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Escherichia coli O157:H7 outbreak linked to salami, British Columbia, Canada, 1999

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SUMMARY

An outbreak of *E. coli* O157:H7 infections was identified in November 1999 with a fivefold increase in the occurrence of laboratory-confirmed cases of *E. coli* O157:H7 infection. A matched case-control study was conducted. Samples of food from cases and from retailers were analysed for the presence of *E. coli* O157:H7. A total of 143 cases were identified over a 12-week period with the same pulsed-field gel electrophoresis (PFGE) pattern. The case-control study found that Company A salami was significantly associated with illness (Mantel–Haenszel matched odds ratio 10·0, 95% CI 1·4–434, $P=0·01$). Company A salami tested positive for *E. coli* O157:H7 and isolates had the same PFGE pattern as case isolates. An immediate voluntary national recall of Company A dry fermented meat products took place. Findings from the investigation of this outbreak suggest that the hold-and-test option may not be adequate to prevent shiga-toxigenic *Escherichia coli* (STEC) infection in salami consumers.

INTRODUCTION

In early November 1999, laboratory surveillance noted an increase in *E. coli* O157:H7 isolates over a 2-week period. An investigation was initiated when the British Columbia Centre for Disease Control (BCCDC) determined the increase to be fivefold above background rates. This paper presents the outcome of the investigation and discusses the implications for fermented meat manufacturers and public health.

Shiga-toxigenic *Escherichia coli* (STEC) infection is an increasingly recognized cause of foodborne illness. Its potential for human pathogenicity was first recognized in 1982 [1]. Most STEC outbreaks in North America have resulted from infection with *Escherichia coli* O157:H7 (*E. coli* O157:H7). Between 1993 and 1998, the number of cases of *E. coli* O157:H7 infection reported annually in British Columbia (BC) ranged from 133 to 206 with 178 occurring in 1998 [2]. Infection often presents as bloody diarrhoea, and can result in haemolytic–uraemic syndrome (HUS) with renal failure in up to 6% of cases [3]. Most illness has been associated with eating undercooked, contaminated ground beef [4–6]. Secondary cases from faecal–oral routes of transmission often occur among family members and in child-care centres [7–11]. Infection has also been associated with other food sources including unpasteurized milk or apple juice, yoghurt, cheese, water and salad products [12]. Transmission via direct or indirect animal contact has been reported [13–15]. The first documented outbreak related to dry fermented meat occurred in 1994 in Washington and Northern California [16]. In Canada, the only documented STEC outbreak related to fermented meat occurred in 1998 [17].

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METHODS

Initial case series and case-control study

Confirmed *E. coli* O157:H7 cases with indistinguishable pulsed-field gel electrophoresis (PFGE) patterns to the outbreak strain were interviewed. The hypothesis-generating questionnaire included information on demographics, food eaten and environmental exposures. The 28-item case-control study questionnaire asked about exposure to a variety of salami and delicatessen products, as well as symptoms. All cases identified between 21 October and 10 November who were infected with the outbreak strain were eligible for inclusion in the study. For each case, one age-matched neighbourhood control was identified either by the case or by random digit dialling. Controls were excluded if they had diarrhoea in the past month. Data entry and analysis were completed using Epi-Info 6.04 [18]. Statistical analysis included matched odds ratios and χ^2.

Laboratory investigations: enteric bacteriology

E. coli O157:H7 isolates were forwarded to the provincial laboratory for serotype confirmation shiga-toxin testing and PFGE. Positive cultures and stool supernatants were inoculated onto Vero cells and observed for cytopathic effects that were neutralized by VT1 and VT2 antisera after 72 h incubation [19]. All isolates serotyped as *E. coli* O157:H7 were subjected to PFGE according to the protocol developed by Centers for Disease Control and Prevention (CDC) [20]. The PFGE patterns of each isolate were classified according to Tenover's criteria to determine their relatedness [21], based on the assumption that genetically related isolates are also epidemiologically related.

Laboratory investigations: foodborne disease laboratory

The selection of food samples was based on the hypothesis generated from the questionnaire. Food samples were sent to the provincial laboratory for the enumeration, detection and isolation of *E. coli* O157:H7 using the modified BAM procedures for direct and enrichment culture [22]. The Visual Immunoprecipitate (VIP) rapid screen test kit [23] was also used as an ancillary test. Food samples were obtained from confirmed cases and unopened food samples which were collected from retail stores linked to implicated food items. *E. coli* O157:H7 isolates from food samples were referred for confirmation and further studies, including PFGE.

