

## ***Salmonella enterica* serotype Javiana infections associated with amphibian contact, Mississippi, 2001**

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### **SUMMARY**

*Salmonella* Javiana is a *Salmonella* serotype that is restricted geographically in the United States to the Southeast. During the summer of 2001, the number of reported *S. Javiana* infections in Mississippi increased sevenfold. To identify sources of infection, we conducted a case-control study, defining a case as an infection with *S. Javiana* between August and September in a Mississippi resident. We enrolled 55 cases and 109 controls. Thirty (55%) case patients reported exposure to amphibians, defined as owning, touching, or seeing an amphibian on one's property, compared with 30 (29%) controls (matched odds ratio 2·8,  $P=0\cdot006$ ). Contact with amphibians and their environments may be a risk factor for human infection with *S. Javiana*. The geographic pattern of *S. Javiana* infections in the United States mimics the distribution of certain amphibian species in the Southeast. Public health officials should consider amphibians as potential sources of salmonellosis, and promote hand washing after contact with amphibians.

### **INTRODUCTION**

Salmonellosis was recently estimated to cause 1·4 million illnesses annually in the United States [1]. Human infection most commonly results from ingestion of contaminated foods of animal origin. However, in several recent outbreak investigations, fresh produce, water and contact with reptiles have also been implicated as vehicles of transmission [2–4].

Most *Salmonella enterica* serotypes that have been linked to commercial food sources are geographically distributed throughout the United States. However, infections caused by some serotypes appear to be geographically clustered in certain areas of the country. *S. enterica* serotype Javiana (*S. Javiana*) is one such serotype that displays a striking geographic distribution. Over the last four decades, areas with the highest rates of *S. Javiana* infection have consistently clustered in the southeastern United States [5], particularly the coastal areas of North and South Carolina, central Arkansas, and the southern counties of Georgia. In addition, there is a marked seasonality

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to the incidence of *S. Javiana* infections that exceeds that of most *Salmonella* serotypes; human *S. Javiana* infections occur almost exclusively in July, August and September. *S. Javiana* also disproportionately affects infants and young children as over 40% of infections occur in those less than 5 years old [5]. By comparison, less than 30% of infections due to other *Salmonella* serotypes occur in children of this age group.

In both relative and absolute terms, *S. Javiana* infections have become increasingly common over the last decade, rising in rank from the eleventh to the fifth most commonly isolated *Salmonella* serotype among humans nationwide. The number of *S. Javiana* isolates reported to CDC increased from 700 in 1990, to over 1100 in 2000 [6]. These reported infections represent only a fraction of the illness caused by *S. Javiana*, since the degree of under-reporting for *Salmonella* infections has been estimated at 38-fold [1]. Despite the increasing rate of infection, risk factors and sources for *S. Javiana* infection remain largely unknown.

In September 2001, CDC was notified of an increase in *S. Javiana* infections in Mississippi. Concurrent with exceptionally high rainfall between June and September, 43 cases of *S. Javiana* were reported to the Mississippi State Department of Health (MSDH), a sevenfold increase over the previous year. The majority of cases occurred in the three counties that comprise the greater Jackson, Mississippi area (Hinds, Madison and Rankin). Initial interviews with patients did not reveal any common food exposures. We therefore conducted a case-control study to identify risk factors and sources for *S. Javiana* infection.

## METHODS

### Case finding

All *Salmonella* isolates are routinely sent to the Mississippi State Public Health Laboratory (MSPHL) for confirmation and serotyping. We reviewed state *Salmonella* surveillance data to obtain demographic information and onset-of-illness dates for all patients with confirmed *S. Javiana* infection reported between January and September 2001.

### Hypothesis generation

To generate hypotheses for potential sources of infection, in-depth face-to-face interviews were

conducted with randomly selected patients with culture-confirmed illness. Patients were questioned about food, water and environmental exposures in the week preceding onset of illness.

