. (1). Enrichment techniques were employed for the isolation of salmonellae. The 435 colonies picked for identification from selective agar plates are shown in Table 1. The most-common isolate found in all samples tested belonged to the genus *Citrobacter*. Salmonellae were identified on 115 occasions and comprised 26% of the enteric isolates. The salmonellae were present in 21% of the frog samples and 25% of the water samples. *Edwardsiella tarda* was identified on four occasions. The 15 isolates of *Enterobacter hafniae* were all lactose negative but weakly *o*-nitrophenyl-β-D-galactopyranosidase positive.

The aquarium water was demonstrated to have from 6×10^6 to 8×10^7 viable aerobes per ml (average, 6.0×10^7; TSA; 37°C) and from 2×10^7 to 8×10^7 organisms capable of anaerobic growth per ml (average, 5×10^7). Of these, an average of 5×10^7 organisms per ml were capable of growth on MacConkey agar, with 8×10^5 lactose fermenters. The homogenized frog samples contained 7×10^5 to 1×10^9 aerobes per g of tissue (average, 3.0×10^7) and 6×10^6 to 2×10^7 mesophiles capable of anaerobic growth (average, 1.5×10^7).

TABLE 1. *Frequency of isolation of Enterobacteriaceae from pet frogs and aquarium water containing frogs*[a]

Species isolated	No. of isolates	No. of samples from which isolated[b]	
		Water	Frogs
Citrobacter diversus	29	7	6
C. freundii	249	20	19
Edwardsiella tarda	4	2	2
Enterobacter cloaceae	6	3	2
E. hafniae	15		4
Klebsiella pneumoniae	10	2	2
Proteus	7	2	2
Salmonella	115	5	4

[a] Isolations were made from bismuth sulfite agar and salmonella-shigella agar.

[b] Total sample number comprised 20 samples of aquarium water and 19 samples of frogs.

The salmonellae isolated belonged to six serotypes as shown in Table 2. The most-common serotype isolated was *S. typhimurium*. Fifty-five of these isolates from two sources were aerogenic strains, phage type 3. *S. hadar* and *S. bovis-morbificans* were also frequent isolates. Only a single serotype was isolated from an individual sample, and the same serotype was isolated from both the frog tissue and the related aquarium water samples.

DISCUSSION

This study has revealed another reservoir and vehicle for salmonellae and suggests that small pet frogs present a risk to the public health. The isolation of salmonellae from the frogs over a period of 9 months and from three different cities suggests that this public health risk may be widespread. The frogs were shedding these pathogens, and the salmonellae were readily recoverable from the water containing the animals as well as from the frog tissues. The organisms were isolated from 2-g samples of frog tissue, in contrast to the 200-g samples needed in the case of aquarium snails (1). Each positive sample in the current study yielded only one serotype, which differed from the snails where more than one serotype was present per sample. The salmonellae isolated from frogs did not include the unusual serotypes previously found in snails (1) and differed from those found by Sharma et al. (7) in wild Indian frogs.

The serotypes isolated from pet frogs in-

TABLE 2. *Frequency of isolation of salmonellae from Hynochinus sp. and aquarium water housing frogs*

Serotype	Source of isolate	No. of isolates	No. of samples from which isolated	Enrichment sequence[a]			
				TBS-SS	TBS-BS	Sel-SS	Sel-BS
S. arizonae (Ar. O26:24-25)	Water[b]	4	1			4	
S. bovis-morbificans (6,8:r:1,5)	Water	20	1	6	5	6	3
S. hadar (6,8:z_{10}:e,n,x)	Water,	12	1			10	2
	Frog[c]	15	1			10	5
S. saint-paul (4,12:e,h:1,2)	Water	4	1			4	
S. typhimurium (4,5,12:i:1,2)	Water,	22 and 7	2			13 and 7	9
	Frog	28	1			14	14
S. worthington (1,13,23:z,l,w)	Frog	7	1	3	4		

[a] TBS, Tetrathionate brilliant green broth; SS, salmonella-shigella agar; BS, bismuth-sulfite agar; Sel, selenite-F broth.
[b] Number of water samples tested = 20.
[c] Number of frog samples tested = 19.

cluded such common species as *S. saint-paul* and *S. typhimurium*; this latter serotype is the most-common isolate in Canada as in most countries. *S. hadar*, a serotype rare to Canada, was also isolated. This serotype was first reported in 1971 from a human source in Ontario. Since then, only four additional isolates have been obtained from humans and one from a river in North Saskatchewan. These five isolates were obtained in 1975 and were all dulcitol negative. The *S. hadar* isolated in the current study was dulcitol positive. The dulcitol reaction of the initial isolate in Ontario was not reported.

With respect to the other bacterial species isolated in this investigation, the finding of small numbers of *E. tarda* associated with some of the frog samples reinforces the findings of Sharma et al. (7). These workers found this potential pathogen to be associated with frogs and suggested that frogs may be treated as suspects in the transmission cycle of *Edwardsiella*. As with the other aquarium samples and species studied (2, 10), the predominant bacterial species isolated was *Citrobacter freundii*.

The ability to isolate salmonellae from the aquarium water is disquieting, since their presence in the water is likely to facilitate continuing distribution of the pathogens from the aquarium to the environment. The organisms have been shown to multiply in the bottom sludge of rivers, lakes, and streams (4) and are likely to do the same in the sand at the bottom of the aquarium, and so contribute to the numbers of salmonellae in the water. The aquarium water will, in fact, represent a dilute liquid culture of a human pathogen. This will obviously present a health risk in the home since the aquarium needs cleaning and servicing, and the water is changed on occasion. It is not uncommon for the water to be poured down a kitchen sink, and this potentially allows the organisms an opportunity to contact foodstuffs in a kitchen.

There are many possibilities for the spread of these pathogens within a retail outlet such as a pet store, especially since workers in such stores do not employ, nor can be expected to employ, aseptic techniques. The pathogen could be picked up by direct physical contact or indirectly via aquarium hardware such as dip nets, aquarium filters, etc. The workers handle other pet animals such as dogs, cats, birds, and guinea pigs, and salmonellae could thus be transferred to these animals. The chance certainly exists for persons working in pet stores or handling the frogs to become carriers of salmonellae. There is even a risk to shoppers in the vicinity of the aquaria. The action of aquarium aerators generates an aerosol, and this represents another possible mode of transmission for salmonellae. It is interesting that aerosols containing salmonellae have been demonstrated to be capable of infecting mammals, and the infective dose in such cases is only 27% of the number necessary via the gastrointestinal tract (6).

Although epidemiological studies have yet to implicate these small pet frogs as a source of human infection, public health officials should be aware that these amphibians can carry salmonellae. Persons handling large shipments of

these animals must be warned of the possible risk of infection. In pet stores, persons handling frogs should wash their hands before dealing with the public. In the home, children should not be allowed to handle frogs or the aquarium water and hardware unless they are responsible enough to wash their hands afterwards. Frog water should not be discharged into the kitchen sink or allowed to contaminate the food preparation area. Only one person who is careful to wash his hands should care for the frogs, and this person should not look after other household pets. The current study suggests that it may become desirable to include frogs in the list of species required to be certified *Salmonella*-free before international and intranational shipment and subsequent sale to the public.

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