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Sporadic *Salmonella enterica* serotype Javiana infections in Georgia and Tennessee: a hypothesis-generating study

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SUMMARY

From 1996 to 2004, the incidence of *Salmonella* Javiana infections increased in FoodNet, the U.S. national active foodborne disease surveillance programme. Contact with amphibians and consumption of tomatoes have been associated with outbreaks of *S.* Javiana infection. To generate and test hypotheses about risk factors associated with sporadic *S.* Javiana infections, we interviewed patients with laboratory-confirmed *S.* Javiana infection identified in Georgia and Tennessee during August–October 2004. We collected data on food and water consumption, animal contact, and environmental exposure from cases. Responses were compared with population-based survey exposure data. Seventy-two of 117 identified *S.* Javiana case-patients were interviewed. Consumption of well water [adjusted odds ratio (aOR) 4·3, 95% confidence interval (CI) 1·6–11·2] and reptile or amphibian contact (aOR 2·6, 95% CI 0·9–7·1) were associated with infection. Consumption of tomatoes (aOR 0·5, 95% CI 0·3–0·9) and poultry (aOR 0·5, 95% CI 0·2–1·0) were protective. Our study suggests that environmental factors are associated with *S.* Javiana infections in Georgia and Tennessee.

Key words: Epidemiology, *Salmonella*, *Salmonella enterica*, salmonellosis, zoonoses.

INTRODUCTION

Salmonellosis is a bacterial illness affecting an estimated 1·4 million Americans each year [1]. Symptoms include fever, bloody diarrhoea, and abdominal pain, and infection can result in hospitalization and death. More than 2500 serotypes of *Salmonella* have been identified, and different serotypes are associated with different sources. In 2004, *Salmonella* serotype Javiana was the fourth most common serotype in the USA, accounting for 5% of all reported *Salmonella* cases [2]. In one tertiary-care hospital in northern Florida, *S.* Javiana was the most common serotype, accounting for 29% ($n = 126$) of all patient *Salmonella* isolates from 1986 to 1992 [3]. The incidence of *S.* Javiana increased significantly in the Foodborne Diseases Active Surveillance Network (FoodNet) sites by 167% from 1996–1998 to 2004 [4]. In 2004, Georgia had the highest 5-year average incidence (2·79/100 000 population) and Tennessee had the second highest 5-year average incidence (0·81/100 000

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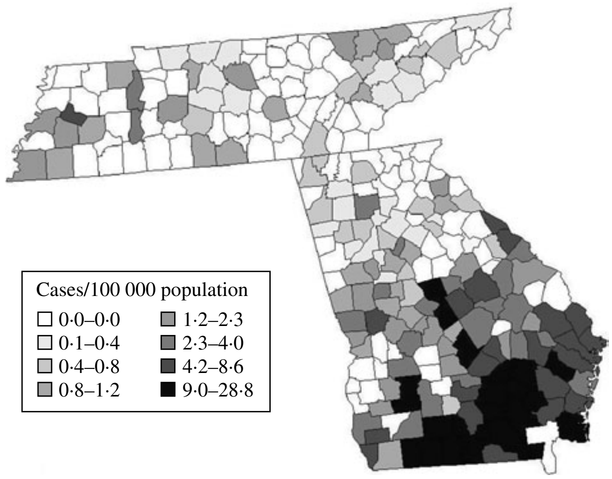

Fig. 1. Average annual incidence rate of *Salmonella* serotype Javiana by county, Georgia and Tennessee, 1996–2005.

population) of *S*. Javiana among FoodNet sites. In Georgia and Tennessee, cases occur most frequently from August to October. In Georgia, *S*. Javiana cases occur more frequently in the southern rural part of the state (Fig. 1).

