Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003–04

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Abstract

Antibiotic resistant *Salmonella* infections are rare in Australia. We investigated an increase in multi-drug resistant *Salmonella* Paratyphi B biovar Java (*S.* Java) infections in Australia during 2003–04. Eighty-two per cent (18/22) of *S.* Java cases enrolled into the study reported that they had been in contact with aquariums housing fish during their incubation period. Seventy-two per cent (13/18) of cases were infected with strains that were resistant to ApSmTcCmSuSp (ampicillin, streptomycin, tetracycline, chloramphenicol, sulfonamides, spectinomycin). Case households commonly reported high risk behaviours, such as cleaning aquaria in sinks. Sixty-one per cent (11/18) of cases reported that fish in their aquarium had been sick or died in the week prior to their illness, and *S.* Java was isolated from the water or gravel of 5 cases. These antibiotic strains are being spread internationally and may become endemic in countries importing tropical fish or result in transfer of resistance to other more common *Salmonella* serotypes. *Commun Dis Intell* 2006;30:222–227.

Keywords: Salmonella, *paratyphi, antibiotics, aquariums, fish, reptiles, zoonoses*

Introduction

Salmonella enterica is an important cause of human illness throughout the world. While *Salmonella* is predominantly transmitted via contaminated food, infected animals are also important sources of infection. *Salmonella* infection in people who have been in contact with reptiles is well recognised.[1,2] Public health investigators have also recorded instances where patients infected with *S.* Paratyphi B biovar Java infection have had an aquarium housing tropical fish in their home.[3,4,5] In Australia, *Salmonella enterica* subspecies *enterica* serotype Paratyphi B biovar Java (*S.* Java) has been isolated from the faeces of ill people and water and gravel samples taken from their tropical fish tanks (D Lightfoot, Microbiological Diagnostic Unit, unpublished data).

During 2003, we observed an increase in human infections of *S.* Java acquired within Australia, and particularly strains that were resistant to several antibiotics. To determine the causes for this increase, we conducted a case series investigation of all infections reported to Australian health departments between March 2003 and April 2004.

Methods

The aim of this case series was to identify environmental and behavioural risk factors for *S.* Java infection acquired in Australia. A case of *S.* Java was defined as a person who had:

- a diarrhoeal illness;
- *S.* Java isolated from a stool culture;
- not travelled in the week prior to illness; and
- not been a case in a recognised outbreak.

Cases of *S.* Java infection were identified through state- and territory-based registers of patients infected with conditions reported under public health legislation.

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Epidemiologists conducted telephone interviews of cases using a standardised questionnaire. Investigators interviewed cases aged between 10 and 18 years old with guardian consent. A surrogate, usually a parent, was interviewed for cases under 10 years of age. The questionnaire collected information on the patient's illness and exposure to animals, particularly tropical fish and reptiles in the thirty days prior to illness. The presence and care of aquariums, along with hand washing practices after exposure to the aquarium was also recorded.

Where investigators identified that cases had prior contact with tropical freshwater fish, they visited the patient's home to sample water and gravel from the fish tank using a standardised protocol. After stirring the tank water, investigators collected 100 ml of tank-water in a sterile container, which was refrigerated and transported to the laboratory within six hours. Investigators also collected at least 25 grams of gravel from the bottom of the tank and fish food, which was transported to the laboratory in the same manner as the water. Water was tested for the presence of *Salmonella* using membrane filtration, while aquarium foods and gravel were tested using standard isolation methods for food.[6,7]

Pathology laboratories send all *Salmonella* isolates to a State reference laboratory for confirmation of species and serotype. Isolates of *S.* Java were sent to the Microbiological Diagnostic Unit public health laboratory at the University of Melbourne for phage typing and antibiotic susceptibility testing.[8] This included *Salmonella* isolated from aquarium environs. We assigned local codes for distinct phage type patterns where isolates of *S.* Java Reacted but Did Not Conform (RDNC) to phage types recognised by the Laboratory of Enteric Pathogens, Colindale. To distinguish these different strains, we assigned additional codes to these Australian RDNC isolates, such as 'Aus 3', 'Aus 4', etc. The method of susceptibility testing used was disk diffusion as described by the National Committee for Clinical Laboratory Standards and covered ampicillin (Ap), streptomycin (Sm), chloramphenicol (Cm), sulphathiazole (Su), trimethoprim (Tp), kanamycin (K), nalidixic acid (Na), spectinomycin (Sp), gentamicin (G), ciprofloxacin (Cp) and cefotaxime (Cf).[9]

