Pork Implicated in a Shiga Toxin-producing *Escherichia coli* O157:H7 Outbreak in Ontario, Canada

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ABSTRACT

Objectives: To describe an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 infection following a four-day family gathering in Ontario. This is the first published account of a STEC O157 outbreak in Canada linked to consumption of pork.

Methods: The outbreak investigation included interviews with food handlers and other key associated persons, inspection of food preparation premises, traceback investigations, case finding, analysis of data from an outbreak questionnaire, and laboratory analysis of samples collected from various sources associated with the outbreak.

Results: Several meals, including pork from a pig roast, were served to the 59 attendees, 29 of whom developed gastrointestinal illness following the event. Six cases developed bloody diarrhoea and seven were hospitalized. Leftover pork served the day after the pig roast was the item most significantly associated with an increased risk of illness ($p<0.001$). STEC O157:H7 was isolated from 11 of the 29 ill attendees, and also from the pork. By pulse-field gel electrophoresis (PFGE), all STEC O157:H7 pork isolates were either identical or closely related to the 11 clinical isolates. No STEC was detected in any other samples. Three *Clostridium perfringens* isolates, unrelated by PFGE, were obtained from two STEC-positive cases and the pork.

Conclusion: This outbreak highlights the need for increased awareness of pork as a potential source of STEC O157 infection, and for enhanced education regarding the safe handling, cooking and storage of food, specifically where large cuts of meat are cooked outdoors at events such as pig roasts, a cultural norm in some communities.

Key words: *Escherichia coli* O157; zoonoses; food-borne illnesses; outbreaks; pigs; pork

La traduction du résumé se trouve à la fin de l'article.

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Shiga toxin-producing *Escherichia coli* O157:H7/NM (STEC O157) are zoonotic pathogens associated with many food-borne and water-borne outbreaks in North America and elsewhere.[1-3] Most STEC O157 infections have been linked to the consumption of beef, produce or water contaminated directly or indirectly by cattle manure.[1,4-7] However, STEC O157 carriage has been reported not only in cattle but also in other animal species, including other ruminants, swine and poultry.[2,8-11]

In October 2011, a local health department in southwestern Ontario was notified of several cases of bloody diarrhoea in persons who had attended a four-day gathering that had ended four days earlier. The 59 attendees had shared several meals prepared by attendees and caterers, including pork from a pig roasted whole by a caterer at a pig roast, served fresh on the second day of the event along with a meal prepared by another caterer, and as cold and reheated leftovers the following day. This report describes the investigation of this outbreak, in which evidence implicated the pork as the source of illness.

METHODS

Case definition

A confirmed case was an attendee reporting enteric symptoms (nausea, abdominal cramps, vomiting, and/or diarrhoea) beginning anytime from day 2 of the event to 10 days after the last day of the event, with laboratory confirmation of STEC O157:H7 infection. A probable case was an attendee reporting enteric symptoms (nausea, abdominal cramps, vomiting, and/or diarrhoea) during the same period without laboratory confirmation of STEC O157:H7 infection.

Case finding and administration of outbreak questionnaire

Menu lists of all meals served were used to produce a questionnaire on demographics, symptoms, onset and recovery dates, and food exposures (consumption of individual menu items) at shared meals. A list of attendees was provided by the host. Those who could be contacted were asked to complete the questionnaire, either by telephone or in person.

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Conflict of Interest: None to declare.

Table 1. Symptoms Reported by Confirmed and Probable Cases, STEC O157:H7 Outbreak, Southwestern Ontario, October 2011 (n=27*)

Symptom	Number of Cases Reporting Symptom†	Percentage
Diarrhoea	24	89%
Abdominal cramps	20	74%
Nausea	7	26%
Bloody diarrhoea	6	22%
Fever	6	22%
Muscle aches	6	22%
Vomiting	5	19%
Blood in urine	1	4%

* Data on symptoms not available for two additional cases.
† Cases include both confirmed and probable cases.

Statistical analysis of outbreak questionnaire data

Questionnaire data were entered in a Microsoft Access database and analyzed in Stata 11.0 (Statacorp, College Station, Texas) and Microsoft Excel. Following descriptive analysis, exposure variables (consumption of meals and individual menu items) were investigated for association with illness by a retrospective cohort approach, using the <cs> command with the <exact> option in Stata 11.0 for computation of Fisher's exact p values, risks (attack rates), risk differences (attack rate differences) and risk ratios. Variables with two-sided p values >0.05 were considered statistically significant. Exposures significantly associated with illness were included in a logistic regression model with illness as the outcome variable, and a final model was built by stepwise backward elimination.

