Salmonellosis is usually associated with foodborne transmission. To identify risk from animal contact, we compared animal exposures of case-patients infected with bovine-associated Salmonella subtypes with those of control-patients infected with non-bovine-associated subtypes. We used data collected in New York and Washington, USA, from March 1, 2008, through March 1, 2010. Contact with farm animals during the 5 days before illness onset was significantly associated with being a case-patient (odds ratio 3.2, p = 0.0008), after consumption of undercooked ground beef and unpasteurized milk were controlled for. Contact
with cattle specifically was also significantly associated with being a case-patient (odds ratio 7.4, \( p = 0.0002 \)), after food exposures were controlled for. More cases of bovine-associated salmonellosis in humans might result from direct contact with cattle, as opposed to ingestion of foods of bovine origin, than previously recognized. Efforts to control salmonellosis should include a focus on transmission routes other than foodborne.

**Salmonella enterica** remains a formidable public health challenge, resulting in \( \approx 1.2 \) million illnesses and 400 deaths annually in the United States alone (1). Disease manifestations include diarrhea, fever, anorexia, abdominal pain, vomiting, and malaise. Although clinical disease generally resolves within 3–7 days, *Salmonella* spp. can also produce potentially fatal invasive infections. The incidence of human salmonellosis has not declined over the past 15 years and is significantly higher than it was during 2006–2008 (2). An estimated 94% of *Salmonella* infections are foodborne (1); common sources include undercooked eggs, poultry, beef, and pork; unpasteurized dairy products; and raw vegetables (3–7). Although some studies have shown that direct contact with infected animals is a risk factor for salmonellosis (8,9), the foodborne route is still regarded as the primary transmission route.

Dairy cattle are considered a key source of several *Salmonella* serovars that are a threat to human health, including multidrug-resistant *S. enterica* serovar Newport and *S. enterica* serovar Typhimurium (8–11). Foodborne transmission can occur through fecal contamination of beef carcasses at the time of slaughter (12) or through contamination of crops, either by manure used as fertilizer or by manure-contaminated irrigation water (13). Milk and other dairy products pose less of a public health threat because of commercial pasteurization, although consumption of unpasteurized dairy products persists. Infection by direct contact is an occupational risk for dairy farm workers and veterinarians. The most recent National Animal Health Monitoring System Dairy Study reports that there were >75,000 dairy operations in the United States in 2006, and the American Veterinary Medical Association reports that there were >5,000 veterinarians engaged either predominantly or exclusively in food animal practice as of 2010. Persons who interact with dairy cattle in public settings, such as open farms, petting zoos, and county or state fairs, are also at risk for salmonellosis through direct exposure (8,9,14,15).

Our objective was to identify significant risk factors for salmonellosis caused by bovine-associated *Salmonella* subtypes (including those within the Newport and Typhimurium serovars) by using the case–case study design (16). We specifically evaluated the role of direct animal contact as a potential route of transmission.

**Materials and Methods**

**Study Population**

This case–case study was conducted by using culture-confirmed human salmonellosis cases reported in targeted geographic areas in the states of New York and Washington, USA. Specimens were collected from March 1, 2008, through March 1, 2010.

**New York State**

Public Health Law in New York requires laboratories and physicians to report all salmonellosis cases to local health departments and to submit isolates to the New York State Department of Health (NYSDOH) Wadsworth Center Public Health Laboratory for diagnostic confirmation and speciation. The local health departments submit all case information, including laboratory data and questionnaire results, to the NYSDOH by a secure electronic data collection system. Within the FoodNet (www.cdc.gov/foodnet/) catchment area of the Centers for Disease Control and Prevention (CDC) Emerging Infections Program, surveillance officers actively monitor clinical microbiology laboratories and contact local health departments to ascertain all laboratory-confirmed salmonellosis cases, and they review case reports for accuracy and completeness. This catchment area includes 34 counties in the Albany, Buffalo, and Rochester areas of New York, representing \( \approx 4.3 \) million residents (22% of the total state population).

