

Fermenting a place in history: The first outbreak of *Escherichia coli* O157 associated with kimchi in Canada

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Abstract

A Canadian outbreak investigation was initiated in January 2022 after a cluster of cases of Shiga-toxin-producing *Escherichia coli* (STEC) O157 was identified through whole genome sequencing (WGS). Exposure information was collected through case interviews. Traceback investigations were conducted, and samples from case homes, retail, and the manufacturer were tested for STEC O157. Fourteen cases were identified in two provinces in Western Canada, with isolates related by 0–5 whole genome multi-locus sequence typing allele differences. Symptom onset dates ranged from 11 December 2021 to 7 January 2022. The median age of cases was 29.5 (range 0–61); 64% were female. No hospitalisations or deaths were reported. Of 11 cases with information available on fermented vegetable exposures, 91% (10/11) reported consuming Kimchi Brand A during their exposure period. The traceback investigation identified Manufacturer A in Western Canada as the producer. One open and one closed sample of Kimchi Brand A tested positive for STEC O157, with isolates considered genetically related by WGS to the outbreak strain. Napa cabbage within the kimchi product was hypothesised as the most likely source of contamination. This paper summarises the investigation into this STEC O157 outbreak associated with kimchi, the first reported outside of East Asia.

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC) O157 is a bacterial pathogen causing gastroenteritis in humans. The typical presentation of STEC O157 infection includes abdominal pain and diarrhoea, with approximately 10–15% of patients developing haemolytic uraemic syndrome [1]. STEC O157 is associated with the fourth largest number of hospitalisations and deaths due to domestically acquired foodborne illness each year in Canada [2]. Although the incidence of STEC O157 in Canada was 1.06 cases per 100 000 persons in 2019 [3], based on estimates of under-reporting, the true rate of illness caused by STEC O157 is likely 20 times higher [4].

Historically, outbreaks of STEC O157 have commonly been associated with leafy greens [5–8], beef products [9, 10], and raw milk products [11–13]. Fermented vegetable products are acidic in nature and are therefore often thought to be an inhospitable environment to pathogens, and an unlikely vehicle for foodborne outbreaks [14]. Kimchi is a traditional Korean side dish consisting of cabbage and other vegetables that are salted and fermented with additional ingredients, such as red pepper powder, garlic, and ginger. Several outbreaks of STEC or other pathogenic *E. coli* in kimchi have been noted in the literature in South Korea [15, 16] and Japan [17–19], indicating that kimchi can serve as a vehicle of transmission for foodborne pathogens.

On 21 January 2022, a cluster of ten STEC O157 cases was identified by Canada's National Microbiology Laboratory via whole genome sequencing (WGS). Cases were reported across two provinces in Western Canada. The objective of this paper is to describe the outbreak investigation, which is the first known outbreak of STEC O157 associated with kimchi to occur outside of East Asia.

Methods

Case definition

A confirmed case was defined as a resident of or visitor to Canada with laboratory confirmation of STEC O157, with isolates matching PulseNet Canada cluster 2201ECWGS-1MP by WGS and a

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symptom onset, collection, or isolation date on or after 1 December 2021. WGS subtyping was conducted using whole genome multi-locus sequence typing (wgMLST) analysis.

Laboratory analysis

In Canada, all clinical STEC isolates are forwarded to provincial public health laboratories or the National Microbiology Laboratory for WGS-based subtyping. WGS is completed using the standardised PulseNet Canada protocol [20]. WGS data is analysed locally and then uploaded to a centralised BioNumerics v7.6 (BioMérieux) database where it is analysed by the PulseNet Canada national database team using wgMLST. Multi-jurisdictional clusters are identified using a threshold of two or more isolates with isolation dates within the past 60 days that are related within 0-10 wgMLST allele differences. To visualise isolate relatedness, unweighted pair group method with arithmetic mean (UPGMA) dendrograms are constructed within BioNumerics v7.6 using a categorical similarity coefficient. Canada and the United States (US) have a bilateral information-sharing agreement that allows the routine exchange of molecular and genomic data. Under this agreement, WGS data was exchanged with PulseNet USA to facilitate the query of the PulseNet USA databases for potential matches in the US as well as on the National Center for Biotechnology Information (NCBI) pathogen detection pipeline.

