

US Outbreak of Human *Salmonella* Infections Associated With Aquatic Frogs, 2008–2011



WHAT'S KNOWN ON THIS SUBJECT: Although amphibians are known *Salmonella* carriers, aquatic African dwarf frogs are specifically marketed toward children, who are especially vulnerable to *Salmonella* infections. Both direct animal contact and indirect contact with animal habitats can lead to human *Salmonella* infections.



WHAT THIS STUDY ADDS: This is the first reported outbreak of human *Salmonella* infections associated with African dwarf frogs, particularly among young children. Parents should be aware of the risk of *Salmonella* infections from both direct and indirect animal contact. Pediatricians should regularly inquire about animal contact and advise families about risks.

abstract

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OBJECTIVE: Although amphibians are known *Salmonella* carriers, no such outbreaks have been reported. We investigated a nationwide outbreak of human *Salmonella* Typhimurium infections occurring predominantly among children from 2008 to 2011.

METHODS: We conducted a matched case-control study. Cases were defined as persons with *Salmonella* Typhimurium infection yielding an isolate indistinguishable from the outbreak strain. Controls were persons with recent infection with *Salmonella* strains other than the outbreak strain and matched to cases by age and geography. Environmental samples were obtained from patients' homes; traceback investigations were conducted.

RESULTS: We identified 376 cases from 44 states from January 1, 2008, to December 31, 2011; 29% (56/193) of patients were hospitalized and none died. Median patient age was 5 years (range <1–86 years); 69% were children <10 years old (253/367). Among 114 patients interviewed, 69 (61%) reported frog exposure. Of patients who knew frog type, 79% (44/56) reported African dwarf frogs (ADF), a type of aquatic frog. Among 18 cases and 29 controls, illness was significantly associated with frog exposure (67% cases versus 3% controls, matched odds ratio 12.4, 95% confidence interval 1.9–infinity). Environmental samples from aquariums containing ADFs in 8 patients' homes, 2 ADF distributors, and a day care center yielded isolates indistinguishable from the outbreak strain. Traceback investigations of ADFs from patient purchases converged to a common ADF breeding facility. Environmental samples from the breeding facility yielded the outbreak strain.

CONCLUSIONS: ADFs were the source of this nationwide pediatric predominant outbreak. Pediatricians should routinely inquire about pet ownership and advise families about illness risks associated with animals. *Pediatrics* 2013;131:724–731

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KEY WORDS

children, pediatric, *Salmonella*, outbreak, zoonoses, amphibians, frogs, pets

ABBREVIATIONS

ADF—African dwarf frog
CDC—Centers for Disease Control and Prevention
CI—confidence interval
CDPH—California Department of Public Health
MLVA—multiple-locus variable number tandem repeat analysis
mOR—matched odds ratio
PFGE—pulsed-field gel electrophoresis

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

(Continued on last page)

Nontyphoidal *Salmonella* causes an estimated 1.2 million human infections, 23 000 hospitalizations, and 450 deaths annually in the United States.¹ Although most *Salmonella* infections are food-borne, animal contact remains an important source of human salmonellosis.² Studies conducted during 1996 to 1997 determined that ~74 000 *Salmonella* infections each year in the United States resulted from reptile and amphibian exposure.³ Reptiles (eg, turtles) and amphibians (eg, frogs) have long been recognized as *Salmonella* carriers.^{3,4} Although there have been multiple multistate outbreaks of human *Salmonella* infections associated with turtles,^{5,6} this report documents the first known nationwide outbreak of *Salmonella* infections associated with amphibians, specifically African dwarf frogs (ADFs) (Fig 1).

In fall 2008, epidemiologists from the Centers for Disease Control and Prevention (CDC) and state and local health departments investigated a cluster of human *Salmonella* Typhimurium infections with an indistinguishable pulsed-field gel electrophoresis (PFGE) pattern predominantly among children. The number of cases decreased to baseline levels by February 2009 before a vehicle could be identified. During April to July

2009, Utah Department of Health epidemiologists identified 5 new cases with the same PFGE pattern, mostly among children, and notified CDC. Because the number of cases increased above baseline again, a multistate investigation was reinitiated in July 2009 to identify the source of the outbreak.

