**Research Paper** 

# Nuggets of Wisdom: *Salmonella* Enteritidis Outbreaks and the Case for New Rules on Uncooked Frozen Processed Chicken

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# ABSTRACT

In 2014 and 2015, three Canadian Salmonella serotype Enteritidis outbreak investigations implicated uncooked, frozen, processed chicken products produced at the same establishment, namely establishment A. In November 2014, a sustained increase in the number of reported domestically acquired Salmonella Enteritidis cases in Ontario led to the first outbreak investigation, which implicated uncooked, frozen, processed chicken products produced at establishment A. In June 2015, the identification of pulsed-field gel electrophoresis patterns that had not been previously reported in Canada led to a national Salmonella Enteritidis investigation. Of 51 cases reported nationally, 35 were from Ontario. Uncooked, frozen, processed chicken products produced at establishment A were identified as the source of the outbreak, and public health action was taken as a result of this second investigation. In September 2015, a sustained increase in the number of domestically acquired Salmonella Enteritidis PT13a cases in Ontario led to a third outbreak investigation, which identified a total of 36 PT13a cases. Uncooked, frozen, processed chicken products produced at establishment A were again identified as the source of the outbreak. Outbreaks have been linked to uncooked, frozen, processed chicken products since the late 1990s. Information collected during the three outbreak investigations, and from other jurisdictions, suggests that the breaded and prebrowned appearance of the product, as well as factors related to product packaging and marketing, result in consumer misperception that this raw product is cooked. This misperception may result in mishandling and improper cooking. The three outbreaks described in this article highlight the potential ongoing risks to consumers from these products and support interventions to prevent contamination at the source level and infection at the consumer level.

Key words: Contamination; Domestically acquired; Frozen processed chicken; Outbreak; Raw uncooked chicken; Salmonella Enteritidis

Salmonella is the second most frequently reported enteric pathogen, accounting for a large burden of disease in the Ontario population. In 2014, 3,042 Salmonella cases were reported, corresponding to an incidence rate of 22.5 per 100,000 population (21). It is estimated that, for each case of Salmonella reported to public health authorities, 13 to 37 cases go unreported (26). Consumption of poultry and poultry products is an important risk factor for Salmonella (9, 12). Studies in Ontario, British Columbia, and Canada have identified uncooked, frozen, processed chicken products as an important risk factor for infection among domestically acquired Salmonella cases (10, 18, 19). These products, which include nuggets, strips, and burgers, are often breaded and appear fully cooked. Similarly, investigations in Australia and the United States have linked outbreaks of Salmonella serovars Typhimurium, Heidelberg, and Enteritidis to uncooked, frozen, processed chicken products, as well as to other types of chicken products that appear fully cooked, such as chicken livers and frozen chicken meals (5, 15, 16, 25). More recently, in 2014 and 2015 in the United States, three *Salmonella* Enteritidis outbreaks were linked to frozen, raw, stuffed, breaded chicken entrees, all of which resulted in voluntary recalls from the retailer and in publicly issued warnings to avoid product consumption (6, 7, 20).

The objective of this report is to describe three outbreak investigations in Canada that linked human illness to uncooked, frozen, processed chicken products. It will highlight the importance of government and industry policies related to uncooked, frozen, processed chicken products to prevent contamination at the source level and infection at the consumer level. Two of the outbreaks occurred in Ontario, and one was investigated nationally. This article will focus on describing Ontario cases and Ontario laboratory testing linked to the three outbreaks. The uncooked, frozen, processed chicken products implicated in all three investigations were produced at the same federally inspected establishment, namely, establishment A, suggesting a common root cause for all three outbreaks.