All STEC isolates recovered from food tested by the Canadian Food Inspection Agency (CFIA) were sequenced by CFIA laboratories, as outlined previously [21], and uploaded to the National Microbiology Laboratory for comparison to clinical isolates.

The Shiga toxin gene profile for isolates recovered from clinical specimens and open food samples collected from case homes was determined in-silico from WGS data using the VirulenceFinder 2.0 database [22, 23]. The Shiga toxin gene profile for food isolates recovered from retention samples tested at the CFIA Laboratory was determined using method MFLP-22 [24], and further characterised for subtype as previously described [25].

Epidemiological investigation

Food and risk factor exposure information for cases was collected through initial interviews with local public health officials using standard provincial *E. coli* questionnaires or the Public Health Agency of Canada (PHAC) *E. coli* hypothesis-generating questionnaire [26]. Cases were asked about exposures in the 10 days prior to illness onset, in alignment with the maximum incubation period for *E. coli*. After initial analyses identified a signal for Grocery Chain A, re-interviews were conducted using the PHAC hypothesis-generating questionnaire with additional questions about exposures related to Grocery Chain A. After kimchi from Grocery Chain A was identified as a suspect source, additional re-interviews were conducted using focused questionnaires to ask more specific questions regarding the brand, lot code, purchase location, and purchase date of the kimchi. Cases were asked for any leftovers of kimchi product. When available, samples from case homes were collected for testing by provincial laboratories.

Data analysis

Case demographic and food exposure information were gathered from interview data and summarised. Data from the 2015 Foodbook population-based telephone survey [27] were used to establish the expected proportion of Canadians that reported eating various

food items in the past seven days. For analyses, Foodbook values were restricted to the provinces where cases were reported, and to the months in which illness onsets were reported. Comparisons between case exposure frequencies and Foodbook values were made using binomial probabilities. All epidemiological data were entered into a Microsoft Access database and descriptive analyses and visualisations were performed in Microsoft Excel and Stata 15 [28].

Food safety investigation

Data collected from the epidemiological investigation were shared with the CFIA to support the food safety investigation. The CFIA completed traceback and traceforward activities on the item of interest, kimchi from Grocery Chain A, during the investigation to identify the manufacturer, the distribution of the product, and details about the manufacturing process, including the origin of ingredients in specific lot codes of product.

A number of unopened retail and retention samples of the kimchi product were collected and tested by the CFIA for STEC O157. The kimchi samples' pH levels were also tested to inform root cause hypotheses and to observe the acidity characteristics of the product.

Results

Case characteristics

A total of 14 cases were identified across two provinces in Western Canada (Province A = 13, Province B = 1; Figure 1). Symptom onset dates ranged from 11 December 2021 to 7 January 2022. The median age of cases was 29.5 (range 0-61), and 64% were female. There were no hospitalisations or deaths reported.

Laboratory analysis

The 14 clinical cases and 3 non-clinical isolates recovered from Kimchi Brand A had the same Shiga toxin gene profile (*stx1* positive and *stx2* positive) and were considered highly related to each other based on WGS, grouping together with 0-5 wgMLST allele differences (Figure 2). The Shiga toxin genes from retention samples of Kimchi Brand A were further characterised as subtypes *stx1a* and *stx2b*. The wgMLST subtype was unique in the Canadian database and no other genetically related isolates were identified. A query of the PulseNet USA databases identified that the Canadian isolates differed from a 2020 US outbreak cluster by 1-4 core genome multi-locus sequence typing (cgMLST) alleles. Although the source of the US outbreak was not identified, many of the outbreak cases reported eating or possibly eating various types of leafy greens. Subsequent sampling conducted external to the 2020 US outbreak investigation identified the outbreak strain in a sample of romaine lettuce from California. No other WGS sequencing matches outside of Canada were identified using the NCBI Pathogen Detection Pipeline.

