

ORIGINAL ARTICLE

A multi-state *Salmonella* Typhimurium outbreak associated with frozen vacuum-packed rodents used to feed snakes

C. C. Fuller¹, S. L. Jawahir², F. T. Leano², S. A. Bidol³, K. Signs³, C. Davis⁴, Y. Holmes⁴, J. Morgan⁴, G. Teltow⁴, B. Jones⁵, R. B. Sexton⁵, G. L. Davis⁶, C. R. Braden⁷, N. J. Patel⁷, M. P. Deasy III⁸ and K. E. Smith¹

¹ Minnesota Department of Health, Acute Disease Investigation and Control Section, 625 Robert St. N., St. Paul, MN 55164, USA

² Minnesota Department of Health, Public Health Laboratory, 601 Robert St. N., St. Paul, MN 55155, USA

³ Michigan Department of Community Health, Bureau of Epidemiology, 201 Townsend St. Lansing, MI 49013, USA

⁴ Texas Department of State Health Services, Health Services Region 7, 2408 South 37th St. Temple, TX 76504, USA

⁵ Texas Feed and Fertilizer Control Service, Office of the Texas State Chemist, Texas Feed and Fertilizer Control Service, 445 Agronomy Road, College Station, TX 77843, USA

⁶ U.S. Food & Drug Administration, Dallas District, Fort Worth Resident Post, Fort Worth, TX, USA

⁷ Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA

⁸ Pennsylvania Department of Health, Division of Infectious Disease Epidemiology, Room 933 Health and Welfare Building, 7th and Forster Streets, Harrisburg, PA 17120, USA

Impacts

- Rodents, like reptiles, should be handled as if they are carrying *Salmonella*.
- Regulation of feed rodent industries and consumer education about the risk of *Salmonella* and feed rodents should be considered.
- This is the second recent multi-state salmonellosis outbreak associated with commercially distributed rodents.

Keywords:

Salmonella Typhimurium; frozen rodents; reptiles; pulsed-field gel electrophoresis

Correspondence:

C. Fuller. Minnesota Department of Health, Acute Disease Investigation and Control Section, 625 Robert St. N., St. Paul, MN 55164, USA. Tel.: 651-201-5414; Fax: 651-201-5082; E-mail: Candace.Fuller@health.state.mn.us

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Summary

From December 2005 through January 2006, the Minnesota Department of Health (MDH) identified four human clinical isolates of *Salmonella* Typhimurium that were indistinguishable by pulsed-field gel electrophoresis (PFGE). During routine interviews, three of the cases reported attending the same junior high school and two handled snakes in the science classroom. MDH collected environmental samples from the school's science classroom for *Salmonella* culturing; these included environmental samples and frozen vacuum-packed mice purchased over the internet to feed the classroom snakes.

Through PulseNet, a national molecular subtyping surveillance network for enteric bacteria, 21 human *S. Typhimurium* isolates with indistinguishable PFGE patterns were identified in the United States since December 2005. Each state determined whether these human cases had recent exposure to snakes fed vacuum-packed rodents. Texas state officials conducted tracebacks of the vacuum-packed mice and collected samples at the breeding facility.

Nineteen of 21 cases were interviewed, and seven reported contact with frozen vacuum-packed rodents from the same internet-based supplier in Texas. In Minnesota, the outbreak PFGE subtype of *S. Typhimurium* was isolated from the snakes, frozen feed rodents, and the classroom environment. Three human cases were identified in Michigan, Pennsylvania, and Wyoming. The outbreak PFGE subtype of *S. Typhimurium* was isolated from the Pennsylvania case's frozen rodents and the Michigan case's pet snake. The outbreak PFGE subtype of *S. Typhimurium* was also isolated from the supplier's rodent facility.

This was a *S. Typhimurium* outbreak associated with frozen rodents. Human transmission likely occurred through direct contact with snakes and

contaminated environmental surfaces. This report represents the second recent multi-state salmonellosis outbreak associated with commercially distributed rodents. Stronger oversight of the commercial rodent industry is warranted.

Introduction

Non-typhoidal *Salmonella* is estimated to cause 1.4 million illnesses, 15 000 hospitalizations, and 400 deaths per year in the United States (Voetsch et al., 2004). Between 1993 and 2005, the two most commonly reported serotypes in the United States were Typhimurium and Enteritidis (CDC, 2005a).