METHODS

Outbreak Identification and Case Finding

State diagnostic laboratories forward *Salmonella* strains from clinical isolates to state public health laboratories for serotyping and PFGE. PFGE patterns are electronically submitted to PulseNet, the national molecular subtyping network for foodborne disease surveillance.⁷ Multiple-locus variable-number tandem repeat analysis (MLVA) may be used to further distinguish *Salmonella* Typhimurium isolates. A case was defined as a person with a *Salmonella* Typhimurium infection whose clinical isolate was indistinguishable from the outbreak PFGE pattern, and whose MLVA pattern matched the outbreak pattern or MLVA unknown, and with an illness-onset date (or isolation date if onset unknown) from January 1, 2008, to December 31, 2011.

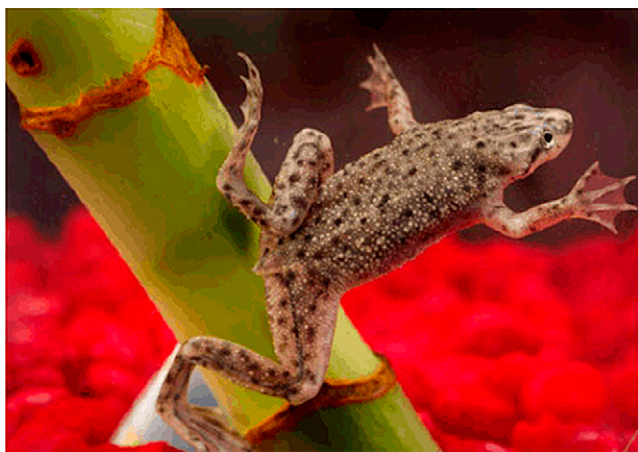


FIGURE 1
African dwarf frog, an exclusively aquatic frog measuring up to 63.5 mm long.

Hypothesis Generation

Patients or their caregivers were interviewed using a structured hypothesis-generating questionnaire and by open-ended interviews about exposures in the 7 days preceding illness. The structured questionnaire consisted of >400 food and animal exposures, including dining and grocery venues. Data from the structured questionnaires were entered into a Microsoft Access 2003 database and analyzed for exposure frequencies.

Open-ended interviews obtained data not included in the structured questionnaire, such as detailed behavior history in the 7 days preceding illness. A constant comparative and iterative approach was used to reinterview cases and gather quantitative data on exposure frequency.

Case-Control Study

Exposures identified through hypothesis generation were tested in a multistate case-control study from November 30 to December 7, 2009. Patients with illness onset from July 15 to November 9, 2009, were enrolled. Controls were persons with recent *Salmonella* infection with strains other than the outbreak strain identified from state enteric databases. Controls with the most recent illness were selected and matched to patients by age group (<5, 5–12, 13–18, 19–59, and >60) and geography. Potential controls were excluded if they traveled out of the country in the 7 days before the interview. Investigators sought to enroll 2 controls per case. Questionnaires focused on the leading hypotheses, including food and animal exposures for 7 days preceding illness for patients and 7 days preceding interview for controls.

Case Study

A retrospective review of PulseNet data indicated cases with the outbreak strain first increased in April 2008. After

completion of the case-control study, any patient with illness onset from April 1, 2008, to December 31, 2011, was eligible to be interviewed using a structured questionnaire focused on exposures to aquatic pets, including frogs, fish, and other water animals. The questionnaire also included questions about place and date of purchase, water filtration, other animals sharing the habitat, and frequency and methods of habitat cleaning. Patients or their caregivers were also asked about awareness of *Salmonella* risk from reptile and amphibian contact.

Statistical Analysis

Data from the case-control and case study were entered into Microsoft Access 2007 and analyzed using SAS 9.2 (SAS Institute, Cary, NC). Matched odds ratios (mOR) and exact 95% confidence intervals (CI) were calculated via conditional logistic regression to examine relationships between exposures and illness.