Risk factors for *S*. Javiana infection are not well characterized. Several outbreaks of *S*. Javiana in the USA have been associated with consumption of tomatoes [5–7]. The source of tomato contamination in one outbreak was contaminated rinse water from South Carolina, suggesting environmental contamination [5]. Other foods, including watermelon [8] and potato chips flavoured with contaminated paprika [9] have been associated with *S*. Javiana infection. Amphibian contact was suspected to be associated with *S*. Javiana infections in Mississippi [10]. In contrast to other *Salmonella* serotypes, *S*. Javiana is rarely isolated from retail meat products. From 1998 to 2005, the United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) isolated *S*. Javiana from $<0{\cdot}5\%$ of broiler chicken and ground beef samples, with no *S*. Javiana identified in ground beef since 2003 [11].

In 2004, an outbreak of 429 cases of multiple *Salmonella* serotypes including *S*. Javiana associated with tomato consumption was investigated in nine states (not including Georgia) [7]. Following the outbreak, the Georgia and Tennessee FoodNet sites conducted an investigation of sporadic *S*. Javiana cases to examine the role of non-foodborne transmission, evaluate the role of tomato consumption, and generate hypotheses for future studies.

METHODS

FoodNet is a CDC-coordinated project that conducts active, population-based foodborne disease surveillance and epidemiological studies in ten sites: Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and selected counties in California, Colorado, and New York [12]. In 2004, the population of the catchment area for this study (Georgia and Tennessee) was 14 730 345 or 5% of the US population. Surveillance for *S*. Javiana cases was conducted through the state public health laboratories in Georgia and Tennessee, which are responsible for serotyping *Salmonella* isolates submitted by clinical laboratories. A case-patient was defined as a Georgia or Tennessee resident that had laboratory-confirmed *S*. Javiana infection identified during August–October 2004. Case-patients were interviewed by telephone or in person using a standard protocol to ensure consistency of interview technique. At least ten attempts were made to contact each case-patient within 45 days of the first positive *Salmonella* culture. At least two attempts were conducted on the weekend and two in the evening, to ensure inclusion of working adults. For case-patients aged <18 years, interviewers spoke with the primary caregiver. Case-patients were compared to control respondents from Georgia and Tennessee interviewed as part of the 2002–2003 FoodNet Population Survey and limited to the months of August–October to control for any possible seasonal effects [12]. The FoodNet Population Survey is a telephone survey conducted on a representative sample of persons from FoodNet sites.

Case-patients were asked about foods consumed, including fruits and vegetables, with specific mention of tomatoes, meats, and dairy items, in the 7 days before illness onset. Animal exposure was defined as having physically touched a frog, toad, snake, salamander, newt, lizard, gecko, iguana, or turtle. Drinking well water was assessed from responses to the question, 'In the 7 days prior to illness, did you drink from the following sources of water: municipal or city water (including filtered water), private well water, or bottled water?' Participation in recreational water activities was assessed from responses to the question, 'In the 7 days prior to illness were you near a lake, pond, creek, spring, or river?' and, if yes, 'Did you do any of the following activities: swim in the water, wade in the water, touch the water, drink or swallow the water?'

Table 1. *Study population characteristics, Georgia and Tennessee, August–October 2004**

	Cases ($n=72$)		Controls ($n=824$)			
Characteristics	n/N	(%)	n/N	(%)	OR	(95% CI)
Age <13 years	44/72	(61)	112/824	(14)	10·0	(6·0–16·7)
Male	44/72	(61)	321/824	(39)	2·5	(1·5–4·0)
White race	51/69	(74)	636/817	(78)	0·8	(0·5–1·4)
Rural residence	18/65	(28)	138/820	(17)	1·9	(1·1–3·4)
College graduate or higher	10/62	(16)	357/820	(44)	0·3	(0·1–0·5)
Insured	65/71	(92)	746/819	(91)	1·1	(0·4–2·5)

OR, Odds ratio; CI, confidence interval.
* Denominator varies due to missing or unknown responses.

