OUTBREAK OF *Salmonella* Enteritidis phage type 13
ASSOCIATED WITH MUNG BEAN SPROUTS IN ONTARIO, 2005

OUTBREAK INVESTIGATION

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ABSTRACT

Introduction: This report details an outbreak of salmonellosis due to contaminated mung bean sprouts that occurred in the province of Ontario in the fall of 2005. This report is truly a collaborative effort - many different individuals from several different agencies contributed to this report, with the aim of having one cohesive document of the outbreak investigation. Compilation of completed chapters, paragraphs and incorporation of comments received was performed by M. Anderson, of the Canadian Field Epidemiology Program, Public Health Agency of Canada.

Background: Through enhanced surveillance in response to higher than expected numbers of SE cases, a change in the demographic profile of SE cases was observed by the Ontario Ministry of Health and Long Term Care. Additionally, a specific phage type, PT13 appeared to be dominant. An epidemiological investigation was undertaken on October 28, 2005 to identify the source of infection.

Methods: A case was defined as laboratory confirmed SE PT13 infection in Ontario between October 1 and December 14, 2005. Interviews were conducted with recent SE PT13 cases. A province-wide matched case-control study was conducted. Controls were matched to cases by age-group and geographic location. Environmental investigations included sampling at the retail, restaurant and production level. Many different agencies were a part of this outbreak investigation. The Ontario Ministry of Health and Long Term Care led the investigation. Health units across the province participated, as did the Ontario Ministry of Food and Rural Affairs, the Public Health Agency of Canada, and the Canadian Food Inspection Agency.

Results: A total of 552 laboratory confirmed SE PT13 cases were identified during the outbreak period in 32 health units. Cases were 59% female (323/552), the median age was 31 (range: 1-92). Thirty cases (5%) were hospitalized. Consumption of mung bean sprouts was documented for 247 cases. Fifty-one case-control pairs were recruited for the case-control study. Consumption of bean sprouts was a significant risk factor for SE PT13 infection (MOR= 13.9, 95% CI=4.2-86.7). No other risk factor was significant. Thirty-three percent of recruited cases reported eating raw bean sprouts. Bean sprout samples taken at two restaurants in different regions were positive for SE PT13. Available traceback data implicated one supplier, Company A.

Conclusions: This is the largest documented Salmonella outbreak associated with mung bean sprouts. The source of contamination is unknown. Previous sprout outbreaks have more often been associated with alfalfa sprouts. As a result of this outbreak, a change in public health messaging in Canada occurred to address cooking mung bean sprouts to reduce the risk of foodborne illness. This report includes details regarding local public health unit investigations, laboratory methods, epidemiological methods, traceback methods, interventions taken during the outbreak and results of the investigation. A discussion of the investigation follows.
CHAPTER 1: INTRODUCTION

*Salmonella* Enteritidis (SE) is the most common *Salmonella* serotype causing foodborne salmonellosis in humans worldwide, particularly in developed countries [1]. In Canada, SE is the second most frequently identified *Salmonella* serovar, [2] second only to *S.* Typhimurium. SE phage type (PT) 13 accounted for 55% of typed SE isolates in 2005 [3]. In Canada and the United States, documented cases of human infection due to SE have been most often related to contaminated poultry and eggs [4-15]. However, more recently, *Salmonella* infection increasingly has been related to other products, such as fruit and fresh produce [16]. In the last decade multiple outbreaks of *Salmonella* spp. linked to seed sprouts have occurred throughout the world [17-20]. In Canada, previous sprout outbreaks have been associated with alfalfa sprouts [21, 22], and mung bean sprouts [23,24].

SE PT13 infections in particular, have had documented associations in Canada and the US with mixed meat lasagna, salad [25], hollandaise sauce [26] and cream filled cakes [4].

This report will detail an epidemiological investigation of an outbreak of SE, PT13, which occurred between October 1 and December 14, 2005 in the province of Ontario, Canada.
CHAPTER 2: BACKGROUND

The Ontario Ministry of Health and Long Term Care (MOHLTC) began monitoring an increase in the reporting of SE PT13 beginning in May 2005; several other provinces reported similar increases. In August 2005, Public Health Agency of Canada (PHAC) coordinated a multi-jurisdictional investigation. The investigation failed to identify a single source, but poultry and eggs were suspected. During the same time period, the Laboratory for Foodborne Zoonoses (LFZ), PHAC identified SE PT13 circulating on poultry farms in Ontario [27].