Laboratory, Environmental, and Traceback Investigations

Isolates of *Salmonella* Typhimurium were PFGE subtyped at state and local public health laboratories and results were electronically submitted to PulseNet. Isolates indistinguishable from the outbreak PFGE pattern were requested by CDC for confirmatory testing by MLVA.

Six clinical isolates were sent to the National Antimicrobial Resistance Monitoring System Laboratory at CDC for antimicrobial susceptibility testing.

Local and state public health departments collected environmental samples from frog vendors, frog distributors, day care centers, and homes of cases or their caregivers for testing by state public health laboratories. Traceback investigations were conducted for ADF purchases associated with patients, including frogs associated with

environmental isolates that tested positive for the outbreak strain. Site visits were made to the ADF breeding facility in December 2009, January 2010, April 2010, and March 2011, and water samples collected on each visit were tested by CDC and California Department of Public Health (CDPH) laboratories.

RESULTS

Outbreak Identification and Case Finding

Between January 1, 2008, and December 31, 2011, 376 patients with the outbreak strain were identified from 44 states; illness-onset dates ranged from January 1, 2008, to December 31, 2011 (Fig 2). Fifty-two percent (186/359) were female; the median age was 5 years (range: <1 year–86 years), and 69% (253/367) were children <10 years. Among those with available outcome information, 29% (56/193) were hospitalized, 51% (28/55) were <5 years, and 69% (38/55) were <10 years; no deaths were reported.

Hypothesis Generation

Three primary hypotheses emerged during hypothesis generation. Among 11 patients interviewed, 100% (11/11) reported consumption of Brand A cheese-flavored crackers, 100% (11/11) reported consumption of Brand B cheese-flavored crackers, and 73% (8/11) reported exposure to an aquatic pet, such as a fish or frog. Seven additional exposures were identified with frequencies >60%, including cold cereal, yogurt, chicken nuggets, peanut butter, chips, pretzels, and apples.

Case-Control Study

Eighteen patients and 29 matched controls were enrolled in the case-control study from 14 states. In bivariate analysis, patients were significantly more likely than controls to report exposure to any aquatic pet, including fish or frogs (78% cases versus 38%

controls; mOR 4.7, 95% confidence interval [CI] 1.2–27.0) (Table 1). More specifically, illness was associated with exposure to frogs (67% cases versus 3% controls; mOR 12.4, 95% CI 1.9–infinity). Illness was not associated with fish or any other food or animal exposures. In a multivariable analysis, including exposure to frogs, fish, and Brand B cheese-flavored crackers, only exposure to frogs was statistically significant with an adjusted mOR of 9.3 (95% CI 1.3–infinity).

Case Study

A total of 112 patients were interviewed with illness onset between April 1, 2009, and December 31, 2011, 18 of whom were enrolled in the case-control study. Among 114 patients interviewed, 76% (87/114) reported exposure to any aquatic animal, 61% (69/114) reported any frog, 17% (18/104) reported frogs only, 14% (15/105) reported fish only, and 85% (90/106) reported both frogs and fish. Among 69 patients reporting any frog exposure, 56 knew the frog type of which 79% (44/56) reported ADFs.

In retrospective interviews, among 25 patients with illness onsets between April 1, 2008, and March 23, 2009, 84% (21/25) reported exposure to any aquatic animal, and 60% (15/25) reported exposure to frogs. Among the 11 who knew the type of frog, 4 identified ADFs. In total, 139 patients had illness onset between April 1, 2008, and December 31, 2011, and 60% (84/139) reported exposure to frogs.

