

Multistate Outbreak of *Salmonella enterica* Serotype Enteritidis Infection Associated with Pet Guinea Pigs

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Abstract

Salmonella causes about one million illnesses annually in the United States. Although most infections result from foodborne exposures, animal contact is an important mode of transmission. We investigated a case of *Salmonella enterica* serotype Enteritidis (SE) sternal osteomyelitis in a previously healthy child who cared for two recently deceased guinea pigs (GPs). A case was defined as SE pulsed-field gel electrophoresis (PFGE) XbaI pattern JEGX01.0021, BlnI pattern JEGA26.0002 (outbreak strain) infection occurring during 2010 in a patient who reported GP exposure. To locate outbreak strain isolates, PulseNet and the US Department of Agriculture National Veterinary Service Laboratories (NVSL) databases were queried. Outbreak strain isolates underwent multilocus variable-number tandem repeat analysis (MLVA). Traceback and environmental investigations were conducted at homes, stores, and breeder or broker facilities. We detected 10 cases among residents of eight states and four NVSL GP outbreak strain isolates. One patient was hospitalized; none died. The median patient age was 9.5 (range, 1–61) years. Among 10 patients, two purchased GPs at independent stores, and three purchased GPs at different national retail chain (chain A) store locations; three were chain A employees and two reported GP exposures of unknown characterization. MLVA revealed four related patterns. Tracebacks identified four distributors and 92 sources supplying GPs to chain A, including one breeder potentially supplying GPs to all case-associated chain A stores. All environmental samples were *Salmonella* culture-negative. A definitive SE-contaminated environmental source was not identified. Because GPs can harbor *Salmonella*, consumers and pet industry personnel should be educated regarding risks.

Key Words: Salmonellosis—*Salmonella enteritidis*—Guinea pigs.

Introduction

AN ESTIMATED ONE MILLION CASES of human *Salmonella* infection occur annually in the United States, resulting in approximately 19,000 hospitalizations and 400 deaths (Scallan et al. 2011). Most human salmonellosis results from consuming foods contaminated with animal feces containing *Salmonella*; however, contact with animals or their envi-

ronments also poses infection risks (Elliot et al. 1985, Schutze et al. 1999, Centers for Disease Control and Prevention 2000, Porcalla and Rodriguez 2001, Finley et al. 2006, Bender and Minicucci 2007, Centers for Disease Control and Prevention 2009, Behravesch et al. 2010, Hoelzer et al. 2011). Among illnesses caused by the seven most common foodborne pathogens, 14% are attributable to animal contact (Hale et al. 2012). Nontraditional pets (*e.g.*,

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reptiles, amphibians, poultry, hedgehogs, and rodents) can carry *Salmonella* and pose risks to humans (Centers for Disease Control and Prevention 1992, Woodward et al. 1997, Mermin et al. 2004, Centers for Disease Control and Prevention 2005, Riley and Chomel 2005, Swanson et al. 2007, Harris et al. 2010, Lowther et al. 2011, Gaffga et al. 2012, Kolker et al. 2012, Loharikar et al. 2012, Centers for Disease Control and Prevention 2013). Nontraditional pet ownership is increasing, which can increase risks for human exposure (American Veterinary Medical Association 2007).

During October, 2010, the Wisconsin Division of Public Health (WDPH) investigated a case of *Salmonella enterica* serotype Enteritidis (SE) sternal osteomyelitis occurring in a child who cared for two pet guinea pigs (GPs) that had recently died. To determine whether the child's illness represented a widespread problem or an isolated case linked with exposure to pet GPs, we conducted an epidemiologic and laboratory investigation that resulted in the detection of a multistate outbreak of SE infections linked with exposure to pet GPs. We provide a report of the patient's illness and the investigation of the multistate outbreak.

