Introduction

This series focuses on investigations of outbreaks caused by commercially distributed food items and detected through pathogen-specific surveillance. The etiologic agents often are *Salmonella*, Shiga toxin-producing *E. coli* (STEC), or *Listeria monocytogenes*, but other pathogens are sometimes responsible. The primary target audience is foodborne disease epidemiologists who investigate (or are training to do so) these types of outbreaks, but others might find this series informative as well.

The primary focus of this series is methods used by epidemiologists (in concert with their co-investigators) to generate, develop, and confirm hypotheses about the outbreak vehicle. Descriptions will generally begin with the detection of a cluster (typically by molecular subtyping of submitted clinical isolates at a public health lab) and end when the food source is identified to a level of certainty/confidence that public health interventions are implemented. While this outbreak occurred when pulsed field gel electrophoresis was the subtyping method used by public health laboratories, the lessons are still applicable now that whole genome sequencing is the subtyping method.

From an epidemiologist’s perspective, the overall goal in these types of investigations is to document a sufficiently specific food exposure in a sufficiently high proportion of cases that one can confidently conclude that the food item of interest is the outbreak vehicle. This series will use outbreak examples that detail the exact process and methods that led investigators to that “threshold of confidence” that prompted them to take action. What were the epidemiologists thinking and doing day-by-day, case-by-case, and step-by-step as the investigation progressed, leading up to the attainment of that threshold of confidence? How were leads identified, and how did investigators decide when and how aggressively to follow a particular lead? The nuances, complexities, obstacles, and decision nodes involved in these types of investigations are nearly impossible to fully describe in the limited space of a peer-reviewed manuscript (plus, many excellent investigations are never published). It is our objective to capture all of the important methodological intricacies of selected particularly speedy or effective investigations using a detailed timeline format. We strongly encourage our audience to read the published investigation manuscript (when one exists) before going through our description. We hope that our descriptions will be a useful, educational supplement to the characterization of the investigation.
E. coli O157:H7 - Multistate Outbreak Associated with Hazelnuts, 2010
FEBRUARY 7 (DAY 1 OF INVESTIGATION)

This story starts with receipt of 2 clinical E. coli O157:H7 (O157) isolates at the Minnesota Department of Health (MDH) Public Health Laboratory (PHL) on January 18 and February 1, 2010 (submission of clinical O157 isolates to MDH is mandatory in Minnesota). By February 7, subtyping of the two cases’ O157 isolates by pulsed-field gel electrophoresis (PFGE) revealed that they were indistinguishable. The MDH PHL notified the foodborne epidemiologists, and a cluster investigation was initiated.

Two restriction enzymes are used routinely on O157 isolates in Minnesota. The isolates were given the Minnesota 2-enzyme subtype designation MN579ECB319 (national PulseNet designation EXHX01.1159/EXHA26.3665).

PFGE-XbaI

Case 1 MN579
Case 2 MN579

What are two of the first questions an epidemiologist should consider once receiving these laboratory subtyping results?

Move to the next page to see what the investigators were thinking…
FEBRUARY 7 (DAY 1 OF INVESTIGATION)

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Two restriction enzymes are used routinely on O157 isolates in Minnesota. The isolates were given the Minnesota 2-enzyme subtype designation MN579ECB319 (national PulseNet designation EXHX01.1159/EXHA26.3665).

How common is the PFGE pattern?
This PFGE pattern had not been seen previously in Minnesota, which suggests that this cluster represents a true common source outbreak; therefore, aggressive follow-up is warranted.

Are there other cases with this PFGE pattern in other states?
A PulseNet search revealed that there were 6 isolates with this PFGE pattern posted in the past 60 days; in addition to the 2 from Minnesota (MN), there was 1 from Michigan (MI) and 3 from Wisconsin (WI) (see epidemic curve below).
FEBRUARY 7 (DAY 1 OF INVESTIGATION)

What could the epidemic curve tell us about the outbreak vehicle?
What characteristics of cases should you examine for possible clues as to what food item is causing the outbreak?
Move to the next page to see what the investigators were thinking...
What could the epi curve tell us about the outbreak vehicle?

The cases are spread out in time, suggesting a vehicle that has a somewhat long shelf life instead of one that is highly perishable (e.g., pre-packaged salad).

*Note: On subsequent slides the epi curve will now be collapsed into weeks to include the investigation time period.*

What characteristics of cases should you examine for possible clues as to what food item is causing the outbreak?