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# MATERIALS AND METHODS

Salmonella surveillance and outbreak investigations. Salmonella infections in Ontario are reportable to the Ontario Ministry of Health and Long-Term Care through the integrated Public Health Information System (iPHIS), the provincial reportable disease database. iPHIS is a passive surveillance system that contains reports from health care providers and laboratories that are required to report designated diseases, including Salmonella, under provincial legislation. Public health units in Ontario follow up with reported cases of Salmonella infection to identify possible sources of disease acquisition (also referred to as exposures), as well as to conduct case management. During routine follow-up of Salmonella cases, public health units are requested to administer provincially standardized questionnaires; however, some public health units may implement their own questionnaires. During outbreak investigations, public health units are required to administer provincially standardized questionnaires. Standardized questionnaires include open-ended and "yes-no" questions pertaining to the cases' food histories in the 3 days prior to illness onset, details of which are also reported provincially through iPHIS. During outbreak investigations, standardized questionnaires are submitted provincially, and additional hypothesisgenerating and/or focused questionnaires are administered to collect further details pertaining to food histories, product information, and purchase location, as well as the cases' food handling and preparation practices. Furthermore, public health units are also requested to collect samples of suspect food items from the homes of cases. Obtaining food samples is dependent on the availability of the suspected food items, which are often requested 2 to 3 weeks after consumption.

Outbreak case definitions. During the first outbreak investigation, a case was defined as a resident or visitor to Ontario with laboratory confirmation of Salmonella Enteritidis, with symptom onset from 1 September 2014 to 19 February 2015 and with no history of travel outside of Ontario during their disease incubation period. For the second investigation, a case was defined as a resident or visitor to Canada with laboratory confirmation of Salmonella Enteritidis or Salmonella Berta and the pulsed-field gel electrophoresis (PFGE) XbaI pattern SENXAI.0257, SEN-XAI.0259, or SENXAI.0266, with symptom onset from 1 January to 6 August 2015 (the PFGE pattern designation for Salmonella Berta was SENXAI.0259, which is a Salmonella Enteritidis designation because it was 100% identical to the Salmonella Enteritidis isolates). For the third investigation, a case was defined as a resident or visitor to Ontario with laboratory confirmation of Salmonella Enteritidis phage type (PT) 13a, with symptom onset from 1 August to 27 November 2015 and with no history of travel outside of Ontario during the disease incubation period.

**Exposure analyses.** Exposures to suspect foods were identified during case interviews, and the proportions of cases exposed to suspect food items were compared using a binomial probability to expected consumption rates for the general Ontario population, as described in "FoodBook," a Canadian-based telephone survey that collected food exposure information (8). Food items with higher than expected consumption rates were further assessed to identify commonalities, such as brand, lot code, and purchase location. Analyses were performed using Microsoft Excel (version 2010, Microsoft Corporation, Redmond, WA). Because this report concerns outbreak investigations, research ethics review was not required.

Laboratory testing for clinical isolates. Clinical diagnostic laboratories in Ontario routinely forward isolates of *Salmonella* to the Public Health Ontario Laboratory (PHOL) for serotyping. Clinical isolates of *Salmonella* are serotyped according to recognized laboratory slide agglutination protocols (*11*, *14*). Phage typing was performed routinely for all *Salmonella* Enteritidis isolates, and PFGE and/or whole genome sequencing (WGS) was performed at the National Microbiology Laboratory in Winnipeg, and PFGE was performed at the PHOL according to standardized PulseNet Canada protocols (*1*). PulseNet Canada is a laboratory-based surveillance system that uses standardized methods to allow for interlaboratory comparison and communication of information pertaining to foodborne illness. PFGE pattern designation and comparison with national data was performed at the National Microbiology Laboratory (*4*).

WGS is also performed at PHOL. Isolates were cultured overnight on blood agar. DNA was extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA), according to the manufacturer recommendations, from a full loop of bacterial growth. The genomes of isolates were sequenced as paired ends (151 bp + 151 bp) with an Illumina MiSeq instrument (New Zealand Genomics Limited, Massey Genome Service, Palmerston North, New Zealand). Short-reads were mapped to the reference P125109 (GenBank accession no. NC\_011294), using Smalt (24). SAMtools was used to convert SAM formatted files of Smalt to Bam files and to sort the Bam files (17). Single nucleotide polymorphisms (SNPs) were called using Freebayes with min-coverage 15, min-base quality 30, min-mapping quality 30, min-fraction of the reads to contain the variant 75% (13). To call high quality core SNPs, repetitive regions on the reference were not included. Variants passing the above conditions were further evaluated against SAMtools mpileup/ BCFTools. Only variants for positions present in all isolates consistent between both sets were kept. Maximum likelihood trees of concatenated SNPs were generated using MEGA 6 with the Tamura-Nei model with uniform rates.

