Mild Illness during Outbreak of Shiga Toxin–Producing *Escherichia coli* O157 Infections Associated with Agricultural Show, Australia

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During a large outbreak of Shiga toxin–producing *Escherichia coli* illness associated with an agricultural show in Australia, we used whole-genome sequencing to detect an IS1203v insertion in the Shiga toxin 2c subunit A gene of Shiga toxin–producing *E. coli*. Our study showed that clinical illness was mild, and hemolytic uremic syndrome was not detected.

Shiga toxin–producing *Escherichia coli* (STEC) is a major cause of serious human gastrointestinal illness and has the potential to cause life-threatening complications, such as hemolytic uremic syndrome (HUS) (1). An average of 0.4 cases of STEC illness per 100,000 persons per year are reported to public health authorities in Australia (2). Disease severity can range from asymptomatic infection to serious and sometimes fatal illness, particularly in young children and the elderly (3,4).

Healthy ruminants, particularly cattle, are the reservoir for STEC (5). Human infection with STEC usually occurs as a result of inadvertent ingestion of fecal matter after consumption of contaminated food, water, or unpasteurized milk; contact with animals or their environments; or secondarily, through contact with infected humans (4,5). In the largest previously reported outbreak of STEC illness in Australia in 1995, which was associated with consumption of mettwurst (uncooked, semidy, fermented sausages), HUS developed in 23 of the 51 case-patients identified, and there was 1 death (6).

The Study

A multidisciplinary incident management team was established to investigate an outbreak of STEC illness associated with an annual agricultural show in Brisbane, Queensland, Australia, in August 2013 (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/10/16-1836-Techapp1.pdf). The incident management team defined primary and secondary outbreak cases (online Technical Appendix). Persons with laboratory-confirmed STEC infection associated with the outbreak and their household contacts were followed up until the point of microbiological evidence of clearance, which was defined as 2 consecutive negative stool samples collected ≥24 h apart (7).

Case-patients and contacts with a high risk for transmission (persons <5 years of age; persons unable to maintain good hygiene; or childcare, healthcare, aged care, or food preparation workers) were advised to avoid childcare and work settings in accordance with Queensland Health Guidelines (7). Enhanced surveillance measures were implemented to assist with case finding (online Technical Appendix). Medical practitioners were requested to avoid use of antimicrobial drugs for suspected case-patients with STEC infections because of previously reported associations between antimicrobial drug use and HUS (online Technical Appendix).

We developed a case–control study to obtain additional information related to animal contact, hand hygiene, and food consumption at the agricultural show (online Technical Appendix). We analyzed data by using Epi Info 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) (online Technical Appendix).

STEC identified from human, environmental, and animal samples were serotyped for O and H antigens (online Technical Appendix). Expression of Shiga toxin 1 (stx1) and stx2 genes was determined for selected isolates (online Technical Appendix). Shiga toxin gene subtyping and whole-genome sequencing (WGS) analysis was performed (online Technical Appendix).

During August 21–September 27, 2013, we identified 57 outbreak-associated laboratory-confirmed case-patients with STEC infection: 54 confirmed primary case-patients, 1 probable primary case-patient, and 2 secondary case-patients (Figure 1). Of the 57 case-patients, 32 (56%) were
Mild Illness during STEC O157 Infections, Australia

female. Case-patients ranged in age from 1 to 77 (median 9) years; 31 (56%) case-patients were <10 years of age. Median incubation period after attending the agricultural show was 4 (range 1–11, 25th–75th percentile 3–5) days.

Case-patients reported diarrhea (96%), abdominal pain (93%), bloody diarrhea (41%), and fever (32%) (Table 1). Seven case-patients were hospitalized. No cases of HUS or deaths were reported.

Public Health Units followed up 40 case-patients until microbiological evidence of clearance; the remaining case-patients were lost to follow-up. Median duration of STEC excretion among primary case-patients was 18 (range 2–52) days (Table 2). After 27 days and 6 recurrent stools positive for STEC, and after acute diarrheal illness had resolved, 1 child was given azithromycin on day 40 for 3 days to hasten decolonization. Two consecutive stool specimens obtained >48 h after treatment with antimicrobial drugs was stopped were negative for STEC in this child. This patient did not have any adverse effects from azithromycin treatment.