Among 84 patients with illness onsets from April 1, 2008, to December 31, 2011, who reported exposure to frogs, only 27% (22/82) reported touching a frog and 46% (38/83) reported feeding a frog, whereas 59% (49/83) reported contact with the frog's habitat and 60% (50/83) reported exposure to water from the frog's habitat. When asked about cleaning the frog habitat, 19%

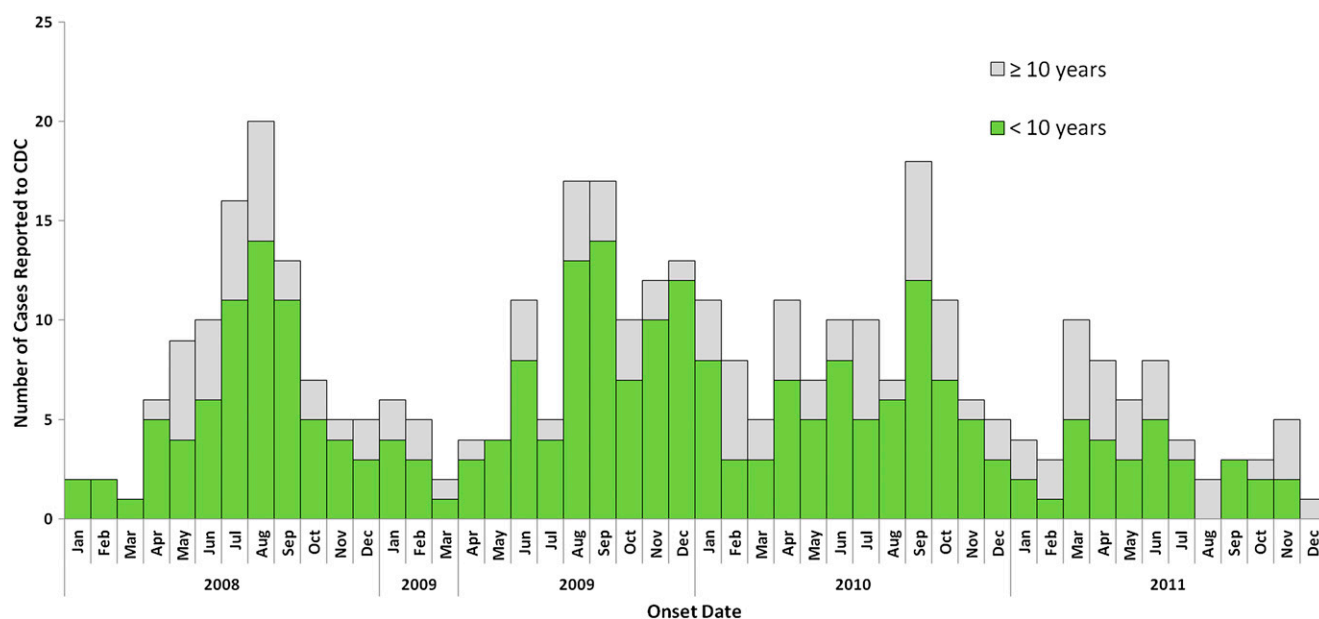


FIGURE 2

Cases by illness onset and age, January 1, 2008, to December 31, 2011; $n = 368$ (for whom age and illness onset was known).

TABLE 1 Bivariate and Multivariate Analysis of Food and Animal Exposures Among Cases of *Salmonella* Typhimurium Infection and Age and Geography-Matched Controls: Multistate Case-Control Study, November 2009–December 2009

Exposure	Cases, %, $n = 18$	Controls, %, $n = 29$	Matched, OR (95% CI)	Adjusted, mOR ^b (95% CI)
Any aquatic pet	78	38	4.7 (1.2–27.0)	n/a^c
Any frog	67	3	12.4 (1.9–unbounded)	9.3 (1.3–unbounded)
Any fish	65	50	1.2 (0.2–8.4)	1.6 (0.1–28.4)
Pretzels	50	17	5.4 (1.0–53.4)	n/a^d
Brand B cheese-flavored crackers	75	30	3.5 (1.0–15.6)	1.9 (0.2–28.5)

^a Interviews were conducted among residents of California, Colorado, Kentucky, Louisiana, Michigan, Minnesota, New Jersey, New Mexico, Ohio, Pennsylvania, Texas, Utah, Virginia, and Washington.