Materials and Methods

Identification and clinical description of initial case

The patient, a previously healthy female aged 7 years old, was hospitalized during October, 2010, with a 5-day history of fever to 39.4°C and chest and thoracic back pain. Prior to hospitalization, she was evaluated in an outpatient clinic and emergency department, diagnosed with a urinary tract infection, and treated with a cephalosporin antibiotic. Two chest radiographs were reported as normal. On the hospital admission day (day 1), the patient had focal chest wall swelling, erythema, sternal tenderness, dyspnea secondary to pain, continued fever, and mild dehydration. She also reported having diarrhea 2 weeks before admission that included one episode of bloody stools. Magnetic resonance imaging of her thoracic spine and chest revealed soft tissue edema and inflammatory changes with fluid collections anterior and posterior to the lower sternum. The patient was diagnosed with sternal osteomyelitis. During day 2, sternal biopsy and abscess drainage were performed, a peripherally inserted central catheter was placed, and the patient received intravenous vancomycin and cefotaxime. Gram-negative rods were isolated from sternum specimens; the antibiotic regimen was changed to oral ciprofloxacin and intravenous ceftriaxone during day 3. The bacterium was identified as *Salmonella*, sensitive to the routinely tested antibiotics. The patient was discharged to home on day 4 to complete a 6-week, twice daily, antibiotic course of 1.5 grams of intravenous ceftriaxone and 500 mg of oral ciprofloxacin. She made a full recovery.

On October 25, 2010, the patient's *Salmonella* infection was reported to her local health department (LHD). Information obtained during a telephone interview of the patient's parent conducted by LHD staff on October 25 indicated patient exposure to two pet GPs that died approximately 3 weeks before her hospital admission. The patient was the primary caregiver of the GPs. The family purchased a GP during early September, 2010, at a national retail chain (chain A) pet store in Wisconsin. The GP, which appeared frail but without signs of disease, died 19 days after purchase.

Two days later, her other GP, owned for 1 year, developed bloody diarrhea and died. The family discarded both GPs.

Following review of all case and interview-related data, WDPH staff requested that the *Salmonella* isolate be sent to the Wisconsin State Laboratory of Hygiene (WSLH) for further analysis. WSLH staff received the isolate on November 12; serotyping and pulsed-field gel electrophoresis (PFGE) subtyping identified it as SE PFGE XbaI pattern JEGX01.0021. On November 16, 2010, the isolate pattern was posted to the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet) database, and WDPH staff expanded the investigation.

Epidemiologic investigation

PFGE was performed using standardized protocols (Ribot et al. 2006). Because the PulseNet-designated SE PFGE XbaI pattern JEGX01.0021 was relatively common, PFGE second enzyme (BlnI) analysis (CDC 2013) and multilocus variable-number tandem repeat analysis (MLVA) testing was used to determine the isolates most likely related to this investigation (Boxrud et al. 2007). A case was defined as a laboratory-confirmed infection caused by SE with PFGE XbaI pattern JEGX01.0021, BlnI pattern JEGA26.0002 (outbreak strain) in a patient having illness onset during 2010 and who reported GP exposure during the 7 days before illness onset. To find cases, the PulseNet database was queried to locate SE PFGE XbaI pattern JEGX01.0021 isolates having specimen collection dates during 2010.

State and local health departments responsible for follow-up of patient isolates identified during PulseNet query were requested to review data from enteric disease patient interviews for reports of GP exposure and reinterview persons who reported GP exposure using a detailed questionnaire to ascertain small animal exposures within and outside the household during the week before the patient's illness onset, pet store visits, illness among small pets, and products used to feed and care for these animals.

Laboratory investigation

State laboratories were requested to send GP-associated or GP-exposure unknown isolates of SE PFGE XbaI pattern JEGX01.0021 to the Centers for Disease Control and Prevention (CDC) for PFGE second enzyme testing, if not completed, and MLVA testing. The US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) National Veterinary Service Laboratories (NVSL) database was queried for GP SE isolates submitted during 2008–2010. These GP isolates underwent PFGE subtyping at NVSL and were sent to CDC for MLVA typing.