### Case-control study

For the purposes of the case-control study, a case was defined as a culture-confirmed *S. Javiana* infection in a Mississippi resident with onset of illness occurring between 1 August and 30 September 2001. Two methods were used to select controls depending on the age of patients. For patients less than 5 years old, controls were randomly selected by using the Mississippi State Birth Registry and were age-matched by month and year of birth, and by county of residence at the time of birth. For patients aged 5 years and older, controls were randomly selected using sequential digit dialling and were matched to the patient by age group and county of residence. Controls for patients between 6 and 20 years old were age-matched to within 2 years of the patient's age. Controls for patients aged 21 years and older were matched to within 5 years of the patient's age.

Persons were excluded as controls if they had any previous personal history of *Salmonella* infection, or a history of diarrhoea (defined as three or more loose stools in a 24-h period) or fever greater than 38 °C with abdominal pain at any time during the 14 days preceding the date of interview. Persons were excluded as controls for patients residing in Hinds, Madison and Rankin counties if they did not reside in the any of these three counties. For patients in other counties, individuals were excluded as controls if they did not reside in the same county as the patient. The goal was to obtain two controls per patient.

We administered a written study questionnaire by telephone from 30 September to 12 October. If a patient or control was less than 15 years old, the interview was conducted with the guardian, assisted by the child when appropriate. Both patients and controls were asked about consumption of food items, sources of drinking water, type of home residence, exposure to lakes and rivers, and exposure to animals. For the purposes of the study, we defined exposure to an animal as keeping or owning an animal, seeing an animal on one's property or yard, or touching an animal outside the home in the week before onset of illness. Patients were also asked about clinical symptoms related to their illness. Patients were questioned about the 7 days before the onset of

illness, while controls were asked about the 7 days preceding the date of interview.

### Laboratory investigation

Culture-confirmed *Salmonella* isolates were serotyped at MSPHL according to the Kauffmann–White scheme [7]. Antimicrobial susceptibility testing was performed on all confirmed *S. Javiana* isolates by disk diffusion according to the methods of the National Committee of Clinical Laboratory Standards to the following antimicrobial agents: ampicillin, amoxicillin–clavulanate, carbenicillin, cefazolin, cefuroxime, cephalothin, ceftriaxone, ciprofloxacin, gentamicin, minocycline, nalidixic acid, norfloxacin, ofloxacin, ticarcillin–clavulanate, tobramycin and trimethoprim–sulphamethoxazole [8]. Molecular subtyping by pulsed-field gel electrophoresis (PFGE) was performed on *S. Javiana* isolates using restriction enzymes *Xba*I and *Avr*II at the Louisiana State Public Health Laboratory and CDC, using previously described methods [9].

### Environmental investigation

Amphibians and reptiles from the neighbourhoods and yards of patients were collected and cultured for the presence of *S. Javiana*. Animals were grouped by species and by date and location of collection and placed in dry terrariums to enhance faecal shedding. After 24 h, approximately 15 ml of sterile water was added to the terrarium and this liquid was recovered after 24 h and submitted to the MSPHL for *Salmonella* culture. The total water sample from each animal specimen was incubated with an equal volume of double-strength Selenite broth at 35 °C for 24 h and subcultured on Hektoen enteric agar and xylose–lysine–desoxycholate (XLD) agar. Hydrogen sulphide-producing colonies were transferred to triple-sugar iron agar slants, and presumptive *Salmonella* colonies were confirmed by biochemical tests. All *Salmonella* isolates were serotyped. All animals were subsequently released to their original habitats.

### Review of national *Salmonella* surveillance data

In order to determine the national distribution of *S. Javiana*, data were reviewed on *Salmonella* isolates reported through the Public Health Laboratory Information System to CDC between 1968 and 2000. We further examined the annual, age-standardized

rates of reported *S. Javiana* isolates, weighted by county population for the period 1980–2000, and assuming that yearly rates were comparable, the counties with the highest rates of *S. Javiana* isolation for the 20-year period were mapped.