Results

During the 14-month period from March 2003 to April 2004 there were 76 cases of *S*. Java notified to Australian health departments. Travel to South East Asia was recorded for 21 per cent (16/76) of cases. Twenty-seven per cent (21/76) of cases were excluded from the study, as they were unable to be contacted by telephone, or were contacted more than 30 days following the onset of their illness. The reason for non-recruitment was unspecified for 17 cases. We enrolled 22 cases of *S.* Java into the study that had acquired their infection in Australia. This represented 29 per cent (22/76) of all cases of *S*. Java notified in Australia during the period.

The median age of cases was three years old (range 4 months-48 years old) and the male to female ratio was 1:1.2. Cases reported diarrhoea (100%), fever (77%), abdominal cramps (86%), vomiting (68%), blood in stool (55%), headaches (55%) and myalgia (50%). The median duration of diarrhoea for cases was eight days with a range of 3–26 days. Thirty-seven per cent (8/22) of cases were hospitalised.

Eighty-two per cent (18/22) of cases reported that they had been in contact with aquariums housing fish during their incubation period. Of the remaining four cases, one case infected with *S.* Java that was fully sensitive to all antibiotics tested had acquired a diamond python in the two months prior to illness. The other three cases not reporting exposure to aquariums or pet fish during the month prior to their illness were also infected with sensitive strains (phage types: Battersea, 3b var, and untypable).

For the 18 cases reporting exposure to aquariums, there were 11 cases of S. Java RDNC Aus 3 infection, followed by two cases each of RDNC Aus 5 and Dundee var 2 (Table 1). There were single infections each due to 1 var 15, 3b var and RDNC Aus 6. In cases infected with *S*. Java RDNC Aus 3, 82% (9/11) reported exposure to tropical fish and patient isolates were resistant to ApSmTcCmSuSp. The other two cases infected with multi-drug resistant *S*. Java were siblings who kept goldfish. Thirteen cases were infected with strains that were resistant to ApSmTcCmSuSp.

Of cases reporting exposure to aquariums housing fish, 72 per cent (13/18) reported that the fish tank was kept in the main room of the house (Table 2). Three cases had aquariums present in the kitchen. All aquariums had been present in cases' homes for at least two months (median 5–12 months) prior to illness. Only two cases had more than one aquarium in their house.

Only one case reported being the person that regularly maintained the aquarium. Forty-four per cent (8/18) of cases reported changing the water for their aquarium more frequently than fortnightly. Fifty per cent (9/18) cases reported discarding aquarium water down their sink. Two cases discarding water down the sink reported using the kitchen sink, while the remainder used the sink in their laundry. Two households also reported cleaning their aquarium filter in the kitchen sink.

Table 1. Demographics of cases of *Salmonella* Java, antibiotic resistance profile and fish ownership

