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Everything but the kitchen sink: The use of multiple hypothesis generation methods to investigate an outbreak of *Salmonella* Enteritidis associated with frozen profiteroles and eclairs

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Abstract

In December 2018, an outbreak of *Salmonella* Enteritidis infections was identified in Canada by whole-genome sequencing (WGS). An investigation was initiated to identify the source of the illnesses, which proved challenging and complex. Microbiological hypothesis generation methods included comparisons of *Salmonella* isolate sequence data to historical domestic outbreaks and international repositories. Epidemiological hypothesis generation methods included routine case interviews, open-ended centralized re-interviewing, thematic analysis of open-ended interview data, collection of purchase records, a grocery store site visit, analytic comparison to healthy control groups, and case–case analyses. Food safety hypothesis testing methods included food sample collection and analysis, and traceback investigations. Overall, 83 cases were identified across seven provinces, with onset dates from 6 November 2018 to 7 May 2019. Case ages ranged from 1 to 88 years; 60% (50/83) were female; 39% (22/56) were hospitalized; and three deaths were reported. Brand X profiteroles and eclairs imported from Thailand were identified as the source of the outbreak, and eggs from an unregistered facility were hypothesized as the likely cause of contamination. This study aims to describe the outbreak investigation and highlight the multiple hypothesis generation methods that were employed to identify the source.

Key results

- In December 2018, an outbreak of *Salmonella* Enteritidis infections was identified in Canada. Eighty-three cases across seven provinces were reported over the course of the investigation.
- Case-onset dates ranged from 6 November 2018 to 7 May 2019. Ages ranged from 1 to 88 years; 60% (50/83) of the cases were female; 39% (22/56) were hospitalized; and there were three deaths reported.
- Brand X profiteroles and eclairs imported from Thailand in October 2018 were identified as the source of the outbreak based on epidemiological, microbiological, and food safety evidence. Eggs supplied from an unregistered facility were hypothesized to be the likely cause of contamination.
- The outbreak investigation was challenging and complex, requiring multiple hypothesis generation methods to identify the source, including various interviewing approaches, analytic approaches, and comparison of genomic sequence data to domestic and international repositories.

Introduction

During foodborne illness outbreak investigations, microbiological, epidemiological, and food safety evidence is collated to identify the source of the illnesses. For complex investigations, this can necessitate the use of multiple, iterative hypothesis generation methods [1]. These methods

may include analysing case questionnaires and open-ended interview data [2, 3] and using population-based food exposure surveys [4, 5] or online surveys [6] to generate reference values for exposures of interest. Additional hypothesis generation methods might include case focus groups [3], analytic studies [7–9], in-person interviews, or shopping trips with cases. Methods such as analysing consumer food purchase records [10–13] and comparing case pathogen isolate sequence data with domestic and international repositories can also help generate hypotheses and identify exposures of interest. As described by Morton et al. (2020), the hypothesis generation process is pivotal in foodborne disease outbreak investigations but often poorly described, representing a missed opportunity for sharing lessons learned [3].

In December 2018, an outbreak of *Salmonella* Enteritidis was identified in Canada via WGS, with cases geographically distributed across multiple provinces. By February 2019, the cluster was growing rapidly, and a collaborative investigation was initiated to identify the source of the illnesses, implement control measures, and prevent future illnesses and deaths [14]. This study describes the complex and challenging outbreak investigation, highlighting the many complementary hypothesis generation methods that were used to identify the outbreak source.

Methods

Case definition

A confirmed case was defined as a resident of or visitor to Canada with laboratory confirmation of *Salmonella* Enteritidis, an isolate related within 0–10 whole-genome multi-locus sequence typing (wgMLST) allele differences, and symptom onset or specimen collection date on or after 1 November 2018. The collaborative investigation was deactivated on 17 June 2019.