### Statistical analysis

Matched univariate analyses using SAS software version 8.2 (SAS Institute, Carey, NC, USA) were conducted and risk factors for infection were expressed as matched odds ratios (mOR) with 95% confidence intervals (CI) for categorical variables. A multivariate conditional logistic regression model was constructed to identify independent variables that were significant risk factors for infection. Variables that were significantly associated with illness in univariate analysis and were biologically plausible risk factors were included in the model. A *P* value of  $\leq 0.05$  was taken as significant.

## RESULTS

### Case finding

A total of 66 cases of *S. Javiana* infection were reported to MSDH between January and September 2001. Cases occurred primarily in the summer months, and peaked in August and September (Fig. 1). More than 70% of the cases occurred in children less than 5 years old.

### Hypothesis generation

We conducted in-person interviews with 11 patients with culture-confirmed illness. Onset-of-illness dates for patients ranged from 1 July to 1 September 2001. Patients did not report any common food or water exposures. However, all interviewed patients did report seeing an increased number of frogs and toads on their property in the 2 weeks before the onset of illness.

### Case-control study

Fifty-five out of 57 eligible patients were successfully enrolled in the case-control study. Thirty-seven patients (67%) were from the greater Jackson area, and 31 (56%) were female. The median age of patients was 24 months (range 3 months to 70 years). Many patients lived in newer suburban housing

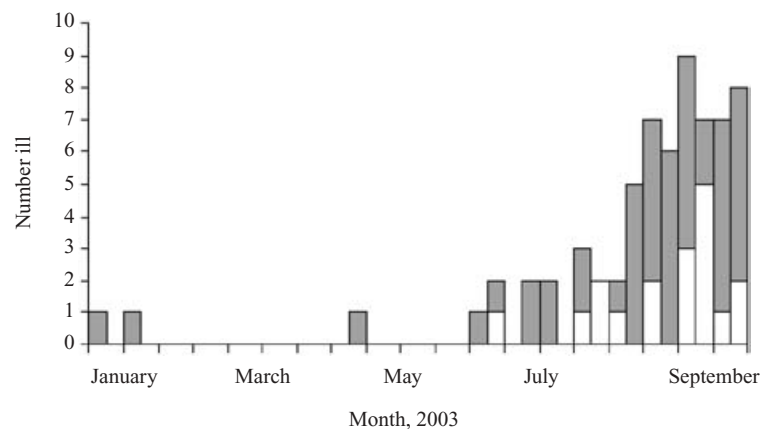


Fig. 1. *S. Javiana* infections by week, Mississippi, 2001. ■, 0–4 years; □, ≥5 years.

Table 1. Clinical characteristic of patients enrolled in the case-control study, Jackson, Mississippi, 2001

Clinical characteristic	Patients ( <i>n</i> = 55)	
	<i>n</i>	(%)
Diarrhoea	55	(100)
Fever	47	(86)
Abdominal pain	45	(83)
Vomiting	26	(48)
Bloody diarrhoea	24	(44)
Received antimicrobial therapy	40	(73)
Hospitalization	9	(16)

developments, in what were previously undeveloped floodplains. Illness was dispersed relatively evenly over the 2-month case definition period, with 53% of infections occurring in September. The clinical characteristics of the study patients are summarized in Table 1. The median duration of illness was 7 days and all patients reported episodes of diarrhoeal illness, with 24 (44%) reporting bloody diarrhoea. Other predominant symptoms included fever (86%), abdominal pain (83%) and vomiting (48%). Forty (73%) patients received antibiotic therapy for their illness and 9 (16%) were hospitalized. There were no deaths.