**Washington State**

As in New York, salmonellosis is reportable in the state of Washington, and clinical laboratories are required to submit all isolates to the Washington State Department of Health (WSDOH) Public Health Laboratories for further characterization. County health departments submit all case information to the WSDOH Communicable Disease Epidemiology Unit. The 6 participating counties in Washington were King, Pierce, Snohomish, Spokane, Whatcom, and Yakima. These included 3 of the most populous counties (King, Pierce, and Snohomish) and 2 counties with the highest concentrations of dairy cattle (Whatcom and Yakima) in Washington. Spokane County comprises an urban population in addition to rural and farming communities. The 6 participating counties represent \( \approx 4.3 \) million residents (65% of the total state population).

**Laboratory Methods**

In New York, serotyping and pulsed-field gel electrophoresis (PFGE) were performed on all *Salmonella* FoodNet isolates received by the NYSDOH during the study period. Typing data were forwarded to Cornell University (Ithaca, NY, USA) for PFGE pattern comparison
by BioNumerics software (Applied Maths Inc., Austin, TX, USA). Confirmed Salmonella isolates of bovine origin, obtained either from clinical samples submitted to the Cornell University Animal Health Diagnostic Center or from field study samples collected from clinically ill and asymptomatic dairy cattle, were sent to the US Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories (Ames, IA, USA) for serotyping by standard protocols. PFGE subtyping of bovine isolates was performed in the Food Science Laboratory at Cornell University. The standard CDC PulseNet protocol (17) was used for subtyping all study isolates.

In Washington, serotyping and PFGE were performed on all human clinical isolates submitted to the WSDOH as described for New York. Bovine isolates, obtained either from clinical samples submitted to the Washington Animal Disease Diagnostic Laboratory or from samples collected during dairy cattle field studies, were also sent to the National Veterinary Services Laboratories for serotyping. PFGE subtyping of bovine isolates was performed at Washington State University, again by using the standard CDC PulseNet protocol.

Questionnaire
As part of the routine investigation of foodborne Salmonella infections, trained interviewers from local health departments in both states administered a standardized questionnaire to each patient by telephone. The NYSDOH Salmonella questionnaire was adapted from a previous version used for investigating all cases of foodborne infection. The standard WSDOH questionnaire was supplemented with an additional set of questions to ensure completeness of exposure data collection and to better align Washington data with New York data. Patient identification data were removed from each dataset before being transferred to the university research group in the respective state. Data collected in each state included demographic information, clinical features, and exposure history during the 5 days before disease onset. Exposure data included animal contacts, food history, food hygiene practices, water use for drinking and recreation, health care or daycare exposures, and travel history. After data collection had ended, both datasets were compiled at Cornell University for analysis.

Case-Patients and Control-Patients
Eligible cases included Salmonella–positive patients from the NYS FoodNet catchment area and the 6 participating Washington counties that were identified during the study period. Patients were excluded if they were associated with an obvious outbreak (as noted by the state health departments) or if they had a typhoidal Salmonella infection (either Typhi or Paratyphi A). For the case–control analysis, case-patients were defined as patients infected with Salmonella isolates that matched contemporary bovine isolates from the respective state by serovar and PFGE pattern. Control-patients were defined as patients infected with Salmonella isolates that were not associated with cattle, according to those criteria. Specifically, all patients infected with S. enterica serovar Dublin were classified as case-patients because this serovar is host-adapted to cattle (18). Patients infected with 6 other serovars (Newport, Typhimurium, Infantis, 4,5,12:i--; Agona, and Montevideo) were classified as potential case-patients because of the importance of these serovars in bovine and human hosts.

According to a recent comprehensive study on the incidence of salmonellosis among dairy herds in New York and other northeastern states, the first 5 serovars just mentioned were among the leading serovars shed by dairy cattle with clinical Salmonella infections (19), and Montevideo is consistently one of the most prevalent serovars shed by asymptomatic cattle (20). All 6 are among the top 20 serovars isolated from human patients with laboratory-confirmed salmonellosis in the United States (21). For patients infected with one of the aforementioned serovars, PFGE patterns from the human isolates were compared with those from cattle. To be considered bovine associated, an isolate had to have a PFGE pattern indistinguishable from that of isolates obtained from ≥2 cattle in the same state from March 1, 2007, through March 1, 2010; patients infected with such isolates were thus classified as case-patients. Human isolates that differed from the most similar bovine isolate by 1–3 visible bands were excluded from the analysis, as were human isolates with a PFGE pattern matching that of just 1 bovine animal. Patients infected with isolates that differed from the most similar bovine isolate by ≥4 visible bands were classified as control-patients.