Microbiological hypothesis generation

In Canada, all *Salmonella* isolates are forwarded to provincial public health laboratories or the National Microbiology Laboratory for WGS-based subtyping using the standardized PulseNet Canada (PNC) protocol [15]. WGS data are analysed locally and uploaded to a centralized BioNumerics v7.6 (BioMerieux) database where it is analysed by the PNC national database team using wgMLST. Isolates within 0–10 wgMLST allele differences were considered genetically related and included in the case definition for this outbreak investigation. WGS data were deposited retrospectively onto the National Center for Biotechnology Information (NCBI) in BioProject PRJNA543337.

Comparison of sequence data to domestic WGS data

The PNC WGS database was reviewed for Canadian clinical and non-clinical historical matches to the outbreak within 0–10 wgMLST allele differences.

Comparison of sequence data to international repositories

Under a bilateral information sharing agreement between Canada and the United States (USA), WGS data were exchanged with PulseNet USA to facilitate the query of the PulseNet USA databases for potential matches. Related US isolates were used to query the NCBI Pathogen Detection Pipeline. Data from potentially related international isolates identified via NCBI were imported into the PNC WGS database to allow for standardized analysis. Following confirmation from the International Food Safety Authorities Network (INFOSAN) that the implicated product was distributed in Australia, PNC shared WGS data with OzFoodNet to facilitate the identification of related clinical cases in Australia.

Epidemiological hypothesis generation

Case interviewing

Local public health authorities conducted initial case investigations by telephone using routine provincial *Salmonella* questionnaires. These questionnaires varied in content and length, but typically included questions on various exposures prior to onset, including travel within and outside Canada, foodborne exposures (within and outside the home), zoonotic exposures, and exposures to high-risk occupations or environments. Questionnaire data were sent to the Public Health Agency of Canada (PHAC) for centralized analysis (i.e. analysis across provinces). Selected cases were re-interviewed by telephone by one of two interviewers, centralized at PHAC, using an iterative, open-ended interviewing approach. Cases were selected for re-interview based on a variety of factors, which included whether they provided consent to be re-contacted, whether they had good recall of exposures at initial interview, and the length of time since their symptom onset (with more recent cases prioritized for re-interview). These interviews were conversational in style and allowed cases to elaborate on typical food habits and preferences.

Case–case analysis

Two case–case analyses were conducted with a subset of cases from British Columbia (BC). One case–case analysis compared the exposures reported by outbreak cases to other *Salmonella* Enteritidis cases in BC from November 2018 to January 2019 that did not match the outbreak strain. The other case–case analysis compared the exposures reported by outbreak cases to all other *Salmonella* cases in BC, excluding *Salmonella* Enteritidis and *Salmonella* Typhi cases. *Salmonella* Enteritidis cases were excluded due to an ongoing outbreak of *S.* Enteritidis in BC associated with poultry and egg products, and *Salmonella* Typhi cases were excluded as *S.* Typhi is not domestically acquired in Canada; excluding these serotypes served to increase the comparability of the groups.

Grocery store site visit

Investigators visited a location of Grocery Store Chain A, a chain reported by several cases. Comparison of case clinical isolate sequence data to international repositories had revealed a potential connection to Thailand. Investigators examined product labels at Grocery Store Chain A to identify foods originating from Thailand as potential exposures of interest.

Thematic analysis

The two centralized interviewers read aloud their open-ended interviewing notes. Investigators recorded food exposures, preferences, habits, and shopping locations on a whiteboard and grouped this information into themes.

Comparison to healthy control groups

Using binomial probabilities, case exposure data were compared to results of the 2015 Foodbook population-based telephone survey, which reports the expected proportion of people in Canada reporting various food exposures in the previous 7 days [4]. Foodbook values were restricted to the provinces where cases were reported and to months in which illness onsets were reported. A Bonferroni correction was used to restrict the

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significant p-value of each individual test and reduce the likelihood of a Type 1 error given the number of simultaneous tests (n = 185).