Patients were compared with 104 age-matched controls. The results of matched univariate analysis are summarized in Table 2. Univariate analysis of food exposures revealed that patients were significantly more likely to have consumed orange juice (mOR 2.9, 95% CI 1.2–6.8,  $P=0.02$ ) than were controls, although patients did not consume any one specific brand of orange juice. Patients were also more likely to have eaten watermelon (mOR 9.1, 95%

CI 1.1–78.4,  $P=0.05$ ) than were controls, although this was an uncommon exposure among patients and could only account for a small percentage (12%) of illnesses. Many other food exposures, including consumption of meat products and eggs, were not associated with illness. There was no association between illness and either previous diarrhoeal illness in a household member or exposure to a child-care setting. Patients were more likely than controls to have private health insurance (mOR 2.4, 95% CI 1.1–5.5,  $P=0.03$ ).

Univariate analysis of environmental exposures revealed that patients were more likely than controls to have visited a lake or pond (mOR 2.8, 95% CI 1.3–5.8,  $P=0.006$ ) and were more likely than controls to have had exposure to snakes (mOR 7.3, 95% CI 1.5–34.7,  $P=0.01$ ), turtles (mOR 6.2, 95% CI 1.7–22.7,  $P=0.006$ ), and frogs or toads (mOR 2.6, 95% CI 1.4–4.9,  $P=0.004$ ). Of the 30 patients who reported exposure to frogs or toads, only one reported actually touching a frog or toad. All other patients and all controls reported that their primary exposure to frogs or toads had been seeing the animals on their property or in their yard. The frequency of exposure to frogs or toads was not substantially different in a sub-analysis stratified by month of illness. This finding suggests ongoing exposure to frogs, toads and turtles throughout the epidemic period. Patients did not live in newer homes than controls. However, patients who were exposed to frogs or toads lived in homes that were built more recently than those of patients without this exposure (median year of construction 1987 vs. 1980,  $P=0.03$ ).

In multivariate analysis, exposure to frogs or toads (mOR 2.5, 95% CI 1.2–5.6,  $P=0.02$ ) and exposure to turtles (mOR 5.1, 95% CI 1.3–21.1,  $P=0.02$ )

Table 2. Univariate analysis of exposures associated with *S. Javiana* infection among cases and matched controls, Jackson, Mississippi, 2001

Risk factor	Patients (n=55)		Controls (n=104)		mOR	95% CI	P value
	n	(%)	n	(%)			
Private insurance	42	(76)	56	(56)	2.5	1.1–5.5	0.03
Eating watermelon*	6	(12)	1	(1)	9.1	1.1–78.4	0.05
Drinking orange juice*	26	(49)	32	(31)	2.9	1.2–6.8	0.02
Visiting a lake/pond*	22	(40)	19	(18)	2.8	1.3–5.8	0.006
Exposure to snakes*†	8	(15)	3	(3)	7.3	1.5–34.7	0.01
Exposure to turtles*†	10	(18)	10	(4)	6.2	1.7–22.7	0.006
Exposure to frogs or toads*†	30	(55)	30	(29)	2.6	1.4–4.9	0.004

\* In the 7 days before onset of illness for cases, in the 7 days before date of interview for controls.

† Exposure defined as keeping or owning an animal, seeing an animal on one's property or yard, or touching an animal outside the home in the week before onset of illness.

mOR, matched odds ratio; CI, confidence interval.

remained independently associated with illness (Table 3). The frequency of exposure to turtles among patients was relatively low (18%), and could only account for a small number of infections. Furthermore, among the 10 patients exposed to turtles, 8 were also exposed to frogs or toads. Private insurance also remained significantly associated with illness in multivariate analysis (mOR 3.2, 95% CI 1.2–8.6,  $P=0.02$ ). Consumption of watermelon was not significantly associated with illness (mOR 10.3, 95% CI 0.9–115.6,  $P=0.06$ ) and consumption of orange juice, exposure to snakes, and visiting a lake or pond were not independently associated with illness.