Informal interviews

Public health inspectors informally interviewed the host and the caterers to review how foods had been produced, prepared, transported, stored and served. As much information as possible was gathered on who had prepared each menu item, the ingredients the meals contained (including brands), and where these had been purchased.

Inspection of food preparation premises and kitchen

Public health staff inspected the premises and kitchen where the catered meal had been prepared, to review menus, refrigerator temperatures, cleaning records, supply records and staff absenteeism records, and to audit the hazard analysis and critical control points (HACCP) systems for all menu items.

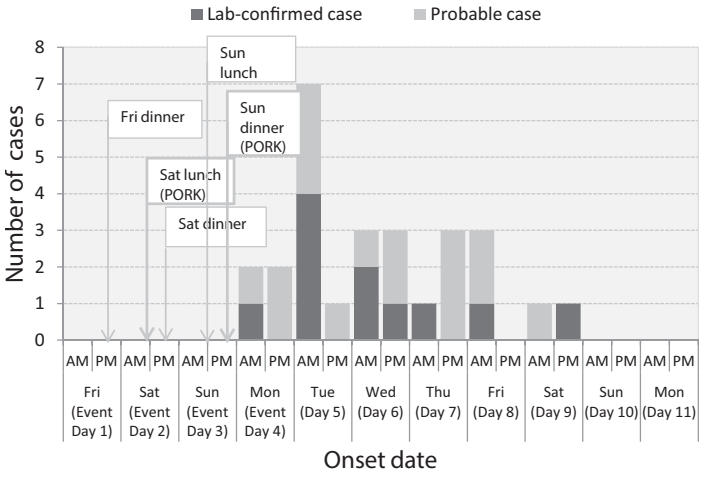
Traceback investigations

A whole, dressed, 42-kg pig had been cooked at the pig roast. Investigations included identification of the plant of origin of the pig, informal interviews with the plant managers, and identification and inspection of the supermarket where the pig had been purchased and the refrigeration unit where it had been stored before being cooked. Traceback investigations were also conducted for meats, salads and other foods served at the event that were considered common sources of food-borne illness.

Collection of samples for laboratory analysis

Stool samples were requested from individuals still ill at the time of their interview. Duplicate samples were submitted in Cary-Blair medium for bacterial testing,[12] and in sodium acetate–acetic acid–formalin (SAF) fixative for parasitic testing.[13]

Figure 1. Epidemic curve of laboratory-confirmed and probable cases, STEC O157:H7 outbreak, southwestern Ontario, October 2011 (n=27)



Note: The dates of the event are indicated by Event Days 1 to 4. No cases were known to have occurred after Day 9. Excluded from the chart are two individuals with unknown exact dates of onset between Days 5 and 7.

Samples of leftover foods from the event (cooked potato, turkey, carrots, beets, and pork) and water samples from the event premises were also collected for testing for enteric pathogens.

Three batches of home-made ice cream served on the second and third days of the gathering had been prepared using eggs from a local hobby farm where goats and poultry shared a common housing area. Therefore, water, environmental (soiled animal bedding) and fecal samples from goats and poultry on this farm were collected for STEC O157 testing.

Laboratory analysis of samples

All samples were tested at the Public Health Laboratory in Toronto, Ontario by routine enteric diagnostic methods, including testing of food and water samples by immunomagnetic separation (IMS) of STEC O157 and selective culture methods based on FDA and Health Canada methods.[14,15] Isolates were confirmed biochemically as STEC O157 and *Clostridium perfringens,* subtyped by pulse-field gel electrophoresis (PFGE), and serotyped (STEC O157). The hobby-farm fecal and environmental samples were also tested at the Public Health Agency of Canada Laboratory for Foodborne Zoonoses, Guelph, Ontario, for any STEC by screening enrichment broths for Shiga toxin by ELISA[16] and the Verocell cytotoxicity assay,[17] and for STEC O157 by IMS.[15]

RESULTS

Summary of cases and results of analysis of outbreak questionnaire data

Descriptive Epidemiological Analysis

Although some ill individuals may have been secondary cases, all had attended the event and had onset dates within the period specified in the case definition. Therefore, in analyzing the questionnaire data, no distinction was made between primary and secondary cases.