An online survey was conducted to gather contemporary exposure information from healthy, population-based controls for foods without comparison values in Foodbook. At the time of the survey, leading exposures of interest included frozen fish and commercially prepared mixed fruit cups. The survey was launched on Canada.ca webpages and promoted through social media channels and was available from 12 April 2019 to 30 April 2019. Residents of Canada that did not report vomiting, diarrhoea, or international travel in the previous 7 days were asked about consumption of unbreaded/unbattered frozen fish, breaded/battered frozen fish, and mixed fruit cups. Survey responses were extracted once per week and compared to case exposure data.

Purchase record analysis

During centralized case re-interview, cases with grocery store loyalty cards were asked for consent for investigators to access their purchase records for the 3 months prior to illness onset. For stores without loyalty card programmes, cases were asked to provide any available receipts for their food purchases. Several confirmed cases resided in long-term care facilities; menus and invoices were requested from these facilities. Analysis of purchase records and invoices were conducted in Microsoft Excel, looking across records for commonalities in categories of foods and specific food items.

Food safety hypothesis testing

Traceback analysis

When suspect outbreak sources were identified, product details (e.g. purchase location, purchase date, product name) were shared with the Canadian Food Inspection Agency (CFIA) to support food safety investigation and traceback activities. CFIA completed traceback on several exposures of interest during the investigation.

Food sample collection and analysis from case homes and establishments

Opened and unopened food samples taken from retail, case homes, and a long-term care facility were tested by CFIA using the MFHPB-20, MFLP-29, or MFLP-40 methods published in Health Canada's Compendium of Analytical Methods for the Microbiological Analysis of Foods [16]. The British Columbia Centres for Disease Control (BCCDC) Public Health Laboratory also tested retail samples using the MFLP-29 method. Health Canada tested recalled products obtained from retail as well as an open sample collected from a long-term care facility using the MFHPB-20 method. WGS was performed on any *Salmonella* isolates recovered from food samples and compared to clinical isolates.

Results

Eighty-three (83) cases in seven provinces were identified as part of this outbreak with onset dates from 6 November 2018 to 7 May 2019 (Figure 1). Ages ranged from 1 to 88 years (median age = 51 years); 60% (50/83) of the cases were female; 39% (22/56) were hospitalized; and three deaths were reported. Figure 2 presents a timeline of all hypothesis generation and testing methods used throughout the investigation.

Microbiological results

Comparison of sequence data to domestic WGS data

All clinical isolates within the outbreak grouped together by 0–10 wgMLST allele differences and were considered genetically related based on WGS. The WGS profile of the outbreak isolates was considered unique within the Canadian national database.

Comparison of sequence data to international repositories

Clinical (n = 29) and food (n = 4) isolates posted on NCBI by the UK Health Security Agency (called Public Health England at the time of the investigation) were found to match the outbreak