Forty-four of 55 primary case-patients and 28 household contacts who attended the agricultural show were included in the case–control study. Median age of case-patients was 8 (range 1–77) years, and median age of controls was 38 (range 1–70) years.

We showed by using univariate analysis that case-patients were not more likely than controls to have entered the animal nursery at the show. Case-patients were more likely than controls to have had contact with lambs or goats, fed the animals, or had their hands licked by animals (online Technical Appendix).

We identified the same multilocus variable number tandem repeat and stx subtype genotype of *E. coli* O157:H–in human case-patients, animal bedding from the animal nursery before disposal, and fecal samples collected from lambs, goats, and calves (online Technical Appendix). WGS and read mapping to an *E. coli* O157 reference genome showed that of the human, animal, and environmental isolates analyzed, all contained an IS1203v insertion that resulted in deletion of the first 494 bp of the stx2c subunit A gene (Figure 2). Expression of stx2 was not detected in these isolates by Immunocard STAT! EHEC (Meridian Bioscience, Cincinnati, OH, USA) and Shiga toxin Quik Chek (Alere, Waltham, MA, USA) lateral flow devices. No additional stx2 genes were identified, and no disruptions were detected in the stx1 gene regions of any of the isolates.
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Conclusions

We found that STEC infection was associated with feeding lambs or goats, feeding animals, and having the hands licked by animals. The course of E. coli O157 infection was relatively mild; no cases of HUS were associated with this outbreak. Heiman et al. found that of 4,928 cases of 390 E. coli O157 illness outbreaks in the United States during 2003–2012, HUS was detected in 299 cases (6% of illnesses) (8). HUS cases with stx1+/stx2–E. coli O157 isolates have been reported (9,10). We speculate that the absence of severe complications in this outbreak might have been caused, in part, by disruption of the stx2 subunit A gene by the IS1203v insertion, which resulted in lack of expression or a nonfunctional Stx2 toxin.

The proportion of case-patients reporting bloody diarrhea (19/46, 41%) was also lower than previously reported. Ethelberg et al. reported that 69% (56/81) of case-patients in Denmark infected with E. coli O157 had bloody diarrhea (11). A recent retrospective cohort study from England reported that 61% (2,027/3,323) of symptomatic case-patients infected with E. coli O157 had bloody diarrhea. Bloody diarrhea was reported to be a risk factor for HUS (odds ratio 2.10; p = 0.001) (12). In the outbreak we studied, children <5 years of age were less likely than older children and adults to report bloody diarrhea. STEC infection should be actively considered for young children with nonbloody diarrhea who were exposed to potential sources of STEC.

In this outbreak, 1 child was given azithromycin for 3 days to hasten decolonization some weeks after the acute diarrheal illness had resolved. Antimicrobial drugs are generally not recommended to hasten STEC decolonization because of major associations with HUS (13). Recommendations to avoid high-risk activities (such as childcare attendance) might place a major socioeconomic burden on STEC carriers and their families. Further studies are required to assess whether WGS can provide useful information for safe administration of antimicrobial drugs for treatment of acute illness caused by STEC, or when chronic shedding becomes established.

Our comprehensive study of a large outbreak E. coli O157 illness, characterized by an IS1203v insertion disrupting the stx2c subunit A gene, showed mild clinical illness and an absence of HUS. Further characterization by virulence studies on isolates with this stx2c subunit A gene disruption might provide further insights into the mild illness caused by this strain.

Acknowledgments

We thank Clare Nourse and Joshua Francis for reviewing draft versions of this article and providing advice about case management; staff at the Metro North, Metro South, Gold Coast, and West Moreton Public Health Units for public health management of STEC cases; Metro North environmental health officers for site investigations of the agricultural show; Queensland Forensic and Scientific Biosecurity Services Laboratory for testing human and animal samples; Biosecurity Queensland inspectors and veterinary officers for performing field work for animal investigation; the Biosecurity Services Laboratory for testing animal samples; and the Royal National Agricultural and Industrial Association of Queensland for assistance with public health investigations.