Table 1: Human SE PT13 isolates in Ontario, 2005 by month

<table>
<thead>
<tr>
<th>MONTH</th>
<th>SE PT13 CASES</th>
<th>SE CASES</th>
<th>% SE PT13</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>19</td>
<td>57</td>
<td>33.3</td>
</tr>
<tr>
<td>June</td>
<td>14</td>
<td>34</td>
<td>41.2</td>
</tr>
<tr>
<td>July</td>
<td>33</td>
<td>55</td>
<td>60.0</td>
</tr>
<tr>
<td>August</td>
<td>16</td>
<td>42</td>
<td>38.1</td>
</tr>
<tr>
<td>September</td>
<td>23</td>
<td>56</td>
<td>41.1</td>
</tr>
<tr>
<td>October</td>
<td>53</td>
<td>71</td>
<td>74.7</td>
</tr>
</tbody>
</table>

Source: Ontario Ministry of Health, Enteric and Zoonotic Diseases Unit, Laboratory SE data, 2005

In October 2005, the Ontario MOHLTC identified a further increase in the number of SE PT13 reports; this increase was observed only in Ontario. An examination of case demographics revealed a shift in the demographic profile of the October cases in comparison to those in May to September, with more adults than children, and more females than males represented. This prompted a concern that a new source may be causing these illnesses.
On October 27, 2005 the Ontario MOHLTC alerted the Outbreak Response Section of the Foodborne, Waterborne and Zoonotic Infections Division (FWZID), PHAC of their findings. A Field Epi was deployed to Toronto on October 28 to assist in the investigation. Appendix 1 details the investigation timelines from October 27 – December 14, 2005.
CHAPTER 3: DESCRIPTIVE EPIDEMIOLOGY

Methods

3.0 Hypothesis generation

Case Definition

A laboratory confirmed case was defined as a case of *Salmonella* Enteritidis phage type 13 reported by the Central Public Health Laboratory (CPHL) between October 1st and December 14, 2005 in Ontario.

Between October 28 and November 15, a standardized hypothesis-generating questionnaire (Appendix 2) was used to interview recent cases and included questions on a broad range of food items, including a three-day food history.

Cases were identified through the CPHL, in collaboration with the National Microbiology Laboratory (NML). Ten of the most recent laboratory confirmed cases were selected for interview in order to maximize food history recall. A single interviewer, the Field Epi, conducted interviews. Contact information was obtained from the health unit in which the case resided; some health units required permission from the case prior to releasing the phone number to MOHLTC.

Case investigation forms were requested from all local health units with laboratory confirmed cases. These forms were reviewed to assess the exposure hypotheses generated from the case interviews.
3.2 Data collection

Each health unit collected data, using the integrated Public Health Information System (iPHIS). Data analyses were conducted using SPSS v.13 (Chicago, IL) and Microsoft Excel.

Results

3.3 Results of Hypothesis Generation

Nine of ten (90%) interviewed cases reported consumption of undercooked chicken, runny eggs or improper handling of raw poultry. The last case interviewed (case #10) was on a restricted diet, and did not eat chicken or eggs. This case reported eating bean sprouts repeatedly in the three days prior to illness onset. In total, three of the ten (30%) cases interviewed reported bean sprout consumption.

A total of 34 case reports were received from 11 health units, 24% (8/34) had consumed undercooked chicken, runny eggs or reported improper handling of raw chicken, and 29% (10/34) had bean sprout exposure. Minimal food histories were available for 50% (17/34) of case reports received, making interpretation of the potential exposure difficult for those cases.

Case clusters occurring in several Ontario health units helped substantiate the hypothesis. Please see Chapter 7: Health Unit Investigations, for more details.

3.4 Descriptive Epidemiology of the outbreak

Between October 1 and December 14, 2005 there were 552 SE PT 13 infections reported in Ontario. Among SE PT13 cases, 30 (5%) were hospitalized, and fortunately, zero deaths occurred.
The epidemic curve includes 524 laboratory-confirmed cases reported between September 22 and December 16, 2005 and shows a peak occurring on November 14 and 15. The pattern is suggestive of a continuous source outbreak, which became markedly worse in November.

Table 2: Reported symptoms of SE PT13 cases (n=525)

<table>
<thead>
<tr>
<th>Symptom</th>
<th># of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>502</td>
<td>95.6</td>
</tr>
<tr>
<td>Abdominal Pain/Cramps</td>
<td>329</td>
<td>62.7</td>
</tr>
<tr>
<td>Fever</td>
<td>293</td>
<td>55.8</td>
</tr>
<tr>
<td>Nausea</td>
<td>177</td>
<td>33.7</td>
</tr>
<tr>
<td>Headache</td>
<td>170</td>
<td>32.4</td>
</tr>
<tr>
<td>Vomiting</td>
<td>118</td>
<td>22.5</td>
</tr>
<tr>
<td>Bloody Diarrhea</td>
<td>93</td>
<td>17.7</td>
</tr>
</tbody>
</table>
Twenty-seven cases with unknown symptomology are excluded in the above table. Of note are 18% of cases that experienced bloody diarrhea, which is often indicative of a more severe illness.