Traceback and environmental investigations

Traceback investigations were conducted by USDA, CDC, and state and local health department and agriculture department staff using information collected from pet stores, brokers, and breeder facilities. During environmental investigations, specimens of feed, stool, bedding, and cage swabs were collected from pet stores, patient homes, and a GP breeder facility. Specimens were sent to state public health laboratories to culture for *Salmonella* using standard procedures (Centers for Disease Control and Prevention April

2013). When *Salmonella* was isolated, serotyping and PFGE subtyping of isolates was conducted; MLVA was conducted at the CDC.

Results

PulseNet query detected 686 SE human isolates from 47 states with PFGE XbaI pattern JEGX01.0021 that were uploaded during January 1 to December 9, 2010. This pattern comprised 8.3% of SE in the PulseNet database and was the fourth most common among 830 patterns. Of those 686 isolates, 10 were from patients reporting prior GP exposure. These patients resided in eight states: Illinois and Wisconsin (two patients each), and California, North Carolina, Oklahoma, Tennessee, Vermont, and Washington. All 10 isolates had an indistinguishable PFGE second-enzyme BlnI pattern JEGA26.0002.

Patient characteristics

Among the 10 patients, six (60%) were aged <12 years (median, 9.5 years; range, 1–61 years), one was hospitalized, and none died. Illness onsets occurred during January, 2010 (Tennessee)–November, 2010 (Wisconsin-2); nine patients had illness onsets during May–November, 2010 (Fig. 1). Three patients (Oklahoma, Tennessee, and Wisconsin-1) purchased GPs from chain A stores, and three illnesses occurred among chain A employees (Washington, Wisconsin-2, and Vermont), whose duties included caring for small animals. Two patients (Illinois-1 and California) purchased GPs from local independent pet stores. The GP purchased by the California patient had an unknown previous owner. Two patients (Illinois-2 and North Carolina) reported GP exposure during initial interview but were lost to follow-up; the sources of GPs to which they were exposed are unknown.

Laboratory investigation

The NVSL database query yielded six SE isolates from GP specimens submitted during 2008–2010, including two submitted from a single chain A store and two submitted from a Texas vendor who distributes directly to chain A. Four isolates, including one from a GP specimen submitted by the Texas vendor to a university laboratory during June, 2010, had PFGE patterns indistinguishable from the outbreak strain. The university laboratory forwarded the isolate to NVSL for serotyping.

The clinical SE isolates from the 10 case patients displayed four very closely related MLVA patterns that were rare to the national database. Three patient isolates (Washington, Tennessee, and California) and six GP isolates had MLVA patterns that were indistinguishable (pattern 1). The remaining seven clinical isolates exhibited three patterns closely related to each other and pattern 1 (Fig. 2).

Environmental investigation

During November, 2010, WDPH staff requested from chain A headquarters information regarding GPs and animal illnesses reported from the Wisconsin store where the index patient's GP was purchased. No documented animal illnesses temporally related to this purchase were reported. Following the request, chain A officials initiated *Salmonella* testing of fecal pellets and stock feed obtained from the Wisconsin chain A store. Additionally, chain A officials requested the stock feed manufacturer to test all feed retention samples for *Salmonella*. Cultures of all store-obtained stock feed, fecal pellets, and manufacturer retention samples tested negative for *Salmonella*.

When possible, state health department staffs collected environmental samples at patient homes. Environmental swabs were obtained on December 3, 2010, from the cage at the index patient's residence; the cage had been rinsed with