Age (years)	Sex	State	Month of isolation	Hospitalised (Y/N)	Duration of illness (days)	*Salmonella* Paratyphi B biovar Java phage type	Antibiotic resistance profile†	Warm water aquarium (Y/N)	Aquarium tested (Y/N)	Fish types
4	F	Qld	Jun-03	Y	8	RDNC Aus 5	Ap	N	N	Goldfish
48	F	Vic	Mar-03	N	7	RDNC Aus 3	ApSmTcCmSuSp	Y	Y	Tropical
4	**M**	**Qld**	**Apr-03**	**Y**	**8**	**RDNC Aus 3**	ApSmTcCmSuSp	N	N	Goldfish
0	**M**	**Qld**	**Jun-03**	**N**	**UK**	**RDNC Aus 3**	ApSmTcCmSuSp	N	N	Goldfish
28	F	Qld	May-03	N	8	3b var	ApSmTcCmSuSp	Y	N	Tropical
3	F	Qld	May-03	N	8	RDNC Aus 3	ApSmTcCmSuSp	Y	N	Tropical
12	M	Vic	May-03	N	UK	RDNC Aus 3	ApSmTcCmSuSp	Y	Y	Tropical
1	F	WA	Jun-03	N	26	RDNC Aus 3	ApSmTcCmSuSp	Y	Y	Tropical
15	F	Qld	Jul-03	N	3	RDNC Aus 3	ApSmTcCmSuSp	Y	N	Tropical
44	F	Vic	Jul-03	Y	17	RDNC Aus 3	ApSmTcCmSuSp	Y	Y	Tropical
48	F	NSW	Aug-03	N	6	1 var 15	ApSmTcCmSuSp	N	N	Goldfish
2	M	Qld	Feb-04	N	4	RDNC Aus 3	ApSmTcCmSuSp	Y	N	Tropical
1	F	NSW	Feb-04	Y	6	RDNC Aus 3	ApSmTcCmSuSp	Y	N	Tropical
2	M	Qld	Feb-04	Y	11	RDNC Aus 3	ApSmTcCmSuSp	Y	N	Tropical
0	M	Qld	Jun-03	Y	8	RDNC Aus 5	Sensitive	N	N	Goldfish
1	M	SA	Apr-04	Y	9	Dundee var 2	Sensitive	Y	N	Tropical
34	F	NT	Mar-03	N	7	RDNC Aus 6	Sm	N	N	Locally caught
1	M	NSW	Oct-03	Y	11	Dundee var 2	SmSuSp	Y	Y	Tropical

* Cases in bold are siblings.

† Ap=ampicillin, Sm=streptomycin, Tc=tetracycline, Cm=chloramphenicol, Su=sulphathiazole, and Sp=spectinomycin

Table 2. Exposures reported by cases of *Salmonella* Paratyphi B biovar Java who had recent exposure to aquariums housing fish.

Exposure	Cases reporting exposure	Proportion exposed (%)
Tank present in home more than 2 months	18	100
Tank in main living room	13	72
Fish tank in kitchen	3	17
Contact with tank water in previous week	11	61
Tank cleaned at least every two weeks	8	44
Water discarded down sink	9	50
Tropical fish (warm water)	12	67
New fish in previous month	5	28
Fish ill in previous month	7	39
Dead fish in month before illness	7	39

One case reported that their aquarium was leaking and another reported adding new rocks in the week before the case's illness. Sixty-one per cent (11/18) of cases reported contact with fish and/or aquarium water in the week before illness, defined as putting hands in water (n=6), feeding fish (n=4) or cleaning the aquarium (n=1). Hand washing with soap and running water following routine maintenance of the aquarium was reported by 78% (n=14) cases.

Cases reported keeping a variety of ornamental cold and warm water fish. Cold water fish—goldfish—were kept by five cases (two of whom were siblings). One case kept fish caught in a creek in the Northern Territory. The other cases kept a variety of tropical freshwater fish including tetras (44%), guppies (39%) and angel fish (28%). Both cold and warm water fish were associated with multi-drug resistant strains of *S*. Java (Fishers Exact Test p=0.176). No cases reported exposure to tropical saltwater fish. Sixty-one per cent (11/18) of cases reported that fish in their aquarium had been sick or died in the week before their illness. Only 28 per cent of cases reported adding new fish to their aquariums in the month before illness. One case reported adding a general purpose antibiotic to their aquarium water, but were unable to identify the specific type.

Aquarium water and gravel was sampled in five homes up to three months after the cases were diagnosed. All samples of water and gravel were positive for *S*. Java. The phage types of *S.* Java isolated from these aquarium environments were the same as those isolated from the cases and were resistant to ApSmTcCmSuSp. One of these case studies was published during the study period.[10] Fish food was sampled from one home one month after their illness and no *Salmonella* was detected. No fish were tested for *Salmonella*.