Demographic characteristics such as gender, age, and race/ethnicity. Strong demographic characteristics in a cluster may suggest a particular food vehicle because certain segments of the population are more likely to eat certain foods. For example, if the cluster cases are predominately adult females, this suggests that a produce item like leafy greens or sprouts is the vehicle.

*Move to the next page to see the demographic characteristics of this cluster...*
**FEBRUARY 7 (DAY 1 OF INVESTIGATION)**

Demographic characteristics of the first six cases in the cluster.

<table>
<thead>
<tr>
<th>State</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Race/ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>MI 78</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>Case 2</td>
<td>WI 15</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>Case 3</td>
<td>MN 55</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>Case 4</td>
<td>WI 70</td>
<td>Female</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>Case 5</td>
<td>WI 62</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>Case 6</td>
<td>MN 64</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
</tbody>
</table>

- 83% Male  
- Median age; 63 years  
- All White/Non-Hispanic

The predominance of older males is noteworthy. What types of foods would this prompt you to consider as the vehicle? Move to the next page to see what the investigators were thinking...
**FEBRUARY 7 (DAY 1 OF INVESTIGATION)**

Demographic characteristics of the first six cases in the cluster.

<table>
<thead>
<tr>
<th>Case</th>
<th>State</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Race/ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MI</td>
<td>78</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>2</td>
<td>WI</td>
<td>15</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>3</td>
<td>MN</td>
<td>55</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>4</td>
<td>WI</td>
<td>70</td>
<td>Female</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>5</td>
<td>WI</td>
<td>62</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
<tr>
<td>6</td>
<td>MN</td>
<td>64</td>
<td>Male</td>
<td>White/Non-Hispanic</td>
</tr>
</tbody>
</table>

- 83% Male
- Median age: 63 years
- All White/Non-Hispanic

What types of foods would this prompt you to consider as the vehicle?

At this point we thought some sort of beef was the best guess given the demographics and that beef is a common vehicle for O157. The demographics certainly suggest that the vehicle was not leafy greens or sprouts.
**February 7 (Day 1 of Investigation)**

Team Diarrhea had attempted to interview the first Minnesota case on January 30, but he had refused to be interviewed. Given that the case was now likely part of an outbreak due to a commercially distributed food, a letter explaining that we needed to speak to him because he was part of an outbreak was drafted and sent on February 7.

Team Diarrhea also interviewed Case 2 by telephone using the MDH Standard Questionnaire for STEC and Salmonella Cases:

- He ate ground beef from Walmart, beef from ¼ cow from a meat shop, and steak from Applebee’s.

<table>
<thead>
<tr>
<th>Ground beef</th>
<th>hamburger gravy</th>
<th>/</th>
<th>1/24/11</th>
<th>Ground beef from Walmart, Sartell</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Ground beef as an ingredient: type of dish</td>
<td>hamburger gravy</td>
<td>/</td>
<td>1/24/11</td>
<td>Ground beef from Walmart, Sartell</td>
</tr>
<tr>
<td>b. Ground beef: raw Y</td>
<td>Y</td>
<td>N</td>
<td>U</td>
<td>medium (pink in middle)</td>
</tr>
<tr>
<td>Other beef (e.g., steak)</td>
<td>X</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

5. Where and when did you purchase any ground beef you ate the week before your illness?

- Sore
- Beef from Hoke Meat Shop, Heumel Piggotee (11/2 days before), sausage (not sure)
- Purchased: What type of ground beef was it? Tubed, Tray, Pre-made Patties

- Prepackaged salad X

<table>
<thead>
<tr>
<th>Prepackaged salad</th>
<th>/</th>
<th>/</th>
<th>/</th>
<th>/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceberg</td>
<td>X</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Salad, sandwich</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Not sure of brand</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Cashwise</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Vons</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

Item Ate Did Not Eat May Have Eaten How Prepared Variety or Brand Date Purchased Grocery Store Where Purchased

**OTHER**

- Nuts (e.g., almonds, pecans, walnuts, peanuts, cashews, other type)
- Specify roasted, raw, in the shell

<table>
<thead>
<tr>
<th>Item</th>
<th>Ate</th>
<th>Did not eat</th>
<th>May have eaten</th>
<th>How prepared</th>
<th>Variety or brand</th>
<th>Date purchased</th>
<th>Grocery store where purchased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelled nuts</td>
<td>X</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>Cashwise</td>
</tr>
<tr>
<td>Pecans, walnuts, hazelnuts</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Scooped out of bin</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Not sure of brand</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

- He also reported eating iceberg lettuce.