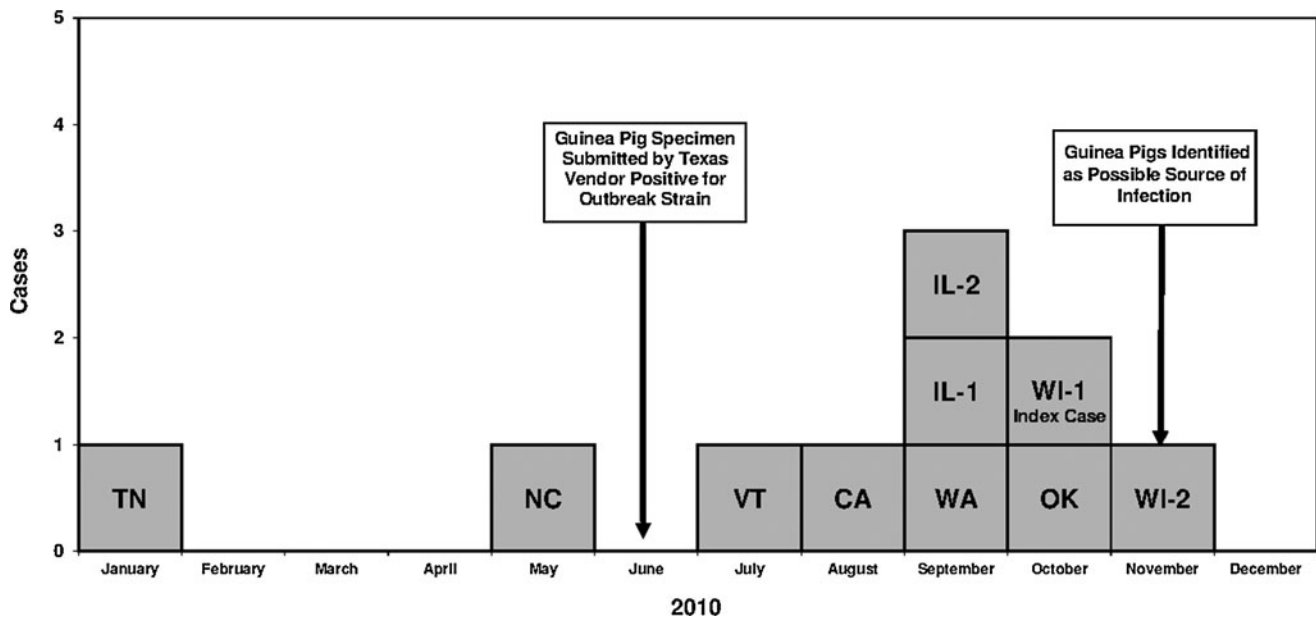


FIG. 1. Guinea pig–associated cases of *S. enterica* serotype Enteritidis infection by month of illness onset and state of residence as follows: Tennessee (TN), North Carolina (NC), Vermont (VT), California (CA), Illinois (IL), Washington (WA), Oklahoma (OK), Wisconsin (WI). Case WI-1 is the index case.