Discussion and conclusions

Our study indicates that the presence of fish aquariums containing tropical freshwater fish in the home is a risk factor for multi-drug resistant *S*. Java infection, particularly in children aged less than 5 years. This is a major concern, as Australia has very low rates of antibiotic resistant *Salmonella* infection (Unpublished Data, The Microbiological Diagnostic Unit Public Health Laboratory, Melbourne). A recent review of multi-drug resistant *S.* Java strains in the United Kingdom found that S. Java isolates (that were not associated with poultry in Europe) had an identical genetic mechanism of resistance to that of the epidemic clone of *S.* Typhimurium DT 104. These results suggest that there is either a common origin or the horizontal transfer of the resistance gene cluster.[11] *S.* Typhimurium DT 104 is not endemic in Australia, but is a major problem for animal industries in the northern hemisphere.[12,13] In the United Kingdom, a 'fish tank' strain was recently isolated from a calves, showing the potential for this strain to become established in primary production settings.[14] These strains of *S.* Java appear to have emerged in other countries, which is a cause for global concern.[14,15]

Pet ownership is common in Australia. In 2002, there were an estimated 12.2 million fish kept as pets.[16] The way that fish are maintained and transferred between tanks allows for *Salmonella* to travel from the country of origin of the tropical fish to the wholesalers, pet stores and finally, to the home. Domestic wastewater, a possible source of *Salmonella*, is used for the cultivation of fish in places in the world that export exotic fish.[17]

S. Java has been previously isolated from water used to import tropical fish from Singapore.[4,18] In Australia, the carriage water is discarded at the quarantining aquarium and the fish are netted into a

new tank environment, suggesting that the original carriage water from the country of origin is not the direct source of illness to humans in Australia. It is quite possible that aquarium tanks and environments used to quarantine tropical fish are a continual source of *S.* Java for new fish sold to the public.

Australian fish importers use antibiotics in fish tanks as a preventative measure against illness from aquatic fish pathogens. Tetras, the most common fish imported from Indonesia and kept by 44 per cent of cases in this investigation, are prophylactically treated in Australia by adding chloromycetin, tetracycline, metronidazole and sulphadiazine to the tank water. These are four of the six antibiotics that *S*. Java show decreased susceptibility to, and may encourage selection of resistant strains. Consideration should be given to the role prophylactic antibiotics may play in promoting the development of antibiotic resistant bacteria in quarantined fish.

Tropical fish can act as bacterial reservoirs and excrete *Salmonella* in their faeces, without displaying any physical symptoms of illness, which may result in the water or environment becoming contaminated.[4] Once in the home, tropical fish aquarium water is rarely emptied in full, allowing for the *Salmonella* to remain in the aquarium water, gravel and equipment even if fish carrying *Salmonella* are removed. In our survey, several cases or their carers' reported poor hygienic practices, including discarding aquarium water down kitchen sinks. There is a clear need to educate the public about the safety of pets held in aquariums, including those housing fish.

We expected to see that cases reported exposure to warm water tropical fish, but were surprised to find some cases only reporting exposure to cold water goldfish. Retail outlets at the point of sale should provide written information on the risk of infection with *Salmonella* and household aquariums with advice regarding hand washing and safe disposal of aquarium water.

Contact with aquarium water, particularly in children, was associated with illness. Pre-school aged children are often tasked with feeding fish, and may 'play' with aquarium water, and in this study are shown to be more likely to be infected than the main carers of the aquarium. This is probably because children do not wash their hands adequately, if at all, after contact with aquarium water. This study was limited by its' ability to assess the hand washing practices of the cases. The question sought information on hand washing following 'cleaning, feeding fish and touching the water'. As most cases were young children the respondents were parents, not cases, and described their own hygiene practices as the main carer of the aquarium. As such this question did not necessarily capture the hand washing practices of the case. Further investigation may be useful to identify specific behavioural exposures.

Salmonella can result in serious illness, particularly in children. In this study 55 per cent of cases reported bloody diarrhoea and 37 per cent required hospitalisation, which is a higher proportion than previous Australian case control studies of patients infected with antibiotic susceptible strains of *Salmonella enterica* (OzFoodNet, unpublished data). Previous studies have shown that people infected with multi-drug resistant strains have a higher likelihood of severe symptoms and hospitalisation.[19,20] Prevention of multi-drug resistant *S.* Java requires knowledge of its potential sources and adherence to good hygiene following contact with aquariums and fish. Australia needs to ensure that these multi-drug resistant strains do not become established or allow genetic transfer of resistance mechanisms to other more common *Salmonella* serotypes.[13] Consideration should be given to preventing the importation of ornamental fish that harbour antibiotic resistant bacteria.

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