The descriptive analyses included data for 52 of the 59 attendees. Of the 52, 48 completed questionnaires, all within 5-11 days after

Table 2. Risk Differences and Ratios of Food Exposure Variables Significantly Associated With an Increased Risk of Illness, STEC O157:H7 Outbreak, Southwestern Ontario, October 2011 (n=48)

Exposure/Food or Beverage	Persons Who Ate (Exposed)				Persons Who Did Not Eat (Unexposed)							
	Ill	Not Ill	Total	Risk of Illness [Attack Rate] (% Ill)	Ill	Not Ill	Total	Risk of Illness [Attack Rate] (% ill)	Attack Rate [Risk] Difference (%)	Risk Ratio	Confidence Interval of Risk Ratio*	p (Fisher's exact, two-sided)
Fri – Event Day 1 (dinner):												
Ate at Fri dinner*	25	17	42	59.5	0*	6	6	14.3*	45.2*	4.2*	0.7-26.0*	0.0082
Garlic bread	23	12	35	65.7	2	11	13	15.4	50.3	4.3	1.2-15.6	0.0029
Sat – Event Day 2 (lunch)†:												
Ate at Sat lunch†	25	23	48	52.1	0	0	0	–	–	–	–	–
Sat – Event Day 2 (dinner):												
Ate at Sat dinner*	25	17	42	59.5	0*	6	6	14.3*	45.2*	4.2*	0.7-26.0*	0.0082
Pizza (various)*	25	17	42	59.5	0*	6	6	14.3*	45.2*	4.2*	0.7-26.0*	0.0082
Sun – Event Day 3 (lunch):												
Ate at Sun lunch*	25	17	42	59.5	0*	6	6	14.3*	45.2*	4.2*	0.7-26.0*	0.0082
Roasted turkey	22	12	34	64.7	3	10	13	23.1	41.6	2.8	1.01-7.8	0.0204
Sun – Event Day 3 (dinner):												
Ate at Sun dinner	22	13	35	62.9	2	10	12	16.7	46.2	3.8	1.03-13.7	0.0078
Leftover pork	18	4	22	81.8	4	14	18	22.2	59.6	3.7	1.5-8.9	0.0003
Buns	18	5	23	78.3	5	11	16	31.3	47.0	2.5	1.2-5.3	0.0072

* For exposures with counts of zero in the "unexposed and ill" cell, estimates of risks, risk differences and risk ratios were computed by substituting a cell count of one in that cell.

† All attendees included in these analyses had eaten at the Saturday lunch, therefore no Fisher's exact p-value could be computed for the exposure "Ate at Sat lunch". In addition, no menu items served at the Saturday lunch were statistically associated with an increased risk of illness (p>0.05).

the end of the event. Information on demographics and symptoms was available for 4 additional known cases who did not complete questionnaires.

Age and sex were known for 50 of the 52 persons: 23 (46%) were male and 27 (54%) were female, ranging in age from 1-84 (median 31, mean 33.5) years. Twenty-nine (55.8%) were identified as cases according to the case definition, of whom 11 were confirmed cases of STEC O157:H7 infection and 18 were probable cases. There was no significant difference in either age or sex between those who reported illness and those who did not (p>0.1).

Illness and Symptoms

Onset dates of illness ranged from 0-5 days following the last day of the event (early on Day 4 of the gathering, to the afternoon of Day 9) (Figure 1).

Table 1 summarizes symptoms reported by 27 of the 29 cases; information on symptoms was unavailable for 2 cases. Recovery dates were available for only 5 cases, for whom the durations of illness were: less than one day (n=1), three days (n=1) and five days (n=3).

Seven (24.1%) of the 29 cases were hospitalized: two adults <60 years, one adult >60 years, and four children aged 1-15 years. Verbal updates from health care professionals and family members indicated that of these, two children and one adult had signs of haemolytic uraemic syndrome; one child and the adult required dialysis and blood transfusions as a result. Another adult who initially reported resolution of enteric symptoms was subsequently hospitalized with pancreatitis suspected to be a complication of STEC O157:H7 infection. No deaths resulting from the outbreak were reported.

Analysis of Exposure Variables

During the four-day event, five communal meals (including 50 individual menu items) were served before the onset date of the earliest case (Day 4). Analysis of questionnaire data from 48 attendees (25 cases and 23 non-cases) for exposures significantly associated with an increased risk of illness (p<0.05) revealed that of the 50 menu items investigated, the leftover pork served on Day 3 (Sunday) produced the highest level of statistical significance (p=0.0003) and the highest attack rate (risk) difference (59.6%; Table 2).