Figure 2: Laboratory-confirmed SE PT13 cases between October 1 and December 14, 2005 by age and sex (N=548)

This outbreak affected more females than males (59% females). More adults, (75% age 20-69) were reported compared to the young (<19) and the elderly (70+).
552 cases were reported in 32/36 (89%) health units across the province of Ontario. Cases had the highest concentration in the Toronto Public Health unit, and the surrounding Greater Toronto Area (GTA), which mimics the population distribution in the province of Ontario.
A total of 247/552 cases reported consuming mung bean sprouts prior to their symptom onset. Mung bean sprout exposure could not be determined for 217 cases. However, there was no statistically significant difference, with respect to sex and age, between SE PT13 cases that ate mung bean sprouts and cases where mung bean sprout exposure could not be determined. (Sex: OR=1.23 95% CI=0.8-1.87, Age: OR=0.65, 95% CI=0.41-1.05)
CHAPTER 4: ANALYTICAL EPIDEMIOLOGY

4.0 Objective

A case-control study was conducted between November 26 and December 5 to test the association between consumption of bean sprouts and infection with SE PT13.

4.1 Methods

Sixty-five (65) cases were randomly selected from the list of most recent laboratory confirmed SE cases in Ontario. Most recent cases were selected in order to increase the likelihood of food history recall. A standard questionnaire (Appendix 3 and 4) designed to collect information on a limited number of exposures identified during hypotheses generation was utilized. Data were collected on chicken, egg and mung bean sprout consumption, including place of purchase, location of consumption and cooking method. Public health personnel in each respective health unit administered case questionnaires.

Controls were matched to cases by age-group (under 15, and 15 and over) and geographic location. The last digit of the case telephone number was increased sequentially to recruit controls. Potential controls were excluded if they had diarrhea, defined as three or more loose stools in a 24-hour period, within three weeks of interview or had travelled outside of Ontario within two weeks of interview. One control per case was enrolled.

Data were entered into EpiData version 3.1. Data analysis was preformed using SAS version 9.1. Matched odds ratios were obtained using conditional logistic regression for matched pairs and statistical significance was assessed at the 95% confidence level.
4.2 Results

Fifty-one (51) case questionnaires were completed, and 51 matched controls were recruited. A total of 59% (30/51) cases reported consuming mung bean sprouts, 39% (20/51) did not eat mung bean sprouts, and 1 case had unknown exposure. Among cases that ate mung bean sprouts, 29/30 (97%) provided information on preparation of bean sprouts; 33% (10/30) reported eating raw mung bean sprouts. Of the controls recruited, 4/51 (8%) reported mung bean sprout consumption and all four reported consuming cooked mung bean sprouts. The following tables provide descriptive information on cases and controls.

**Table 3**: Demographics of cases and controls (n=102)

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 15 (29.4%)</td>
<td>18 (35.3%)</td>
</tr>
<tr>
<td></td>
<td>Female 35 (68.6%)</td>
<td>32 (62.7%)</td>
</tr>
<tr>
<td>Age</td>
<td>Median 28</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td>Range 3-77</td>
<td>2-77</td>
</tr>
</tbody>
</table>

**Table 4**: Symptoms and outcome of cases (n=51)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>50 (98%)</td>
</tr>
<tr>
<td>Bloody diarrhea</td>
<td>21 (41%)</td>
</tr>
<tr>
<td>Fever</td>
<td>39 (77%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>33 (65%)</td>
</tr>
<tr>
<td>Visited emergency room</td>
<td>20 (39%)</td>
</tr>
<tr>
<td>Admitted to hospital</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>
Table 5: Matched pairs of bean sprout exposure among cases & controls (n=48 matched pairs)*

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ate bean sprouts</td>
</tr>
<tr>
<td>Ate bean sprouts</td>
<td>2</td>
</tr>
<tr>
<td>Did not eat bean sprouts</td>
<td>2</td>
</tr>
</tbody>
</table>

MOR=13.9, 95% CI= 4.2-86.7
*Three pairs were excluded due to unknown bean sprout exposure

Table 6: Media awareness of controls (n=51)

| Media awareness | Not aware of warnings issued by MOHLTC re: consumption of mung bean sprouts | 28 (55%) |

Cases were **14 times** more likely to have eaten mung bean sprouts compared to controls. (MOR= 13.9; 95% CI=4.2 – 86.7). Three cases and their matched control were excluded from the analysis due to unknown bean sprout exposure.

Other exposures did not indicate a significant association with infection. These exposures were: consuming raw or runny eggs (MOR= 0.86; 95% CI=0.28-2.6), and handling raw chicken (MOR=0.39; 95% C.I. = 0.12-1.02).
Ontario health units performed the initial traceback investigation. On November 22, the Kingston-Frontenac & Lennox and Addington Health Unit submitted to the Ontario MOHLTC an invoice for the shipment of bean sprouts from Company A to Restaurant A in Kingston on November 9. This shipment of sprouts immediately preceded the dates of consumption of bean sprouts by 10 people who ate in this restaurant and subsequently became ill with SE PT13; another five people ate bean sprouts from this restaurant and became ill but were not laboratory-confirmed cases.

By November 24, the MOHLTC had been notified of 13 separate clusters of SE cases in eight health units that had consumed mung bean sprouts in restaurants, or purchased from grocery stores for home consumption, that were eventually traced back to Company A.