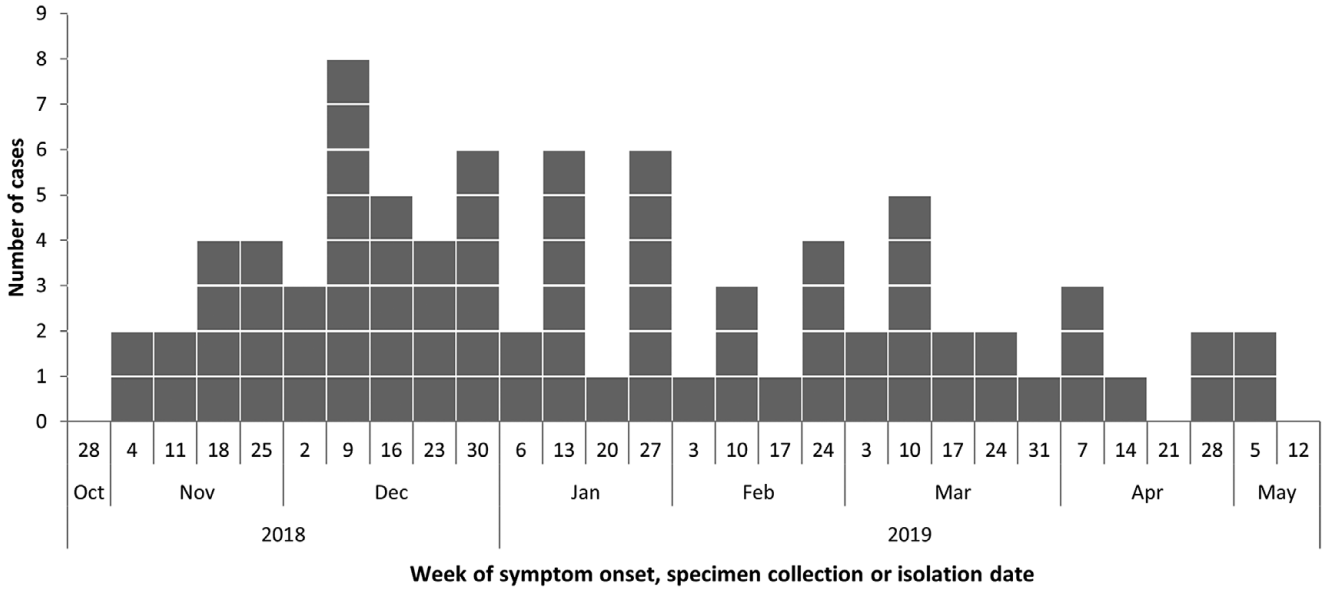

Figure 1. Number of confirmed outbreak cases of *Salmonella* Enteritidis by symptom onset, specimen collection, or isolation date (n = 82). One case did not have a reported onset, specimen collection, or isolation date and is not included in this figure.

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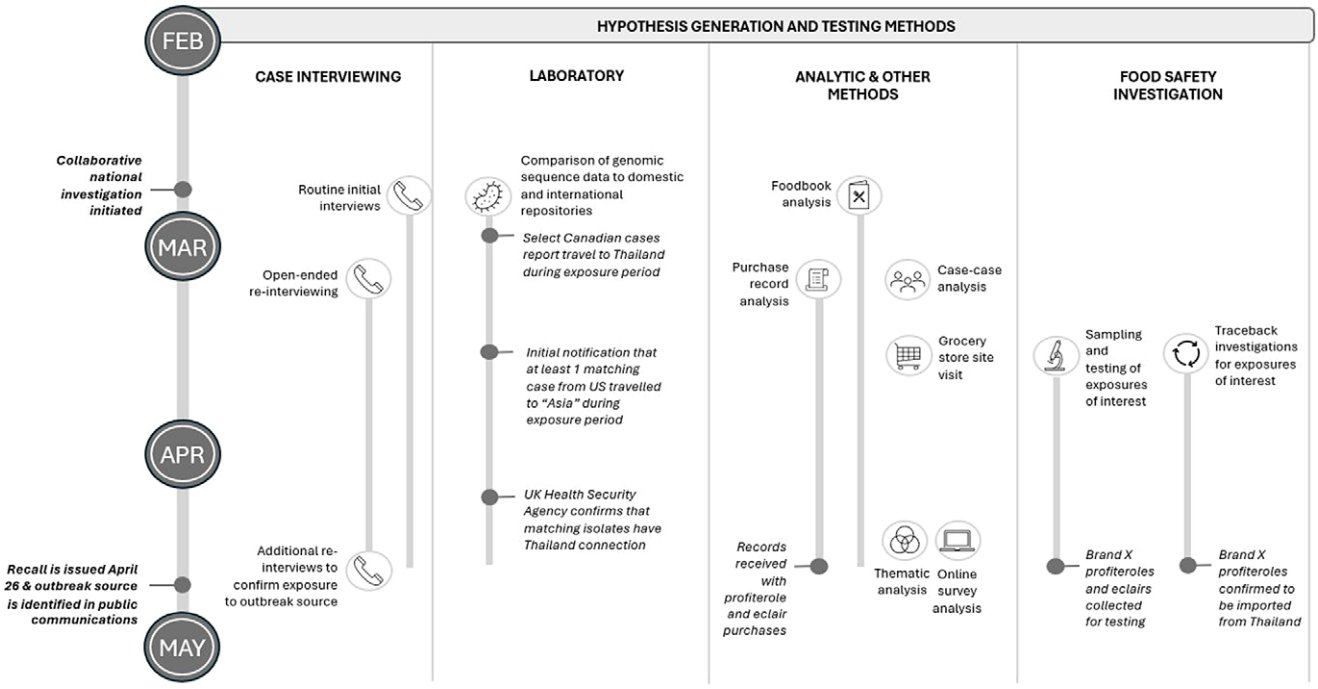