Population Survey controls were asked about foods consumed, animal exposure, well water consumption, and participation in recreational water activities in the 7 days prior to interview. Animal exposure was assessed from responses to the question, 'In the past 7 days, did you have contact with a reptile or amphibian, such as a snake, turtle, or frog?' Drinking well water or recreational water was assessed from responses to the question, 'In the past 7 days, did you drink any water directly from the following sources: lake, pond, river, or stream; private well?' Demographic information was also collected. Because of the design of the survey, the proportion of respondents that were asked each question changed according to the exposure question.

Case-patient data were entered and cleaned in Epi-Info 3.3 for Windows. Control data were available in a SAS 9.1 file. Case-patient and control data were analysed using SAS 9.1 for Windows. For several exposures, responses to several questions were combined into a composite variable for comparability to the control questionnaire. Reptile and amphibian exposures were combined into a composite variable because control data were not available for individual animal exposures. Case-patients and controls that travelled internationally in the 7 days prior to illness were excluded from analysis. We assumed younger children and pre-teens had different types of outdoor activities than older children and adults and therefore dichotomized age at 13 years in the analyses. Rural residence was defined as response to a question about home location of 'rural area not on farm' or 'on a farm in rural area'. Baseline characteristics including demographic variables were compared using χ^2 or Fisher's exact tests with an alpha of 0·05. Case-patients and controls were compared by exposure to foods, reptiles or amphibians, well water, and recreational water activities, and stratified by age, gender, and rural residence using the Breslow–Day test of homogeneity. Logistic regression analyses were performed to control for age, gender, and rural residence.

RESULTS

Seventy-two (62%) of 117 *S.* Javiana case-patients identified during the study period were enrolled. No significant differences in age, gender, or race were identified between 72 enrolled and 45 non-enrolled case-patients. Study controls were the 824 persons in Georgia and Tennessee interviewed for the FoodNet population survey in the months of August–October. Case-patients were more likely than controls to be male (61% *vs.* 39%, $P<0·01$) (Table 1). Case-patients were more likely to be aged <13 years than controls (61% *vs.* 14%, $P<0·01$), with a median case-patient age of 5 years and a median control age of 41 years. Seventy-four per cent of case-patients were white compared with 78% of controls ($P=0·45$). Ethnicity was not analysed because only one case-patient was Hispanic. Case-patients were more likely than controls to live in a rural area (28% *vs.* 17%, $P=0·03$), and less likely to have completed college (16% *vs.* 44%, $P<0·01$). Nearly all case-patients and controls had health insurance (92% *vs.* 91%, $P=0·90$).

Case-patients were less likely than controls to have reported consumption of tomatoes [odds ratio (OR) 0·3, 95% confidence interval (CI) 0·2–0·5], poultry items including chicken and turkey products (OR 0·5, 95% CI 0·2–0·9), and shell eggs (OR 0·6, 95% CI 0·4–1·1) (Table 2). Fewer case-patients than controls consumed cantaloupe, scallions, sprouts, or lettuce, with a statistically significant difference between

Table 2. *Food and environmental risk factors associated with* Salmonella *Javiana infection, Georgia and Tennessee, August–October 2004**

	Cases ($n=72$)		Controls			
Exposure	n/N	(%)	n/N	(%)	OR	(95% CI)
Uncooked tomatoes	27/67	(40)	285/413	(69)	0·3	(0·2–0·5)
Sprouts	2/70	(3)	22/413	(5)	0·5	(0·1–2·3)
Scallions	7/69	(10)	92/414	(22)	0·4	(0·2–0·9)
Lettuce	37/70	(53)	273/413	(66)	0·6	(0·4–1·0)†
Cantaloupe	15/70	(21)	107/413	(26)	0·8	(0·4–1·4)
Poultry	54/70	(77)	364/413	(88)	0·5	(0·2–0·9)
Shell eggs	31/64	(48)	245/409	(60)	0·6	(0·4–1·1)
Recreational water	3/67	(4)	2/407	(0·4)	9·5	(1·6–57·9)
Well water	18/68	(26)	37/405	(9)	3·6	(1·9–6·8)
Reptile or amphibian contact‡§	9/50	(18)	32/413	(8)	2·6	(1·2–5·9)
Frog/toad	5/44	(11)				
Snake	1/49	(2)				
Salamander/newt	1/49	(2)				
Lizard/gecko	4/46	(9)				
Iguana	0	(0)				
Turtle	2/48	(4)				

OR, Odds ratio; CI, confidence interval.