Many of the exposure variables were highly correlated. All individuals included in the analyses had attended the pig roast (lunch) on Day 2, and all except two had consumed the pork at that meal.

Multivariable logistic regression, using backward elimination to produce a final model from an initial model that included exposures to all five menu items listed in Table 2 as independent variables, resulted in only leftover pork being retained in the model. In this final model, the odds ratio associated with consumption of leftover pork was 9.0 (95% CI: 1.8-45.3; p=0.008).

Similar results were obtained when the exposure analyses were repeated without five possibly secondary cases with onset dates later than Day 7.

Informal interviews with food handlers, inspection of food preparation premises, and traceback investigations

No concerns were identified on inspection and HACCP audit of the meal caterer's kitchen or of food storage or preparation, and there were no reports of illness or unexplained absenteeism among food handlers.

Served foods initially considered high risk for causing food-borne enteric illness included salads, beef lasagne, turkey, pork from the pig roast, and the home-made ice cream. However, except for the pork, no obvious concerns about preparation of those foods arose from investigations.

According to the pig roast caterer, the pig had been turned continuously for 12h on a spit positioned 1.5 to 2 feet (approximately 0.5 m) above the coals. Temperature probe readings taken at several sites in the meat during cooking were reportedly 182°C, but probe type, placement and readings had not been documented. After cooking, the pork had been sliced into hot chafing dishes for immediate serving. Leftover pork was refrigerated in large aluminium baking pans of unknown dimensions. A potential concern

was that the 42kg carcass had been roasted in cool air temperatures (minimum 4°C overnight) above the heat source and without a shelter, potentially resulting in inadequate cooking of internal parts of the meat. Further, leftover pork was stored in a refrigerator full of other leftovers, which may not have allowed for quick cooling of the meat to optimal storage temperatures.

The pig had originated from a government-regulated and -inspected plant that processed only pigs. No concerns arose from interviews with the plant managers or provincial inspection authorities, or from inspection of the supermarket where the pig had been purchased, including the supermarket holding units. During inspection, the temperature of pig carcasses in the store cooler was 3°C. Pig carcasses were delivered through a separate entrance into a walk-in cooler for only pigs and ducks, suggesting minimal potential for cross-contamination between pig carcasses and other meats at the store.

Laboratory analysis

STEC O157:H7 was isolated from 11 of 25 stool samples from symptomatic attendees, and from the leftover pork – the only tested food item positive for enteric pathogens. Isolates from eight cases and the pork had the same PFGE pattern: (ECXAI.0221/ECBNI.0012). The PFGE pattern of isolates from the three other cases (ECXAI.2684/ECBNI.0012) differed from that of the other isolates by only one band, indicating that they were closely related.[18] *Clostridium perfringens* was isolated (but not quantified) from two STEC-O157:H7-positive cases, and was also found at high levels (1.7×10^6 organisms per gram) in the pork. By PFGE, the three *C. perfringens* isolates were all unrelated to each other.

The remaining 14 stool samples, other foods, water and hobby-farm samples submitted for testing were negative for STEC or enteric pathogens.

DISCUSSION AND CONCLUSION

Many ingredients of the meals for this event had been purchased from large retailers, and/or were popular, locally-available brands. However, review of surveillance data from the local area during the months spanning the outbreak period indicated no concurrent increase in reported STEC O157:H7 infections in the general population, nor isolation of STEC O157:H7 with the PFGE patterns identified in this outbreak. Therefore the source of STEC O157:H7 that caused this outbreak appeared to have been limited to this event.

Several possible sources of STEC infection at the event were considered. High-risk food items were identified from assessment – based on information from interviews and inspections – of the potential for contamination of food items on the menu lists, and from statistical analysis of food exposures. In addition, as small ruminants and poultry may carry STEC O157:H7,[8,9,11] and as at least one STEC outbreak has been linked to consumption of home-made ice cream,[19] samples from the hobby farm that provided ingredients for the ice cream were tested for STEC. However, of all of the potential sources investigated, STEC O157 was isolated only from the pork.

The isolation of identical or closely-related strains of STEC O157:H7 from 11 confirmed cases and the pork, as well as the negative results for other possible sources tested, suggests that the pork was the source of infection. *Clostridium perfringens* was also identified at clinically significant levels in the pork, and was isolated from two STEC O157:H7-positive cases. Though the three isolates were unrelated by PFGE,[20] infection or co-infection by *C. perfringens* could have caused symptoms experienced by some of the cases – probable or confirmed.