Figure 2. Timeline of hypothesis generation and testing methods employed throughout the outbreak investigation in 2019.

cluster within 0–12 wgMLST alleles. In early April, it was reported that of the UK cases, the majority had travel to Thailand indicated on their laboratory forms. The four UK food isolates were frozen raw chicken and frozen salted chicken from Thailand, which were tested in late 2017 and mid-2018. Seven clinical isolates from the USA were identified as grouping within 0 to 14 wgMLST allele differences to the Canadian outbreak cluster, with isolation dates ranging from February 2018 to March 2019. By early May, it was confirmed that five of the US cases reported travel during their exposure period, with three cases travelling to Thailand, one to Asia (country not specified), and one to Mexico. One additional case did not report travel, but a household contact travelled to Thailand. In late May, Australian state public health laboratories ran independent analyses of *Salmonella* Enteritidis sequences from their jurisdictions to identify cases related to the representative outbreak sequence provided by PNC. All related Australian cases reported travel to Thailand prior to illness onset.

Epidemiological results

Case interviewing

Exposure information was available for 89% of cases (74/83). Of the 74 cases with exposure information, five reported travel to Thailand or Southeast Asia during their exposure period and were excluded from food frequency analyses. Of the nine cases without exposure information, five were lost to follow-up and four were not interviewed.

Of the 74 cases with exposure information, 41 cases were interviewed using a routine provincial *Salmonella* questionnaire only. Thirty-three cases were additionally interviewed throughout March and April by one of two PHAC interviewers using an open-ended approach. PHAC conducted 64 open-ended interviews with these 33 cases, contacting each case one to five times throughout the investigation as additional exposures of interest were identified. Exposure to cream-filled pastries including profiteroles and eclairs was not included on any routine *Salmonella* questionnaires in Canada at the time of this outbreak. Consequently, only two cases reported exposure to profiteroles or eclairs (unprompted) when interviewed using routine provincial *Salmonella* questionnaires, and only two more cases reported this exposure (unprompted) in their first open-ended re-interview.

After additional re-interviews conducted in late-April 2019, 81% (21/26) of cases who were directly asked about exposure to profiteroles and eclairs reported consuming these products in the 7 days prior to illness onset. An additional three cases who resided in the same personal care home were not asked about profiterole or eclair exposure; however, it was later confirmed in late-April that the personal care home had received a shipment of Brand X profiteroles prior to the residents' illness onset dates, and 'cream puffs' were listed on the residents' menu.

Case–case analysis

The case–case analysis was conducted in early March. Consumption of chicken nuggets or strips, other chicken or poultry, raw eggs, beef, pork, cucumbers, nuts, and contact with animals were reported with significantly higher frequency among outbreak cases in comparison with other *Salmonella* Enteritidis cases in BC. In comparison with other *Salmonella* cases in BC (excluding *Salmonella* Enteritidis and *Salmonella* Typhi), exposures reported with significantly higher frequency among outbreak cases were contact with animals or raw pet food treats, consumption of chicken pieces or parts, chicken nuggets or strips, raw eggs, beef, pork, cucumbers, leafy greens, and nuts. Because profiteroles and eclairs are not included in the routine *Salmonella* questionnaire of BC, these exposures were not included in this analysis.

Grocery store site visit

The visit to Grocery Store Chain A, a chain reported by several outbreak cases, was conducted in mid-March and identified several

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products of interest that originated from Thailand. These products included frozen fish, canned fish, imitation crab meat, frozen breaded shrimp, and shelf-stable commercially prepared mixed fruit cups.

