Sporadic Infections of *Salmonella* Paratyphi B, var. Java Associated with Fish Tanks

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ABSTRACT

Objective: Identify a source of contamination to explain the increase in the number of cases of *S*. Paratyphi B var. Java infection.

Method: A descriptive study was conducted to gather more information about cases and potential risk factors for infection. Between January 2000 and June 2003, we identified and interviewed by questionnaire 53 people with *S.* Paratyphi B infection. Due to the suspected link with fish tanks, an environmental investigation was conducted for each case at patients' homes as well as at pet stores and wholesalers. Various samples were taken from fish tanks. The fish were purchased at different pet shops. The pulsed field gel electrophoresis method was used to compare the human and aquarium strains.

Results: Of those infected, 60% had bought or had contact with an aquarium or tropical fishes before becoming ill. More than 50% of the samples taken from fish tanks in homes, pet shops, and wholesaler operations contained a number of serotypes of *Salmonella* in addition to *Salmonella* Paratyphi B var. Java. There were similarities between the PFGE patterns of human strains and aquarium samples.

Discussion: Contact with fish tanks could be a risk factor for developing a *Salmonella* infection and the public may be unaware of this risk. It was recommended that information be produced and distributed to pet shops and their customers to inform them of safety precautions in order to reduce the risk of contamination from bacterial pathogens that may be present in aquarium water.

MeSH terms: Salmonella Paratyphi B; sporadic infections; fish tanks

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in the number of cases of *Salmonella* Paratyphi B, var. Java infection in the province of Québec.¹ A descriptive study was conducted to gather more information about cases and potential risk factors for infection.

Past paratyphoid fever outbreaks have been linked to the consumption of unpasteurized goat cheese,² certain fish and seafood,³ alfalfa sprouts,⁴ and smoked fish.⁵ A number of cases appear to be linked to travel.⁶⁻⁹ A huge surge in cases of *Salmonella* Java was observed in poultry in the Netherlands without a corresponding rise in infections among humans.¹⁰ Only one study mentions the handling of tropical fish.¹¹ The outbreak of *Salmonella* Paratyphi B, var. Java described here raises the issue of probable contamination of aquariums by different serotypes of *Salmonella*.

Context

In the period following the establishment of the registry of mandatory notifiable diseases (MADO) in 1990, only one or two cases of Salmonella Paratyphi B, var. Java were reported yearly.1 In September 2000, an increase in the number of cases was reported by Laboratoire de santé publique du Québec (LSPQ). Since the outbreaks occurred over a short period of time, an alert was issued and an investigation was launched by Québec's regional public health departments (DSP). At that time, six cases of Salmonella Paratyphi B, var. Java had been reported to Ministère de la Santé et des Services sociaux (MSSS). This pathogenic agent is rare in Québec, but the cases all occurred within a four-week period. The initial investigation by the DSPs revealed that all of the individuals in whom Salmonella Paratyphi B, var. Java was isolated had purchased or been in contact with an aquarium or tropical fishes in the weeks prior to falling ill. Under an agreement on zoonoses, veterinarians from Ministère de l'Agriculture, des Pêcheries et de l'Alimentation (MAPAQ) conducted the environmental investigation and, in cooperation with the inspectors, took samples from the aquariums in the homes of the infected persons. The samples enabled them to identify Salmonella Paratyphi B, var. Java in 3 of the 6 cases.12

From January 2001 to June 2003, the number of cases of infection increased,

which led to several investigations. During this period, an investigative methodology similar to the ones used in 2000 was employed for every case.

METHODOLOGY

Epidemiological investigation

A descriptive study was conducted on all cases reported to the regional Public Health Department (DSP) from January 2000 to June 2003. The investigators at the DSP launched the investigation as soon as they were notified. Cases were contacted by telephone and asked to respond to a questionnaire. The questions dealt with demographics, details of symptomatology, and identification of potential risk factors (foodborne or environmental), as well as questions about whether they had had contact with an aquarium in the weeks preceding the symptoms.

We issued a Canada-wide alert in 2001 to recruit other cases across the country.

Environmental investigation

Because an epidemiological link was suspected between the disease and aquarium ownership or a recent purchase of tropical fishes, the homes of the infected persons, the pet shops where they bought the fishes, and the fish wholesalers supplying the pet shops in question were investigated. Water samples, filtering material from the aquariums and, in some cases, whole fishes, were taken at each location by veterinarians and MAPAQ inspectors. At the pet shops and wholesalers, MAPAQ inspectors gathered information on the delivery dates of new fishes, the place of origin of the fishes, the kind of filtration systems in the tanks, and whether the tanks were linked by a common filter.

Laboratory analysis

Human Strains

The isolation of *Salmonella* from the patients' fecal samples was done in their local hospitals and sent for serotyping to the LSPQ, where the biotype was determined for the strains from 2002 and 2003. The phagetyping was done on all strains from 2000 to 2003 at the National Laboratory for Enteric Pathogens (NLEP) in Winnipeg. Pulsed field gel electrophoresis (PFGE) was performed on every strain from 2000 to 2003 at the LSPQ.