^b In all other models including a frog, there was no significant association between illness and any suspect-foods or other animals.

^c Any aquatic pet was not included in the multivariable model because it included frog and fish.

^d Pretzels were not included in the multivariable model because of 8 cases with exposure, all reported different brands of pretzels.

(11/57) reported never cleaning the habitat; 23% (16/69) reported cleaning the habitat in the bathroom sink, and 35% (24/69) reported cleaning the habitat in the kitchen sink. The median number of days between purchase of a frog and illness onset was 30 days (range 5–2310).

Among 84 patients exposed to frogs, 38% (31/82) reported awareness of *Salmonella* infection risk from reptile exposure, but only 17% (14/81) reported awareness of the *Salmonella* risk from amphibian contact.

Laboratory, Environmental, and Traceback Investigations

Before this outbreak, the outbreak strain PFGE *Xba*I pattern made up 0.79% of all *Salmonella* Typhimurium isolates in the PulseNet database. During the outbreak period, 328 human isolates were MLVA tested and 30 (9%) were excluded as non-MLVA matches. Thirty isolates not sent to CDC for MLVA testing were classified as MLVA unknown. Six clinical isolates were tested and found to be pansusceptible to a panel of 15 antimicrobial agents (Table 2).

Eleven environmental samples from 8 patient homes with ADFs in 8 states, an ADF festival vendor, a large-scale ADF distributor, and a day care center with ADFs yielded isolates indistinguishable from the outbreak strain (Table 3). Traceback investigations conducted for 25 ADFs associated with *Salmonella* infections, including the 11 that yielded environmental samples with the outbreak strain, all converged to a single source, an ADF breeding facility in Madera County, California. No other source of ADFs was identified.

Investigation of ADF Breeding Facility

The ADF breeding facility was investigated in December 2009 by Madera County Public Health and Environmental Health (MCPHEH) officials, and in January 2010 by a team consisting of members from the CDC, CDPH, and the MCPHEH. The facility only bred ADFs and no ADFs were imported into the facility in the past decade. ADFs in the facility were raised from eggs to adults. Adult ADFs were shipped to distributors nationwide but were not sold directly to pet stores or the public. Environmental

TABLE 2 Antimicrobial Susceptibility Results for 6 Outbreak-Associated *Salmonella* Isolated From Patients.

Antimicrobial Agent	Range of MICs ($\mu\text{g/mL}$) ^a
Amikacin ^b	≤ 0.5 –2
Amoxicillin/clavulanic acid	≤ 1 –2
Ampicillin	≤ 1 –2
Cefoxitin	2–4
Ceftiofur ^b	0.5–2
Ceftriaxone	≤ 0.25
Chloramphenicol	4–8
Ciprofloxacin	≤ 0.015 –0.03
Gentamicin ^b	≤ 0.25 –1
Kanamycin ^b	≤ 8
Nalidixic acid	2–8
Streptomycin ^b	≤ 32
Sulfisoxazole	≤ 16 –32
Tetracycline	≤ 4
Trimethoprim/sulfamethoxazole	≤ 0.12

MIC, minimum inhibitory concentration.

^a All MIC results were interpreted as susceptible according to clinical interpretive criteria established by the Clinical and Laboratory Standards Institute (CLSI) or according to criteria used by the National Antimicrobial Resistance Monitoring System (NARMS) that are based on MIC distributions and presence of known resistance genes or mutations.

^b NARMS tests these antimicrobial agents for surveillance purposes but clinical interpretive criteria established by CLSI are not available for these agents either because they are not used in human medicine or are not effective clinically for *Salmonella* infections.

samples obtained from the facility yielded the outbreak strain from multiple sources, including ADFs, water from tanks containing ADFs, tank-cleaning equipment, water filter media, and floor drains (Table 4). Environmental tests of source well water and all frog food tested negative for the outbreak strain. The investigation team provided recommendations over multiple visits and teleconferences to the owner of the ADF breeding facility regarding biosafety, water systems, and animal husbandry.