Dr. Vasant is a public health physician at Queensland Health, Brisbane, Queensland, Australia. Her research interests include public health management of communicable and noncommunicable diseases, health of indigenous and minority communities, and public health response to climate change.

Table 2. Clearance of STEC from stool samples of persons, by age group, during outbreak of Shiga toxin–producing Escherichia coli illness associated with an agricultural show, Brisbane, Queensland, Australia, 2013*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All, n = 40</th>
<th>1–4 y, n = 12</th>
<th>5–14 y, n = 13</th>
<th>&gt;15 y, n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median clearance, d (range)</td>
<td>18 (2–52)</td>
<td>29 (5–37)</td>
<td>23 (2–52)</td>
<td>12 (2–28)</td>
</tr>
<tr>
<td>Mean clearance, d (SD)</td>
<td>19 (12)</td>
<td>24 (9)</td>
<td>24 (12)</td>
<td>12 (9)</td>
</tr>
</tbody>
</table>

*Time to clearance was based on onset of diarrhea. STEC, Shiga toxin–producing Escherichia coli.

Figure 2. Alignment of genomic region from a representative isolate (EC_4844) showing insertion of IS1203v in the Shiga toxin 2 (stx2) gene region of Shiga toxin–producing Escherichia coli associated with an agricultural show, Brisbane, Queensland, Australia, 2013. CDS, coding DNA sequence.

References


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**Marburg [mahr’boork] Virus**

Ronnie Henry, Frederick A. Murphy

In August and September 1967, an outbreak of a viral hemorrhagic fever occurred among laboratory workers in Marburg and Frankfurt, Germany, and Belgrade, Yugoslavia (now Serbia) who were processing kidneys from African green monkeys that had been imported from Uganda. (These kidneys were used in the production of polio vaccine.) Of 25 primary and 6 secondary cases, 7 were fatal.

A new virus, named Marburg virus, was isolated from patients and monkeys, and the high case-fatality ratio called for the best biocontainment of the day. The Centers for Disease Control and Prevention (CDC) borrowed a mobile containment laboratory from the National Institutes of Health and set it up in the CDC parking lot; it provided approximately biosafety level 2+ containment. A few isolated, sporadic cases were reported in the following decades until a 1998 outbreak in the Democratic Republic of the Congo affected 154 people with a case-fatality ratio of 83%, and a 2004 outbreak in Angola affected 227 people with a case-fatality ratio of 90%.

**Sources**


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**etymologia**

Ronnie Henry, Frederick A. Murphy

Negative contrast electron microscopy of Marburg virus, from original monkey kidney cell culture propagation done at CDC in 1967, magnification ≈40,000x. Image courtesy of Frederick A. Murphy.

Image courtesy of Frederick A. Murphy.

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**Technical Appendix**

**Outbreak**

On August 21, 2013, Queensland Health Forensic and Scientific Services (QHFSS), the public health reference laboratory in Coopers Plains, Queensland, Australia, informed public health authorities of 2 case-patients with Shiga toxin–producing *Escherichia coli* O157 (STEC) illness in Brisbane. The next day, 2 additional case-patients were reported. All 4 case-patients attended the annual agricultural show in Brisbane, the capital of Queensland, a state of Australia, and had no other exposures in common. The agricultural show was open to the public during August 8–17. The show provided opportunities for visitors to see and pet farm animals. More than 400,000 persons visited the show in 2013.

On August 23, after identification of the common exposure, the Queensland Communicable Diseases Unit convened an incident management team consisting of public health units, QHFSS, and OzFoodNet (a national network that investigates foodborne disease outbreaks) (1). Biosecurity Queensland (the Queensland Government agency that leads efforts to prevent, respond to, and recover from pests and diseases threatening agricultural prosperity, the environment, social amenity and human health) and Workplace Health and Safety Queensland (a government agency that enforces workplace health and safety laws) provided interagency support.

