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Enteric pathogens in tropical aquaria

By D. SANYAL, S. H. BURGE

Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff CF4 4XW

AND P. G. HUTCHINGS

City of Cardiff Environmental Health Department, Caerwys House, Windsor Lane, Cardiff CF1 3LA

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SUMMARY

A total of 100 tropical aquaria from six pet shops were sampled for the presence of *Salmonella*, *Campylobacter* and *Aeromonas* species. Eight fish tanks contained unusual serotypes of salmonella. None had been used to house terrapins. One hundred and three isolates of *Aeromonas* species were obtained from 98 tanks. One tank contained *Plesiomonas shigelloides*. No *Campylobacter* species were isolated. It is suggested that a tropical aquarium may constitute an infection hazard.

INTRODUCTION

The association between salmonellas and terrapins is well documented (Leading Article, 1981; Cohen *et al.* 1980). Numerous outbreaks in humans have been described in this and in other countries (Gumsley, 1968; Williams & Helsdon, 1965; Fujita, Murono & Yoshioka, 1981). The causative organism has often been *Salmonella java* (Orton & Henderson, 1972; Leading Article, 1981). In addition, *Campylobacter* and *Aeromonas* species have been isolated from cold-blooded animals (McCoy & Seidler, 1973; Harvey & Greenwood, 1985). We are not aware of any similar study involving tropical fish. *Salmonella* spp. are known to contaminate fresh fish intended for human consumption (Gulasekharam, Velaudapillai & Niles, 1955). Experimental infection of freshwater carp with *Salmonella enteritidis* has also been described (Heuschmann-Brunner, 1974). With this in mind, we investigated 100 aquaria from pet shops in Cardiff for the presence of *Salmonella*, *Aeromonas* and *Campylobacter* species. The aquaria all contained tropical warm-water fish which were maintained at an average temperature of 25 °C. None of the tanks selected was used to contain terrapins. Tropical fish require an acid pH (approx. 6–7), whereas terrapins prefer alkaline conditions. Samples of fish-feed were also tested for the presence of enteric pathogens.

MATERIALS AND METHODS

The survey lasted from October 1986 to February 1987, during which time environmental health officers obtained 300 ml samples from 100 tropical aquaria

in six pet shops. None of the tanks was resampled. Specimens were submitted by the environmental health officers to the laboratory within 6 h of sampling.

A questionnaire was also completed for each specimen detailing species of fish, frequency of water changes in the tank, use of cleaning additives, temperature and pH of water.

Samples were examined by the membrane filtration method (DHSS, 1982). Each 300 ml sample was split into three aliquots of 100 ml. Each aliquot was then passed through a membrane filter (pore size 0·45 μl) and treated as below. No attempt was made to assess the presence of organisms quantitatively.

Salmonella *species*

The membrane was placed in 25 ml of buffered peptone water and incubated overnight at 37 °C. Ten microlitres in a standard loop was then subcultured into Rappaport's selective medium and incubated overnight at 37 °C. Further subcultures were made on to Brilliant Green MacConkey agar after 24 and 48 h incubation.

Non-lactose-fermenting colonies were tested by slide agglutination with salmonella O and H antisera, and further biochemical analysis was carried out with the API 20E system (API Laboratory Products Ltd, Basingstoke, Hants). Serological analysis was completed at the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale.

Aeromonas *species*

The membrane was placed in 25 ml of alkaline peptone water and incubated overnight at 37 °C. Subcultures were made on to Xylose Desoxycholate-citrate agar at 24 and 48 h. Non-xylose fermenters were then subcultured on to blood agar and subjected to a battery of tests (including an API 20E) for identification and speciation of aeromonas (Lee & Donovan, 1985).

Campylobacter *species*

The membrane was placed in 20 ml of Preston broth (Bolton & Robertson, 1982) and incubated at 43 °C. Subcultures were made at 24 and 48 h on to Skirrow's selective agar (Skirrow, 1977).

RESULTS

All the aquaria samples were found to contain *Salmonella* or *Aeromonas* species. The organisms were present in moderate numbers after 24 h incubation in enrichment medium. *Campylobacter* sp. was not isolated. Salmonella serotypes were isolated from 8 tanks and aeromonas from 98 tanks. Six aquaria contained both salmonella and aeromonas. Five tanks contained two species of *Aeromonas*, making a total of 103 isolates. One tank also contained *Plesiomonas shigelloides*.

None of the fish-feeds yielded potential enteric pathogens. The salmonella isolates were unusual. The serotypes identified are only rarely found in humans. The same serotype was usually found in more than one tank. No particular species of fish was especially associated with these isolates. The serotypes were unrelated to *Salmonella java* and are not generally associated with terrapins (Table 1).