Thematic analysis

The thematic analysis was conducted in mid-April and revealed key themes among the outbreak cases' reported food exposures. Cases tended to be 'plain' eaters preferring milder flavours and starchy products and also tended to be bargain shoppers, purchasing 'whatever was on sale' rather than any specific brands or flavours. Cases also tended to reside outside of large urban areas and shop at smaller or discount grocery stores.

Comparison to healthy control groups

Comparisons to Foodbook reference values were conducted throughout March and April and revealed significant results ($p < 0.001$) for turkey, bacon, pork pieces or parts, ground beef, whole cut beef products, and sausage. Profiteroles and eclairs do not have corresponding Foodbook values and were therefore not included in this analysis.

In mid-April, frozen fish and shelf-stable commercially prepared mixed fruit cups were included in the online survey because they were commonly reported among cases during open-ended interviews and had been identified as potentially originating from Thailand during the visit to Grocery Store Chain A. The online survey received 283 complete responses between 12 April 2019 and 30 April 2019. The mean age of respondents was 38 years with a range of 16 to 72 years, and 75% of respondents were female. A larger proportion of outbreak cases reported frozen unbreaded/breaded fish (52.2%/47.8%) and commercially prepared mixed fruit cup exposure (55.5%) when compared to survey respondents (14.2%/11.0% and 7.8%, respectively). Each of these comparisons was statistically significant (Table 1).

Following a traceback investigation, frozen fish was determined to be an unlikely source due to lack of convergence in brands, processors, and suppliers across the exposures reported by cases. Discussion with food safety partners revealed that shelf-stable commercially prepared mixed fruit cups were not a plausible outbreak source as they would undergo a thermal treatment sufficient to inactivate *Salmonella* Enteritidis. Therefore, frozen fish and mixed fruit cups were excluded as possible hypotheses.

Table 1. Online survey results and comparison to outbreak case exposures[a]

Exposure	Outbreak cases exposed (%)	Online survey respondents exposed (%)	Odds ratio (95% CI)
Frozen unbreaded/unbattered fish	12/23 (52.2%)	40/282[b] (14.2%)	6.60 (2.46–17.61)
Frozen breaded/battered fish	11/23 (47.8%)	31/283 (11.0%)	7.45 (2.70–20.04)
Commercially prepared mixed fruit cups	10/18 (55.6%)	22/283 (7.8%)	14.83 (4.65–47.30)

[a]Numerators represent cases or online survey respondents responding 'Yes' or 'Probably' to a given exposure during the time period of interest, and denominators represent cases or online survey respondents specifically asked about a given exposure.
[b]One online survey respondent replied 'Don't know' to exposure to frozen unbreaded/unbattered fish and was excluded from analysis of that question.

Purchase record analysis

Twenty sets of purchase records were collected from 15 cases throughout March and April, representing purchases at 13 different grocery stores. Invoices were collected from a care home where one case resided and a personal care home where three cases resided. Ultimately, purchase records obtained in late-April for three cases from a single grocery store location indicated that each case had purchased Brand X classical profiteroles, egg nog profiteroles, or mini chocolate eclairs in the 2 months prior to their illness onset. This unusual commonality provided the initial lead for additional investigation into these products via additional interviewing (described above) and traceback investigation (described below).

Food safety results

Traceback analysis

Several exposures of interest were identified through the epidemiological investigation, resulting in traceback being conducted for almonds, frozen fish, sausage, and deli meat. Traceback analysis helped to rule out these items as suspect sources due to lack of convergence to a common supplier.

The traceback investigation for profiteroles and eclairs provided strong evidence that these were the source of the outbreak. A single manufacturer was identified for Brand X products. These products were manufactured in Thailand, connecting the Canadian outbreak to the microbiological data shared by the USA, England, and, later, Australia, that were linked to Thailand. The products were imported into Canada for the first time in October 2018, aligning with the symptom-onset dates of initial cases in November 2018. The products were stored frozen, had a 15-month shelf life, and were distributed to grocery chains in the provinces where illnesses were reported. Of note, these grocery chains were generally smaller chains or discount grocery stores, consistent with what was reported during open-ended case interviewing. Additionally, the personal care home where three cases resided had received a shipment of Brand X profiteroles prior to the residents' illness onset dates, and 'cream puffs' were listed on the residents' menu.