### Laboratory investigation

Antimicrobial susceptibility testing revealed that all isolates of *S. Javiana* were susceptible to all antimicrobials tested. Molecular subtyping of 51 isolates yielded 18 distinct PFGE patterns, indicating that this was not a point-source outbreak. The most common PFGE pattern was found in 20 isolates (39%), suggesting that clusters of infection may exist. However, a further analysis of these 20 patients revealed no epidemiological link or common exposure unique to this group.

### Environmental investigation

A total of 32 reptiles and amphibians were collected between 2 and 18 October 2001 and grouped in 21 separate terrariums. The 21 amphibians came from two species: Fowler's toad (*Bufo woodhouse fowleri*)

Table 3. Multivariate analysis of exposures associated with *S. Javiana* infection among cases and matched controls, Jackson, Mississippi, 2001

Risk factor	mOR	95% CI	P value
Frogs/toads	2.5	1.2–5.6	0.02
Turtles	5.1	1.3–21.1	0.02
Private insurance	3.2	1.2–8.6	0.02
Watermelon	10.3	0.9–115.6	0.06

mOR, matched odds ratio; CI, confidence interval.

and the Southern cricket frog (*Acris gryllus gryllus*). The collected amphibians were grouped in 10 separate terrariums. The species of the 11 reptiles collected were green anole (*Anole carolinensis*), red-eared slider turtle (*Trachemys scripta elegans*), ground skink (*Scincella lateralis*), and common musk turtle (*Sternotherus odoratus*). *S. Javiana* was not recovered from any of the animal specimens collected during the study. *S. Newport* was isolated from one Fowler's toad.

### Review of national *Salmonella* surveillance data

Review of national *Salmonella* surveillance data indicated that the majority of *S. Javiana* infections occurred in the Southeast region. Between 1980 and 2000, several counties in the southeastern United States have reported consistently high rates of *S. Javiana* isolation. Counties with rates of *S. Javiana* isolation in the highest 10th percentile for this 20-year period are shown in Figure 2. Certain counties in