Food product investigation

Plant inspection was carried out by the Canadian Food Inspection Agency (CFIA). The inspection identified and reviewed critical-control points in the production of the product. Environmental swabs, samples of the salami product and pre-processed ingredients, where available, were obtained for culture and analysed following the same methods as described for the clinical specimens.

Enhanced surveillance

An enhanced surveillance questionnaire was developed for follow up of all confirmed cases of *E. coli* O157:H7 infection in BC occurring between 1 October 1999 to 31 December 1999. The questionnaire asked about consumption of Company A products, clinical symptoms, and treatment. For purposes of enhanced surveillance, the definition for primary cases was: a resident of BC between 1 September and 31 December 1999 who had either: (1) the outbreak PFGE strain isolated from their stool; or (2) shiga toxin in their stool and consumption of Company A Hungarian, Gypsy, Pepper or Cervelat salami. The definition for secondary cases was: a resident of BC between September and 31 December 1999 who had either: (1) the outbreak PFGE strain isolated from their stool; or (2) shiga toxin in their stool and contact with an outbreak-associated primary case.

RESULTS

Initial case series and case-control study

Over a 2-week period (21 Oct.–4 Nov.), 19 *E. coli* O157:H7 cases were identified through laboratory surveillance. Twelve (63%) had a common PFGE pattern while 7 cases had unrelated patterns. The hypothesis-generating questionnaire was administered to 9 (75%) of these 12 cases and 7 (77%) reported eating salami prior to symptom onset. No other common exposures were identified. A product wrapper provided by a case on 9 November identified Company A Hungarian salami.

By 10 November, 29 cases with the common PFGE pattern were identified, and 19 were available for

Table. *Results of case-control study* (*age and neighbourhood matched*) (*n* = *19*)

Risk factor	Number of all cases who ate product (%)	Number of controls who ate product (%)	OR	95% CI	*P*
Company A Hungarian salami	11 (58·0%)	0 (0%)	10	1·42–434	0·006
Company A Cervelat salami	6 (31·6%)	1 (5·3%)	6	0·73–276	0·06
Hungarian or Cervelat salami	17 (89·5%)	1 (5·3%)	13	1·95–552	0·001

OR, Odds ratio; CI, confidence interval.

inclusion in the case-control study. A significant association was found between illness and consumption of Hungarian salami alone and either Hungarian or Cervelat salamis produced by Company A (see Table).

Laboratory investigations

Laboratory surveillance identified 143 cases during the course of the outbreak: *E. coli* O157:H7 with a common PFGE pattern, defined as the outbreak strain, was isolated from the stools of 135 cases, while shiga-toxin-producing *E. coli* (serotype undetermined) was isolated from the stool of 8 additional cases who had consumed Company A salami. The provincial laboratory processed 57 food samples. Forty-three of the 57 were Company A products (32 Hungarian, 7 Cervelat and 4 other types of sausage). The enrichment culture yielded growth of *E. coli* O157:H7 from nine of the Company A products. The same nine Company A products also tested positive with the VIP rapid screen test. The PFGE pattern from these nine isolates from salami samples were identical to the outbreak strain. Both Hungarian and Cervelat salami samples were found to be positive.

Food product investigation

The environmental investigation of the plant did not identify any breach in the manufacturing practices which CFIA felt contributed to the outbreak. Six isolates of *E. coli* O157:H7 were identified from four salami samples produced on four different dates ranging from 6 August 1999 to 17 September 1999. Five of the six isolates had the same PFGE pattern identified in case isolates. The sixth had a completely different pattern. As pre-processed ingredients from implicated production dates were not available for testing, a precise source of contamination was not identified. Two of the positive isolates were from production dates in August 1999. A small portion of the entire lot was exported to the United States, it was tested as part of the hold-and-test programme and there was no growth of *E. coli* O157:H7 prior to export.

The salami was produced from raw beef, raw beef suet, and raw pork which were combined, along with other ingredients, into a batter. The batter was placed into casings, and then fermented and dried. The individual or combined ingredients were not cooked during production of the salami. The company did, however, follow a CFIA-recognized Hazard Analysis and Critical Control Point (HACCP) plan that required water activity and pH to be maintained within specified ranges. A review of the company's records showed no deviations from these ranges during production of the implicated lots.