Samples from Aquariums (water, filtering material, and fish)

The samples were submitted to Laboratoires d'expertises et d'analyses alimentaires (LEAA) and to the MAPAQ's Laboratoire de pathologie animale in Saint-Hyacinthe for detection of Salmonella using a standardized technique. The water and filtering material samples taken in the pet shops were from tanks containing the same species of fish as the infected person had at home. As with the human strains, serotyping, biotyping, phagetyping, and PFGE were performed on every strain by LSPQ and NLEP.

RESULTS

Epidemiological investigation

Between January 2000 and June 30, 2003, 53 cases of *S.* Paratyphi B, var. Java were reported in Québec. The main symptoms were diarrhea, stomach cramps, fever, and bloody diarrhea Among the cases, 17 were children under the age of 11. Among the 20 other cases reported between 2000 and June 2003, no other risk factors were identified. Over 50% of the cases required hospitalization.

Table I provides an overview of cases of S. Paratyphi B, var. Java infection in Québec during this period. The province of Québec is divided into 18 regions. Depending on the year, cases were reported in 4 to 9 regions in Québec. Among those infected, 33 (62.2%) owned an aquarium or had purchased tropical fishes in the weeks preceding the onset of symptoms, however, only 31 of these cases still had their aquariums available for testing at the time of this survey.

In 2000 and 2001, the outbreaks occurred primarily in September and October, whereas in 2002 and 2003, cases were reported throughout the year.

Laboratory analysis

From January 2000 to June 2003, samples were taken from most of the aquariums (31/33) in the homes of the infected persons (Table I). Among them, 21 (67.7%) water or filtering material samples tested positive for various *Salmonella* serotypes, including *S.* Paratyphi B, var. Java. During the four-year period, *S.* Paratyphi B, var. Java was found in the water or filtering

material of 18 aquariums tested in the homes of the infected persons. Other *Salmonella* serotypes identified included Blockley, Matopeni, Anatum, and Typhimurium phagetype 104.

Over the same period, veterinarians and MAPAQ inspectors identified the pet shops where the infected persons had purchased their fish. They visited a total of 34 pet shops where they took samples from the aquariums containing the same species of fish as those purchased by the infected persons. Eighteen (53%) of the pet shops visited tested positive for Salmonella. Of that number, 8 (44%) tested positive for S. Paratyphi B, var. Java and 10 (56%) for other Salmonella serotypes. The other Salmonella serotypes identified in the pet shops were Agona, Anatum, Blockley, Bovismorbificans, Cubana, Hadar, Kallo, Larochelle, Matopeni, Oslo, Schwarzengrund, Stanley, Virchow, Wandsworth, and Weltevreden. S. Paratyphi B was also identified at one of the wholesalers, as was S. Hvittingfoss.

The pulsed field analysis revealed that the human strains and the strains from the aquariums tested in the homes of the infected persons had similar profiles.

Environmental investigations and shipping of tropical fish

In Québec, tropical fishes are imported from Singapore, Thailand, Florida, and other places. They are sent to the wholesalers in bags containing water from the source fish tanks and are then transferred into large tanks containing numerous fishes. The original water is usually discarded and the fishes are transferred into the wholesalers' tanks where they may or may not undergo a quarantine period. Tank water is mechanically and biologically filtered. Some tanks are also equipped with ultraviolet sterilizers.

The fishes are shipped to the pet shops in bags containing water from the whole-salers' tanks. These fish are then transferred to the pet shop tanks. Certain pet shops transfer the fishes using a dipper, then discard the water from the whole-salers' bags. Others transfer the entire contents of the bag into their own tanks. Some of these tanks are linked by common filtration systems. When fishes are purchased from the pet shop, they are put in plastic bags with water from the pet shop aquari-

TABLE I
Sporadic Cases of *S.* Paratyphi B, var. Java in the Province of Québec from January 2000 to 2003

| Year | Number of Cases of <i>S.</i> Paratyphi B, var. Java | Number of Regions Affected in Québec | Number of Infected Persons Owning an Aquarium | Number of Home Aquariums That Tested Positive for Salmonella (N=31†) | Period During Which All Cases Occurred |
|-------|---|--|---|---|--|
| 2000 | 6 | 4 | 6 | 3 | AugSept. |
| 2001 | 15 | 7 | 8 | 5 | AugNov. |
| 2002 | 15 | 8 | 7 | 4 | JanDec. |
| 2003* | 17 | 9 | 12 | 9 | JanJune |
| Total | 53 | | 33 | 21 | |

- * Partial data from January 2000 to June 2003
- † Samples were obtained from only 31 of the 33 fish tanks because two cases no longer had their fish at the time of the survey.

um, which is then usually transferred to the home aquarium.

These outbreaks were reported to the National Enteric Surveillance Program. No other province in Canada reported outbreaks of *Salmonella* Paratyphi B, var. Java in connection to aquariums.