Because of evidence of an ongoing problem in late March 2011, local health department officials visited the facility again and collected additional environmental samples. Multiple water samples tested in CDC laboratories yielded isolates indistinguishable from the outbreak strain. Eight environmental samples tested by the CDPH laboratory, such as filter media, cleaning

brushes, and additional water samples obtained from the ADF breeding facility yielded isolates with the outbreak strain (Table 4).

In April 2011, the owner of the ADF breeding facility voluntarily and temporarily stopped shipping ADFs. In May 2011, the CDPH sent a letter to the facility's direct customers recommending they discontinue distribution and sale of these frogs and decontaminate tanks or aquariums in which frogs were kept. In June 2011, after instituting sanitary and husbandry interventions of unclear impact, the owner resumed shipping ADFs to its customers. MCPHEH and a veterinary consultant specializing in aquatic animals continued to work with the owner to enforce interventions and ongoing testing and monitoring of this facility.

DISCUSSION

Epidemiologic, traceback, and laboratory findings between 2008 and 2011, link this outbreak of human *Salmonella* Typhimurium infections to a single ADF breeding facility in Madera, California. This is the first known nationwide outbreak of human *Salmonella* infections associated with amphibians, specifically ADFs. This outbreak caused illnesses predominantly among children and spanned at least 3 years. Illnesses will likely continue unless an enhanced biosecurity program and an ongoing *Salmonella* monitoring program developed in consultation with a veterinarian specializing in aquatic animals is maintained indefinitely at this ADF breeding facility. The owner of the facility was cooperative, temporarily and voluntarily suspending distribution of ADFs in April 2011, to implement the previous mentioned programs; distribution of ADFs resumed in June 2011. Although the number of human illnesses with this outbreak strain declined, transmission continued. As of May 30, 2012, 5 human illnesses with

this outbreak strain were reported to CDC. Because ADFs have a life expectancy of 5 to 18 years, infected ADFs from the implicated facility may remain in households and could continue to cause human illness. As of October 31, 2011, ill persons who reported exposure to ADFs reported purchasing ADFs before interventions at the implicated facility were fully implemented. Tank water may become increasingly contaminated over time, resulting in higher *Salmonella* levels that can lead to human infections. Because ADFs are not typically handled directly, indirect frog contact, such as exposure to their contaminated tank water or habitat played a role in transmission of *Salmonella* to humans in this outbreak. Only 27% of patients reported directly touching the frog, whereas 60% reported coming in contact with water from the frog's aquarium or indirect contact. It is possible that ill persons unknowingly came into contact with contaminated water from the frog's habitat through cross-contamination, such as cleaning the frog's aquarium in the kitchen sink and then preparing food.

ADFs are small, exclusively aquatic frogs. Sold as aquarium pets since the 1960s, they have become more popular in recent years. Although ADFs are often purchased from pet stores, they are increasingly found at fairs, festivals, and novelty, educational, and toy stores and are marketed as good pets for young children or school classrooms. Children are likely at higher risk for acquiring *Salmonella* infection because of their decreased likelihood to practice good hand-washing, as well as increased risk of hand-to-face contact, and contact with the environment.^{2,8} In addition, young children, particularly infants and children <5 years old, and the immunocompromised are at greatest risk for severe or complicated disease. In this outbreak, 29% of patients

TABLE 3 Type and Sources of Environmental Samples Yielding the Outbreak Strain of *Salmonella* Typhimurium, December 2009–April 2011

Date of Collection	State	Sample Collection Site	Sample Type
December 2009	CO	Patient's home	ADF #1
			ADF #2
	NM	Patient's home	Rocks in ADF aquarium
			Water from ADF aquarium
			Filtration system from ADF aquarium
			Gravel from ADF aquarium
OH	Patient's home	Water from ADF aquarium	
		Lid of ADF aquarium	
		Edge of ADF aquarium	
UT	Patient's home	Container used to hold ADF while ADF aquarium cleaned	
UT	ADF Vendor	Filters from 4 ADF aquariums	
SC	ADF Distributor	Water from 8 ADF tanks	
January 2010	CA	Patient's home	Water from aquarium containing fish purchased from pet store aquarium containing ADFs
March 2010	GA	Day care and patient's home ^a	Water from ADF aquarium in patient's home
			Water from 2 ADF aquariums at patient's daycare
March 2011	NY	Patient's home	Water from ADF aquarium
April 2011	MI	Patient's home	ADF
			Water from ADF aquarium
			Rocks in ADF aquarium