Although most human *Salmonella* infections are thought to be acquired through consumption of contaminated food (Mead et al., 1999), many human cases of *Salmonella* infection are acquired through animal contact. *Salmonella* outbreaks have been associated with contact with chicks, ducklings, cats, livestock, hedgehogs, guinea pigs, commercially distributed pet rodents, and most commonly amphibians and reptiles (Fish et al., 1968; CDC, 1995a, 2000, 2005b; Friedman et al., 1998; Sanchez et al., 2002; Merritt and Herlihy, 2003; Mermin et al., 2004; Wells et al., 2004; Swanson et al., 2007).

In this report, we describe a multi-state outbreak of *S. Typhimurium* infections among individuals that interacted with constrictor snakes that were fed commercially bred and euthanized rodents. These rodents were obtained via the internet and shipped frozen and vacuum-packed. This is the second recent outbreak of salmonellosis in the United States associated with commercially distributed rodents.

Materials and Methods

Recognition of outbreak in Minnesota

Minnesota state communicable disease rules mandate that all *Salmonella* cases be reported to the Minnesota Department of Health (MDH) and that all *Salmonella* isolates be sent to MDH for confirmation. All *Salmonella* isolates are subtyped by pulsed-field gel electrophoresis (PFGE) (Bender et al., 2001). All *Salmonella* cases are interviewed by MDH about food consumption, animal exposures, and other potential exposures as a part of routine surveillance. Interviews of *Salmonella* cases that are indistinguishable by PFGE are compared to identify potential common exposures.

From December 2005 through January 2006, four human case isolates of *S. Typhimurium* with an uncommon PFGE pattern, (PulseNet National Database Pattern JPXX01.0129) were identified by the MDH Public Health Laboratory through routine surveillance. All four cases

resided in the same county. This PFGE pattern was uncommon; a query to the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet) *Salmonella* Database in January 2006 revealed that the PFGE pattern of these isolates represented 0.2% (45 of 29 000) of all *S. Typhimurium* isolates obtained since 1996. Prior to December 2005, only one case isolate of this PFGE pattern had been seen in Minnesota among 2169 of all *S. Typhimurium* human isolates subtyped in the state since 1994. During routine interviews, three of the cases reported attending the same junior high school, and the fourth had a sibling at the school. Three cases reported observing or handling two constrictor snakes, a ball python and a fox snake, in the science classroom at the junior high school.

On March 6, 2006 two MDH epidemiologists collected six environmental and two fecal samples from the two snakes kept in the science classroom at the school. The primary food source for the snakes was frozen vacuum-packed mice ordered from an internet vendor (Supplier A) in Texas. Seven frozen feed mice were collected from four vacuum-packed bags received in a recent bulk shipment. School officials were interviewed about snake husbandry practices, management of reptiles, and handwashing. Student contact with the reptiles was also assessed.

Case ascertainment and investigations in other states

The Minnesota outbreak investigation prompted a national search for additional *S. Typhimurium* cases with the outbreak PFGE pattern. We queried the PulseNet National *Salmonella* Database for human patient isolates that matched the outbreak strain by one enzyme, Xba I. The state of residence for the patients infected with the outbreak strain was identified, and state health departments were contacted for more information about these patients (i.e., recent exposure to reptiles or rodents). States retrieved rodent purchase and traceback information and collected frozen rodents and reptile samples from case households for testing at their state public health laboratories. State officials conducted tracebacks of the mice and collected frozen rodent samples and environmental samples at the rodent breeding facility. If a patient reported contact with a reptile, additional questions about source of feed were asked. A case was defined as a person from whom the outbreak PFGE subtype of *S. Typhimurium* was isolated from December

2005 through August 2006 and who had direct or indirect exposure to rodents from Supplier A within the 7 days before onset of illness.

CDC, the United States Food and Drug Administration, and local and state health departments participated in an investigation into the reptiles and the source of animal feed in each individual case. Investigations and environmental sampling at the rodent distributor in Texas were conducted by the Office of the Texas State Chemist Feed and Fertilizer Control Service and Department of State Health Services. Animal and environmental isolates were sent to respective state public health laboratories for serotyping and PFGE subtyping.