**Enhanced Surveillance**

On August 23, the Queensland Communicable Diseases Unit alerted all pathology laboratories, hospital emergency departments, infectious diseases physicians, and general practitioners in the Brisbane metropolitan area about the outbreak. Medical practitioners were
requested to submit bloody stool specimens for STEC testing and to avoid use of antimicrobial drugs for potential cases because of previously reported associations between antimicrobial drug use and hemolytic uremic syndrome (HUS) (2). Pathology laboratories were requested to review test results for recently collected bloody stool specimens and forward these specimens to QHFSS for STEC testing. Case finding was assisted by a media release on August 23 that alerted the public about cases of STEC associated with the agricultural show and advised persons who attended the show and in whom bloody, severe, or persistent diarrhea subsequently developed to seek medical attention (3).

**Case Definition**

A confirmed primary case was defined as a case in a person in whom all 4 virulence genes (Shiga toxin \( [stx] \), intimin \([eaeA]\), enterohemolysin \([ehxA]\), and autoagglutinating adhesion \([saa]\)) were detected by PCR in a stool specimen and who had visited the annual agricultural show and whose onset of illness or whose stool specimen collection date was within 14 days of attendance. A probable primary case was similarly defined, except for differences in the STEC strain and virulence genes. A secondary case was also similarly defined, except that the case was epidemiologically linked to a primary case, and that the secondary case-patient did not attend the agricultural show or the onset of illness was \( >14 \) days after attending the agricultural show. The case definition excluded case of STEC illness that were not associated with the agricultural show. The end of the outbreak was defined by an absence of new cases during 2 incubation periods (28 days) of the onset of illness in the last confirmed outbreak-associated case of STEC illness.

HUS was defined as the presence of microangiopathic hemolytic anemia, thrombocytopenia (i.e., platelet count \( <150,000/\mu\text{L} \)), and renal insufficiency. Renal insufficiency was defined as a creatinine level greater than the upper limit of the reference range for age (4).

**Case Investigations and Case-Control Study**

Public health units administered the standard Queensland Health STEC case questionnaire to all case-patients identified in this outbreak (5). The proportion of case-patients who reported symptoms was based on persons for whom data for the specific field was available. For the case–control study, a supplementary questionnaire was developed to obtain additional
information related to animal contact, hand hygiene, and food consumption at the agricultural show.

The supplementary questionnaire was administered to primary case-patients. Controls for the case–control study were asymptomatic household contacts who visited the agricultural show and had negative test results for STEC infection. The parent or guardian were interviewed for case-patients and household contacts ≤14 years of age. For persons 15–17 years of age, verbal consent of the parent or guardian was obtained before the interview was conducted.

**Statistical Analysis**

Data were analyzed by using Epi Info 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Univariate analysis generating crude odds ratios with 95% CIs was used to investigate associations between potential risk factors and STEC infection (Technical Appendix Table 1). Variables with a p value ≤0.05 were included in multivariable logistic regression models for further assessment. Potential collinear variables were assessed by using the Cramer V statistic (Stata version 11.0; StataCorp LLC, College Station, TX, USA). To assess the effect of using other household members as controls, we also performed matched analyses adjusting for age and sex.

**Environmental Investigation**

Environmental health officers reviewed infection prevention and control measures at the agricultural show animal pavilion, including handwashing facilities, signs encouraging visitors to wash their hands, and animal waste disposal. Environmental samples (including remaining composite straw, shavings, and visible manure) were obtained from the animal nursery for laboratory testing after the show had ended. Biosecurity Queensland coordinated a risk assessment of the animal contact areas, traced animals that had been on display, and subsequently collected animal fecal samples for STEC testing.