From November 24 to December 9, the MOHLTC collected invoices from the health units obtained during their investigations into the origin of bean sprouts consumed by SE cases. The traceback of bean sprouts from restaurants revealed that restaurants purchased sprouts directly from three main sources: sprout producers, intermediate distributors, and/or local grocery stores. Where possible, a complete set of invoices was collected, from the original supplier to the restaurant.

Although many large chain grocery stores claimed to have received their bean sprouts from a single source, the possibility that sprouts might be obtained from different sources at different times existed. The MOHLTC collected information on store location and date of purchase for the bean sprouts consumed by SE cases in order to identify the
source of the particular sprouts associated with illness. This information was supplied to the Canadian Food Inspection Agency (CFIA) as evidence to support a recall.

The information received by the MOHLTC from the traceback investigations conducted by the health units was amalgamated into a summary table (Table 7). The summary gives the total number of store/restaurant locations from which traceback data were received, and the number of SE PT13 cases for which traceback was completed. The numbers are then divided into those that traced back to Company A only, to Company A and another producer, and to another producer only.

Table 7: Number of store/restaurant locations and SE PT13 cases linked to various sprout producers via traceback

<table>
<thead>
<tr>
<th>Sprout producers</th>
<th>Locations</th>
<th>SE PT13 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company A only</td>
<td>90 (67.7%)</td>
<td>128 (62.7%)</td>
</tr>
<tr>
<td>Company A &amp; other source</td>
<td>29 (21.8%)</td>
<td>59 (28.9%)</td>
</tr>
<tr>
<td>Other source only</td>
<td>14 (10.5%)</td>
<td>17 (8.3%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>133</td>
<td>204</td>
</tr>
</tbody>
</table>

*Note: One location in the Company A category is associated with a lab confirmed secondary case via a non-lab-confirmed case that ate at the restaurant. Only lab confirmed SE PT13 cases are included.*

It was noted that at a primary distribution level, records from Company A showed product mainly going to the GTA and Southern Ontario. Very few cases occurred where less product was distributed. In North West Ontario, zero cases occurred and no product from Company A was distributed to those regions. Distribution of SE PT13 cases matched the distribution pattern of the product.

Traceback data implicates Company A, as the likely source of contaminated mung bean sprouts, since most of the sprouts consumed by SE PT13 cases appear to have originated with Company A.
CHAPTER 6: ENVIRONMENTAL INVESTIGATIONS

The environmental investigations were performed by both the CFIA and TPH.

6.0 Toronto Public Health report

Background

Toronto Public Health (TPH) received notification from MOHLTC on Wednesday November 23, 2005 of a traceback investigation that implicated bean sprouts produced by Company A. Company A is located within the jurisdictional boundaries of TPH. Company A distributes dry goods and produce, and produces and distributes mung bean sprouts. Additionally, Company A had a poultry processing division, which operates next door to the sprout production and distribution building. A summary of the public health inspections and investigations at Company A by TPH follows.

Environmental investigation

On November 23, the district public health inspector (PHI) conducted a compliance inspection at 1:30 pm. Four samples of raw mung bean sprouts were taken from Company A and submitted to CPHL.

On November 24, the PHI met with representatives from CFIA, OMAFRA, PHAC, and representatives of Company A at the facilities of Company A. The outbreak and the ongoing traceback were discussed. Issues were raised regarding possible cross contamination from staff of poultry plant working in bean sprout production, shipping bean sprouts in the same vehicle as raw chicken, repair to water main serving the plant. Two environmental swabs were taken of the delivery vehicle.
On November 25, a Section 13 order was filed against Company A by TPH. This order halts distribution of product. Further food and environmental samples were taken at Company A by TPH that day. These were: 2 environmental samples taken of grey totes, 2 dry mung bean samples, 4 mung bean sprouts samples, 2 raw chicken breasts, 1 water sample from hand sink in harvesting area, and 1 water sample from water tank.

On November 26, TPH met with a representative from Company A to ensure premises remain closed. At this time progress was being made toward discarding product from the rooms that are used for growing sprouts.

On November 27, TPH met with a representative from Company A to ensure premises was closed. All bean sprouts had been removed from the plant and were in garbage bins for disposal. Cleaning and sanitizing of rooms and equipment was in progress.

Seeds from lot # 062905 were planted at 5:00 pm in chamber room 3 in anticipation of the section 13 Order being revoked prior to completion of the seven-day growth cycle.

On November 28, TPH met with a representative from Company A to ensure premises were closed. The garbage disposal bins were empty, all cleaning and sanitizing had been completed. A list of Company A employees was provided to TPH Communicable Disease Control staff for interview. Toronto Water confirmed that a temporary water line was installed in front of Company A as a result of a routine upgrade to water supply system. This occurred between October 5 and October 21, 2005. A backflow prevention device that prevents back siphonage was in place at that time.
On November 29, TPH met with Company A and a lawyer representing Company A, CFIA, and OMAFRA. It was agreed that TPH would collect spent water samples from chamber room 3 on November 30.