Results

Case information

Twenty-one matching human clinical isolates of *S. Typhimurium* from humans were identified in the US with onsets between December 2005 and August 2006. Nineteen of these patients were interviewed, nine patients (47%) in six states reported recent contact with snakes or rodents. Seven of the nine patients (four from Minnesota and one each from Michigan, Pennsylvania, and Wyoming) had direct or indirect contact with frozen vacuum-packed rodents ordered from the same internet-based rodent supplier (Supplier A) to use as reptile food (see below for investigation details in each state). Data for the seven identified cases are as follows. The months of illness onset ranged from December 2005 through July 2006 (Fig. 1). The median age of cases was 13 years (range, 9–50). Symptoms included fever (71%), vomiting (71%), and diarrhea (100%). The median duration of diarrhea was 8 days (range, 5–14 days). One patient was hospitalized for 7 days. There were no deaths.

Minnesota investigation

In Minnesota all four outbreak cases were associated with a single junior high school. Two of the four cases reported direct contact with snakes in the science class-

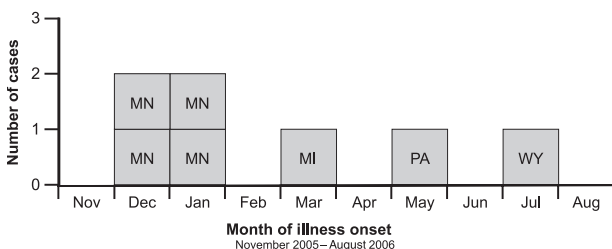


Fig. 1. *Salmonella* Typhimurium cases associated with frozen vacuum-packed rodents by state and month of illness onset.

room. One case did not report handling snakes but was frequently in the same science classroom. The fourth case had a sibling at the school. The science classroom was set up with a long table lining the back wall of the room. Two constrictor snakes, a ball python and a fox snake, were housed in separate terrariums on this table. Directly in front of the snakes' terrariums was a large island counter where classroom supplies, including paper, assignments, and writing utensils were placed. Desks were centered in the room; some desks were just a few feet from the island counter. The classroom also had a sink that was used for cleaning the snake terrariums. At the time of inspection debris from the snake terrariums was visible at the bottom of the sink. The teacher was interviewed and did not know that snakes can carry *Salmonella* and reported not his washing hands after cleaning the snake terrariums, which were cleaned weekly in the classroom sink. The snakes were fed adult vacuum-packed mice ordered via the internet from Supplier A. The teacher reported ordering frozen rodents from Supplier A for many years, with the most recent shipment being received in January 2006. The teacher reported that the students rarely handled the snakes themselves, but were not instructed to wash their hands after coming into contact with them. The students did not directly handle the feed rodents.

The outbreak PFGE subtype of *S. Typhimurium* and *Salmonella* somatic group C1 were isolated from the fox snake's fecal sample, and *S. enterica* subspecies *Diarizonae* was isolated from the ball python's fecal sample (Table 1). *Salmonellae* were also isolated from the environmental samples and feed mouse samples. Specifically, the outbreak PFGE subtype of *S. Typhimurium* and *Salmonella* serogroup C1 were isolated from two different areas of the countertop where the snakes were stationed, the center island counter top in front of the terrariums, and the classroom sink (Table 1).

Seven feed mice from four separate vacuum-packed bags were collected from the January 2006 frozen rodent shipment. These mice were necropsied and cultured for *Salmonella*. Three of the mice had gross pathologic lesions. Cultures of these three mice yielded pure growth of *S. Typhimurium* from the liver, spleen, and intestines. PFGE subtyping was performed on these isolates and yielded two subtypes, one of which was indistinguishable from that of isolates from the cases, snakes, and environmental samples (Table 1). In addition, two of these mice were also positive for *Salmonella* Group B 4,5,12:-:- with a PFGE subtype that differed from the outbreak PFGE subtype of *S. Typhimurium* by one band. The *S. Typhimurium* isolates matching the PFGE outbreak subtype by one restriction enzyme were further tested by PFGE using a second restriction enzyme, Bln I (Roche, Basel,