Table 1. *Salmonella isolates*

Type of fish	Salmonella
Oneline Tetras *Hemigrammus unilineatus*	*S. haifa*
Spotted Barbs *Barbus bipotatus*	
Gouramies *Osphronemus goramy*	*S. haifa*
Zebra Danios *Brachydanio rerio*	*S. haifa*
Corydoras *Corydoras bondi*	
Swordtails *Xiphophorus helleri*	*S. javiana*
Catfish Callichthys callichthys	*S. javiana*
Keyhole cichlids *Aequidens maroni*	*S. wandsworth*
Oscar cichlids *Astronotus ocellatus*	*S. wandsworth*
Guppies *Poecillia reticulata*	*S. litchfield*
Angelfish *Pterophyllum scalare*	

Table 2. *Number of isolates from different shops*

Shop	No. of tanks sampled	Salmonella	*A. hydrophila*	*A. sobria*	*A. caviae*
A	24	5 (21%)	12	8	8
B*	8	2 (25%)	3	2	3
C	21	1 (5%)	10	8	5
D	17	—	11	10	2
E	11	—	8	4	1
F	7	—	5	3	0
	100	8	49	35	19

* One isolate of *Plesiomonas shigelloides* also obtained at this shop.
Figures in parentheses represent the percentage of tanks positive for salmonella.

The finding of aeromonas was virtually universal in aquaria from all six shops. The frequency of isolation of aeromonas was independent of that of *Salmonella* species. Of the 103 isolates of aeromonas, 49 (48%) belonged to *A. hydrophila* species, 35 (43%) were *A. sobria* and 19 (18%) were *A. caviae*. These results are shown in Table 2.

The efficacy of the three methods that were used to ensure the cleanliness of the tanks was compared. The methods were: (i) simple topping-up of tanks without the use of biocides or filters (shops A and B); (ii) a fortnightly water change with the use of a biocide but not a filter (shop C); and (iii) a weekly water change with the use of a biocide and a filter (shops D, E and F). Table 2 shows that salmonellas were not isolated from the shops in which the third system was used.

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DISCUSSION

Salmonella organisms have been isolated from fresh fish (Floyd & Jones, 1954; Gulasekharan, Velaudapillai & Niles, 1955) obtained from markets and intended for human consumption, but there are no records of similar isolation from fish kept as pets.

That none of the fish feeds was contaminated with pathogens testifies to the efficacy of the pasteurization process. It also indicates that potential pathogens in tropical fish are probably endogenously derived, presumably from the intestinal flora.

Salmonella spp. are not pathogenic in fish but may have a saprophytic role. These fish may be caught in conditions where they are affected by human and animal pollution in shallow seas or lagoons.

Fish living in such waters can thus acquire human and animal faecal pathogens (Guélin, 1952). The presence of the same serotype in more than one aquarium (as occurred in shops A and B), could be due either to cross-contamination via the tank water or be due to a common source of infection of many fish in their original habitat.

Salmonellas have been shown to persist experimentally in fish for up to 38 weeks (Heuschmann-Brunner, 1974). Apparently culture-negative fish may be latent carriers and become culture-positive during the stress of transport. This has been demonstrated in turtles (Duponte, Nakamura & Chang, 1978).

Although there was an appreciable number of salmonella isolates (8%), they were only obtained from enrichment and were therefore present, initially, only in small numbers. This source probably does not constitute as great an infective hazard to man as does *Salmonella java* in terrapins. Terrapins may be allowed to roam free in the house and may be handled by younger members of the family. Tropical fish are kept in an enclosed tank and probably only pose a risk when the tank is being cleaned or the water changed. The staff at shops A and B were screened for salmonella but none was found. Nevertheless, the PHLS Communicable Disease Surveillance Centre are aware of at least one outbreak of salmonellosis where tropical fish were implicated (S. E. Young, personal communication). Although the serotypes isolated in this study were uncommon, they have been implicated in human infections. Further outbreaks from this kind of source may have gone unnoticed, especially if a common serotype was implicated.

The comparison of hygienic practices, though a very small study, suggests that the use of a biocide and a biological filter will at least control the salmonellas in the tank water and may presumably prevent or reduce the potential spread from fish to fish. Whether there is any longer-term effect on the carriage in fish is not known. It would seem reasonable to recommend the regular use of water changes, additives and filtration.

Aeromonas has previously been isolated from a wide variety of aquatic sources (Hazen, Fliermans & Hirsch, 1978), and also from many fish (Austin & Allen-Austin, 1985). Its presence in 98% of the aquaria examined is therefore not surprising. Most of the isolates were *Aeromonas hydrophila*. This species has been most frequently observed in fresh and sea water (Austin & Allen-Austin, 1985). *Aeromonas hydrophila* may be a pathogen in fish, causing loss of stock especially during the stress of intercontinental transport.

Aeromonas spp. may be found in patients with diarrhoea (Gracey, Burke & Rockhill, 1982; Motyl & Janda, 1983), and with septicaemia in immunocompromised patients (McCracken & Barkley, 1972). *Aeromonas hydrophila* and *A. sobria* are believed to be pathogenic, especially the latter (Wilson *et al.* 1985), but *A. caviae* is probably not (Millership, Barer & Tabaqchali, 1986). Of the aeromonas isolates, 82% belonged to *A. hydrophila* and *A. sobria* species. *Aeromonas hydrophila* has been isolated from turtle water suspected of causing gastroenteritis in a small child (Janda, Bottone & Reitano, 1983). Infection with this organism has also caused tenosynovitis following a fish tank-associated abrasion in a child (Warrier *et al.* 1984).

This study has shown that *Salmonella* and *Aeromonas* species can be found in tropical fish tanks. As sales of tropical fish are increasing greatly, it is suggested that more attention should be paid to cleaning the water in the tanks and that the possibility of human infection arising from such tanks should be borne in mind.

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