- He reported eating in-shell pecans, walnuts, and hazelnuts purchased at a grocery store from a bulk bin.

- He didn’t report consuming unpasteurized milk or sprouts, and didn’t have any contact with animals.
February 7 (Day 1 of Investigation)

After the two Minnesota case isolate PFGE patterns were uploaded to PulseNet, the Wisconsin Division of Public Health (WDPH) called MDH on February 7 to discuss the cluster.

The Michigan case and all three Wisconsin cases had been interviewed with their own state-specific questionnaires:

- 2 of 4 reported consuming ground beef.
- 2 of 4 reported consuming lettuce.
  - Both cases consumed bagged lettuce; one case reported Dole, and one case was not sure about the brand.
  - One case also reported consuming iceberg lettuce.
- 2 of 4 reported consuming nuts.
  - One case reported consuming mixed nuts.
  - One case reported consuming hazelnuts and cashews.
- Recall that the Minnesota case reported consuming ground beef, iceberg lettuce, and in-shell mixed nuts.

What are you thinking at this point?

*Move to the next page to see what the investigators were thinking...*
How could you test if the observed exposures were associated with illness?

Move to the next page to see what the investigators were thinking…

Assessment:
At this point there is not an extremely compelling signal. The demographics of the cases don’t fit with a lettuce outbreak. Ground beef is a very plausible vehicle and warrants follow-up to get additional detailed information on the source. Finally, the nuts are an interesting commonality for 3 cases; getting additional detail on the specific types of nuts consumed is warranted.
FEBRUARY 9 (DAYS 3 OF INVESTIGATION)

The second Minnesota case called MDH after receiving the letter and learning he was part of an outbreak.

- He reported eating private kill beef from a local farm that was processed at a local meat processor.
- He said he may have eaten Dole prepackaged salad, and ate iceberg lettuce on a sandwich at a party.
- He reported consuming a variety of mixed in-shell nuts including, pecans, Brazil nuts, hazelnuts, almonds, peanuts, and macadamia nuts from Walmart.

<table>
<thead>
<tr>
<th>Item</th>
<th>Ate</th>
<th>Did not eat</th>
<th>May have eaten</th>
<th>How prepared</th>
<th>Variety or brand</th>
<th>Date purchased</th>
<th>Grocery store where purchased</th>
</tr>
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<tbody>
<tr>
<td>OTHER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts (e.g., almonds, pecans, walnuts, peanuts, cashews, other type)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The MN and WI cases were re-interviewed to get additional specific details on ground beef, lettuce, and nuts (in-shell or not in the shell, from a bulk bin or not, nut types, where purchased, and date or date range of purchase). Including the MI case, who had just been interviewed once:

- 5 of 6 cases reported consuming ground beef, and 3 report eating beef roasts.
  - However, two cases’ only beef exposures were private kill beef from different local farms.
- 4 of 6 cases reported consuming lettuce.
  - 2 cases ate prepackaged lettuce.
  - 1 case said they may have eaten prepackaged lettuce.
  - 2 cases reported consuming iceberg lettuce.
- 5 of 6 cases report consuming in-shell nuts.
  - All 5 cases reported consuming in-shell hazelnuts.
    - 2 reported that the only nuts consumed were hazelnuts.
  - 4 of 5 cases reported purchasing the nuts from bulk bins.
  - No cases had any nuts remaining to test.

What are you thinking at this point?
Move to the next page to see what the investigators were thinking…
Assessment:

- Beef was not considered a compelling source of the outbreak because 2 of the cases consumed only local beef obtained through a private slaughter arrangement; also, beef sources for the other cases did not appear to have been purchased from the same retail chains. This evidence vastly diminished the likelihood that the vehicle was commercially distributed beef.

- Lettuce also was not considered a compelling source. The demographics don’t fit well, and no cases are reporting the same type of product.

- The hazelnut exposure was becoming increasingly intriguing; even though nuts had never before been implicated as a vehicle for O157, they have been for Salmonella, so hazelnuts were considered a plausible vehicle.

What could you do to determine whether the observed hazelnut exposures are worth investigating further?

What is your next step?