**Laboratory Investigation**

**STEC Detection**

All specimens were inoculated into *E. coli* enrichment broth (Difco, Franklin Lakes, NJ, USA) for 16–24 h at 37°C, which was then plated onto MacConkey agar for 16–24 h at 37°C. Resulting growth was screened for the *stx1, stx2, eaeA, ehxA*, and *saa* genes (6). Cultures positive for *stx1* and/or *stx2* were subcultured to isolate pure growth for further testing. The
expression of \textit{stx1} and \textit{stx2} was determined on selected isolates by using Immunocard STAT! EHEC (Meridian Bioscience, Cincinnati, OH, USA) and Shiga toxin Quik Chek (Alere, Waltham, MA, USA) tests according to the manufacturer’s instructions. All \textit{stx1} and/or \textit{stx2} gene–positive \textit{E.coli} isolates were serotyped for O and H antigens (H typing performed by the Microbiological Diagnostic Unit, Melbourne, Victoria, Australia).

Molecular Characterization

Shiga toxin gene subtyping for \textit{stx1} and \textit{stx2} was performed for all isolates available (7–10). Multilocus variable number tandem repeats analysis (MLVA) was also performed by using 2 schemes, 1 specific for all \textit{E. coli} isolates and 1 specific for to serogroup O157 isolates (11,12).

Whole-genome sequencing was performed for selected isolates and demonstrated the molecular profile associated with the outbreak (3 human isolates, 1 ovine isolate, 1 caprine isolate, 1 bovine isolate, 1 bedding isolate). A total of 300 ng of genomic DNA was sheared by ultrasonification to 300-bp fragments by using an S220/E220 ultrasonicator (Covaris, Woburn, MA, USA).

Samples were prepared into barcoded fragment libraries by using the Ion Plus Fragment Library Kit and IonXpress barcode adaptors, and sequenced on an Ion Torrent PGM by using the Ion PGM Hi-Q Sequencing Kit, the Ion PGM Hi-Q Chef Kit, and 316v2 chips (Life Technologies, Waltham, MA, USA) according to the manufacturer’s instructions. Libraries were assessed by using TapeStation 2200 with High Sensitivity D1000 Screen Tape (Agilent Technologies, Santa Clara, CA, USA). Quality check filtering, trimming, and adaptor sequence removal was performed by Torrent Suite software (Life Technologies, Waltham, MA). Raw reads are located in the sequence read archive under BioProject PRJNA342737.

FASTQ sequences were mapped to the reference genome of \textit{E. coli} O157:H7 strain Sakai (NC_002695) by using Geneious R7 (http://www.geneious.com/). De novo assemblies produced by the Geneious R7 assembler were used to identify in silico multilocus sequence typing alleles in Ridom SeqSphere+ according to a standard scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) (13).
Ethics

Ethical approval was not required because all activities contributed to the public health response in identifying, characterizing, and controlling disease. Outbreak prevention and control measures are covered under the Public Health Act 2005, Queensland (14).

Results

Environmental Investigation

The 2013 agricultural show displayed >10,000 animals and included sections where direct contact between visitors and animals could occur. The animal boulevard included a large animal nursery where visitors could pat and feed farm animals, including goats, lambs, calves, piglets, chicks, ducklings, donkeys, and turkeys. A milking demonstration took place in an area adjacent to the animal nursery and visitors were invited to milk a cow. Unpasteurized milk was not served. Visitors could also view the birth of lambs that took place in an enclosed booth. The birthed lambs were available for supervised petting after ≥24 h after veterinary clearance. Other animals displayed in the animal boulevard and other pavilions were less accessible to the public for direct contact.

The number of visitors in the animal nursery was not restricted. Limited unsupervised handwashing facilities were available opposite the exit of the animal nursery. Hand sanitizers were available in other areas. Signs in animal contact areas encouraged visitors to wash their hands. Staff at the agricultural show regularly removed animal waste from animal contact areas.