On November 30, five spent water samples were taken from Company A by TPH and sent for testing to the University of Guelph Laboratory Service.

The use of ice to maintain the bean sprouts was identified as a concern. On December 1, the CFIA inspector for the poultry division of Company A was interviewed and it was determined that ice is not made or used in their product. All ice received with raw chicken is disposed of into a floor level basin. In addition the CFIA inspector was not aware of any staff from the bean sprout division working at the poultry plant.

Ice is used by Company A during the warm weather to assist in cooling of bean sprouts, the ice machine is not used after September. On December 2, the ice machine was activated and samples were taken. These samples were: 2 environmental swabs of the ice machine chute and 1 ice sample.

On December 4, TPH attended the plant at 7:00 am to sample finished product from chamber room 3. Samples taken were: 3 samples from chamber, 3 samples from harvest machine, 1 sample from midpoint in chamber room.

Since the Section 13 Order was still in effect, all mung bean sprouts were to be disposed of on Monday Dec. 5.

On December 6, TPH Incident Manager requested an investigation regarding birds and bird droppings at Company A. No evidence of birds or bird droppings was found in the bean sprout production area. Live birds and bird droppings were found in the storage warehouse used for seeds and other packaged food products. Further samples
were taken: 2 swabs from exhaust vent in chamber room 1, 1 swab from area of ceiling above chamber room 1, 2 swabs of bird dropping in the storage area.

All samples taken by TPH at Company A were negative for Salmonella.

On December 9, TPH received notice of a requested hearing with Health Services Appeal and Review Board from a lawyer representing Company A.

On December 13, TPH rescinded the Section 13 order against Company A.

On December 23, MOHLTC informed TPH that Company A had resumed distribution of product during the week of December 19 to 23. TPH also received a letter from a lawyer representing Company A, addressing the status of requirements that TPH had outlined in their letter, dated December 13.

6.2 Canadian Food Inspection Agency report

The CFIA issued a health hazard alert for mung bean sprouts produced by Company A on November 24, 2005 and December 24, 2005. The alert advised consumers not to eat mung bean sprouts produced by Company A. Company A voluntarily recalled their product.

6.3 Summary

While the source of the SE PT13 infection is known to have been mung bean sprouts, the source of the contamination of the sprouts remains unknown. Previous outbreaks have most often implicated the sprout seeds; however in this investigation, environmental contamination could not be ruled out; however, disinfection of Company A prior to the opportunity for thorough environmental sampling precluded further exploration of this hypothesis. There was no laboratory evidence of SE PT13 isolated from seeds, or from the environment at Company A.
CHAPTER 7: HEALTH UNIT INVESTIGATIONS

There were 32 health units with SE PT13 cases during the outbreak period. Three health units were selected based upon their contribution to the investigation. Their respective investigations are highlighted here with detailed summaries available in Appendix 5, 6 and 7.

7.0 Middlesex-London report

Critical factors contributing to outbreak identification

- Standard *Salmonella* investigation protocol at the health unit including questions regarding fresh produce and unpasteurized products as well as chicken and egg consumption.

- Surveillance capability that includes comparison of monthly case numbers to a ten-year average for the month, allowing increases beyond expected values to be quickly identified and investigated.

MLHU received a total of 7 laboratory-confirmed *Salmonella* cases (six later confirmed SE PT13) and two epi-linked cases between November 7-13, 2005 when in the last 10 years the average number of cases in November had been 3.

Hypothesis generation

Investigation of two clusters (n=4) resulted in exposure hypotheses of raw bean sprouts, green onions, broccoli, cilantro or other fresh produces used in pad thai and spring rolls. This information was shared with MOHLTC in response to their request for data on November 17 and contributed to the development of bean sprout consumption as an exposure hypothesis.
On December 14 there were 30 laboratory-confirmed SEPT13 cases reported in MLHU and of these 19 (63%) reported bean sprout consumption.

**Traceback**

The bean sprout producers were identified for all 19 cases with bean sprout exposure and traced back to three Ontario sprouting operations. Company A was the original source of bean sprouts for 14/19 (73.7%) of cases who ate sprouts. Company B was the original source of bean sprouts for 2/19 (10.5%). For one case (5.3%), bean sprouts were consumed in a meal prepared by a restaurant. This restaurant is supplied in two ways: 1) Company A or 2) A sub-distributor that is supplied by Company B and Company Z. The invoice closest to the case’s exposure date was from the sub-distributor, therefore this case was not considered to be associated with Company A. Finally, 2/19 (10.5%) cases purchased bean sprouts from a retail grocery chain that is supplied by both Company A and Company B.

**Environmental investigations**

No environmental samples were gathered by the MLHU. A total of four food samples were collected and submitted to the Public Health Laboratory for testing. Three bean sprout samples were collected from grocery stores. One sample of peanuts used to garnish pad thai was also collected prior to the provincial outbreak being declared.

**Public health messaging**

The MLHU advised the local newspaper of the MOHLTC press release of November 25, 2005 and issued its own press release on the same day. The MLHU media release
cautioned the public to avoid eating bean sprouts. A more detailed list of public health actions arising out of this outbreak investigation in MLHU can be found in Appendix 6.