Given the high overall attack rate in this outbreak and the lack of evidence for cross-contamination from other foods during slicing and serving of the pork, it is likely that contamination of the pork existed at the time of roasting on Day 2, possibly originating at the time of slaughter from STEC O157:H7 infection in the living pig or from another source. Improper cooking, cooling and storing, followed by inadequate reheating before leftovers were served on Day 3, may have allowed *C. perfringens* and STEC O157:H7 to survive and possibly proliferate. A few attendees may have been infected by consuming the pork on Day 2; however, freshly-cooked pork consumed on Day 2 was likely carved mainly from the surface of the carcass, which would have been cooked more thoroughly than the inner parts of the meat if a significant temperature gradient from the outside to the inside of the carcass existed during cooking. However, if cooling of the leftover meat occurred too slowly following refrigeration, significant bacterial growth could have occurred, causing the inner portions of the pork served as leftovers, some cold, on Day 3, to contain significant levels of pathogens. This hypothesis is supported by the highly significant association between illness and the consumption of leftover pork on Day 3, with no such association being found for the freshly roasted pork served on Day 2 when only a few attendees may have been exposed to the pathogen. Additionally, the incubation period in this outbreak, assuming exposure on Day 2 or Day 3, was 1-7 days with a median of 3-4 days: within the reported ranges for STEC O157:H7 of 1 to >7 days, with a median of 3-4 days.[21-24]

Although most commonly found in ruminants, pigs may also carry STEC O157.[10,11,25-27] However, there are few reports of STEC O157 outbreaks implicating pork as the likely source.[28] The results of this investigation, including the fact that the plant that had processed the pig did not process cattle, emphasize the importance of considering other meat sources besides beef when investigating outbreaks or clusters of STEC O157 infection.

This investigation also highlights the need for enhancement of local and provincial educational resources for the public and for food handlers on proper food handling and storage, to reduce the risk of food-borne illness at events such as the one described here. Specifically, there is need for clear provincial guidelines pertaining to the safe handling and cooking of whole carcasses and large cuts of meat outdoors on open spits at events such as a pig roasts, a cultural norm in many rural communities in Ontario.

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RÉSUMÉ

Objectif : Décrire une éclosion d'infections à Escherichia coli producteur de toxine dysentérique (STEC) O157:H7 après une réunion familiale de quatre jours en Ontario. Il s'agit du premier compte rendu publié d'une éclosion de STEC O157 liée à la consommation de porc au Canada.

Méthode : L'enquête sur l'éclosion a compris des entretiens avec les manipulateurs d'aliments et autres personnes liées, l'inspection des installations de préparation des aliments, des enquêtes de retraçage, la recherche des cas, l'analyse des données d'un questionnaire sur l'éclosion et des analyses de laboratoire sur des échantillons prélevés de diverses sources associées à l'éclosion.

Résultats : Plusieurs repas, y compris du cochon cuit à la broche, ont été servis aux 59 participants, dont 29 ont contracté une maladie gastrointestinale après la fête. Six cas ont eu une diarrhée sanglante et sept ont été hospitalisés. Les restes de porc servis le lendemain de la cuisson à la broche ont présenté la corrélation la plus significative avec le risque accru de maladie (p<0,001). STEC O157:H7 a été isolé chez 11 des 29 participants malades, ainsi que dans le porc. Selon l'électrophorèse sur gel en champs pulsé (EGCP), tous les isolats de STEC O157:H7 du porc étaient soient identiques, soit étroitement liés aux 11 isolats cliniques. La présence de STEC n'a été détectée dans aucun autre échantillon. Trois isolats de *Clostridium perfringens*, non liés selon l'EGCP, ont été obtenus à partir du porc et de deux cas positifs pour le STEC.

Conclusion : Cette éclosion montre qu'il faut davantage surveiller le porc comme source d'infection potentielle par le pathogène STEC O157 et sensibiliser la population aux techniques de manipulation, de cuisson et d'entreposage sans danger des aliments, en particulier lorsque de grandes coupes de viande, comme le porc, sont cuites à l'extérieur à la broche (ce qui fait partie de la culture de certaines collectivités).

Mots clés : Escherichia coli O157; zoonoses; toxi-infections alimentaires; flambées épidémiques; cochon; porc