Laboratory Investigation

Stool samples from 56 of 57 case-patients showed identical virulence gene profiles, consisting of stx1, stx2, eaeA, and ehxA. The virulence gene profile of the remaining probable primary case-patient was only stx2 and ehxA. Twenty bovine, 4 ovine, and 2 caprine fecal samples were tested from animals traced to other properties after the show had ended. Serotype O157:H– was confirmed from 51 of the human cases, and also from ovine, caprine, and bovine feces, and the animal bedding sample. All O157:H– isolated from animal and environmental sources displayed the same MLVA profiles (6_8_2_9_4_7_8_2_3_8 and 11–7–13–4–5–6–4–9) (Technical Appendix Table 2), stx1a and stx2c subtypes, and sequence type ST11, and 2/51 of human isolates differed by 1 allele in 1 of the MLVA profiles. Although E. coli O157 has
frequently been reported to belong to sequence type 11 (13), the MLVA profiles were novel to the Queensland collection of previously typed STEC isolates (n = 112).

References


Technical Appendix Table 1. Univariate analysis of selected animal and environmental exposures for Escherichia coli O157:H–isolates obtained during outbreak associated with agricultural show, Australia, 2013

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Cases-patients</th>
<th>Controls</th>
<th>Unadjusted odds ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction with animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attend animal boulevard</td>
<td>42/44 (95.5)</td>
<td>24/28 (85.7)</td>
<td>3.5 (0.6–20.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Attend animal nursery</td>
<td>39/43 (90.7)</td>
<td>23/26 (88.5)</td>
<td>1.3 (0.3–6.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pet/touch lambs</td>
<td>32/37 (86.5)</td>
<td>13/22 (59.1)</td>
<td>4.4 (1.2–15.8)</td>
<td>0.03†</td>
</tr>
<tr>
<td>Pet/touch calves</td>
<td>30/38 (79.0)</td>
<td>13/22 (59.1)</td>
<td>2.6 (0.8–8.2)</td>
<td>0.10</td>
</tr>
<tr>
<td>Pet/touch piglets</td>
<td>4/31 (12.9)</td>
<td>2/18 (11.1)</td>
<td>1.2 (0.2–7.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pet/touch goats</td>
<td>30/36 (53.3)</td>
<td>9/17 (50.0)</td>
<td>5.0 (1.4–17.9)</td>
<td>0.02‡</td>
</tr>
<tr>
<td>Pet/touch chicks</td>
<td>9/37 (24.3)</td>
<td>2/18 (11.1)</td>
<td>2.6 (0.5–13.4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Pet/touch ducklings</td>
<td>5/37 (13.5)</td>
<td>2/18 (11.1)</td>
<td>1.3 (0.2–7.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pet/touch donkeys</td>
<td>9/35 (25.7)</td>
<td>3/18 (16.7)</td>
<td>1.7 (0.4–7.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Pet/touch turkeys</td>
<td>1/37 (2.7)</td>
<td>1/17 (5.9)</td>
<td>0.4 (0.0–7.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Pet/touch llamas/llamas</td>
<td>6/34 (17.7)</td>
<td>3/19 (15.8)</td>
<td>1.1 (0.3–5.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pet/touch cattle at open stalls</td>
<td>14/41 (34.2)</td>
<td>8/27 (29.6)</td>
<td>1.2 (0.4–3.5)</td>
<td>0.70</td>
</tr>
<tr>
<td>Contact with animal manure</td>
<td>10/26 (38.5)</td>
<td>5/19 (25.3)</td>
<td>1.8 (0.5–6.4)</td>
<td>0.53</td>
</tr>
<tr>
<td>Attend milking barn</td>
<td>4/42 (9.5)</td>
<td>0/27 (0.0)</td>
<td>ND</td>
<td>0.15</td>
</tr>
<tr>
<td>Attend little miracles</td>
<td>14/42 (33.3)</td>
<td>7/26 (26.9)</td>
<td>1.4 (0.5–4.0)</td>
<td>0.79</td>
</tr>
<tr>
<td>Pet/touched newborn lamb</td>
<td>1/14 (7.1)</td>
<td>0/7 (0.0)</td>
<td>ND</td>
<td>1.00</td>
</tr>
<tr>
<td>Fed animals by hand</td>
<td>27/37 (73.0)</td>
<td>5/21 (23.8)</td>
<td>8.6 (2.5–29.8)</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Animals licked hands</td>
<td>23/33 (69.7)</td>
<td>4/20 (20.0)</td>
<td>9.2 (2.4–34.6)</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