* For cases, denominator varies for each variable due to missing or unknown responses; for controls, some of the variables were asked of only half of the respondents, so denominator varies according to skip patterns.

† The upper limit of the confidence interval was 0·959 prior to rounding.

‡ Missing exposure data for 24 cases for types of reptiles and amphibians.

§ Specific reptile/amphibian questions were only asked of cases, and some individuals had contact with multiple types.

groups for scallions and lettuce. Case-patients were more likely than controls to have had contact with reptiles or amphibians (OR 2·6, 95% CI 1·2–5·9) (Table 2). For case-patients, the most frequently reported contacts were with frogs or toads ($n=5$) and lizards or geckos ($n=4$). Case-patients were more likely than controls to have consumed well water (OR 3·6, 95% CI 1·9–6·8) or to have swallowed water during recreational water activities (OR 9·5, 95% CI 1·6–57·9). Stratified analyses (data not shown) found no significantly different exposures by age, gender, or rural residence.

Logistic regression models were used to identify associations between the various food and environmental exposures as independent variables and infection as the dependent variable, while controlling for age, gender, and rural residence (Table 3). In the multivariate analyses, consumption of tomatoes remained protective [adjusted odds ratio (aOR) 0·5, 95% CI 0·3–0·9] and consumption of well water remained associated with infection (aOR 4·3, 95% CI 1·6–11·2). Consumption of poultry items approached significance as a protective factor (aOR 0·5, 95% CI 0·2–1·0), as did contact with reptiles or amphibians as a risk factor (aOR 2·6, 95% CI 0·9–7·1), each with P values of 0·06.

Table 3. *Multivariate analyses of food and environmental exposures, controlling for age, gender, and rural residence, Georgia and Tennessee, August–October 2004*

Exposure	aOR	(95% CI)
Uncooked tomatoes	0·5*	(0·3–0·9)
Sprouts	0·9	(0·2–4·3)
Scallions	0·7	(0·3–1·7)
Lettuce	0·8	(0·5–1·6)
Cantaloupe	0·9	(0·4–1·9)
Poultry	0·5†	(0·2–1·0)
Shell eggs	0·7	(0·4–1·3)
Recreational water	4·8	(0·5–44·3)
Well water	4·3*	(1·6–11·2)
Reptile or amphibian contact	2·6†	(0·9–7·1)

aOR, Adjusted odds ratio; CI, confidence interval.

* Odds ratio significant at $P=0·05$.

† $P=0·06$.

DISCUSSION

In our analysis of demographic characteristics, food exposures, and environmental exposures for *S*. Javiana case-patients in Georgia and Tennessee during August–October 2004, we identified that consumption of well water was associated with an increased risk of *S*. Javiana infection, and consumption of uncooked tomatoes was associated with a decreased risk of infection. Consumption of poultry items and handling of reptiles or amphibians were nearly significant, possibly indicating insufficient power in this initial study. Not all confounding effects may have been controlled for due to small numbers, but will be accounted for in future studies.

The association of consumption of private well water with *S*. Javiana infection has implications for residents of the study area. An estimated 95% of rural Georgians rely on wells as their primary water supply [13]. Private wells, unlike public drinking water supplies, are not routinely monitored for contamination. In Georgia, 41% of private wells tested from 2002 to 2004 were contaminated with faecal coliforms, which are indicators of possible contamination with other organisms such as *Salmonella* [14]. In addition, exposure to recreational water was associated in univariate analyses with *S*. Javiana infection in the study population. Presumably some contaminated water is consumed while swimming in untreated water such as lakes, rivers, and streams. The source of *S*. Javiana water contamination is unknown but could be related to reptiles or amphibians [15, 16]. Another possibility is agricultural run-off containing animal waste, which can contaminate water bodies with faecal coliform bacteria [17]. Although the environmental characteristics of *S*. Javiana specifically are not well described, *Salmonella* is a stable bacterium that can survive up to 30 months in dried faeces [18] and several months in aquatic sediments [19]. *Salmonella* species are adaptable to conditions like acidity, temperature changes, moisture, and water turbidity [15, 20].