Patients infected with Salmonella serovars other than those previously listed were classified as control-patients if the serovar was not detected in cattle in the same state during that time frame. If the serovar was detected in cattle, the human isolate had to differ from the most similar bovine isolate by ≥4 visible bands in order for that patient to be considered a control-patient; otherwise, the human isolate was excluded from the analysis. A total of 422 bovine isolates from New York and 447 bovine isolates from Washington were used for PFGE pattern comparison.

Data Analysis
Data were imported into a commercially available statistical software program (SAS, version 9.2; SAS Institute Inc., Cary, NC, USA) for variable coding and analysis. Age was converted into a categorical variable.
Animal, food, and other exposures were analyzed as dichotomous variables (yes/no). The variables “farm animal contact” and “bovine contact” were created to most effectively capture data from 2 state health department questionnaires that were not identical. In the New York dataset, farm animal contact was considered “yes” if the patient reported an occupation of animal farming or a history of farm animal contact; bovine contact was considered “yes” if the patient specified cattle as the type of farm animal. In the Washington dataset, farm animal contact was considered “yes” if the patient reported a history of living or working on a dairy or other farm type or reported a history of contact with cattle, sheep, goats, horses, or pigs; bovine contact was considered “yes” if the patient specified cattle as the type of farm animal.

Analysis was performed to compare exposures between case-patients and control-patients. Univariable descriptive analysis was performed on all explanatory variables. Bivariable analysis with the χ^2 test was used to determine whether each variable was independently associated with case or control status. Multivariable logistic regression models were used to identify risk factors for infection with bovine-associated subtypes; case or control status was used as the dichotomous outcome variable. Initial selection of variables was based on the bivariable analysis screening (p<0.25), and a backward elimination approach was used to identify relevant 2-way interaction terms (involving exposure variables retained in the final model, demographic variables, and state) were also investigated for significance within each model. Consumption of undercooked ground beef and unpasteurized milk in the 5 days before disease onset were included in each model as potential confounders. The population attributable fraction (PAF), defined as the proportion of disease in a population that can be attributed to a given exposure, was calculated for variables retained in each model by using the formula PAF = P(OR_{adj} – 1)/OR_{adj} (where P = the proportion of case-patients exposed to the risk factor and OR_{adj} = the adjusted odds ratio for that factor) (22).

### Results

From March 1, 2008, though March 1, 2010, the NYSDOH received nontyphoidal Salmonella isolates from 835 patients within the NYS FoodNet catchment area. According to our criteria, 40 (4.8%) of these were classified as case-patients and 356 (42.6%) as control-patients. Among case-patients, 20 (50.0%) were female; among control-patients, 215 (60.4%) were female. The median age among case-patients was 31.5 years, whereas that among control-patients was 33 years. The most common serovar among case-patients was Typhimurium (51.7%), and the most common serovar among control-patients was Enteritidis (40.4%, Table 2).

The datasets from each state were combined to yield a total of 127 case-patients and 784 control-patients.

| Serovar                      | No. (%) |
|------------------------------|---------|
| Case-patients, n = 40        |         |
| Typhimurium                  | 27 (67.5) |
| Dublin                       | 7 (17.5)  |
| Newport                      | 5 (12.5)  |
| Infantis                     | 1 (2.5)   |