^a Samples collected during unrelated *Salmonella enteritidis* outbreak investigation.

were hospitalized with a median hospitalization of 4 days (range 1–9 days).

Contaminated water has been the source of infection during disease outbreaks associated with other animals, such as small turtles, for many years.^{9,10} Repeated outbreaks, predominantly among children, led to a 1975 federal ban on the sale of small turtles, causing a dramatic decline in salmonellosis among children.¹¹ As of June 2012, the distribution of ADFs was unregulated by federal or state agencies.

Control recruitment for young children is difficult using conventional random digit dialing methods. In recent multi-state case-control studies conducted at the CDC, on average, more than 300 phone calls are typically required to enroll a single child control (CDC, unpublished data, 2009). To expedite

identification of controls in this outbreak of predominantly young children, we selected geographic and age-matched controls from local and state health department databases of patients who had been recently ill with a nonoutbreak strain of *Salmonella*. This method required fewer than 2 to 3 calls per control, leading to rapid identification of the outbreak source, with less demand on already strained public health resources. One limitation of this control recruitment method is decreased likelihood of finding an association with the outbreak vehicle, because controls infected with nonoutbreak strains of *Salmonella* may have had similar exposures. Nevertheless, we still found a strong statistical association with frog exposure.

Awareness of the risk of salmonellosis from reptile and amphibian contact is

low. Of patients interviewed, only 38% were aware of the risk of *Salmonella* illness from exposure to reptiles, such as turtles, and only 18% were aware of the risk of *Salmonella* infection from amphibians, such as frogs. Pediatricians have the unique opportunity during well-child visits to discuss the risk of illness from exposure to reptiles and amphibians and their habitats or water. Health care providers should ask families about direct and indirect exposures to pets and other animals and provide education about the risk of salmonellosis from animals particularly marketed toward children, such as reptiles (eg, turtles); amphibians (eg, frogs); and chicks, ducklings, and other live poultry.^{5,6,12–17} The pet industry, pet stores, and others who sell ADFs should provide education about the risk of salmonellosis from ADFs and other amphibians and reptiles at the point of sale. Educational flyers on *Salmonella* infections associated with animal contact are available on CDC's Healthy Pets Healthy People Web site.¹⁸ Exposure may be indirect, such as through aquariums or habitats and may include locations outside of the home, such as at a day care center or caregiver's or relative's home. Parents should be provided education about good hand-washing techniques, including how to assist children during hand-washing. Children <5 years old are at higher risk for serious *Salmonella* infections and should avoid contact with ADFs, their water, and their habitats (eg, tanks or aquariums). Children <5 years should not be allowed to clean the frog's habitat. Kitchen sinks, bathroom sinks, and bathtubs should not be used to empty or wash the frog's habitat; if possible, tanks should be emptied and washed outside of the home, using disposable gloves. Frogs should not be allowed in child care facilities or schools with children <5 years.² All high-risk persons, including children <5 years,

TABLE 4 Environmental Samples Yielding the Outbreak Strain at the ADF Breeding Facility, December 2009–April 2011

Date	Sample Descriptions ^a
December 2009	Filter media/gravel Water from frog tanks ^b Water from frog shipping bags
January 2010	Scourpad Cleaning brush bristles Drains Filter media/gravel Water from egg/tadpole tank Water from breeding frog tank Water from adult frog tanks Water from shipping frog tanks
April 2010	Water from juvenile/adult frog tanks Water from large adult frog tank
March 2011	Filter media/gravel Frog eggs ^c Cleaning brush bristles Water from egg tank Water from juvenile frog tank Water from juvenile frog tanks Water from breeding frog tanks Water from shipping frog tanks Water from egg sample container

^a In most cases, multiple samples of each type were tested.