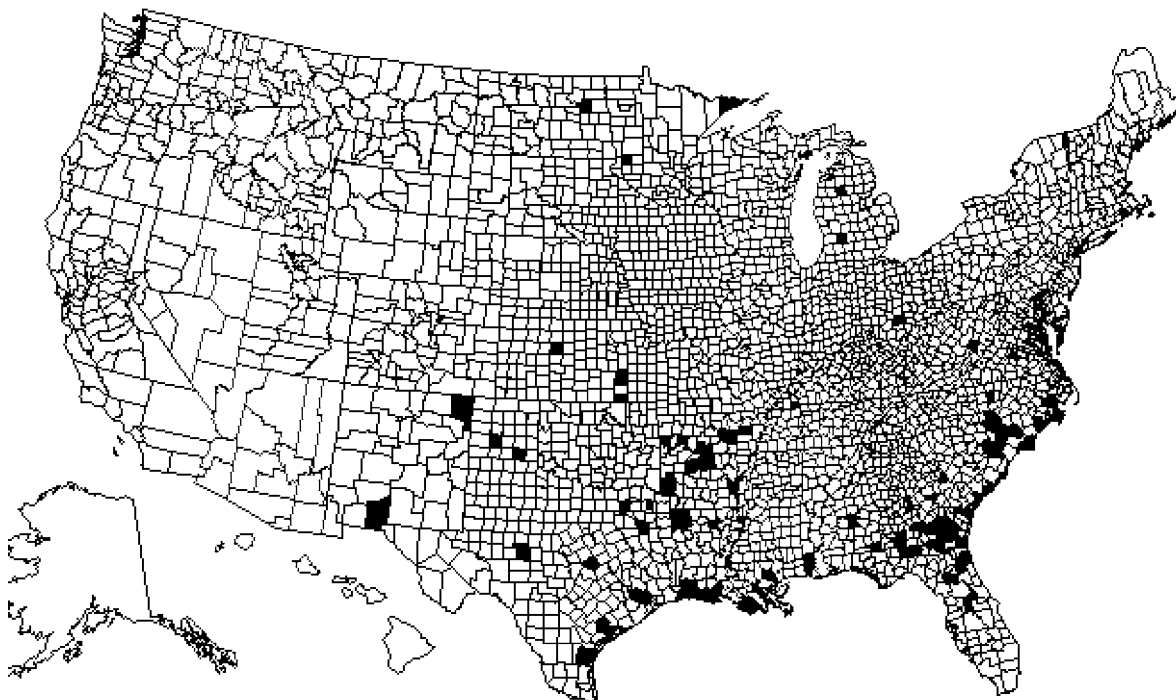


Fig. 2. Counties reporting rates of *S. Javiana* isolation in the highest 10th percentile, 1980–2000.

southern Georgia, along the Gulf Coast, and central Arkansas have reported high rates of infection since 1980, while rates of infection in counties in Virginia and, North Carolina along the southern Atlantic coast have markedly increased in the last decade.

## DISCUSSION

This investigation provides the first epidemiological evidence that human infection with *S. Javiana* is associated with direct and indirect exposure to amphibians. Previous studies have demonstrated that frogs and toads are frequent carriers of *Salmonella* of multiple serotypes, including *S. Newport*, *S. Saintpaul*, *S. Bareilly*, *S. Poona* and *S. Weltevreden* [10–14]. These animals can shed the organism intermittently for long periods of time, acting as effective reservoirs of salmonellae and contaminating their local environment with these pathogens [15].

An animal reservoir may help explain several distinguishing traits of human *S. Javiana* infections, including the striking geographic distribution of infections in the southeastern United States. The diversity of PFGE patterns and the lack of antimicrobial resistance detected among isolates of patients in this investigation are consistent with the possibility of a wild animal reservoir for *S. Javiana* infection. The

geographic distribution of some amphibian species closely mimics that of *S. Javiana* infection [16]. Isolation of *S. Javiana* from a frog or toad captured in the Jackson metropolitan area would have strengthened our epidemiological findings. However, we collected samples in early October, when environmental conditions may have differed considerably from those existing when patients were exposed. The limited enrichment procedures we used may have further limited our ability to detect *Salmonella* from environmental samples. Microbiological testing of animal specimens in our investigation was limited to only two species. One species that we were unable to collect and test is the green tree frog (*Hyla cinerea*), which is often attracted to human habitations and was reportedly seen by many patients. Like *S. Javiana* infections, the distribution of this frog species clusters in the southeastern United States [16].

The marked seasonal pattern of *S. Javiana* infections may be related to the seasonality of amphibian life cycles. The activity of frogs and toads generally peaks between July and the end of September, corresponding with the peak in *S. Javiana* infections. The numbers of some amphibian species in the environment tend to be at their highest during periods of increased precipitation, potentially explaining the disproportionate number of frogs and toads many

patients reported seeing in Mississippi during and after periods of record rainfall in August 2001 [16, 17]. The increased number of animals during this period probably contributes to a higher level of environmental *Salmonella* contamination than in other seasons. Heat and humidity, common in August and September, are also likely to support the growth and survival of *Salmonella enterica* serotypes in the environment [18].

Increasing urbanization may also be a factor in the emergence of infections caused by *S. Javiana*. Many of the patients in this outbreak lived in newer homes that bordered streams, lakes and marsh areas on the outskirts of Jackson, Mississippi. The association between illness and private insurance may reflect the higher socio-economic status of patients living in these newer residential developments, and private insurance may also be associated with better access to health care. The encroachment of housing and commercial development on traditional animal habitats may result in greater interaction between human beings and the animals that may act as a reservoir for *S. Javiana*. This expanding development may provide the level of contact with an animal or animal environment that is necessary to increase the risk of infections.