Laboratory testing for food samples. Suspect food samples were collected by public health units from clinical cases' homes. Food samples submitted to PHOL for *Salmonella* analysis were tested by PCR using AOAC Research Institute method 031001 and the selective culture method Health Canada MFHPB-20 (2, 23). Food isolates of *Salmonella* were serotyped and subtyped by the same methods as described above.

## RESULTS

**Outbreak 1.** In November 2014, a sustained increase in the number of reported domestically acquired *Salmonella* Enteritidis cases in Ontario led to an outbreak investigation. Uncooked, frozen, processed chicken products were identified as an important risk factor contributing to the observed increase. The investigation determined that 44.9% of 32 *Salmonella* Enteritidis cases who were reinterviewed reported uncooked, frozen, processed chicken exposures, compared with an expected frequency of 18.5% for storebought breaded chicken for the Ontario population, based on FoodBook data. Assuming a background consumption frequency of 18.5%, the probability that 44.9% or more of outbreak cases would consume uncooked, frozen, processed chicken was less than 5% (P < 0.05).

As part of the investigation, 11 uncooked, frozen, processed chicken samples were collected from five outbreak cases and were submitted to PHOL for testing. Of the 11 samples submitted, *Salmonella* Entertiidis was detected in

	Case laboratory results			Food testing results <sup>b</sup>					_
		PFGE			Production		PFGE		
Case	PT	(SENXAI/SENBNI) <sup>d</sup>	WGS	Brand	date	PT	(SENXAI/SENBNI)	WGS	Interpretation <sup>c</sup>
Outbreak 1. Food samples submitted by outbreak-confirmed cases									
Case 1 <sup>e</sup>	13	.0038/.0016	Pair A	А	23 July 2014	13	.0038/.0016	Pair A	Case-food match
Case 2	8	.0003/.0292	Pair B	В	NA	8	.0003/.0292	Pair B	Case-food match
Case 2	0	.0003/.0232		В	7 Aug. 2014	13	.0062/.0106	NA	Does not match
Case 3	13a	.0006/.0007	Pair C	В	11 Sep. 2014	13a	.0006/.0007	Pair C	Case-food match
Outbreak 2. Food samples submitted by outbreak-confirmed cases <sup>f</sup>									
Case 1	13a	.0259/	NA	D	22 Jan. 2015	NA	.0257/ <b>.0259</b> /	NA	Case-food match Outbreak match
				D	22 Jan. 2015	NA	.0257/ <b>.0259/</b>	NA	Case-food match Outbreak match
Outbreak 3. Food samples submitted by outbreak-confirmed cases									
Case 1	13a	.0006/.0007	Cluster 1	C	9 July 2015	13a	.0006/.0007	Cluster 1	Case-food match Cluster 1 match
Case 2	13a	.0006/.0007	Cluster 1	С	9 July 2015	19	.0006/.0007	Cluster 1	Case-food match Cluster 1 match
Case 3	13a	.0006/.0007	Cluster 1	С	NA	13	.0062/.0016	No cluster	Does not match
Case 4	13a	.0006/.0007	Cluster 1	С	9 July 2015	13a	.0006/.0007	Cluster 1	Case-food match Cluster 1 match
Outbreak 3. Food samples submitted by nonoutbreak cases (i.e., cases that did not meet the outbreak definition)									
Case 1	8	.0003/.0003	Cluster 2	С	9 July 2015	<b>8,</b> 19	<b>.0003/.0003</b> .0007/.0005 .0006/.0007	Cluster 1	Case-food match Cluster 1 match
Case 2	8	.0003/.0003	Cluster 2	С	9 July 2015	8	.0225/.0003 .0006/.0007 .0007/.0005	Cluster 1 Cluster 2	Case-food match Clusters 1 and 2 match
Case 3	8	.0003/.0003	Cluster 2	В	NA	8	.0003/.0003	Cluster 2	Case-food match Cluster 2 match
Case 4	8	.0003/.0003	Cluster 2	В	6 July 2015	8	.0003/.0003	Cluster 2	Case-food match Cluster 2 match

TABLE 1. Summary of Salmonella Enteritidis–positive uncooked, frozen, processed chicken food samples submitted by cases from three outbreak investigations<sup>a</sup>

<sup>*a*</sup> PT, phage type; PFGE, pulsed-field gel electrophoresis; WGS, whole genome sequence; NA, results or information not available (i.e., sample was not typed or sequenced or product information was not available).