Table 1. *Salmonella* culture and pulsed-field gel electrophoresis (PFGE) results for environmental, reptile, and mouse samples in Minnesota, Michigan, and Pennsylvania

State	Location/subject of sample	Sample type	Sample description	Culture result	Serotype	CDC pulsenet designation
MN	Counter top beside aquariums	Environmental		Positive	Typhimurium	JPXX01.0129
MN	Fox Snake	Feces		Positive	Typhimurium	JPXX01.0129
MN	Counter top beside Fox Snake	Environmental		Positive	Typhimurium	JPXX01.0129
MN	Classroom sink	Environmental		Positive	Typhimurium	JPXX01.0129
MN	Mouse 3 Adult	Spleen	Abnormal	Positive	Typhimurium	JPXX01.0129
		Liver	Abnormal	Positive	Typhimurium	JPXX01.0129
		Intestine	Abnormal	Positive	I 4,5,12:-:Serogroup B, Non-motile	JPXX01.1276
MN	Mouse 4 Adult	Spleen	Abnormal	Positive	Typhimurium	JPXX01.0129
		Liver	Normal	Positive	Typhimurium	JPXX01.0129
		Intestine	Abnormal	Positive	Typhimurium	JPXX01.0129
MN	Mouse 5 Adult	Spleen	Abnormal	Positive	I 4,5,12:-:Serogroup B,	JPXX01.1276
		Liver	Normal	Positive	Non-motile	JPXX01.1276
		Intestine	Abnormal	Positive	I 4,5,12:-:Serogroup B, Non-motile	JPXX01.0129
MI	Milk Snake	Feces		Positive	Typhimurium	JPXX01.0129
PA	Pooled fuzzy and pinky mouse intestine	Intestine	Normal	Positive	Typhimurium	JPXX01.0129

Switzerland). Three of six of these isolates differed by one band by second enzyme and also were resistant to streptomycin and tetracycline. All other isolates were pan-susceptible to all antimicrobials tested. The January 2006 shipment from the online frozen rodent supplier was received after at least two of the case onset dates, indicating that *Salmonella* in the rodents from this supplier may have been a problem before January.

Michigan investigation

The Michigan Department of Health reported a 50-year-old case that owned a constrictor milk snake prior to illness onset on March 22, 2006. This case reported feeding the snake frozen mice from Supplier A. The Michigan Department of Agriculture collected fecal samples from the milk snake and also collected three frozen mouse samples from the case household. The mice were necropsied and tested for *Salmonella* at the state animal health laboratory. The snake samples were positive for the outbreak PFGE subtype of *S. Typhimurium*; however, the mice were culture-negative for *Salmonella*. A rodent shipping box from the case household was used to identify a lot number and shipping date. The frozen rodents had been shipped from Supplier A in December 2004, and had since been kept in the household freezer and used for reptile feeding.

Pennsylvania investigation

Pennsylvania reported a 14-year-old case that owned multiple constrictor snakes including a corn snake, a California king snake, a milk snake, and a ball python. This case fed the snakes mice from Supplier A. The case had an illness onset date of May 2, 2006. The Pennsylvania Department of Health collected five packs of frozen vacuum-packed mice from the case household and cultured them for *Salmonella*. The packs contained newborn mice (referred to as pinkies) and week-old mice (referred to as fuzzies). Three mice were selected from each of the five packs for testing. The mice were dissected and the intestines from each were pooled, blended, and cultured. Cultures of these mice yielded pure growth of *S. Typhimurium* of the outbreak PFGE subtype. A packing slip or packing box was not available for traceback activities.

Wyoming investigation

The Wyoming Department of Health reported a 29-year-old case with a history of handling an albino corn snake during the week prior to illness. The case reported ordering frozen vacuum-packed rodents from Supplier A and stored the rodents in the kitchen freezer with food. The case had an onset date of July 21, 2006. Not long after the illness began, the snake escaped and the case

discarded the rodents and packaging. Consequently, public health officials were not able to collect reptile or rodent samples from the case for laboratory testing.