Move to the next page to see what the investigators were thinking…
What could you do to determine whether the observed hazelnut exposures are worth investigating further?

Some type of analytic study is appropriate to quantify the significance of the hazelnut exposures and therefore inform further efforts to investigate them. Historically, a community case-control study, often with controls matched by age group and geography (anchored on the case’s phone number or address) would have been a frequent choice. However, such a study can be extremely labor extensive and time consuming. Furthermore, even if a statistically significant association with hazelnuts was found, we’d still want more evidence to conclude that hazelnuts were the vehicle and do an intervention. So, why not do an easier, quicker type of analytic study instead? Read on……
**February 11 (Day 5 of Investigation)**

MDH and WDPH epidemiologists decided to conduct 2 types of “quick and dirty” analytic studies: 1) case-case comparison studies; and 2) used a binomial model to compare case consumption rates against estimated background consumption rates.

**Case-case comparison study**

This type of study requires that you use the same hypothesis-generating questionnaire for cases with the pathogen of interest and cases with other pathogens (which will serve as the comparison group). In this instance, we used Salmonella cases interviewed during the outbreak time period as the comparison group. Since we did not ask specifically about hazelnuts in our initial questionnaire, we used non-peanut nut consumption as the variable of interest in our comparison group.

- In Minnesota, *E. coli* O157 outbreak cases were significantly more likely to consume hazelnuts than Salmonella cases were to consume non-peanut nuts (3 of 3 vs. 7 of 34; p = 0.015).
- WDPH also conducted a case-case comparison using Salmonella cases from the same time period as the comparison group and also found a significant association.
MDH and WDPH epidemiologists decided to conduct 2 types of “quick and dirty” analytic studies: 1) case-case comparison studies, and 2) used a binomial model to compare case consumption rates against estimated background consumption rates.

### Binomial model using estimated background consumption rates

- MDH used walnut consumption (as a proxy for Hazelnut consumption) from the 2006-2007 FoodNet Population survey (34%) as the estimate of background hazelnut consumption. We thought that the true background consumption rate of in-shell hazelnuts was likely lower, but it is good to be conservative and err on the high side when using background consumption estimates in this way.

- Consuming in-shell hazelnuts was significantly associated with illness (p = 0.02).

What do you need to do now to firm up the link with hazelnuts, to the point that you are ready to tell the world that they are the source of the outbreak?

Move to the next page to see what the investigators were thinking...
FEBRUARY 11 (DAY 5 OF INVESTIGATION)

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**Assessment:**

Testing of hazelnuts and demonstrating that they have the outbreak strain would be a good answer, but none of the cases had nuts left to test, and we didn’t know specifically what to test from retail locations. However, if a traceback of hazelnuts consumed by the cases demonstrated that they came from a common source or had a distribution point in common, in our minds we could confidently conclude that hazelnuts were indeed the vehicle.

MDH and WDPH epidemiologists asked the Minnesota Department of Agriculture (MDA) and Wisconsin Department of Agriculture to conduct a traceback investigation of in-shell hazelnuts consumed by cases to determine if the hazelnuts consumed by the cases had a common source.
February 23 - 28 (Days 17 - 22 of Investigation)

February 23
- The Michigan case was re-interviewed on February 18, but denied consuming nuts. However, he called back on February 23 to report that he had in fact consumed in-shell hazelnuts, purchased from a bulk bin at a grocery store.
- The Michigan Department of Agriculture immediately began to traceback the in-shell hazelnuts eaten by the Michigan case.

February 28
- A third Minnesota case was identified using a different subtyping method, multiple locus variable-number tandem repeat analysis (MLVA). At this time MLVA was routinely performed on all O157 isolates in Minnesota.
- The PFGE pattern for the case’s isolate was 1 band different from the outbreak PFGE pattern but matched by MLVA.
- This case also reported consuming in-shell hazelnuts and had leftover nuts that MDA collected for testing.

All seven cases now reported consuming in-shell hazelnuts, and two cases reported consuming only in-shell hazelnuts (i.e., and not other types of nuts).
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All seven cases now reported consuming in-shell hazelnuts, and two cases reported consuming only in-shell hazelnuts (i.e., and not other types of nuts).