Epidemiological investigation

Exposure information was collected from the initial interviews for 100% (14/14) of cases, and an early signal for Grocery Chain A was identified. PHAC conducted centralised re-interviews of 12/14 cases; the remaining two cases were lost to follow-up for re-interview. The PHAC *E. coli* hypothesis-generating questionnaire was used to

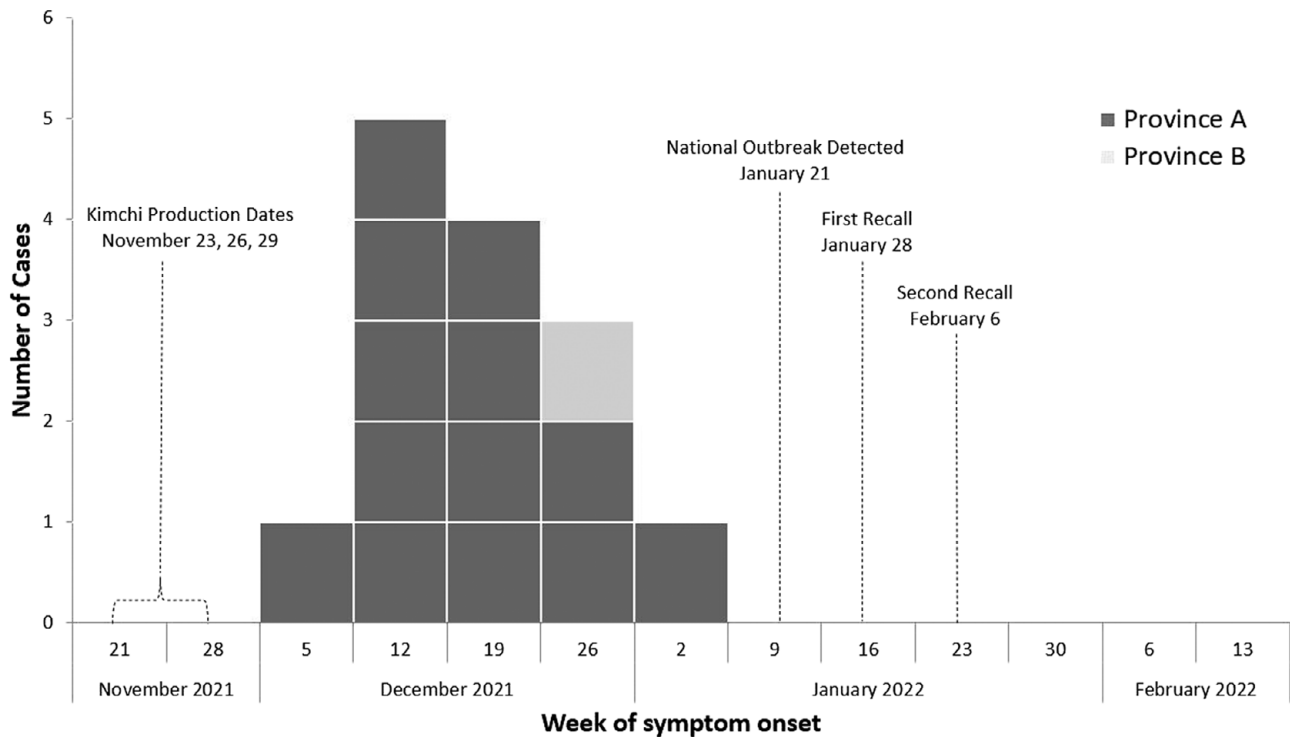


Figure 1. Number of confirmed outbreak cases of STEC O157 (n=14), by week of symptom onset — Canada, December 2021 to January 2022.