Enhanced surveillance

A total of 143 cases of *E. coli* O157:H7 infection were associated to the outbreak. Three cases could not be contacted or refused to be interviewed. Therefore, the questionnaire was administered to 140 cases. Of these cases 135/140 (96%) were primary cases, 3/140 (2%) were secondary cases and two cases could not be classified.

Fifty-six per cent (*n* = 80) of cases were female. Case age range was 1–89 years with a median age of 12 years. Symptoms reported by cases included diarrhoea (98%), abdominal cramps (91%), bloody diarrhoea (79%), nausea (50%), vomiting (44%), headache (34%) and fever (29%). Forty-two

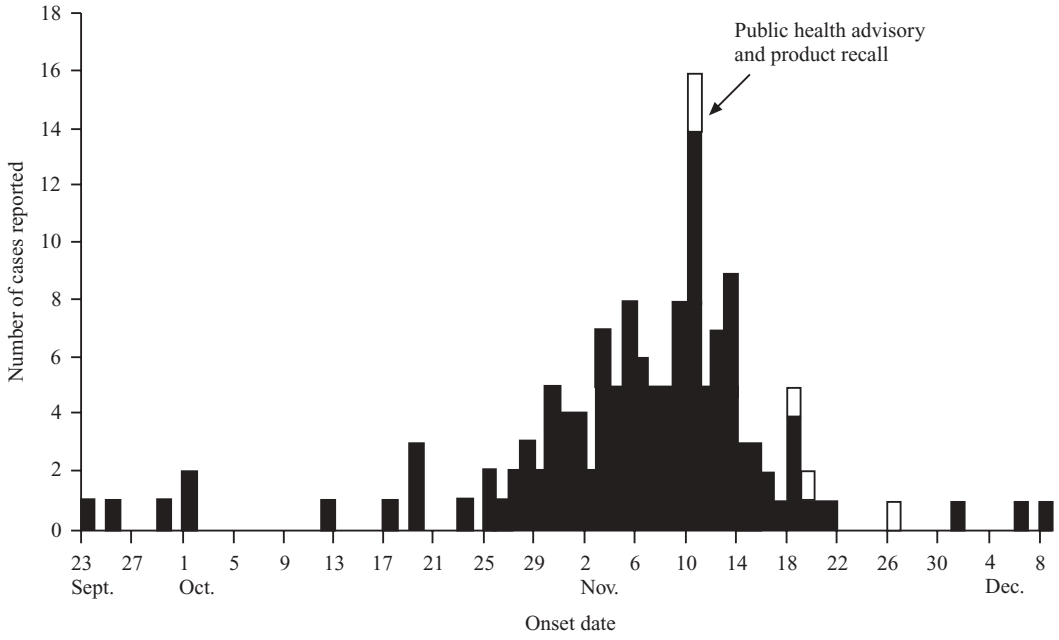

Fig. Epidemic curve. ■, Primary; □, secondary.

individuals were hospitalized and six cases of HUS were reported. No deaths occurred. Forty-two cases were admitted to hospital with an average length of stay of 3 days. Another 16 cases report lengthy stays in emergency departments. The Figure shows the epidemic curve of known onset dates for cases associated with the outbreak. In retrospect, five cases from late September and early October were identified. These cases were related to the contaminated product that was on the market prior to the identification of the outbreak. Of those contacted, 116 out of 140 (83%) reported consuming Company A Hungarian or Cervelat salami.

Public health action

The BCCDC issued a public health advisory on 10 November 1999. Based on the evidence from the case series, the manufacturer and CFIA ordered an immediate voluntary Class 1 Food Recall of all their dry fermented meat products on 10 November 1999. Class 1 recalls involve products that have a reasonable probability of causing serious adverse health consequences or death.