7.1 Kingston, Frontenac and Lennox & Addington

Outbreak identification

A syndromic surveillance system in the local emergency room department identified an increase in gastrointestinal illness on Nov 16 and 17, 2005 and stool samples were requested. On November 19, isolates came back as positive for Salmonella sp. and the local Medical Officer of Health (MOH) was notified.

Hypothesis generation

Initial cases reported eating at the same restaurant and on Nov 21, 2005 additional cases identified themselves to the health unit and also reported eating at the implicated restaurant. The suspect food was pad thai. This information was shared with MOHLTC and contributed to the development of bean sprout consumption as an exposure hypothesis.

There were 16 laboratory-confirmed SEPT13 cases with reported bean sprout consumption in KFL&A.

Traceback

See detailed table in Appendix 7.

Environmental Investigations

On November 23rd four bean sprout samples from a restaurant were collected. Three of the four samples were laboratory confirmed to contain SE PT 13. There were no cases associated with this restaurant.
Public health messaging

KFL&A health unit issued a press release on November 24 and made the decision that all bean sprouts should be removed from all food premises in the health unit, until the source of the contamination could be determined. A more detailed list of public health actions specific to KFL&A arising out of this outbreak investigation can be found in Appendix 7.

7.2 Toronto Public Health

Outbreak identification

The investigation began on October 28 when the Field Epidemiologist worked with TPH to obtain case phone numbers for the purposes of hypothesis generation. The investigation was ongoing from that point forward.

Hypothesis generation

There were 51 laboratory-confirmed SEPT13 cases with reported bean sprout consumption in TPH and 110 cases with ‘unknown’ exposure.

Traceback

Please see Chapter 6: Traceback for details.

Environmental investigations

Please see Chapter 5: Environmental Investigations for details.
CHAPTER 8: LABORATORY INVESTIGATIONS

Ontario CPHL and the laboratory at the CFIA undertook laboratory investigations. Additionally, PHAC’s National Microbiology Laboratory (NML) performed all phage typing of SE isolates. Laboratory specimens included food and environmental samples.

8.0 Ontario Central Public Health Laboratory (CPHL) Methods

Stool Sample Laboratory Investigations (Human isolates)

Stool samples submitted for the isolation of enteric pathogens are processed according to standard microbiological methods for the isolation and identification of enteric pathogens and consist of the following processes.

Samples for the investigation of enteric pathogens are received in the laboratory as:

1. Enteric Diagnostic Laboratory – stool sample for culture
2. Enteric Reference Laboratory – enteric pathogen isolate for confirmation, speciation and serotyping.

Enteric Diagnostic Laboratory

Sample: stool sample submitted in Cary Blair transport medium.

Sample Processing: Sample processing consists of two steps.

1. Selective Enrichment: The stool sample is inoculated into a Selenite enrichment broth and incubated at 35 ± 1.0 C for 12-18 hours.
2. Selective Plating: The stool sample is inoculated onto a variety of selective and differential media for the isolation of enteric pathogens. Plates are incubated at various temperatures and times depending upon pathogen requirements.

Isolation/Identification: The Selenite enrichment broth is subcultured after 12-18 hours onto a selective medium. Selective and differential plates are examined for typical colonies and screened biochemically for Salmonella and other enteric pathogens.
Biochemically suspicious isolates are transferred to the Enteric Reference Laboratory for further testing.

*Enteric Reference Laboratory methods*

Sample: Isolate received for confirmation, speciation and serotyping.

Confirmation: Selected biochemicals inoculated for confirmation of isolate.

Serological Identification: Isolates are tested with somatic (O) and flagellar (H) anti-sera in order to obtain a serological identification.

*Food Sample methodology*

Food samples submitted for the isolation of *Salmonella* were processed according to the Public Health Laboratories Branch – Environmental Bacteriology Laboratory Procedures Manual.

Sample/Pre-enrichment: A minimum sample size of 25 g of submitted food product is placed and homogenized in a pre-enrichment broth of 225 ml 1% buffered peptone water which is incubated at 35 ± 0.5C for 24 ± 2 hours.

Selective Enrichment: After 24 hours incubation, a 1.0 ml amount of the pre-enrichment homogenate is transferred to a Tetrathionate Brilliant Green (TBG) enrichment broth and incubated at 42 ± 0.5C for 48 ± 2 hours.

Selective Plating: After 48 hours incubation, the TBG enrichment broth is plated onto two selective agar plates, Brilliant Green Sulfa Agar (BGS) and Novobiocin Brilliant Green Glucose Agar (NBG). Plates are incubated at 35 ± 0.5 C for 24 hours.
Isolation/Identification: Plates are examined for typical colonies and screened biochemically for *Salmonella*. Biochemically suspicious isolates are confirmed as pure cultures and further identified using a commercial enteric bacterial identification system.

Serological Identification: Isolates are tested with somatic (O) and flagellar (H) anti-sera in order to obtain a serological identification.