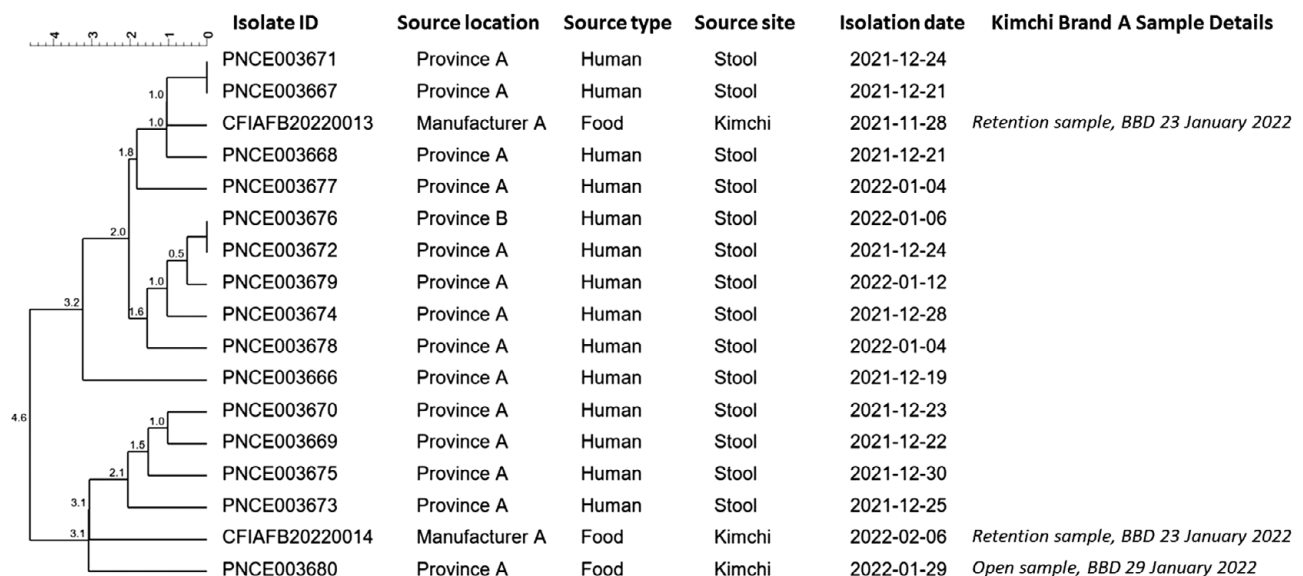


Figure 2. An unweighted pair group method with arithmetic mean (UPGMA) dendrogram of whole genome multi-locus sequence typing (wgMLST) results for clinical and non-clinical (kimchi) isolates included in the outbreak investigation, generated using BioNumerics v7.6 (bioMerieux). Abbreviations: BBD, best before date.

re-interview 3/12 cases to gather more specificity on case exposures, with additional interest in exposures related to Grocery Chain A. Initial analyses revealed many cases reporting fermented vegetable consumption in the 10 days prior to becoming ill, which led to the early identification of kimchi as a suspect source on 27 January 2022. The focused questionnaire was used to re-interview the remaining 9/12 cases to collect more specific details for kimchi.

Of the 14 cases, 93% (13/14) reported shopping at Grocery Chain A. Of the 12 cases asked during re-interview, 91% (10/11)

reported consuming fermented vegetables; one case reported ‘don’t know’ to fermented vegetable exposure, and was excluded from the analysis. Of the 10 cases that reported consuming fermented vegetables, 100% (10/10) reported exposure to kimchi, specifically Kimchi Brand A from Grocery Chain A produced by Manufacturer A. Of the 10 cases that reported exposure to Kimchi Brand A, 50% (5/10) specified consuming the product with a best-before date of 29 January 2022 during their exposure period. The remaining 5 of these 10 cases did not know which best-before date of Kimchi Brand

A they consumed during their exposure period, but 3/5 had an open container with the 29 January 2022 best-before date in their home at the time of re-interview.