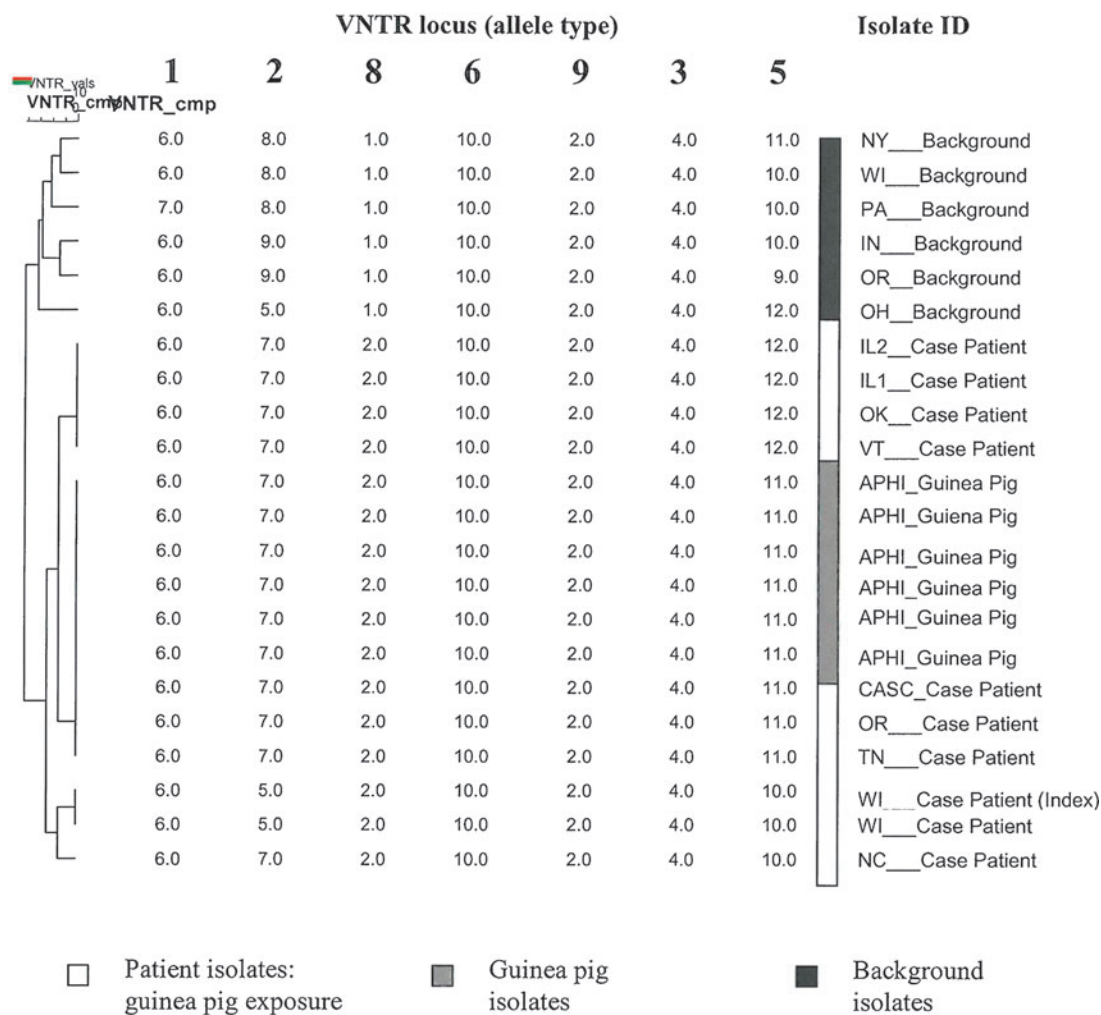


FIG. 2. Multilocus variable-number tandem repeat (VNTR) analysis (MLVA) results of guinea pig (GP) and human *S. enterica* serotype Enteritidis isolates. Three patient isolates (Washington, Tennessee, and California) and six GP isolates had MLVA patterns that were indistinguishable (pattern 1). The remaining seven clinical isolates exhibited three MLVA patterns; five differed from pattern 1 only at VNTR locus 5 by one repeat unit and two differed from pattern 1 at loci 2 and five by a total of three repeats. Loci 2 and 5 are highly variable loci that often evolve during an outbreak. Each of 90 clinical isolates (background isolates) of SE from patients with no history of GP exposure displayed one of six MLVA patterns that were not closely related to pattern 1.

water and not disinfected. State health department staff in Oklahoma and Tennessee collected feed and fecal specimens from patient homes. Local and state health department staff in Wisconsin and Oklahoma conducted site visits at pet stores where patients purchased GPs. Environmental samples obtained included fecal specimens from the sales floor cages, quarantine cages, isolation cages, and stock feed samples. Cultures of all environmental samples collected were negative for *Salmonella* (Table 1).

Traceback investigation

The traceback investigation to determine the distribution and ultimate source of GPs associated with this outbreak was complex. Officials from USDA APHIS conducted site inspections at seven GP broker or vendor facilities. This investigation identified 37 USDA Class A licensed (Table 2) breeders, 48 unlicensed breeders, 14 USDA Class B licensed brokers, and one animal auction as possible GP sources for

pet stores associated with this outbreak; notably, 86% of breeders and brokers identified were located in Pennsylvania. For the six chain A-associated cases, four primary vendors located in four states distributed GPs to the case-associated stores (Fig. 3). Primary vendors supplying the chain A case-associated stores received GPs from 92 breeders and brokers in eight states (Fig. 3). Although no common GP source for all cases was identified, one Pennsylvania breeder (breeder A) supplied GPs to the Texas vendor and to a Pennsylvania broker who supplied other vendors (Fig. 3). During 2010, breeder A shipped GPs to brokers. Thus, GPs from breeder A might have been supplied to five of six pet stores known to be associated with outbreak-related cases.