<sup>b</sup> For each food sample submitted, up to six isolates were tested. PT, PFGE, and WGS results may vary for the tested isolates and only unique results are shown.

<sup>c</sup> The interpretation is based on considering all the isolates associated with the case. Case clinical isolates and the submitted food samples are considered a match if at least one laboratory result was the same (PT or PFGE) or indicates relatedness (WGS) for at least one of the isolates tested for each sample. Laboratory results (PT, PFGE, or WGS) that indicate a case-food sample match or relatedness are shown in bold. WGS cluster 1 or 2 matches were defined based on the food sample result. If at least one of the food isolates for each sample tested was related to the outbreak cluster, the sample was considered a match.

<sup>d</sup> SENXAI and SENBNI are the naming conventions used by PulseNet Canada to identify PFGE molecular designations for enteric pathogens. SEN denotes the *Salmonella* serotype, in this case *Salmonella* Enteritidis, XAI represents the first enzyme used (*XbaI*) in the PFGE process, and BNI (*BlnI*) represents the second enzyme used.

<sup>e</sup> Case 1 from outbreak 1 submitted a total of six frozen processed chicken food samples (including brands A, B, E, and F products). *Salmonella* Enteritidis was detected in one of the six samples.

<sup>*f*</sup> For outbreak 2, PFGE was completed for the first enzyme only. All PFGE patterns for case and food testing results shown in the table for outbreak 2 are SENXAI.

four sample isolates (Table 1). Three of the four food sample isolates from which *Salmonella* Enteritidis was detected matched the PFGE and PT patterns of the respective case's clinical isolate. However, the PFGE pattern combinations and PTs detected in the food sample isolates differed from each other. Furthermore, WGS results supported the relatedness of each of the three cases' clinical isolates to their respective food sample isolates but again indicated that the food sample isolates differed from each other (Fig. 1). The three food

samples (sold under two different brand names: brands A and B) were produced at establishment A.

**Outbreak 2.** On 9 June 2015, the national identification of closely related PFGE patterns (SENXAI.0257, SENXAI.0259, and SENXAI.0266) that had not been previously reported in Canada led to a national *Salmonella* Enteritidis outbreak investigation. Of 51 cases reported, 35 were from Ontario. Similar to the previous investigation, a 706 HOBBS ET AL.

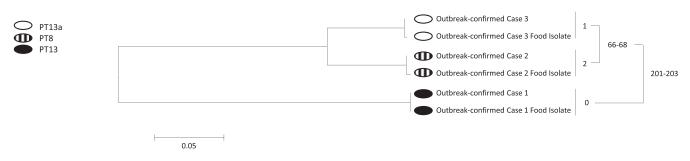


FIGURE 1. Phylogeny of six Salmonella Enteritidis clinical and food isolates from outbreak 1. Maximum likelihood tree based on 238 high quality core genome single nucleotide polymorphism (SNP) positions identified among three clinical and three food isolates using the GenBank reference genome of P125109. The numbers provided indicate the number of SNP differences within case and food isolates and among cases. Epidemiologically related isolates had zero to 16 WGS-SNP differences.

higher than expected proportion (42.3%) of Ontario outbreak cases reported consumption of uncooked, frozen, processed chicken products compared with the expected frequency (18.5%) for the Ontario population. The probability that 42.3% or more of outbreak cases would consume uncooked, frozen, processed chicken was less than 5% (P < 0.05).

Cases were interviewed with focused questionnaires, and they reported consuming various brands of uncooked, frozen, processed chicken products, the majority of which were produced by establishment A (e.g., brands B, C, and D). One Ontario case submitted two brand D samples produced on 22 January 2015 at establishment A for testing (Table 1). Both food sample isolates were a PFGE match (SENXAI.0259) to the clinical case (Table 1), and the case reported preparing the product as per the package directions. In addition, two brand C food samples collected from two cases in other provinces were positive for one of the outbreak PFGE patterns. One of these samples was also produced on 22 January 2015 by establishment A, using the same manufacturing line as the brand D products (results not shown). In summary, all three available brand D and C food samples submitted by cases with a production date of 22 January 2015 tested positive for one of the outbreak PFGE patterns.

Public health action was taken as a result of the investigation. On 28 June 2015, the Public Health Agency of Canada issued a public health notice regarding uncooked, frozen, processed chicken products. On 1 July 2015, establishment A issued a voluntary food recall warning for brand C and D products produced on 22 January 2015, which resulted in the products being withdrawn from retail and the issuance of public warnings to avoid consuming the product (*3*).