| Control-patients, n = 356    |         |
|------------------------------|---------|
| Enteritidis                  | 36 (10.1) |
| Typhimurium                  | 36 (10.1) |
| Heidelberg                   | 32 (9.0) 
| Newport                      | 21 (5.9)  |
| Braenderup                   | 16 (4.5)  |
| Javiana                      | 14 (3.9)  |
| Saintpaul                    | 13 (3.7)  |
| Hadar                        | 12 (3.4)  |
| B,5:i:–                      | 11 (3.1)  |
| Murenchen                    | 10 (2.8)  |
| Agona                        | 8 (2.2)   |
| Berta                        | 8 (2.2)   |
| Paratyphi B var. L-tartrate+ | 8 (2.2)   |
| B,5:b:–                      | 7 (2.0)   |
| Poona                        | 7 (2.0)   |
| Stanley                      | 7 (2.0)   |
| Hartford                     | 6 (1.7)   |
| Miami                        | 6 (1.7)   |
| Montevideo                   | 6 (1.7)   |
| Schwarzengrund               | 5 (1.4)   |
| Bovismorbificans             | 4 (1.1)   |
| Derby                        | 4 (1.1)   |
| Ealing                       | 4 (1.1)   |
| Manhattan                    | 4 (1.1)   |
| Mississippi                  | 4 (1.1)   |
| Other serovars               | 67 (18.8) |

| Excluded patients, n = 439   |         |
|------------------------------|---------|
| Enteritidis                  | 195 (44.4) |
| Typhimurium                  | 99 (22.6) |
| Thompson                     | 22 (5.0)  |
| Oranienburg                  | 16 (4.1)  |
| Newport                      | 14 (3.2)  |
| Montevideo                   | 8 (1.8)   |
| Infantis                     | 7 (1.6)   |
| Tennessee                    | 7 (1.6)   |
| Panama                       | 6 (1.4)   |
| Other serovars               | 46 (10.5) |
| Not typed                    | 17 (3.9)  |
Table 2. Distribution of *Salmonella* serovars among 562 patients, Washington, USA, March 1, 2008–March 1, 2010

| Serovar            | Case-patients, n = 87 | Control-patients, n = 428 | Excluded patients, n = 47 |
|--------------------|-----------------------|---------------------------|---------------------------|
| Typhimurium        | 45 (51.7)             | 29 (6.8)                  | 7 (14.9)                  |
| Montevideo         | 14 (16.1)             | 11 (2.6)                  | 6 (12.8)                  |
| Newport            | 13 (14.9)             | 11 (2.6)                  | 6 (12.8)                  |
| 4,5,12:i:–         | 9 (10.3)              | 8 (1.9)                   | 2 (4.3)                   |
| Dublin             | 5 (5.7)               | 4 (0.9)                   | 2 (4.3)                   |
| Infantis           | 1 (1.1)               |                           |                           |
| Enteritidis        | 173 (40.4)            |                           |                           |
| Typhimurium        | 29 (6.8)              |                           |                           |
| Paratyphi B var. L-tartrate+ | 23 (5.4) |                           |                           |
| Javiana            | 11 (2.6)              |                           |                           |
| Montevideo         | 11 (2.6)              |                           |                           |
| Stanley            | 10 (2.3)              |                           |                           |
| Braenderup         | 9 (2.1)               |                           |                           |
| Litchfield         | 9 (2.1)               |                           |                           |
| Thompson           | 9 (2.1)               |                           |                           |
| 4,5,12:i:–         | 8 (1.9)               |                           |                           |
| Heidelberg         | 8 (1.9)               |                           |                           |
| Muenchen           | 7 (1.6)               |                           |                           |
| Sentenberge        | 7 (1.6)               |                           |                           |
| Virchow            | 7 (1.6)               |                           |                           |
| Agona              | 6 (1.4)               |                           |                           |
| Potsdam            | 6 (1.4)               |                           |                           |
| Saintpaul          | 6 (1.4)               |                           |                           |
| Oranienburg        | 5 (1.2)               |                           |                           |
| Other serovars     | 84 (19.6)             |                           |                           |

Bivariable analysis indicated that more case-patients (11.0%) than control-patients (3.8%) reported a history of farm animal contact during the 5 days before disease onset (p = 0.0004). More case-patients (6.3%) than control-patients (1.0%) also reported a specific history of bovine contact during the 5 days before illness (p = 0.0001). Attendance at an open farm/petting zoo/fair was more common (p = 0.05) among case-patients (11.8%) than control-patients (7.0%), and more case-patients (12.6%) than control-patients (6.5%) reported a history of contact with animal manure (p = 0.01). Fewer case-patients (3.1%) than control-patients (13.9%) reported a history of international travel before illness (p = 0.0006). Case-patients and control-patients did not differ significantly with respect to sex, age group, eating undercooked ground beef, or drinking unpasteurized milk.