^b Frog life stage was not identified at time of collection.

^c Separated from water and surface treated with dilute iodine solution.

older persons, pregnant women, or immunocompromised persons, such as patients with cancer and those undergoing chemotherapy or organ transplantation, should avoid contact with ADFs, their water, and their habitats.¹⁹

CONCLUSIONS

Our investigation identified ADFs as the source of this predominantly pediatric outbreak. Epidemiologic, traceback, and laboratory findings between 2008 and 2011 link this nationwide outbreak

of human *Salmonella* Typhimurium infections to a single ADF breeding facility in Madera County, California. ADFs colonized with the outbreak strain likely remain in households, as they have an average life expectancy of 5 to 18 years. This outbreak highlights the ongoing public health problem of salmonellosis among children from exposure to certain high-risk animals, such as amphibians (eg, frogs), reptiles (eg, turtles), and baby poultry.^{14,20}

Pediatricians are uniquely qualified to provide education to young patients and their families about the risk of *Salmonella* infection from animals. Education regarding salmonellosis risk should be expanded to include risk for salmonellosis from frogs and other amphibians. In particular, consumers should be educated that exposure may be indirect, such as contact with aquarium water containing frogs or turtles, and on simple hygiene precautions.

ACKNOWLEDGMENTS

The *Salmonella* Typhimurium Outbreak Investigation Team members were as follows: Akiko Kimura, MD, Ben Sun, DVM, Curtis Fritz, DVM, PhD, Debra Gilliss, MD, Duc Vugia, MD, MPH, Gregory B. Inami, Janice Lidgard, and Frank Ni (California Department of Public Health); Phil Hudecek, BS (Madera County Environmental Health Department); Marilee Poulson, BS (Utah Department of Health); Shaun Cosgrove, BS, Laura Fawcett, BS, and Jennifer Sadlowski (Colorado Department of Public Health and Environment); Paul

Torres, MS (New Mexico Department of Health); Stacey Short (Cuyahoga County Board of Health, Ohio); Patrice Fraker (Toledo-Lucas County Health District, Ohio); Madhu Anand, MPH, CPH (New York State Department of Health); Sally A. Bidol, MPH (Michigan Department of Community Health); April Johnson, DVM, MPH, PhD (Purdue University); Eija Hyttia-Trees, PhD, DVM, Cheryl A. Bopp, MS, and Mike S. Humphrys, MS (Division of Foodborne, Waterborne, and Environmental Diseases, Enteric Diseases Laboratory Branch, CDC); Suresh Pai, PhD, Bonnie Mull, MPH, Amy Kahler, MS, Chandra Schneeberger, Tina Lusk, MSPH (Division of Foodborne, Waterborne, and Environmental Diseases, Waterborne Disease Prevention Branch CDC); Linda G. Capewell, VMD, MPH, Leslie Hausman, BSN, RN, MPH, and Jeshua Pringle, MPH (Division of Foodborne, Waterborne, and Environmental Diseases, Outbreak Response and Prevention Branch, CDC).

We gratefully acknowledge the epidemiology, environmental, and laboratory teams from the following states that assisted with this investigation: Alaska, Alabama, Arizona, California, Colorado, Connecticut, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Kentucky, Louisiana, Massachusetts, Maryland, Michigan, Minnesota, Missouri, Mississippi, Montana, North Carolina, Nebraska, New Hampshire, New Jersey, New Mexico, Nevada, New York, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Dakota, Tennessee, Texas, Utah, Virginia, Vermont, Washington, Wisconsin, and West Virginia.

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www.pediatrics.org/cgi/doi/10.1542/peds.2012-2031

doi:10.1542/peds.2012-2031

Accepted for publication Nov 29, 2012

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: No external funding.

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Pediatrics 2013;131;724

DOI: 10.1542/peds.2012-2031 originally published online March 11, 2013;

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