During outbreak investigations the laboratory uses two epidemiological markers for determining isolate relatedness. These tests are pulsed-field gel electrophoresis (PFGE) and phage typing (PT). PFGE is performed in the Toronto CPHL and PT testing is performed at the National Microbiology Laboratory in Winnipeg.

8.1 National Microbiology Laboratory Methods

Phage typing to further characterize *Salmonella* Enteritidis isolates received from Central Public Health Laboratory, Ontario was performed using international phage typing scheme at the National Microbiology Laboratory, Winnipeg following standard procedures approved by ISO 17025 [28].

8.2 Ontario Central Public Health Laboratory Results

*Food Samples*

A total of 186 food samples were submitted to the CPHL related to the outbreak. Foods by type are summarized in Table 8. There were 12 samples collected directly from Company A and all other food samples were received from various health units across Ontario.
Table 8: List of food samples submitted to CPHL by location

<table>
<thead>
<tr>
<th></th>
<th>All locations*</th>
<th>Company A only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mung Bean – sprout</td>
<td>89</td>
<td>8</td>
</tr>
<tr>
<td>Mung Bean – seed</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Sprouts – other</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Poultry products</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Eggs</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>186</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>

* All locations = restaurants, grocery stores, and private residences

Table 9: Food Samples positive for *S. Enteritidis* PT13

This table lists only those food samples that were positive for SE PT13 during the outbreak period - 184 food samples were negative.

<table>
<thead>
<tr>
<th>#1*</th>
<th>Turkey pot pie</th>
<th>Bakery</th>
<th>Peel HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2**</td>
<td>Spanish Onion</td>
<td>Home of ill person</td>
<td>Halton HU</td>
</tr>
<tr>
<td>#3</td>
<td>Bean sprouts</td>
<td>Restaurant</td>
<td>Toronto HU</td>
</tr>
<tr>
<td>#4</td>
<td>Bean sprouts</td>
<td>Restaurant</td>
<td>Kingston HU</td>
</tr>
</tbody>
</table>

*Not associated with the outbreak
** Most likely contaminated by ill food handler

Three raw chicken samples were positive for *Salmonella* (not Enteritidis). One chicken sample was positive for *Salmonella* Montevideo PT1 and PT untypable. The second chicken sample was positive for *Salmonella* Kentucky PT1 and the third chicken sample was positive for *Salmonella* Kentucky PT2.
Environmental Samples

Table 10: Environmental sample submissions

<table>
<thead>
<tr>
<th></th>
<th>All locations*</th>
<th>Company A only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental Swabs</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Water</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>21</strong></td>
<td><strong>14</strong></td>
</tr>
</tbody>
</table>

* All locations = restaurants, grocery stores, and private residences

All of the environmental samples collected and tested were negative for *Salmonella*.

One of the positive samples, submitted to CPHL on November 23 was obtained from a Toronto restaurant where cases of SE PT13 were reported to have eaten prior to their illness. The other was a sample submitted to CPHL on November 25 from a restaurant in Kingston. The sprouts were collected as an open sample on November 23. Three (3) samples of sprouts tested positive for SE PT13 out of 4 samples submitted from the restaurant. The sample was taken as part of their investigation after identifying a cluster linked to a different local restaurant. There were no reports of illness linked to the restaurant where the sample was obtained.

In both of the above situations, the traceback investigation could not definitively link the origin of the sprouts to a single supplier. For the Toronto restaurant there were 2 possible suppliers and in Kingston there were 3. Company A was one of the possible suppliers for each. This finding is particularly important when considered in light of the epidemiological evidence linking cases to Company A, as well as the majority of traceback data implicating Company A.
CHAPTER 9: INTERVENTIONS

The following chapter details the public health interventions undertaken during the investigation, which included but were not limited to: press releases to the public, recall of affected product, and levying orders against Company A.

9.0 Communications

On November 25, the MOHLTC issued a media release stating that a Toronto producer of bean sprouts had been implicated as the source of the contaminated sprouts and distribution of the product halted. The media release warned the public to avoid consuming bean sprouts.

Health Units varied in their messages to the public. The messages could be broadly categorized as follows: 1) warned the public not to consume any bean sprouts, 2) warned the public not to consume bean sprouts from the implicated sprout producer, 3) recommended that bean sprouts not be consumed raw, and 4) no message given (for health units without SE PT13 cases).

On December 14, 2005 the MOHLTC issued a media release stating that the outbreak appears to be over.

9.1 Interventions taken by Toronto Public Health

Production and Distribution

On November 25, a Section 13 Order was served to stop production and distribution of bean sprouts produced by Company A and to dispose of the existing mung bean sprouts
growing in the six chamber rooms. The premises were monitored daily to ensure the Order was in effect.

*Environmental sanitation*

TPH gave verbal direction to Company A to clean and sanitize the chamber rooms, seed beds and equipment using 200 ppm chlorine.