**Outbreak 3.** In September 2015, a sustained increase in the number of domestically acquired *Salmonella* Enteritidis PT13a cases in Ontario led to a third outbreak investigation, which identified a total of 36 PT13a cases. In this investigation, WGS was used to further characterize outbreak cases. WGS results supported the relatedness of 18 of 22 outbreak cases tested by WGS (cluster 1) and suggested the presence of an additional cluster (cluster 2) that contained mainly PT8 cases (Fig. 1).

Similar to the previous two investigations, a higher proportion (58.6%) of Ontario outbreak cases reported consumption of uncooked, frozen, processed chicken products compared with the expected frequency (12.6%) for the Ontario population for the months of August to October, inclusive (corresponding to the range of outbreak case onset dates). The probability that 58.6% of outbreak cases or greater would consume uncooked, frozen, processed chicken was less than 5% (P < 0.05).

Cases were interviewed using the same focused questionnaire implemented in the second outbreak to collect detailed product information and information on the cases' handling and preparation of uncooked, frozen, processed chicken products. Public health units were also asked to submit food samples from cases' homes if available. Reinterviewed cases reported consuming various brands of uncooked, frozen, processed chicken products, primarily brands B and C, both produced by establishment A. Four outbreak cases submitted brand C food samples for testing, three of which had the same lot code and a production date of 9 July 2015; product information was not available for the fourth sample (Table 1). The three samples with the 9 July 2015 lot code were a match to the clinical isolates by PFGE, and WGS results supported the relatedness of the food sample isolates with the clinical isolates and also with each other (the cases and food isolates were all in cluster 1, based on WGS results) (Fig. 2). Two of the three cases reported preparing the product as per the package directions. The fourth brand C sample (for which product details were not available) was not a match to the case by PFGE, PT, or WGS.

Additional food samples were submitted by *Salmonella* cases reporting consumption of uncooked, frozen, processed chicken products. These cases did not meet the outbreak case definition; however, they were of interest to the investigation because they were likely the result of a fourth outbreak. Five *Salmonella* Enteritidis PT8 cases from cluster 2 submitted food samples, including two brand C samples with a production date of 9 July 2015 and three brand B samples, all produced by establishment A. *Salmonella* Enteritidis was detected in four of the five food samples—the two brand C samples with a production date of 9 July 2015 and two of the three brand B samples. Isolates from the *Salmonella* Enteritidis—positive brand C food samples were