Texas traceback investigation

Texas officials determined that the Supplier A, which supplied rodents to consumers across the US via an internet website, was an unlicensed rodent breeding and distribution facility in central Texas. Texas Feed and Fertilizer Control Service (TFFCS) regulates animal feed facilities in the state and at the start of this investigation had not been aware of this facility. The Texas Commercial Feed Control Act which is enforced by TFFCS requires animal feed operations to hold a license and appropriate feed labeling (Texas, 2005). Rodent shipping procedures were examined. Rodents were euthanized, vacuum-packed, and frozen, and then were shipped either overnight or by second day mail to customers in an insulated container filled with dry ice. Officials collected environmental samples from rodent trays, feed specimens, fecal/shaving materials, and air vents. Frozen rodent samples were also collected. Cultures of these samples yielded pure growth of *S. Typhimurium* of the outbreak PFGE subtype. Officials noted that there were dead and ill rodents being held in the same trays as well rodents, and that shaving materials did not appear to be changed regularly.

The owner of the establishment voluntarily depopulated the mouse rooms and was required by Texas authorities to put labels on all vacuum packs that included a statement about washing hands. Culled mice were not discarded but were frozen and shipped to customers. Sales and distribution records at the facility were poorly maintained and no traceforward could be conducted. The feed rodent industry at the time of the investigation was not required by any regulating agency to keep distribution information. These findings prompted Texas Feed and Fertilizer Control do an extensive review of rodent facilities in Texas.

Discussion

This was an outbreak of *S. Typhimurium* infections among people exposed to snakes and the frozen rodents used to feed them. The snakes acquired the outbreak strain of *S. Typhimurium* from frozen feed mice that were purchased via the internet. Human transmission likely occurred not only through direct contact with snakes or their feed, but also through contact with contaminated environmental surfaces. Of the 19 interviewed patients nationwide who had the outbreak PFGE subtype, only seven were definitively linked to the frozen feed mice. While some of the other cases may have been unre-

lated, it is possible that some had either unrecognized exposures to reptiles or frozen rodents, were secondarily infected (as was apparently the one Minnesota sibling), or were reluctant to admit to known exposures.

This is the second outbreak in as many years due to commercially distributed rodents. In the previous outbreak (Swanson et al., 2007), the source was live mice, rats, and hamsters that were purchased both as pets and as feed for snakes. Our outbreak investigation provides further evidence that commercial rodents -dead or live- may be an important source of *Salmonella* for humans.

This investigation also further demonstrated the utility of combining PFGE subtype information and rapid detailed case interviews in surveillance for detecting outbreaks. This method greatly improves the sensitivity and specificity of surveillance and can detect widely distributed (both geographically and temporally) outbreaks with very few cases (Bender et al., 2001). The national sharing of PFGE patterns through PulseNet was critical in identifying the scope and ongoing nature of this outbreak because cases occurred over time and in several states. *Salmonella* surveillance faces many challenges due to a number of factors including the self-limiting nature of many salmonellosis infections, care seeking behaviors, physician practices, reporting, and laboratory practices. Therefore, it is estimated that only one in 38 salmonellosis cases are identified by public health departments (Voetsch et al., 2004). In addition, the PulseNet database was only queried back through December 2005; it is possible that prior to December 2005 there were some outbreak associated cases that were never identified. Thus, it is likely that our outbreak investigation only detected a small proportion of the cases that actually occurred.

The finding of *Salmonella* among the snakes was expected; most reptiles (>90%) shed the organisms intermittently throughout life (Burnham et al., 1998). However, *S. Typhimurium*, the most common serotype from human clinical infections in the United States, is not generally thought of as a 'reptile-associated serotype' (Mermin et al., 2004). In this outbreak the commercially distributed feed rodents were the source of *S. Typhimurium* for the snakes, and the ultimate source for the human cases. Rodents, whether frozen or live, like reptiles, should be handled as if they are carrying *Salmonella*. Rodents are susceptible to *S. Typhimurium* and *S. Enteritidis*; both serotypes have been isolated from wild and captive rodents (Sparrow, 1980; Healing, 1991). *S. Typhimurium* causes an illness in mice that resembles typhoid fever in humans caused by *S. enterica* serotype Typhi (*S. Typhi*). Similar to *S. Typhi* in humans, a small percentage of rodents that recover from *S. Typhimurium* associated illness, between 1 and 20 percent become chronic carriers, and a single rodent can easily infect

an entire mouse colony (Habermann and Williams, 1958; Santos et al., 2001). Public health professionals should consider rodents, either pet rodents or those used as feed for other pets, as a potential source of salmonellosis.