On May 25, 2011, USDA APHIS, Pennsylvania Department of Health, and Pennsylvania Department of Agriculture officials inspected the breeder A premises. No evidence of ill GPs was noted during the visit. Cultures of the 55 environmental samples collected at breeder A facilities were negative for *Salmonella* (Table 1).

TABLE 1. ENVIRONMENTAL SAMPLES COLLECTED AND CULTURED FOR THE PRESENCE OF *SALMONELLA* BY STATE OF COLLECTION AS FOLLOWS: TENNESSEE (TN), OKLAHOMA (OK), WISCONSIN (WI), AND PENNSYLVANIA (PA)

State (total number of samples)	Collection date	Location of sample collection	Sample (number collected)	Salmonella result
TN (5) ^a	12/10/2010	Patient home	Stool	Negative
			Cage (plastic) swab	Negative
			Cage (wire) swab	Negative
			Bedding	Negative
OK (9) ^{b,c}	12/29/2010	Chain A store	Feed	Negative
			Stool	Negative
			Bedding	Negative
			Cage (isolation) swab (3)	Negative
		Patient home	Cage (new arrival) swab	Negative
			Stock feed	Negative
			Stool	Negative
WI (11) ^{d,e}	12/22/2010	Chain A store	Feed	Negative
			Stool (5)	Negative
			Feed (2)	Negative
			Cage swab (4)	Negative
PA (55) ^f	12/03/2010	Index patient home	Stool (18)	Negative
	5/25/2011	Breeder A facilities	Feed (11)	Negative
			Water (8)	Negative
			Bedding (15)	Negative
			Hay (1)	Negative
			Bedding or stool (2)	Negative

^aTennessee Department of Health.

^bOklahoma State Department of Health Public Health Laboratory.

^cOklahoma Department of Agriculture, Food, and Forestry Laboratory Services Division (feed sample testing).

^dWisconsin State Laboratory of Hygiene.

^eWisconsin Department of Agriculture, Trade and Consumer Protection Bureau of Laboratory Services (feed sample testing).

^fPennsylvania Department of Health Bureau of Laboratories.

One Illinois independent pet store received GPs from two primary brokers. Each primary broker received GPs from two sources, one licensed breeder and one secondary broker. These four sources were located in four states. Although one secondary broker and one breeder supplied GPs to two different vendors that in turn supplied GPs to chain A, no single-source link was detected between the Illinois pet store where the Illinois patient (Illinois-1) purchased a GP and the other illness-associated stores (Fig. 3).

Discussion

We describe the first documented multistate outbreak of SE infections associated with exposure to GPs. A previously reported outbreak of SE infection in Canada involved one

family exposed to an infected colony of GPs (Fish et al. 1968). The outbreak we describe was detected during investigation of a severe SE infection in a child who cared for two pet GPs. Use of molecular techniques and PulseNet and NVSL databases resulted in detection of nine additional infections among persons with GP exposure and at least four infections among GPs caused by the outbreak strain. MLVA analysis revealed that SE isolates from persons with GP-associated infections and from GPs were either indistinguishable or closely related.

GPs can be an ideal vehicle for pathogen transmission to children. GPs can harbor asymptomatic infection, making identification of *Salmonella*-infected GPs challenging. Treatment of *Salmonella*-infected GPs does not reliably eliminate carriage. Characteristics making GPs popular pets

TABLE 2. US DEPARTMENT OF AGRICULTURE CLASS OF LICENSES: ANY PERSON OPERATING OR DESIRING TO OPERATE AS A DEALER MUST HAVE A VALID CLASS A (BREEDER) OR CLASS B (BROKER) LICENSE

Class license type	Requirement
Class A license	Dealer with a Class A license may conduct business involving <ul style="list-style-type: none"> • only animals that are bred and raised on his or her premises in a closed or stable colony, and • animals acquired for the sole purpose of maintaining or enhancing the breeding colony
Class B license	Dealer with a Class B license may conduct business involving <ul style="list-style-type: none"> • animals bred and raised on his or her premises • the purchase or resale of any regulated animal • negotiating the purchase or sale of any regulated animal • the exhibition of regulated animals as minor part of his or her business • the transportation in commerce for compensation animals not bred and raised on his or her premises