DISCUSSION

This was the largest foodborne outbreak of STEC infection ever documented in Canada. The associated morbidity was substantial with 30% of cases requiring hospitalization and 4% developing HUS. The size of this outbreak can be attributed to the large amount of contaminated product, and the broad geographic market of the producer. Epidemiological and laboratory investigations identified consumption of contaminated, dry fermented, salami as the cause of the outbreak. Our case-control study found a strong association between illness and consumption of salami made by a single producer. *E. coli* O157:H7 was isolated from leftover salami which cases had eaten, and from unsold salami of the same lot number. The majority of study interviews were completed prior to the announcement of the product recall. It was felt that the public health advisory did not bias the study results. *E. coli* O157:H7 isolates from cases' stools and from the salami had a common PFGE pattern. The geographic distribution of cases was representative of the market distribution of the contaminated salami.

An environmental investigation and a review of plant records did not determine how the implicated salami in this outbreak became contaminated with *E. coli* O157:H7.

It has previously been demonstrated that fermentation and drying will not completely eliminate *E. coli* O157:H7 from salami [24, 25]. If present in raw batter, the pathogen will survive processing and persist into the finished, ready-to-eat product. Because of this,

the United States Department of Agriculture (USDA) requires commercial producers of dry and semi-dry fermented sausages in the United States to follow 1 of 5 safety options:

(1) Achieve a 5-log kill using a heat process (63 °C for 4 min).
(2) Develop and validate individual 5-log inactivation treatment plans.
(3) Conduct a hold-and-test programme for finished product. Depending on type of product, 15–30 individual chubs must be sub-sampled per lot.
(4) Propose combinations that demonstrate a collective 5-log kill.
(5) Initiate a hazard analysis critical-control point system that includes raw batter testing and a 2-log inactivation in fermentation and drying [26].

At the time of this outbreak, these safety options were not required for dry fermented sausage produced and distributed within Canada.

Our findings suggest that the third USDA safety option – a hold-and-test programme – may not be effective in ensuring that salami is free of STEC. Forty-three cases had leftover samples of the company A salami they consumed prior to becoming ill. We tested all of these samples but were able to isolate *E. coli* O157:H7 from only nine. Therefore *E. coli* O157:H7 could not be isolated from 34 of 43 samples that are known to have been contaminated. Furthermore, the company conducted a hold-and-test programme for the product from two earlier production dates. *E. coli* O157:H7 was not isolated and the product was exported to the United States. After identification of the outbreak in Canada, products from these production dates were re-tested by the CFIA and found to contain *E. coli* O157:H7, necessitating a recall in the United States.

The results of our investigation suggest that a background level of STEC infections related to consumption of salami could exist at a sufficiently low level to escape identification through routine surveillance. Although most cases in this outbreak were related to salami produced on 17 September, five geographically and temporally dispersed cases were related to lots with earlier production dates. These cases were identified in retrospect because the PFGE pattern of their isolates matched the outbreak strain. It is likely that salami lots from production dates earlier than 17 September contained less viable *E. coli* O157:H7, and were therefore associated with fewer cases. A coordinated network for comparing PFGE patterns did not exist in Canada at the time of this outbreak.

In summary, this was the largest foodborne outbreak of STEC infection in Canada. A coordinated outbreak response allowed for early identification of salami as the cause, and a health advisory and product recall to be initiated. Standard production processes for dry fermented sausages cannot be relied upon to prevent contamination with STEC. Subsequent to this outbreak, all registered meat plants producing dry fermented sausages in Canada are to follow 1 of 5 additional safety options [27]. Findings from the investigation of this outbreak suggest that the hold-and-test option may not be adequate to prevent STEC infection in salami consumers. Our findings also suggest that a contaminated lot of salami may cause only a few STEC infections making epidemiological linkage to a common source difficult.

Recommendations

(1) Dry fermented sausages produced should be subject to heat treatment or equivalent processes that will result in acceptable reductions of STEC and other pathogens.
(2) The effectiveness of the hold-and-test option should be re-evaluated.
(3) Individuals who are at high risk of serious outcomes following infection with STEC, including children, the elderly, and individuals with immuno-compromising conditions, should not consume uncooked, dry fermented sausage products.
(4) Questions about exposure to dry fermented sausages should routinely be asked of all reported cases of *E. coli* O157:H7 and other STEC infection.
(5) Clinical STEC isolates from all community and hospital laboratories should routinely be referred to a provincial reference laboratory.
(6) An electronic network for comparing PFGE patterns should continue to be developed.

Since this outbreak, such a network has been developed in Canada, 'PulseNet Canada'. This network is linked to the corresponding network in the United States, 'PulseNet'.

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