Multivariable logistic regression analysis showed that a history of farm animal contact during the 5 days before disease onset was significantly associated with being a case-patient (odds ratio [OR] 3.2, 95% CI 1.6–6.4, p = 0.0008), after consumption of undercooked ground beef and unpasteurized milk was accounted for (Table 3). A specific history of bovine contact during the 5 days before illness was also significantly associated with being a case-patient (OR, 7.4, 95% CI 2.6–20.9, p = 0.0002), according to estimates from a separate logistic regression model that also controlled for those food exposures (Table 4). International travel was negatively associated with being a case-patient in each of the models. No significant interaction was found between farm animal/bovine contact and state, sex, or age group in the respective models. The PAF, applied here as the proportion of *Salmonella* infections among the source population of laboratory-confirmed cases that can be attributed to a certain exposure, was calculated to be 7.6% for farm animal contact and 5.4% for bovine contact in particular.

To perform a sensitivity analysis for testing the effect of our strict case definition, we repeated multivariable logistic regression models under 2 extreme scenarios; all potential case-patients that were excluded (because the isolate differed from the most similar bovine isolate by 1–3 visible bands or because its PFGE pattern matched that of just 1 bovine animal) served alternatively as case-patients (scenario 1) and control-patients (scenario 2). The parameter estimates and ORs from these hypothetical models were comparable to those obtained from our original analyses (the ORs under scenarios 1 and 2 were 3.2 and 2.3 for farm animal contact, 6.1 and 4.0 for bovine contact, respectively).

Discussion

The case–case study design proposed by McCarthy and Giesecke is an adaptation of the conventional case–control approach (16). It has been used to study risk factors and clinical features associated with particular subtypes of *Salmonella* spp. (8,23–26), *Campylobacter* spp. (27,28), and *Clostridium difficile* (29). One of its main advantages is the removal of selection bias imposed by the surveillance system; case-patients and control-patients were subjected to the same selection process in order to be detected by a state health department as a laboratory-confirmed case. Another advantage is the negation of recall bias (a form of information bias); because case-patients and control-patients had salmonellosis, their recall of exposures should have been similarly affected by attitudes regarding causation.
was not significantly associated with being a case-patient in this study, it is logical to believe that visiting such a facility might increase the risk for salmonellosis, on the basis of our other findings. S. enterica is transmitted primarily by the fecal–oral route. Direct contact with the feces of infected cattle can occur through feeding, petting, or otherwise handling them; contaminated clothing or footwear, animal bedding, barriers, or other environmental surfaces can also be sources of infection (15,31). This threat is underscored by the recent finding that the median duration of fecal Salmonella shedding following clinical disease among dairy cattle is 50 days (32).

The negative association between recent international travel and salmonellosis caused by a bovine-matched subtype was anticipated. Although travel outside the United States is a well-known risk factor for Salmonella infections (33) (observed in a significantly higher proportion of control-patients in this study), it would not be expected to have an association with salmonellosis caused specifically by subtypes shared by dairy cattle in New York and Washington.

The percentage of Salmonella infections in the United States that are foodborne was recently estimated at 94% (1). The results of our statistical analyses suggest that this percentage might be an overestimate, at least for bovine-associated Salmonella subtypes, although our PAF results (which also take into account the frequency of exposure) are more consistent with this estimate. It also must be noted that the effect of animal exposure observed in New York and Washington might not be representative of the rest of the country. Nevertheless, more human infections originating from bovine sources might result from direct contact with cattle (as opposed to foods of bovine origin) than previously recognized. Clear evidence for the role of direct farm animal contact as a source of human salmonellosis indicates that it is imperative for Salmonella control efforts to include a focus on transmission routes other than foodborne. The efficacy and public health impact of addressing nonfoodborne transmission of Salmonella spp. have been demonstrated by studies of direct contact transmission from pet turtles to humans, particularly children. In response to studies that established turtles as an important source of human salmonellosis (34,35), federal legislation in 1975 prohibited the sale and distribution of turtles <4 inches in

A potential limitation of the case–case study design is that the control-patients might not represent the exposure prevalence in the source population on account of the unique exposures that led them to become infected. However, we believe that we addressed this issue by including a diverse array of serovars and PFGE types in the control group, assuming that their associated exposures were presumably also diverse and thus more representative of the total spectrum of exposures associated with nonbovine Salmonella strains. Another possible drawback of this study design is that case-patients and control-patients share a certain subset of exposures that pose a risk for Salmonella infections in general; such exposures will therefore remain unidentified or at least be underestimated as risk factors.