Open samples of Kimchi Brand A with a best-before date of 29 January 2022 were collected from four homes where cases resided. Of the 4 samples tested, 1 tested positive for STEC O157 and was a WGS match to the outbreak cluster (Figure 2). The remaining 3 open samples did not have STEC O157 detected.

A number of exposures were reported by cases in higher proportions than expected compared to the Foodbook reference values ($p < 0.05$). These included exposure to bell peppers, any fish, spinach, fresh garlic, whole-cut beef products, bacon, or raw/undercooked eggs; contact with a domestic animal; or handling of animal feed. After analysing the details of each exposure, no significant commonalities were identified. There is no comparison value available in Foodbook for kimchi or fermented vegetables, and thus references could not be made to the expected proportion of the healthy population that report exposure to kimchi.

Food safety investigation

Kimchi Brand A from Grocery Chain A was produced by Manufacturer A in Western Canada without any heat treatment steps, which is a common practice for kimchi processing. The product contained Napa cabbage, radish, red pepper powder, garlic, sweet rice powder, green onions, onion, salt, anchovy extract, salted shrimp, ginger, and sugar water.

Thirty-three retail and retention samples of Kimchi Brand A, representing 61 units from 31 production dates, were collected for testing. Best-before dates of these samples ranged from 22 January 2022 to 25 March 2022. Of the tested samples, two isolates from a single retention sample were positive for STEC O157. The remaining samples were not detected for STEC O157. The positive sample was a retention sample from Manufacturer A with a best-before date of 23 January 2022. The sample had a pH of 4.1, and the two STEC O157 isolates recovered from the sample matched the clinical isolates associated with the outbreak by wgMLST (Figure 2). The pH testing of various kimchi samples resulted in a range of pH from 4.1 to 5.3, with older samples having lower pH.

Kimchi Brand A with a best-before date of 29 January 2022 was only sold at Grocery Chain A in four provinces in Canada, including Province A and Province B, with the majority of the product distributed in Province A. The production dates for this product were 26 and 29 November 2021. Kimchi Brand A with a best-before date of 23 January 2022 was only sold at Grocery Chain A in one province in Western Canada; this province did not report any associated illnesses. The production date for this product was 23 November 2021. Production dates for both products occurred prior to the earliest case onset of 11 December 2022.

Napa cabbage was hypothesised to be the likeliest source of contamination in the kimchi product. This hypothesis was supported by four pieces of evidence. First, the Napa cabbage was the only raw vegetable ingredient that received no sanitising wash, and as per common practice for kimchi processing, no lethality steps. Second, the same shipment of imported Napa cabbage grown at Farm A in Washington state was used in the two lots of Kimchi Brand A found to contain STEC O157. This convergence was not identified for any other fresh ingredient in Kimchi Brand A. Third, this same shipment of Napa cabbage from Farm A was a new source temporarily used by Manufacturer A during the production of the two implicated lots; the manufacturer did not receive any additional shipments from this source after this period. Lastly, Napa cabbage

comprised approximately 70% of the kimchi formulation by weight. This root cause hypothesis was unable to be confirmed, due to lack of appropriate samples. No connection was identified between the California romaine lettuce sample that matched the outbreak case isolates by wgMLST, and the Napa cabbage from Washington that is the suspected, although unconfirmed, source of the current outbreak.

Control measures

A Public Health Alert was posted on the Canadian Network for Public Health Intelligence to provide information on the outbreak investigation, including case definitions and the initial recall notice, to public health professionals across Canada. A food recall warning was issued on 28 January 2022 for the kimchi product with the best-before date of 29 January 2022. A second food recall warning was issued on 6 February 2022 for the product with the best-before date of 23 January 2022. A Public Health Notice was posted on 29 January 2022 to inform people in Canada of the outbreak and to not eat, sell, or serve the recalled kimchi. This Public Health Notice was updated on 8 February 2022 to reflect the second food recall warning.