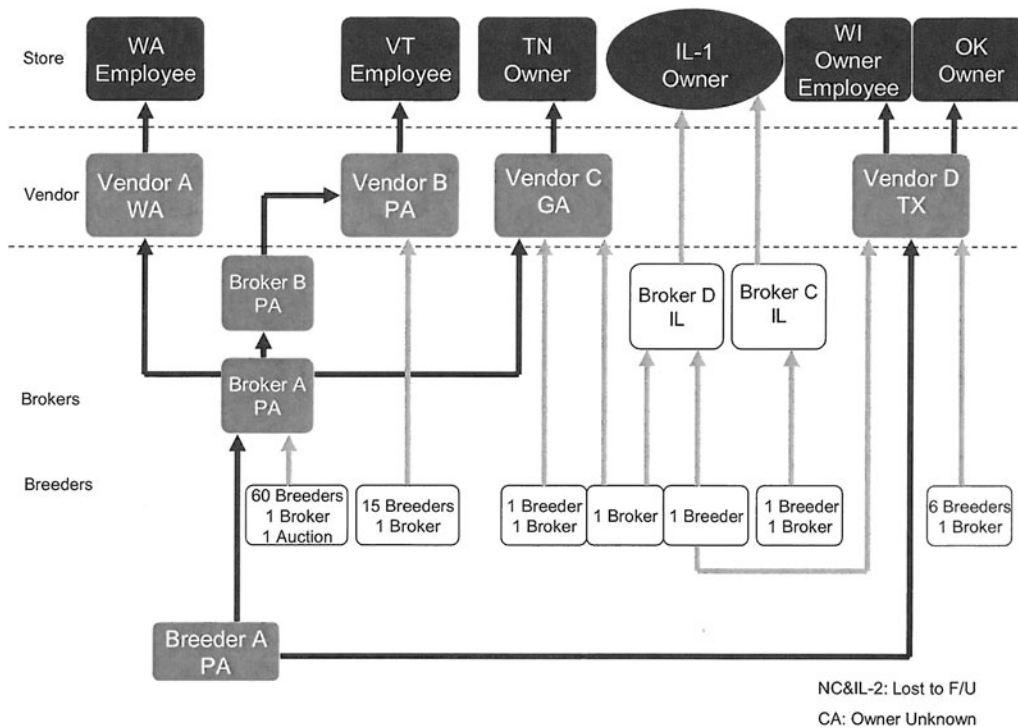


FIG. 3. Routes of guinea pig distribution from breeder to broker or vendor to store for guinea pig-associated cases of *S. enterica* serotype Enteritidis infection. The Washington (WA), Vermont (VT), Tennessee (TN), Wisconsin (WI), and Oklahoma (OK) cases are associated with a national pet store chain (chain A). The Illinois-1 case was associated with an independent pet store.

for children (small size, sociability, comfort with being held, and their soft and cuddly nature) might also place children at increased risk for infection. Finally, the use of pets, including GPs, as central characters in movies and advertisement campaigns might influence demand for these animals as pets (Osterhoudt 2012).

Results of investigations conducted by the USDA, local and state health departments, and state departments of agriculture plus our traceback activities detail the breadth and complexity of GP distribution from breeder to broker or vendor, broker or vendor to pet store, and pet store to consumer. This investigation identified 37 USDA licensed and 48 unlicensed breeders, 14 USDA-licensed brokers, and one animal auction as possible GP sources for the outbreak-associated pet stores; 86% of the breeders and brokers identified during this investigation were located in Pennsylvania.

Because detailed record keeping of individual GPs was not routinely conducted throughout the distribution chain, identifying breeders or brokers of ill GPs or infected breeding stock was challenging. Improved record keeping and understanding of the distribution systems are needed to facilitate detection of illness and zoonotic pathogen transmission among GPs. Accountability of brokers and vendors to the end vendor (e.g., pet stores) is also needed to enable tracking to facilitate prevention and control of pathogen transmission.