Although this study design precludes the study of general risk factors for salmonellosis, it is useful for investigating exposures that are serovar or subtype specific.

Other studies have found an association between salmonellosis and having previous contact with either cattle or a farm environment (8,9,30). Our study investigated this association with a case–case approach that used a strict case definition. Another strength of this study was the use of sporadic cases of salmonellosis rather than cases associated with outbreaks. Insight regarding the epidemiology of sporadic Salmonella infections has traditionally been limited because specific sources of enteric illness are seldom identified when not occurring as part of an outbreak.

Direct contact with dairy cattle or their environment during the 5 days before illness onset was significantly associated with salmonellosis caused by a bovine-matched subtype in New York and Washington. Because there was no interaction between state and the animal contact variables in the models, we concluded that the effect estimate was consistent across the 2 states. The ORs for farm animal contact and specific bovine contact in each state were also similar to those obtained from analysis of the combined dataset (data not shown). In addition, our sensitivity analysis led us to decide that we still would have reached the same conclusions with modified case criteria. These results have important implications for dairy farm workers and their families, veterinarians and veterinary staff, and those who interact with dairy cattle in public settings.

Although attendance at an open farm/petting zoo/fair

| Variable                        | Odds ratio (95% CI) | p value |
|--------------------------------|---------------------|---------|
| Farm animal contact            | 3.2 (1.6–6.4)       | 0.0008  |
| Undercooked ground beef        | 1.5 (0.7–3.1)       | 0.3     |
| Unpasteurized milk             | 0.5 (0.1–4.2)       | 0.5     |
| International travel           | 0.2 (0.1–0.6)       | 0.002   |

*Estimated by a logistic regression model.
carapace length. Still in effect today, this ban coincided with an 18% reduction in Salmonella infections among children 1–9 years of age (36).

A number of measures can be taken to minimize the likelihood of becoming infected with Salmonella spp. from direct contact with farm animals. Washing hands with soap and water is a simple yet highly protective step that can be taken after contact with animals or feces (15,37). Children <5 years of age, elderly adults, and immunocompromised persons are at increased risk for invasive salmonellosis (38,39) and thus should pay special attention to hygiene or avoid certain animal contacts altogether. Veterinarians should teach cattle owners and farm employees to wash well after work or before eating, to disinfect boots and equipment, and to keep coveralls out of the house. If treating infected cattle, veterinarians must specifically counsel their clients about the risk for zoonoses. In particular, high-risk groups should avoid contact with infected cattle. Veterinarians should instruct their staff members to protect themselves by using appropriate infection control procedures (40), especially if working with livestock. Increased physician awareness of the role of direct farm animal contact in transmitting Salmonella spp. and other enteric zoonotic pathogens is likewise needed. Physicians should educate their patients, particularly those at increased risk for severe disease, regarding the potential threat posed by animal contact and the importance of hand hygiene after such contact. Patients with diarrheal illness should be questioned about their exposures to cattle, farm environments, and other animal species. It is also essential that those who operate open farms and other animal exhibits adhere to current guidelines by equipping such areas with handwashing facilities, preventing food and drink in these areas, maintaining an adequate cleaning and disinfection protocol, and providing visitors with educational materials on disease prevention (37). In conclusion, prevention of salmonellosis should include a focus on safe animal contact in addition to food safety measures.

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Dr Cummings is an assistant professor of epidemiology at Texas A&M University College of Veterinary Medicine and Biomedical Sciences. His research focuses on diseases of importance to public health, in particular Salmonella spp. and other foodborne pathogens.

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Address for correspondence: Kevin J. Cummings, Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, 4458 TAMU, College Station, TX 77843-4458, USA; email: kcummings@cvmed.tamu.edu

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