The outbreak was declared over on 29 March 2022, based on the last recall date of 6 February 2022, a 10-day maximum incubation period for STEC O157, and the 90th percentile reporting delay of 41 days.

Discussion

A total of 14 cases of STEC O157 were identified in this outbreak across two provinces in Western Canada. The source of the outbreak was determined to be Kimchi Brand A, with Napa cabbage hypothesised as the source of contamination. This was the first outbreak of its kind to be reported outside of East Asia, and highlights the potential food safety risks of fermented vegetable products like kimchi. The alignment of the epidemiological, food safety, and laboratory findings in this outbreak investigation helped in the rapid identification of the source, and its removal from the Canadian market.

Although the hypothesis could not be confirmed, the investigation for this outbreak resulted in the identification of the Napa cabbage as the most likely source of contamination. Microbial testing in South Korea has indicated that salted Napa cabbage, such as that used in kimchi production, can be a major source of coliform bacteria and *E. coli* [14]. Outbreaks associated with kimchi in East Asia have prompted several research studies on the microbiological quality of Napa cabbage [29, 30], and one research study was able to isolate *E. coli* from irrigation water used in Napa cabbage cultivation, albeit not STEC O157 [31]. Notably, no connection could be made between the Napa cabbage from Washington – the suspected source of contamination in this outbreak – and the romaine lettuce from California that was sampled during a previous US investigation. As such, this investigation supports the possibility that the same *E. coli* strain can be found in different leafy greens, in geographically distinct areas. Interestingly, the head of Napa cabbage is similar in shape to that of a head of romaine lettuce; the shape of romaine lettuce has been theorised to play a key role in STEC O157 contamination [5, 7]. As romaine lettuce heads are relatively open, both outer and inner leaves have the potential for STEC O157 exposure from various sources, such as contaminated irrigation water [5]. The unique shape of romaine is thought to be a factor as to why a greater number of outbreaks of STEC O157 are

linked to romaine lettuce compared to other, more compact, leafy greens. The similar shape of Napa cabbage could also play a role in its vulnerability to STEC O157 contamination.

Given that kimchi is acidic in nature, typically with a pH less than 4.5 [14], it is often assumed to be an unlikely source for foodborne outbreaks. However, the present outbreak, and those noted above from East Asia, have called this assumption into question. Interestingly, pH testing in this outbreak revealed that pH decreased over time throughout the product's shelf life. With decreased pH thought to reduce pathogenic load, it is notable that cases in this outbreak had consumption dates closer to the beginning of the product's shelf life, when pH was likely higher, and the product was less acidic. It is also notable that a positive result for STEC O157 in this outbreak was found in a product with a pH result of 4.1, indicating that this strain of STEC O157 may have also been acid-tolerant, surviving exposure to this pH. Research is ongoing to determine if this may have been the case. This outbreak serves as a reminder that kimchi is a potential source of foodborne outbreaks, and that mitigating measures to prevent pathogen introduction and growth should be considered in the manufacturing process.

There were several investigative strengths of this outbreak to highlight. Initial interviews at the local public health level were key to the early identification of cases reporting exposure related to Grocery Chain A. In addition, the quick completion of re-interviews at the federal level allowed for the rapid identification of a source and its removal from the market. In this outbreak, re-interviewing was expedited as a result of a standard process in Province A, whereby consent for re-interview by provincial or national public health authorities is sought at the time of initial interview. This negated the need to seek case consent for re-interview once an outbreak has been identified, as is the usual process in many jurisdictions in Canada. Similarly, the inclusion of a question about fermented vegetables on the PHAC *E. coli* hypothesis-generating questionnaire was also crucial for the quick identification of this exposure among the outbreak cases. This variable was added to the PHAC *E. coli* hypothesis-generating questionnaire in the fall of 2018 during a periodic review process, based on a comparison to the variables included in questionnaires in other jurisdictions. Given the turnaround time for WGS results, it is often the case that leftovers are not available from case homes for sampling at the time of re-interview by federal public health officials, or that product may no longer be available at retail to test. However, in this outbreak, the investigative team was able to identify the outbreak strain both in an open sample of kimchi from a case home (best-before date 29 January 2022) and in a retention sample from the manufacturer (best-before date 23 January 2022). Good record-keeping practices at the manufacturer were also helpful to identify the ingredients used in specific lots, and their sources, which was helpful for hypothesising about the root cause. Ultimately, the investigation resulted in a strong alignment of epidemiological, food safety, and laboratory evidence to implicate Kimchi Brand A as the source of the outbreak.