Although tomatoes, scallions, sprouts, and lettuce have been implicated in US *Salmonella* outbreaks, this study found these items to be protective. The protective effect of tomatoes was interesting, especially in the context of the multistate tomato-associated *S*. Javiana outbreaks that occurred before and during the time of our study. A potential for bias in our results is that only the case-patients were interviewed during the time of the outbreak investigation and knowledge of the outbreak may have led to decreased consumption of tomatoes. However, few Georgia cases were associated with this outbreak, it was linked to a specific type of tomato and supplier fairly quickly, and the Food and Drug Administration never advised the public to avoid consuming tomatoes [7, 21] Scallions, lettuce, and poultry were also found to be protective, supporting the idea that tomatoes being protective is probably not related to the outbreak. It is possible that these exposures are associated with an unidentified confounder. As FoodNet sites, Georgia and Tennessee have been involved in dialogue about the protective effects of produce for certain foodborne pathogens in recent years and this informed a formal study conducted in 2007. Moreover, previous FoodNet studies have found that fruits and vegetables may be protective [22], but the mechanisms of this are unknown [23–25]. An association between *S*. Javiana and exposure to reptiles and amphibians would be consistent with a previous investigation linking *S*. Javiana to amphibians [10].

A number of limitations were present in this investigation. First, the large number of FoodNet Population Survey interviews used as controls may give the impression of precision in the confidence interval estimates. Second, certain factors associated or nearly associated with infection, i.e. reptile or amphibian contact and consumption of well water, account for fewer than 50% of case-patients (see Table 2). One explanation is that this is a sporadic case-control study, not an outbreak investigation. In outbreak investigations, the assumption is that one is investigating a single disease stemming from a single source. In sporadic case-control investigations, it is probable that one is investigating multiple diseases with multiple sources. Precisely estimating risks for each disease source is much more challenging in the latter scenario, because of this fundamental difference in study type.

An additional limitation was that case-patients and controls were interviewed at different times using different methods. The water and animal exposure items were more detailed on the case instrument, possibly producing more complete answers and thus biasing the results away from the null. Moreover, the likelihood of cases and controls being selected may have varied due to the data collection methods. Using data from different years is not expected to introduce bias because the incidence of *S*. Javiana, rates of participation in recreational water activities, and eating habits are not expected to change much between years, and, compared to a 5-year mean, the number of *S*. Javiana cases in Georgia did not vary by more than 10% from 2002 to 2004. These were the closest years for which data were available. The small case-patient sample also limited the ability to conduct several subset analyses. Finally, differences in rates of rural/urban residence between cases and controls would have biased cases more towards rural areas.

The results of our study suggest that exposure to the outdoor environment, either through contact with reptiles or amphibians or consumption of well water, may be a source of many *S*. Javiana infections in humans. In addition to direct contact with the environment, persons may become ill with *S*. Javiana by consumption of foods that have been contaminated by water or faeces from the animal reservoir. The strong geographic association of *S*. Javiana with the southeastern United States [26] suggests an animal source with that regional habitat. We recommend further study of the environmental exposures. Microbiological testing of reptiles and amphibians and other environmental sources may help to determine the environmental reservoir for *S*. Javiana. Our findings suggest a need for educating people about behaviours that increase the risk of *S*. Javiana infection, with an emphasis on hand hygiene, especially among children handling reptiles or amphibians, well water testing and decontamination, and caution during and after recreational water activities.

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DECLARATION OF INTEREST

None.

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