Assessment:
At this point in the investigation, we were very confident that in-shell hazelnuts were the outbreak vehicle. However, hazelnuts have never been associated with an O157 outbreak before. It made sense to wait for the results of the traceback investigation to confirm that the hazelnuts traced back to a common source and then use that information to guide the intervention and public messaging.
**MARCH 2 (DAY 22 OF INVESTIGATION)**

- Final results from the traceback investigation, displayed in a formal document below, were available. The in-shell hazelnuts consumed by all 7 cases traced back to the same distributor in California. The lack of internal traceability at Distributor C prevented the identification of a single packer, but it had to be one of two packers.

- A multistate conference call was held between the states and federal partners to discuss the traceback results and further steps.
Assessment:

We have now reached that “threshold of confidence” that prompts us to implement a public health intervention. All seven cases report the relatively rare exposure of consuming in-shell hazelnuts, and these nuts traced back to the same distributor. Additionally, in-shell hazelnuts have a long shelf life so likely still represented an ongoing risk to the public.

- A conference call was held and investigators agreed to issue a press release warning consumers not to eat hazelnuts from the implicated distributor.
- The distributor was contacted and agreed to issue a voluntary recall of hazelnuts and mixed nuts with hazelnuts.

Multi-state investigation links *E. coli* O157:H7 cases to eating in-shell hazelnuts
Epilogue

On March 5, O157 was cultured from hazelnuts collected from the third Minnesota case’s home. On March 7, PFGE subtyping results indicated that the hazelnut isolate matched the outbreak strain of O157. Hazelnuts that were returned to distributors in Wisconsin and California also tested positive for the outbreak PFGE subtype of O157. Waiting for final product testing results would have resulted in unnecessary additional exposure to the product among the public. In the end, 8 outbreak cases were identified in Michigan, Minnesota, and Wisconsin. No cases developed HUS. Ultimately, the close collaboration between public health and agriculture agencies in multiple states, CDC, and FDA allowed the identification of a novel vehicle for an O157 outbreak, with a very small number of cases.


Summary of Key Investigation Lessons:

- The PFGE subtype of isolates in this cluster was rare, which indicated that this cluster represented a common source outbreak and warranted aggressive follow-up. The third MN case was not originally recognized as being part of the outbreak because the isolate was 1 band different by PFGE. Only because MDH routinely tested all O157 isolates by MLVA was this key case associated with the outbreak. See: CIFOR Guidelines for Foodborne Disease Outbreak Response Chapter 4.2.9.2

- The epidemiologic curve and demographic characteristics of the cases can give clues to the vehicle. Whereas these factors didn’t help predict nuts as the vehicle, they definitely supported the conclusion that hazelnuts were the vehicle.

- Obtaining detailed product information is key in evaluating exposures reported by the majority of cases in initial interviews (in this outbreak, beef, lettuce, and nuts).

- It is often necessary to call cases back (often more than once) to ask about an exposure again or to get more specific information (brand, type, point of sale, purchase date) on an exposure. Most people don’t report all foods on 1st interview! See: CIFOR Guidelines for Foodborne Disease Outbreak Response Chapter 4.2.10.3

- A letter or text message to a case saying that they are part of an outbreak and you need their help to solve it and remove the responsible food from commerce may persuade them to be interviewed if at first they refused or didn’t answer calls.

- Analytic studies like case-case comparisons and binomial model comparisons using background food consumption rates can be used to quickly assess potential associations between reported foods and illness, and whether further efforts should be expended to confirm a hypothesis (e.g., through tracebacks, food testing, etc.). See: CIFOR Guidelines for Foodborne Disease Outbreak Response Chapter 5.2.4.1.5

- Traceback investigations can be used to increase the specificity of generic commodity exposures reported by cases (in this instance, i.e., in-shell hazelnuts from a bulk bin), and to confirm hypotheses in foodborne outbreak investigations. “Informational” tracebacks are increasingly important for this purpose. See: CIFOR Guidelines for Foodborne Disease Outbreak Response Chapter 5.2.4.1.7 and White Paper on Product Tracing in Epi Investigations

- Outbreaks can be solved with very few cases. To do this, very specific exposure details need to be obtained, and close collaboration between many different agencies is often required.

Acknowledgments:

Minnesota Department of Agriculture, Wisconsin Division of Public Health, Wisconsin Department of Agriculture Trade and Consumer Protection, Michigan Department of Community Health, Michigan Department of Agriculture and Rural Development, California Food Emergency Response Team, Centers for Disease Control and Prevention, United States Food and Drug Administration

http://mnfoodsafetycoe.umn.edu/