Although *Salmonella* was not isolated from environmental specimens, we believe it was likely that a single source of infected GPs was associated with this outbreak. All six patient illnesses associated with chain A GPs might have resulted from contact with GPs that chain A stores received through intermediate brokers or vendors from a single GP

breeder in Pennsylvania. The PFGE patterns of the SE isolates from all 10 patients were indistinguishable and matched those of the four GP isolates, including one GP isolate submitted during 2010 by the Texas GP vendor. Furthermore, MLVA of 16 human and GP isolates revealed four closely related rare patterns, in contrast to the variety of MLVA patterns reported among 90 SE PFGE XbaI pattern JEGX01.0021 isolates from humans without known GP associations.

Although we identified a potential single source of GPs for the six cases associated with chain A stores, cultures of all environmental specimens were negative for *Salmonella*. This was likely associated with inherent delays regarding outbreak detection. Notably, the index patient's illness onset was among the latest during this outbreak; the environmental sampling occurred 3–12 months after associated patient illness onsets, and breeder A sampling was conducted approximately 6 months after the last case was identified. Intermittent shedding of *Salmonella* by animals, often associated with animal stress including stress related to their transportation (Owen et al. 1983, Corrier et al. 1990, Isaacson et al. 1999, Wray and Wray 2000, Wright et al. 2005, Cummings et al. 2010), might explain our inability to detect *Salmonella* in environmental specimens. We noted GPs were transported over varying distances, the longest involved transcontinental transport from Pennsylvania to Washington State. Further, no GPs were transported directly from a breeder site to a retail pet store. Thus, *Salmonella* shedding among GPs could occur at multiple distribution levels.

Three patients were pet store employees whose duties included caring for small animals. Education regarding general

hygiene measures and encouraged use of these measures were detailed to WDPH investigators by chain A and Wisconsin chain A store employees. Documents obtained did not indicate any illness among GPs during the dates of GP purchase by the index case family or the Wisconsin chain A employee's illness.

Our investigation had multiple limitations. Ascertainment of GP exposure among *Salmonella*-infected patients varied among state and local health departments; we could not determine the extent of the response by other health departments to review enteric disease records. Additionally, because of resource constraints, we could not perform environmental sampling at all GP broker or vendor facilities; we opted to test a possible single-source breeder if located. Therefore, environmental sampling was not conducted at all levels of GP distribution. Because certain identified brokers were not inspected, possible single-source breeders might still exist. Finally, although our traceback investigation did not detect a link between GPs shipped to the Illinois independent pet store and the GPs shipped to the chain A stores, this does not preclude such a link.

Although GP distribution was complex, pet store proprietors should become knowledgeable regarding breeding sources of GPs that they sell. Methods to efficiently track GPs from breeders to brokers or vendors to pet stores will enhance animal illness surveillance and increase the likelihood of timely detection of foci of infection among GPs, and timely use of this information to prevent infection among animals and humans. Because pet rodents likely are an under-recognized source of human *Salmonella* infection (Swanson et al. 2007), pet store staff should educate customers regarding risks of *Salmonella* infection associated with GPs as well as other risks of small animal ownership, and ensure that all employees are educated regarding risks of *Salmonella* infection during their employment duties. This is particularly important for employees caring for animals or tending to animal environments. Pet store staff should promote optimal hygiene practices that include hand washing after handling small animals, caring for their environments, and discarding pet feces. Supervision of children, particularly those aged <5 years, when handling and caring for small animals is important. Health care providers and veterinarians should counsel patients and clients regarding the risks of pet ownership and simple precautions to protect patients and their families. Finally, small animal owners and pet store employees should be educated regarding practices to be discouraged. These include eating food while handling small animals, handling small animals in food preparation areas, and kissing or holding small animals close to their mouths.

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Author Disclosure Statement

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the United States Department of Agriculture. No competing financial interests exist.

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