In Part 2 of our piece on epidemiology, a hypothetical epidemiological investigation has been constructed to provide insight into the roles of various public health professionals and their activities during the identification and examination of a food borne illness. This case is typical of many that the produce industry has witnessed over the last several years and describes the protocols and procedures that are followed by health care professionals as the local, county, state and federal levels.

The Cast
George, MD, MPH - County Health Officer
Amanda, PHN – County Public Health Nurse
Julie, REHS – County Registered Environmental Health Specialist
Frank, PhD – State Senior Epidemiologist
Barbara, DVM, MPH – CDC Epidemic Intelligence Officer
SuLyn, MS – CDC PulseNet Microbiologist
Mary – CDC Public Information Office, Media Relations
Maria, RS – Food Safety Officer, National Restaurant Chain X

SCENE 1
Friday, May 1 – A Medium-Sized County in California
At approximately 10am on Friday morning, Amanda, a public health nurse in the County Epidemiology Unit, receives the third report of hemolytic uremic syndrome (HUS) in a week from the local Children’s Hospital. She discusses the reports with Dr. George, her supervisor and County Health Officer. On average, they usually see only 3 HUS cases during the whole year. Suspicious that there may be an outbreak due to shiga toxin-producing E. coli (STEC), which is the leading cause of HUS in the US, they call the physician at the local Children’s Hospital to find out if stool culture results are available. Children with unexplained bloody diarrhea and/or HUS should be cultured for STEC using specialized tests. The hospital informs the Health Officer that they just confirmed isolation of E. coli O157 from two of the children’s’ stool samples; the third child tested negative, but had been receiving antibiotics for several
days (antibiotics may eliminate the bacteria from the gut and produce negative culture results – (there is also conflicting evidence in the medical literature suggesting that antibiotics may be a trigger of HUS during an STEC infection).

*E. coli* O157 and HUS are legally reportable diseases and conditions in Amanda’s state, which mandates that the health care provider report cases by telephone to the local health department immediately, and the laboratory report a positive culture within one working day. Amanda gathers together the Confidential Morbidity Reports sent in by the Children’s Hospital (see example below) and starts a line list of the patients, including contact information for the parents. She also goes through the file and finds all of the reported HUS and *E. coli* O157 cases in the county over the past two months.

Amanda calls Dr. Frank, the on-call Phone Duty Officer at the State Health Department to discuss the 3 HUS cases and isolation of *E. coli* O157 from two of the children’s’ stools. Dr. Frank tells her that three other counties in Northern California are seeing an increase in *E. coli* O157 cases. He asks Amanda to request that the County Public Health Laboratory coordinate with the Hospital Laboratory to forward the patient *E. coli* O157 positive cultures to the State public health laboratory for confirmation. The State will conduct PFGE subtyping and submit the DNA patterns to CDC’s PulseNet, the national molecular subtyping network for foodborne disease surveillance.

Confidential Morbidity Report

![Confidential Morbidity Report](image-url)
Amanda gets off the phone with the state, and calls the parents of the children in the hospital to complete the 9 page E. coli O157/HUS Case Report (see below). The Case Report form includes:

- Patient demographics (age, gender, ethnicity, contact information)
- Clinical information
- Group setting (day care, nursing home, etc.)
- Signs and Symptoms
- Past medical history
- Hospitalization details
- School/work absence
- Treatment
- Outcome
- Laboratory results
- Food history including restaurants
- Food History
- Groceries (may include frequent-shopper card information)
- Outside home dining
- Events (farm visits, petting zoo, etc.)
- Animal exposures
- Travel
- Household contacts
- Ill contacts

It is worth noting that the exposure history in the pathogen-specific Case Report form is based on the most well-known vehicles of transmission for STEC infection. The Case Report form serves as an initial screen for common exposures among the patients and hypothesis generation (food, event, restaurant, grocery store, etc.). Information about ill contacts such as siblings, classmates, or co-workers is also collected at this time. States update their standardized Case Report forms as new emerging food vehicles are identified.

By the end of the day, Amanda has reached the parents of the hospitalized children, and conducted her interviews and completed the Case Report forms. She also found two more possibly related E. coli O157 infections during the last 2 weeks. She classifies the two children with laboratory isolation of E. coli O157 as “probable,” and the HUS case with no laboratory confirmation as “suspect” per the definitions provided on the Case Report Form as shown below. She faxes the report forms to Dr. Frank at the state.

Amanda makes note that 5 of the 5 patients ate at the same chain restaurant (Chain X) during the 7 days before becoming ill; she notifies Dr. Frank at the state of this finding before going home for the day. Dr. Frank sends a notification via the California Health Alert Network (HAN) describing the three cases of HUS clustered in time and location, all with a history of eating at
the same chain restaurant. The HAN alert was sent to all local health department directors, other state health department staff and managers, and designated contacts in the state’s health care system. The recipients were asked to conduct heightened surveillance for diarrheal illness and submit appropriate specimens for diagnosis of STEC. The alert was also posted on the **Epidemic Information Exchange (Epi-X)**, the CDC’s secure national communication network for public health professionals.
SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) (2010)

CLINICAL DESCRIPTION: An infection of variable severity characterized by diarrhea (often bloody) and abdominal cramps. Illness may be complicated by hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP); asymptomatic infections also may occur and the organism may cause extraintestinal infections.