Two shipments of Brand X products were recalled in Canada on 26 April 2019. The CFIA notified Thailand of the recall via INFOSAN. INFOSAN contacts in Thailand confirmed that products produced by the Brand X manufacturer at the same time as those distributed to Canada were also distributed to Australia. Brand X products were recalled in Australia on 1 May 2019 [17].

The Thai Food and Drug Administration (Thai FDA) initiated a food safety investigation at the Brand X manufacturer. This investigation revealed that the eggs used in the production of the implicated product were sourced from a non-registered farm due to shortages in the usual supply from registered farms monitored by the Thai FDA. The eggs from the non-registered farm are hypothesized to be the source of the outbreak. Following a thorough audit, the Thai FDA identified several corrective actions for the manufacturer, including only purchasing eggs directly from a company regulated by the Thai FDA, designating the room where pastries were filled after baking as a high-risk area, changing the layout of the factory to protect the pastry filling room, and protecting the transport of baked profiteroles to the filling room.

Food sample collection and analysis from case homes and establishments

Throughout the course of the outbreak, samples of almonds, frozen fish, chicken, meatballs, fruit cups, deli meat, dry spices, sausage,

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and pepperoni were collected from case homes and establishments for testing; none had *Salmonella* detected. Three profiterole samples tested by Health Canada were positive for *Salmonella* Enteritidis, and the recovered isolates were genetically related to the outbreak cases based on WGS. Further analyses have since characterized the matrix of the recalled frozen profiteroles as permissive to the growth of *Salmonella* [18].

Discussion

Eighty-three cases were identified in this outbreak, with the active investigation taking place between February and April 2019. The outbreak was associated with Brand X profiteroles and eclairs imported into Canada from Thailand, with contaminated eggs hypothesized as the root cause. Although this was not the first outbreak or product recall linked to profiterole and eclair-style products [19–21], this outbreak investigation was challenging and complex and required the use of several hypothesis generation methods to identify the source.

The microbiological hypothesis generation methods employed in this outbreak investigation provided significant contextual clues for investigators. Initial comparison to other clusters of *Salmonella* Enteritidis in Canada made it clear that this cluster was unique and genetically distinct from previously identified clusters associated with poultry. International WGS matches demonstrated a clear connection to Thailand, with clinical isolates from individuals who had travelled to Thailand and non-clinical isolates from products originating from Thailand. Five outbreak cases also reported travel to Thailand or Southeast Asia, strengthening this connection for investigators. This investigation emphasizes the value of countries routinely posting isolates and their associated metadata on public repositories to facilitate rapid comparisons during outbreak investigations, as well as the value of established information sharing agreements, such as the bilateral agreement between PulseNet-USA and PNC.

Open-ended centralized case re-interviewing was the primary epidemiological hypothesis generation method used in this investigation. This strategy was employed after routine questionnaires did not reveal common exposures across outbreak cases. As has been observed previously [22], centralized case re-interviewing allowed for quick identification of commonalities by interviewers as they shared interview results in real time and iteratively modified questions for future interviews. The open-ended interviewing approach was also able to aid case recall challenges in the context of a lengthy delay between exposure and case re-interview, as the unstructured approach permitted investigators to fully explore case food histories, including routine diets, 'one-time' exposures, and foods not included in routine *Salmonella* questionnaires. While the result was rich contextual information, this approach was resource-intensive and produced data which required adjustments in data management strategies and new analytic approaches, including the thematic analysis employed in this investigation. Although the outbreak source was not identified after the first round of open-ended re-interviewing, the data gathered during those interviews and the patterns identified via the thematic analysis provided leads for further hypothesis generation methods (i.e. the grocery store site visit) and supporting evidence for the outbreak source once identified (i.e. a mild-flavoured, starchy food sold at smaller, discount grocery store chains).