Hygiene

| Washed hands upon exiting                     | 37/38 (97.4)   | 18/21 (85.7) | 6.2 (0.6–63.5) | 0.13    |
| Used running water only                       | 1/44 (2.3)     | 0/28 (0.0)   | ND             | 1.00    |
| Used soap and running water only             | 29/38 (76.3)   | 14/20 (70.0) | 1.4 (0.4–4.6) | 0.75    |
| Used running water and hand gel              | 1/43 (2.3)     | 2/28 (7.1)   | 0.3 (0.0–3.5) | 0.56    |
| Used hand gel only                           | 6/44 (13.6)    | 2/28 (7.1)   | 2.1 (0.4–1.0) | 0.47    |

Selected food exposures

| Dagwood dog†                                    | 15/40 (37.5)   | 10/26 (38.5) | 1.0 (0.3–2.7) | 0.94    |
| German sausage                                 | 5/40 (12.5)    | 4/26 (15.4)  | 0.8 (0.2–3.2) | 0.73    |
| Strawberry sundae                              | 23/43 (53.5)   | 12/27 (44.4) | 1.4 (0.5–4.8) | 0.46    |
| Italian meat balls                             | 0 (0.0)        | 0 (0.0)      | ND             | NA      |
| Beef burger                                    | 0 (0.0)        | 0 (0.0)      | ND             | NA      |
| Steak sandwich                                  | 0 (0.0)        | 0 (0.0)      | ND             | NA      |

*NA, not applicable; ND, not defined because of no persons in the case-patients or control groups.
†These associations remained significantly different when analysis was restricted to children <18 y of age.
‡An audience observed the birth of lambs within an enclosed booth. After 24 h and clearance by a veterinarian, the lambs were available for petting.
§In the final unmatched multivariable model, having hands licked by animals (adjusted odds ratio [OR] 11.7, 95% CI 2.4–58.4) was found to be associated with infection after adjusting for all other variables in the model, including age and sex. Matched analyses using conditional logistic regression produced a similar estimate of effect (adjusted OR 12.5, 95% CI 0.95–162.9), although not quite reaching significance for having hands licked by an animal.
¶A hotdog sausage deep-fried in batter and served on a stick.

Technical Appendix Table 2. Molecular typing of environmental, animal, and patient Escherichia coli O157:H– isolates obtained during outbreak associated with agricultural show, Australia, 2013

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>stx gene subtype</th>
<th>E. coli generic MLVA profile</th>
<th>O157 MLVA profile</th>
<th>MLST†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (49/51 from whom isolates were identified)</td>
<td>stx1a, stx2c</td>
<td>6_8_2_9_4_7_8_2_3_8</td>
<td>11–7–13–4–5–6–4–9</td>
<td>ST11</td>
</tr>
<tr>
<td>Bovine feces</td>
<td>stx1a, stx2c</td>
<td>6_8_2_9_4_7_8_2_3_8</td>
<td>11–7–13–4–5–6–4–9</td>
<td>ST11</td>
</tr>
<tr>
<td>Caprine feces</td>
<td>stx1a, stx2c</td>
<td>6_8_2_9_4_7_8_2_3_8</td>
<td>11–7–13–4–5–6–4–9</td>
<td>ST11</td>
</tr>
<tr>
<td>Animal nursery bedding</td>
<td>stx1a, stx2c</td>
<td>6_8_2_9_4_7_8_2_3_8</td>
<td>11–7–13–4–5–6–4–9</td>
<td>ST11</td>
</tr>
</tbody>
</table>

*MLST, multilocus sequence typing; MLVA, multilocus variable number tandem repeats analysis; ST, sequence type; stx, Shiga toxin gene.
†Extrapolated from sequencing data obtained from representative isolates for each environmental, animal, and patient group.