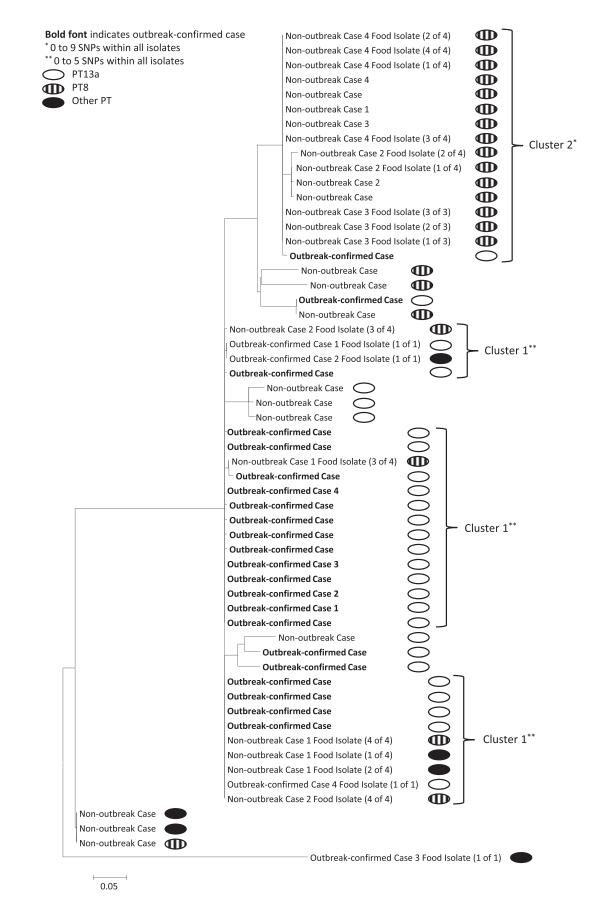


FIGURE 2. Phylogeny of 57 Salmonella Enteritidis clinical and food isolates from outbreak 3. For each food sample submitted, up to six isolates were tested. PT, PFGE, and WGS results may vary for the isolates. Furthermore, WGS was not necessarily completed for all six isolates (e.g., if all isolates had the same PFGE result, only one isolate was sequenced using WGS). The clinical isolate and food sample numbers correspond to isolates and samples shown in Table 1. Maximum likelihood tree is based on 310 high quality core genome SNPs. GenBank reference as in Figure 1.

part of cluster 1 for one case and part of both clusters 1 and 2 for the other case. Isolates for the two *Salmonella* Enteritidis–positive brand B products were all in cluster 2, as were the clinical isolates for the cases who submitted them (Fig. 2). Product information was available for one of the brand B samples but not the other; thus, it could not be determined whether another common lot was also implicated, which would have confirmed a fourth outbreak. In summary, all five available brand C food samples with a production date of 9 July 2015 tested positive for *Salmonella* Enteritidis, with isolates in either cluster 1 or in both clusters 1 and 2. Public health action was not taken as a result of this investigation.

# DISCUSSION

This report describes Ontario cases implicated in three outbreak investigations in Canada (two in Ontario and one national) that linked human illness to uncooked, frozen, processed chicken products. Although outbreaks linked to uncooked, frozen, processed chicken products from various manufacturing plants have been well described, evidence from the three Canadian investigations implicated products from the same establishment, which suggests a common root cause for all three outbreaks. In two of these investigations, specific lots of products were implicated. The June 2015 outbreak resulted in public communication and a recall to mitigate the health risk to the Canadian population.

In addition, laboratory findings in the three investigations identified multiple PT and PFGE patterns and/or WGS results in food sample isolates tested, demonstrating that processed chicken products can be contaminated with multiple *Salmonella* subtypes, which presents a challenge in identifying case and food sample matches.

Furthermore, results from the Public Health Agency of Canada's FoodNet Canada suggest that *Salmonella* contamination of uncooked, frozen, processed chicken products in Canada is an ongoing concern. Tests of random samples of uncooked, frozen, processed chicken nuggets from grocery stores in three Canadian provinces conducted by FoodNet Canada in 2014 and 2015 found that the proportion of chicken nugget samples positive for *Salmonella* and *Salmonella* Enteritidis was as high as or higher than the proportion of raw chicken breasts that were positive (22).

Similar to evidence from other jurisdictions, information collected during the three outbreak investigations suggests that the breaded and prebrowned appearance of uncooked, frozen, processed chicken products may lead to consumer misperception that the product is cooked, possibly resulting in mishandling and improper cooking (5, 10, 15, 16, 18, 19, 25). Although the outer package states that the product is uncooked and some cases reported handling the product as per the package directions, the ongoing number of *Salmonella* cases linked to this product suggests that mishandling and/or improper cooking did occur.

As previously described, the potential for mishandling and improper cooking could also be fostered by other factors related to product packaging and marketing (5, 10, 15, 16, 18, 19, 25). These other factors include the picture on the package, which often shows a cooked product; the small size of the labeling (relative to other package labeling) that indicates that the product is uncooked; the fact that the outer box may be discarded to facilitate storage in the freezer, leaving the product in only the inner plastic bag, which may not indicate that it is an uncooked product and may not have handling or cooking instructions; the placement in the grocery store of both uncooked and fully cooked products in close proximity; and the fact that the products may be marketed as a quick and easy dinner option, implying that they require minimal preparation.

Given the unique features of this type of raw product that appears cooked, the following practices may assist in preventing infection and outbreaks (5, 10, 15, 16, 18, 19, 25), although the feasibility of implementing the practices described is not known: (i) implement acceptable limits of Salmonella in these types of commercial products; (ii) sell only fully cooked or irradiated product; (iii) ensure that labeling and cooking instructions clearly indicate that the product is raw or uncooked; (iv) ensure that labeling and cooking instructions clearly indicate that the product should be cooked to a specific internal temperature; (v) ensure that handling and cooking instructions are clearly indicated on the inner package, should it be separated from the outer package; (vi) separate cooked and uncooked products in grocery store freezer sections; (vii) recall product when an outbreak is linked to a particular lot of uncooked, frozen, processed chicken product based on case-food sample matches and/or retention sample matches, particularly when several matches are detected or when using laboratory subtyping methods that identify uncommon PFGE patterns or clustering by WGS; and (viii) provide public messaging about the existence of an outbreak linked to uncooked, frozen, processed chicken products and the implicated product, if one has been identified.