According to the American Pet Product Manufacturers Association (APPMA), 4.4 million (4%) of households in the United States contained one or more pet reptiles or amphibians in 2004, and 830 000 (1%) households owned a snake (APPMA, 2005). Forty-five percent of snake owners and 22% of lizard owners responding to the survey reported buying feeder rodents for their pets (APPMA, 2005). Therefore, demand for convenience reptile feed products such as frozen vacuum-packed rodents is apparent. In this outbreak, the implicated rodent supplier was internet based. Internet based commerce makes the acquisition of feed rodents easier, but it also increases the opportunity for multi-state outbreaks; in a matter of days, diseased, frozen, vacuum-packed rodents from a small rodent breeding facility can be transported throughout the United States.

Reptile contact is well recognized as a common source of human salmonellosis (CDC, 1995b, 1999a,b, 2003, 2005b; Mermin et al., 1997; Friedman et al., 1998). Reptile exposure is associated with both sporadic cases and outbreaks, and approximately 74 000 *Salmonella* infections are attributed to reptile or amphibian contact annually in the United States (Mermin et al., 2004). Reptiles carry salmonellae in the gastrointestinal tract as part of their normal flora, and 40% of all *Salmonella* serotypes have been cultured predominantly from reptiles (Mermin et al., 2004). *Salmonella* can be transmitted directly from reptiles to humans through handling of reptiles, or indirectly through contact with objects or surfaces contaminated by a reptile or reptile feces (Friedman et al., 1998; Wells et al., 2004). *Salmonella* can persist in the environment after being shed by reptiles, and cases of reptile-associated salmonellosis where no direct contact with a reptile has occurred are documented frequently (CDC, 1995b, 1999a,b, 2003, 2005b).

In addition to being the primary source for what are considered reptile-associated *Salmonella* serotypes (Ackman et al., 1995; Mermin et al., 2004), reptiles have also been linked with other *Salmonella* serotype infections in humans. For example, the two most common serotypes in humans, Typhimurium and Enteritidis, are thought to be transmitted primarily through food. However, even these common serotypes have also been associated with reptile contact (Friedman et al., 1998; Mermin et al., 2004). In this outbreak snakes shed serotypes of *Salmonella* considered to be reptile-associated (Ackman et al., 1995; Mermin et al., 2004), as well as *S. Typhimurium*. *S. Typhimurium*, which was acquired

by the snakes through feed rodents, was the serotype transmitted to humans.

In this outbreak, the staff at the junior high school was unaware that snakes often carry *Salmonella*. This outbreak, along with other recent outbreaks of salmonellosis associated with animals or animal products (such as owl pellets) in schools or preschools (Smith et al., 2005; CDC, 2007), illustrate the need for education of staff in these facilities with regard to the zoonotic disease risks associated with animal activities.

The lack of regulation for commerce in pet and feed rodents likely increases opportunities for outbreaks to occur. There were poor animal husbandry practices in the rodent facility involved in this outbreak. Although the Texas Feed Control Act requires the facility to have a license, these regulations were not directly applicable to rodent husbandry practices; therefore, enforcement of recommended infection control practices was problematic. In general, the responsibility for regulation of this industry in the United States is not well defined. Technically, each state is responsible for the industries in their jurisdiction, but the United States Food and Drug Administration has jurisdiction over interstate commerce of feed rodents. The Animal Welfare Act does not apply to rodents raised for animal consumption (AWA, 2003) and therefore can not be used to correct poor husbandry practices. This outbreak, along with the previously reported outbreak (Swanson et al., 2007), demonstrate the need for more detailed oversight of the commercially distributed rodent industry. We recommend that states assess the feed rodent facilities in their states and require that proper infection control procedures be maintained. Efforts need to be made to educate rodent breeders and distributors about proper infection control and biosecurity practices, and to develop regulations of this industry that will facilitate control of zoonotic diseases in rodents. Public education regarding the risk of salmonellosis after handling rodents, especially in vulnerable populations at risk for severe complications (Angulo and Swerdlow, 1995; Koehler et al., 2006), is another key component in preventing such outbreaks in the future.

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