*Recommendations*

On December 13, the Section 13 Order was lifted and a letter from TPH containing the following recommendations was delivered to Company A. Please see Appendix 8 for a copy of the letter.

1. Ensure finished product is protected from contamination/cross contamination
   - Transport sprouts and raw chicken in separate vehicles, unless measures are taken to prevent cross contamination
   - Store raw chicken and sprouts separately in cold storage facility
   - Use liners in crates for packing finished products
2. Take measures to prevent the entry of birds in the dry storage area.
3. Install back flow prevention devices in the water supply system.
4. Clean and disinfect all areas in Company A including the vent in Chamber Room

In addition the letter listed the following recommendations in keeping with Good Manufacturing Practices and Codes of Practice [29] for the sprout industry:
1. Comply with CFIA Guidelines with respect to the receipt and disinfecting of seeds. According to the US FDA, calcium hypochloride at 20,000 ppm is considered the gold standard.

2. Test seeds and spent irrigation water from each batch of sprouts for *Salmonella* and E. coli O157:H7 prior to distribution of the finished products. Where spent irrigation water is being tested, samples should be taken 48 hours after the start of the germination process and analyzed before the finished product is ready for distribution.

3. Implement a record keeping system that will facilitate traceback and recall of product should that be necessary.

On December 23, a letter of response was received from Company A addressing the requirements and recommendations and indicating that corrective action had been taken.

On December 24, an inspection of Company A was conducted to confirm that the recommendations had been implemented as indicated. Compliance for some items could not be confirmed until after production was resumed.

On December 28, a second letter from TPH to Company A outlining the results of the December 24 inspection and repeating the recommendations was delivered. Please see Appendix 9 for a copy of the letter.

On January 10, the installations of backflow prevention devices on the water supply lines and vent system for the exhaust fan in chamber room 1 were confirmed with Company A.
CHAPTER 10: DISCUSSION AND CONCLUSION

An outbreak of *Salmonella* Enteritidis phage type 13, during the fall of 2005 in Ontario, was the largest salmonellosis outbreak associated with mung bean sprouts documented in Canada. Only laboratory confirmed cases were included in the case definition. Since many persons with gastrointestinal illness do not seek medical attention nor submit a stool sample for laboratory testing [30], the actual number of salmonellosis infections during the outbreak period was likely much higher.

Previous sprout outbreaks in Canada have mainly involved sprouts that are consumed in a raw state, such as alfalfa sprouts. Mung bean sprout-associated *Salmonella* outbreaks have been documented in the past [17]. From 1996-2004, the US FDA documented 27 sprout-related outbreaks. Of these, 85% were associated with alfalfa sprouts, and the remaining 15% were associated with mung bean sprouts [31]. Mung beans differ from other types of sprouts since they can be served cooked. As with other foods, cooking may make the sprouts safer for the consumer. As a result of this outbreak, a change in public health messaging regarding the consumption of sprouts was required to address this difference. The messaging now urges consumers to cook mung bean sprouts to reduce their risk for foodborne illness. Additionally, the elderly, children and those that are immunocompromised are advised to avoid eating any mung bean sprouts.

There are several issues related to mung bean sprouts that make this type of investigation difficult. First, cases may misclassify their exposure because sprouts are usually served as part of mixed dishes, thus the consumer may not recall or even know they have eaten them. Sprouts could also contaminate food preparation surfaces, which
are used to chop other raw produce, cross-contaminating other dishes that do not include sprouts. Second, like other produce commodities, information available for traceback investigations is often limited. The product is not usually packaged with a label that identifies for the consumer the brand or lot number. Finally, microbiological confirmation of a pathogen in the seeds is notoriously a challenge.

The epidemiological and traceback investigations implicated a single mung bean sprout producer, Company A. This producer distributed the majority of their product in Ontario, with only a few accounts in another province. This could explain the absence of cases in the other Canadian provinces.

The source of the contamination of mung bean sprouts in this outbreak is unknown. Seeds have been implicated in past sprout outbreaks as the cause of contamination, however in this outbreak, SE PT13 was not isolated from the seeds taken from Company A. This may not necessarily mean that SE PT13 was not present in the seeds. Studies have demonstrated that seed sampling is not the most effective method of pathogen detection [32].

There were reasons to be concerned about the environment at Company A. The production facility was closely associated with a poultry plant. While cross contamination could not be proven at that time, it could not be ruled out either. Company A was given several recommendations aimed at eliminating any possible cross contamination.

As a result of this outbreak, Canadians are now advised to cook their mung bean sprouts to reduce their risk of foodborne illness. Children, the elderly and immunocompromised individuals avoid eating mung bean sprouts. Currently, specific
regulations for the sprout industry in Canada are not available. A ‘Code of Practice’ was
developed by CFIA, which provides recommendations and guidelines for best practices.
Several outbreaks associated with the sprouting industry, including this large one
associated with mung bean sprouts in Ontario, have been documented in Canada and
elsewhere. In light of the number of sprout-associated outbreaks, adherence to
recommended manufacturing practices of this industry should be reviewed, and stronger
regulation considered.

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