There are a number of limitations with regard to outbreak investigations such as those described here. Reported cases represent only a small fraction of the number of people with *Salmonella* infection; therefore, cases, and potentially outbreaks due to uncooked, frozen, processed chicken, may go unrecognized (26). In addition, subtyping (other than phage typing) is not routinely performed on *Salmonella* clinical isolates in Ontario, which limits the ability to detect outbreaks, including those related to uncooked, frozen, processed chicken. Finally, unlike the case definitions for the second and third outbreak investigations, the case definition for the first investigation was not specific, capturing an increase in domestically acquired *Salmonella* Enteritidis cases, rather than a specific cluster based on subtyping results.

Information collected during outbreak investigations is limited by the cases' ability to recall relevant exposures because cases are often interviewed up to 2 weeks or more after symptom onset. Furthermore, due to delays in reporting, food samples were not available for many cases. Thus, brand and lot information could not be verified, samples could not be tested, or if the food was available, the original packaging had been discarded, making it difficult to identify the potential source of illness.

The market share of establishment A is not known. It is possible that establishment A produces the majority of

uncooked, frozen, processed chicken products available in Ontario and, thus, would be more frequently identified in outbreak investigations.

Outbreaks have been linked to uncooked, frozen, processed chicken products since the late 1990s. The recent outbreaks in Ontario and Canada further demonstrate that outbreaks from this type of product will continue unless interventions are implemented that prevent contamination at the source level and infection at the consumer level.

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### REFERENCES

- Anderson, E. S., and R. E. Williams. 1956. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J. Clin. Pathol.* 9:94–127.
- Balachandran, P., Y. Cao, L. Wong, M. R. Furtado, O. V. Petrauskene, and R. S. Tebbs. 2011. Evaluation of Applied Biosystems microSEQ real-time PCR system for detection of *Salmonella* spp. in food. *J. AOAC Int.* 94:1106–1116.
- Canadian Food Inspection Agency. 2016. The Canadian food safety system: food recalls. 5 January 2016. Available at: http://www. inspection.gc.ca/about-the-cfia/newsroom/food-safety-system/foodrecalls/eng/1332206599275/1332207914673. Accessed 7 July 2016.
- Centers for Disease Control and Prevention. 2002. Standard operating procedure for PulseNet PFGE of *Escherichia coli* 157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. Available at: https://www.cdc.gov/pulsenet/pdf/ ecoli-shigella-salmonella-pfge-protocol-508c.pdf. Accessed 13 September 2016.
- Centers for Disease Control and Prevention. 2013. Multistate outbreak of *Salmonella* Chester infections associated with frozen meals—18 states, 2010. *Morb. Mortal. Wkly. Rep.* 62:979–982.
- Centers for Disease Control and Prevention. 2015. Multistate outbreak of drug-resistant *Salmonella* Enteritidis infections linked to raw, frozen, stuffed chicken entrees produced by Barber Foods. 16 October 2015. Available at: http://www.cdc.gov/salmonella/frozenchicken-entrees-07-15/index.html. Accessed 7 July 2016.
- Centers for Disease Control and Prevention. 2015. Outbreak of Salmonella Enteritidis infections linked to raw, frozen, stuffed chicken entrees produced by Aspen Foods. 16 October 2015. Available at: http://www.cdc.gov/salmonella/frozen-chicken-entreespart2-07-15/index.html. Accessed 7 July 2016.
- Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Infectious Diseases Prevention and Control Branch, Public Health Agency of Canada. 2015. FoodBook report: protecting Canadians from illness. Public Health Agency of Canada, Ottawa, Ontario.
- Chai, S. J., P. L. White, S. L. Lathrop, S. M. Solghan, C. Medus, B. M. McGlinchey, M. Tobin-D'Angelo, R. Marcua, and B. E. Mahon. 2012. *Salmonella enterica* serotype Entertitidis: increasing incidence of domestically acquired infections. *Clin. Infect. Dis.* 54:S488–S497.
- Currie, A., L. MacDougall, J. Aramini, C. Gaulin, R. Ahmed, and S. Isaacs. 2005. Frozen chicken nuggets and strips and eggs are leading

risk factors for *Salmonella* Heidelberg infections in Canada. *Epidemiol. Infect.* 133:809–816.