Consumption of a food item that is contaminated by contact with an infected animal is another possible route of infection, and may explain the marginal association between watermelon and illness in this investigation. Watermelon, which has been implicated in a previous *S. Javiana* outbreak, is often not washed prior to eating, and may be contaminated in the field, where it is grown close to the soil [19]. Animal contamination of food and water has been demonstrated in previous *Salmonella* outbreaks; tree frogs were the likely route of drinking-water contamination in a 1999 *S. Saintpaul* outbreak [20], and tree frogs and southern toads may have introduced *S. Hartford* into unpasteurized orange juice that was associated with a multi-state outbreak in 1995 [21, 22].

According to our survey responses, most patients in our investigation did not have direct contact with a frog or toad. However, it is difficult to obtain accurate exposure data for young children, who comprised the majority of our patients and controls. Young patients may have had direct animal contact without the knowledge of their parents, who provided exposure data for our study. Frogs and toads may also be surrogate markers for better-camouflaged animals, such as turtles, that may act as a reservoir for

infection. *S. Javiana* has been isolated from the aquariums of turtles and iguanas, reptiles that are well associated with salmonellosis [23].

Nevertheless, the sighting of frogs or toads by parents suggests, at the very least, environmental contamination. Direct contact with an amphibian may not be necessary for transmission of *Salmonella*. In the early 1990s, investigations of both outbreaks and sporadic cases of reptile-associated salmonellosis among infants and children indicated that the primary risk factor for the majority of infections was indirect reptile contact [24–26]. Surfaces and objects contaminated by animal excreta can remain culture-positive for long periods of time [18, 25, 27], and potentially serve as vehicles of disease transmission long after an infected animal has actually been in contact with the surface. Moreover, contamination of indoor household surfaces such as carpets may occur if animal faeces are brought into the home on the soles of shoes or on a contaminated object. Young children, who are at greater risk of salmonellosis [28, 29], are likely to have more contact with floor surfaces and may be more affected by household contamination of floors and carpets, potentially explaining the increased incidence of *S. Javiana* in this age group. Investigators in Arkansas cultured samples from the home environments of children with culture-confirmed *Salmonella* infection within 4 days of diagnosis, and found *Salmonella* with a serotype identical to those in the index patient in almost 25% of homes, suggesting an association between contamination of the household environment and the risk of salmonellosis [30]. Investigators in another recent home environment study cultured vacuum cleaner bags to screen households for contamination with *Salmonella enterica*, and found higher levels of household carpet contamination in the homes of persons with occupational exposure to *Salmonella* [31]. Such inadvertent contamination of the household may pose a potential risk of *Salmonella* infection to young family members. Contamination of the home or play environment may also be associated with infections due to other bacterial enteric pathogens. In a recent prospective study of risk factors for *Campylobacter* infection in The Netherlands, investigators identified an association between illness among children and playing in an area with bird droppings [32]. Environmental exposures, such as contact with a farm environment and likely contact with animal excreta, have also been associated with *E. coli* O157 infections [33, 34].

Exposure to environmental and animal reservoirs of *Salmonella*, rather than exposure to a contaminated commercial food, may help to explain the striking geographic distributions of several other *Salmonella* serotypes. Infections caused by some of these geographically restricted serotypes, such as *S. Rubislaw*, *S. Mississippi*, and *S. Bareilly*, also tend to cluster in the southeastern United States, while *S. Weltevreden* infections occur almost exclusively in Hawaii [5]. Like infections caused by *S. Javiana*, infections caused by several of these serotypes also occur predominantly in young children, and similarly display sharp summer seasonality [5]. An amphibian reservoir could potentially explain the unique epidemiology of these serotypes, and warrants further investigation. Furthermore, as the risk of infection from *Salmonella* serotypes commonly found in meat, poultry, and eggs is further mitigated through the successful implementation of food safety measures, man-made changes in the environment, including urban development of wetlands and river plains, may become increasingly important in shaping the epidemiology of human salmonellosis. Public health officials should promote proper hand washing after contact with amphibians and their environments. Further environmental studies may help to better define the distribution of *Salmonella* serotypes in amphibian reservoirs and guide future control measures.

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