DISCUSSION

The presence in tropical fish aquariums of pathogens that are dangerous to humans has been reported elsewhere.^{3,14-16} An article published in 1992 reported *S.* Paratyphi B, var Java infection in two children from the United Kingdom. They were infected by the water used to import tropical fish from Singapore.¹⁴

Salmonella is not known to be pathogenic for tropical fishes. However, the fish may act as bacterial reservoirs for many weeks. 3,14,16,17 During periods of stress, these fishes can excrete Salmonella in their feces - although no symptoms are visible and the water in the tanks or aquariums can become contaminated. 15,18 The tanks are seldom emptied completely, in order to maintain their biological balance. Therefore, if the water from the country of origin is contaminated, it may be passed on to wholesalers, pet shops, and eventually customers who buy from the pet shops, posing a potential risk for customers as well as pet shop staff. In addition, the filtration systems in some pet shops may recirculate the water between aquariums, contaminating a number of tanks in the process.

It is estimated that one million Canadian families own aquariums. ¹⁷ A survey conducted in the province of Québec in April 2003 revealed that 15% of Quebecers had owned an aquarium at some time in the two years preceding the survey. Certain subgroups of the population appear more likely to own an aquari-

um than others, notably people aged 25 to 44 (25%), families with 4 members (23%) or more (29%), and women (18%).¹⁸

The sporadic infections of S. Paratyphi B, var. Java brought to light the frequent contamination of aquariums with over 15 Salmonella serotypes. This situation suggests that the acquisition and care of an aquarium may constitute a risk factor for Salmonella infection, and should be documented more thoroughly. Contamination can occur when cleaning the aquarium, touching the water, or feeding the fish. Twenty cases had not had contact with an aquarium. In the questionnaire, there were no questions asking if the infected persons had been in touch with somebody who owned an aquarium, which would have explained the possibility of crosscontamination.

There appeared to be a seasonal trend in the 2000 and 2001 outbreaks. However, the 2002 outbreak, which continued on through 2003, suggests this may not always be the case. These infections raise a number of questions about fish contamination. For instance, are some fish shipments more contaminated than others? Are some countries more widely affected by contamination? We know that fish can excrete Salmonella when under stress. Is the risk of infection still present several weeks after the fish are purchased?

Following the outbreak in 2000, a joint public health advisory was issued by MAPAQ, MSSS, and the Pet Industry Joint Advisory Council to pet shops across Québec informing them of the situation and making recommendations. Following the 2001 outbreak, a press release and veterinary warning reiterating these recommendations were issued by MAPAQ in November 2001. It was recommended that information posters and leaflets be produced and distributed to pet shops and their customers to heighten awareness of

the risks and inform them of safety precautions to reduce the risk of contamination from bacterial pathogens that may be present in aquarium water.

Given that this phenomenon shows no sign of letting up, it may be worthwhile to further document the frequency of aquarium contamination at pet shops and wholesaler operations to gain a clearer idea of the scope of the problem and the steps that should be taken to minimize its impact.

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RÉSUMÉ

Objectif: Déceler une source de contamination pouvant expliquer l'augmentation du nombre de cas de *Salmonella* paratyphi B de type Java.

Méthode : Une étude descriptive a été effectuée sur tous les cas déclarés de S. paratyphi B de janvier 2000 à juin 2003.

Résultats : Cinquante-trois cas de cette infection ont été déclarés alors que l'on observait antérieurement seulement de deux à quatre cas par an. Près de 60 % des cas ont été en contact avec un aquarium ou avaient acheté des poissons tropicaux dans les semaines précédant la maladie. Étant donné qu'un lien était suspecté entre les cas et les aquariums, une investigation environnementale a aussi été amorcée dans les maisons des personnes possédant un aquarium, dans les animaleries où avaient été achetés les poissons ainsi que chez les fournisseurs. Plusieurs échantillons d'eau et de matières filtrantes ont été prélevés. Plus de 50 % des échantillons prélevés démontraient la présence de salmonelles de différents sérotypes ainsi que de S. Paratyphi B. Les souches provenant des humains et des aquariums ont été comparées à l'aide de la méthode d'électrophorèse en champ pulsé. Cette méthode a démontré une similarité entre les souches chez les patients et dans les aquariums.

Discussion : Le contact avec un aquarium peut représenter un facteur de risque pour l'acquisition d'une infection à salmonelle. Des interventions préventives ont été effectuées auprès des fournisseurs et des animaleries pour les informer de ce risque. Des brochures destinées à la population qui achètent un aquarium ont été préparées et distribuées dans les animaleries.

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pastille qui est ensuite brûlée et introduite dans le doseur). Les résultats de cette première évaluation peuvent être globalement considérés comme satisfaisants. Toutefois, des études supplémentaires doivent être envisagées afin de confirmer ces résultats.

J'adhère donc à l'appel lancé par Haydon et Fisher mais je ne souscris pas à leur optimisme sur le rôle de la connaissance scientifique dans les décisions en matière de santé publique à l'égard de l'usage de drogues. La relation entre évidence scientifique et meilleures décisions publiques n'est pas si évidente¹¹, particulièrement dans le domaine de la consommation de drogues^{12,13}. L'histoire du développement des programmes de méthadone et d'échange de seringues dans une diversité de contextes géographiques en est un clair exemple^{12,14,15}.

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