- Ewing, W. H. (ed.). 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York.
- Fearnley, E., J. Raupach, F. Lagala, and S. Cameron. 2011. Salmonella in chicken meat, eggs and humans: Adelaide, South Australia, 2008. Int. J. Food Microbiol. 146:219–227.
- Garrison, E., and G. Marth. 2012. Haplotype-based variant detection from short-read sequencing. Available at: https://arxiv.org/abs/1207. Accessed 13 September 2016.
- Grimont, P. A. D., and F. X. Weill (ed.). 2007. Antigenic formulas of the *Salmonella* serovars, 9th ed. World Health Organization Collaborating Centre for References and Research on *Salmonella*, Institut Pasteur, Paris.
- Hanson, H., W. T. Hancock, C. Harrison, L. Kornstein, H. Waechter, V. Reddy, J. Luker, M. Malavet, P. Huth, L. Gieraltowski, and S. Balter. 2014. Creating student sleuths: how a team of graduate students helped solve an outbreak of *Salmonella* Heidelberg infections associated with kosher broiled chicken livers. *J. Food Prot.* 77:1390–1393.
- Kenny, B., R. Hall, and S. Cameron. 1999. Consumer attitudes and behaviours: key risk factors in an outbreak of *Salmonella* Typhimurium phage type 12 infection sourced to chicken nuggets. *Aust. N. Z. J. Public Health* 23:164–167.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The sequence alignment/ map format and SAMtools. *Bioinformatics* 25:2078–2079.
- MacDougall, L., M. Fyfe, L. McIntyre, A. Paccagnella, K. Cordner, A. Kerr, and J. Aramini. 2004. Frozen chicken nuggets and strips: a newly identified risk factor for *Salmonella* Heidelberg infection in British Columbia, Canada. *J. Food Prot.* 67:1111–1115.
- Middleton, D., R. Savage, M. K. Tighe, L. Vrbova, R. Walton, Y. Whitfield, C. Varga, B. Lee, L. Rosella, B. Dhar, C. Johnson, R. Ahmed, V. G. Allen, and N. S. Crowcroft. 2014. Risk factors for sporadic domestically acquired *Salmonella* serovar Enteritidis infections: a case-control study in Ontario, Canada, 2011. *Epidemiol. Infect.* 142:1411–1421.
- Minnesota Department of Health. 2014. Salmonella cases linked to raw, frozen chicken entrees. 23 October 2014. Available at: http:// www.health.state.mn.us/news/pressrel/2014/salmonella102314.html. Accessed 7 July 2016.
- Ontario Agency for Health Protection and Promotion (Public Health Ontario). 2014. Reportable disease trends in Ontario, 2011. Available at: http://www.publichealthontario.ca/en/eRepository/Reportable\_ Disease\_Trends\_in\_Ontario\_2011.pdf. Accessed 7 July 2016.
- 22. Public Health Agency of Canada (FoodNet Canada). 2016. Personal communication.
- Reed, A. 2009. Isolation and identification of *Salmonella* from foods and environmental samples. MFHPB-20. Health Products and Food Branch, Food Directorate, Health Canada. Available at: http://www. hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume3-eng.php. Accessed 13 September 2016.
- SMALT. 2015. Wellcome Trust Sanger Institute, Genome Research Limited. Available at: http://www.sanger.ac.uk/science/tools/smalt-0. Accessed 13 September 2016.
- Smith, K. E., C. Medus, S. D. Meyer, D. J. Boxrud, F. Leano, C. W. Hedberg, K. Elfering, C. Braymen, J. B. Bender, and R. N. Danila. 2008. Outbreaks of salmonellosis in Minnesota (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken products. J. Food Prot. 71:2153–2160.
- Thomas, M. K., S. E. Majowicz, F. Pollari, and P. N. Sockett. 2008. Burden of acute gastrointestinal illness in Canada, 1999–2007: interim summary of NSAGI activities. *Can. Commun. Dis. Rep.* 34:8–15.