Other epidemiological hypothesis generation methods, including case–case analyses, comparisons to healthy control groups, and the site visit to Grocery Chain A, did not identify the source of the outbreak. Ultimately, purchase record data from a small grocery chain identified a common product purchased by multiple cases, which led investigators to identify the outbreak source. This grocery chain was amenable to sharing the requested records; however, unlike many major retailers in Canada, they did not have an existing data-sharing agreement with PHAC. As a result, this necessitated a new data-sharing agreement to be developed ad hoc during the investigation, and the process of compiling the records proved labour-intensive for the retailer in the absence of an established or automated system. Many additional methods of epidemiological hypothesis generation were considered but ultimately not employed in this investigation, as the outbreak source was identified before they were initiated. These methods included in-person re-interviews at case homes, in-person grocery store visits with cases, a focus group of cases who resided in the same small city, and a cohort study including cases that were guests at a dinner party.

The food safety investigation was essential to identifying the outbreak source. Throughout the course of the investigation, the epidemiological data implicated many exposures of interest. Traceback activities were invaluable in ruling out each suspect item by searching for convergence in suppliers, manufacturers, distributors, and farms and finding none. Food sample collection and analysis activities throughout the investigation also provided additional evidence to rule out suspect sources as they were identified. Ultimately, while the epidemiological evidence pointed towards profiteroles and eclairs as the cause of the outbreak, and both microbiological and epidemiological evidence indicated a connection to Thailand, it was information received from purchase records and the ensuing food safety investigation that confirmed this link and supported product action in Canada. Analysis of recalled products later provided a microbiological link between recalled product and outbreak cases [18]. Collaboration between Canadian and Thai food safety authorities was also key to arriving at a suspected root cause, which resulted in meaningful public health action at the manufacturer. This outbreak is a reminder of the importance of global partnerships such as INFOSAN [23] in the protection of public health in a world of complex global food trade.

Limitations

There are limitations to reflect upon when considering employing the methods described here in future investigations. While many hypothesis generation methods were used, some produced false leads that may have prolonged the investigation. For example, the case–case analysis, comparison to Foodbook, and online survey all resulted in significant values for foods that were not the outbreak source. These analyses faced challenges due to characteristics of outbreak cases that were likely not shared with these comparison groups, including a strong preference for 'plain' flavours and shopping at smaller discount grocery chains. Because routine *Salmonella* questionnaires and the Foodbook study did not include questions on profiteroles or eclairs, these analyses alone would never have resulted in the identification of the outbreak source. Continual updating of routine questionnaires and population-based food consumption surveys such as Foodbook is important to ensure newly identified outbreak vehicles and changing food habits are reflected in these tools and data sources. Similarly, although searching for international sequencing matches to the Canadian outbreak did provide helpful clues in the link to Thailand, it also resulted in matches of frozen chicken

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isolates, another misleading clue in the investigation. Other methods used, such as open-ended interviews and thematic analysis, provided useful context, but did not identify a specific source of illness. This investigation highlights the importance of considering data from all hypothesis generation methods holistically and to be cognizant of the potential for false leads that might distract the investigation.

Conclusion

The outbreak investigation was challenging and complex, requiring multiple hypothesis generation methods to identify the source, which was Brand X profiteroles and eclairs imported into Canada from Thailand. The hypothesis generation methods employed included closed-ended and open-ended interviewing, thematic analysis of interview data, various comparisons of case data to healthy control groups, a case–case analysis, a grocery store site visit, purchase record analysis, and comparison of genomic sequence data to domestic and international repositories. Each outbreak investigation is unique, and different methods may prove helpful in each one; however, this investigation serves to demonstrate the importance of employing various hypothesis generation methods simultaneously and iteratively, especially in instances where identification of the source proves difficult.

Data availability statement. WGS data for this outbreak are available on the NCBI in BioProject PRJNA543337. Epidemiological data from this study are not publicly available due to privacy concerns and legislative requirements. Please contact the corresponding author (CRS) for additional information on the data.

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