There are several limitations to consider in the context of this outbreak investigation. First, while there is support for the hypothesis that Napa cabbage was the source of contamination of the kimchi, this was not able to be confirmed and thus potential preventive measures at the farm level could not be implemented. Second, the product's increasing acidity over time may have impacted the survival and detection of STEC O157 throughout the shelf life of the product. Only one of the four open samples from case homes had STEC O157 detected. However, these products were tested towards the end of their shelf life, when they were

likely to be more acidic and less conducive to the survival of bacteria. For this reason, it is unclear whether all 'not detected' results truly mean that each product lot was free from STEC O157 at the beginning of its shelf life. Third, only one retention sample was available at Manufacturer A for many production dates, and therefore these single samples were likely not representative of the whole lot. Furthermore, kimchi is comprised of large pieces of vegetables which could contribute to heterogenous subsamples. Fourth, the distribution of the kimchi products went beyond the two provinces with identified cases. Although fewer cases of product were distributed in the other provinces, underreporting, especially as exacerbated by the COVID-19 pandemic, could also explain this result. Lastly, not all re-interviewed cases confirmed exposure to kimchi, and therefore the source of illness could not be confirmed for all outbreak cases. One case reported 'don't know' to kimchi exposure, and one case reported 'no' to kimchi exposure. Although kimchi exposure cannot be explained for these two cases, it is rare within a foodborne outbreak investigation to account for every case's exposure to the outbreak source. Of note, the case reporting 'no' to kimchi exposure was interviewed via proxy, and the case reporting 'don't know' was admittedly a frequent kimchi eater. Lastly, there was an additional two cases that were lost to follow-up for re-interview, and their exposure to kimchi could not be ascertained.

This paper outlines an outbreak of STEC O157 in Canada associated with kimchi, the first known outbreak to occur outside of East Asia. The investigation highlights the potential food safety risks of kimchi, a fermented food often thought to be an inhospitable environment to pathogens. Future research will aim to further understand the food matrix of kimchi and the impact of the microbiota over time. As a known outbreak vehicle, outbreak investigators are encouraged to consider fermented vegetables, such as kimchi, as potential sources when investigating future outbreaks of STEC O157.

Data availability statement. The data from this paper are not publicly available due to privacy concerns and legislative requirements.

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Author contribution. Conceptualization: A.K., A.M., C.R.S., H.B., K.C., L.C., L.H., M.G., V.M., Y.O.L.; Data curation: A.K., A.M., H.B., K.C., L.C., L.H., M.G., V.M., Y.O.L.; Investigation: A.K., A.M., C.R.S., H.B., K.C., L.C., L.H., M.G., V.M., Y.O.L.; Methodology: A.K., A.M., C.R.S., H.B., K.C., L.C., L.H., M.G., V.M.; Validation: A.K., A.M., H.B., K.C., L.C., L.H., M.G., V.M., Y.O.L.; Writing – review & editing: A.K., A.M., C.R.S., H.B., K.C., L.C., L.H., M.G., V.M., Y.O.L.; Formal analysis: A.K., A.M., H.B., L.C., M.G., Y.O.L.; Resources: A.K., L.C., M.G.; Visualization: A.K., A.M., H.B.; Writing – original draft: A.K., C.R.S., H.B.; Project administration: C.R.S.; Supervision: C.R.S.

Competing interest. The authors declare none.

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