LABORATORY CRITERIA FOR DIAGNOSIS: Isolation of Shiga toxin-producing Escherichia coli from a clinical specimen. E. coli O157:H7 isolates may be assumed to be Shiga toxin-producing. For all other E. coli isolates, Shiga toxin production or the presence of Shiga toxin genes must be determined to be considered STEC.

CASE CLASSIFICATION:
- **Confirmed**: A case that meets the laboratory criteria for diagnosis. When available, O and H antigen serotype characterization should be reported.
- **Probable**: A case with isolation of E. coli O157 from a clinical specimen, without confirmation of H antigen or Shiga toxin production, or a clinically compatible case that is epidemiologically linked to a confirmed or probable case, or Identification of an elevated antibody titer to a known Shiga toxin-producing E. coli serotype from a clinically compatible case.
- **Suspect**: A case of post-diarrheal HUS or TTP (see HUS case definition), or identification of Shiga toxin in a specimen from a clinically compatible case without the isolation of the Shiga toxin-producing E. coli.
SCENE 2
Monday, May 1 – CDC PulseNet Headquarters, Atlanta, Georgia (earlier in the day)

On the other side of the country, SuLyn, a CDC microbiologist, alerts her branch chief about a cluster of 12 *E. coli O157* subtypes showing an unusual PFGE pattern (DNA fingerprint) on PulseNet. The indistinguishable strains originated from patients in 4 western states (California, Oregon, Washington, and Utah).

An email notification with an attached file showing the PFGE pattern using a primary enzyme (*Xba*-1), and relevant epidemiological information, is sent to PulseNet participants in state and local health departments around the nation.

Epidemiologists in all 4 states (CA, OR, WA, UT) mobilize over the weekend to review Case Report forms and identify any common exposures among recent *E. coli O157* cases.

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SCENE 3
Week of May 4 – CDC Holds a Conference Call with State Health Departments in CA, OR, WA, and UT

By the end of the weekend, the investigators have identified an additional 20 cases, and a possible common restaurant (Chain X) exposure among the patients. Everyone on the call agrees to conduct an outbreak investigation.

**Step 1. Prepare to investigate**

Dr. Barbara, a CDC Epidemic Intelligence Officer (EISO), is assigned Team Lead for the outbreak investigation. As an EISO, she will coordinate with the state and local health officials to conduct the multi-state investigation at the national level. Other staff recruited onto the state investigation Team include infectious disease epidemiologists, microbiologists, public health nurses, environmental health specialists, and public information officers.

Environmental health specialists in the affected states review past inspection reports for the Chain X stores where the patients ate. They also visit each of these restaurants to identify if there are any immediate food safety violations. Julie in California and the investigators from the other states find no critical violations that pose an imminent risk to public health at the restaurants. At this point, the suspect food or drink has not been identified. The local managers and the restaurant chain’s Food Safety Officer, Maria, are notified of the findings from the investigation.

**Step 2. Verify the Diagnosis and Confirm the Outbreak**

SuLyn verifies that the 2-enzyme (*Xba*-1 and *Bln*-1) PFGE analysis of available *E. coli O157* strains shows that the patterns are unique, and represent less than 1% each of the total *E. coli O157* patterns.
in the PulseNet database. This is designated the “outbreak strain.” The public health laboratories also report that the outbreak strain serotype is non-motile: *E. coli* O157:NM

**Step 3. Case Definition**
The Outbreak Team develops a case definition.

A **suspect** case is defined as illness in a person who ate at Chain X with onset of diarrhea (three or more loose stools in a 24 hour period) on or after April 1 through the present for a duration of two days or longer without another known cause.

A **confirmed** case is defined as a suspect case with laboratory confirmed STEC and a PFGE pattern indistinguishable from the outbreak strain pattern, or HUS clinically diagnosed even if culture negative.

A **probable** case is defined as a suspect case with an epidemiological link to a confirmed case.

**Step 4. Case Finding**
A line listing of cases is maintained at the CDC. State Case Report forms are sent to Dr. Barbara and her team to review exposures and contacts. There are now 66 culture confirmed and 18 suspect cases. The medical and surrounding public health communities are updated on the outbreak and the case definition through additional HAN and Epi-X alerts. They are asked to report possibly related cases, and submit *E. coli* O157 isolates for subtyping and comparison with the outbreak strain.

**Step 5. Perform Descriptive Epidemiology**
Dr. Barbara plots an epidemic curve of the cases, to date, and maps their distribution. The “epi curve” findings suggest that the outbreak may have peaked in mid-April and be on a slow decline now. The age, gender, and number of hospitalizations are also described in a preliminary report shared with the medical and public health communities in the affected and surrounding states.

| Person ◊ | Place ◊ | Time |
Epidemic curve of confirmed (blue) and suspect (red) cases of *E. coli* O157:NM by date of illness onset, as of April 17 (N=84).

Geographic distribution of *E. coli* O157:NM cases as of April 17 (N=84).

**Step 6. Hypothesis Generation**
A review of the Case Report forms in all 4 states also shows that the majority of patients reported eating a chicken salad sandwich or a salad at Chain X.
Step 7. Evaluate the Hypothesis through Statistical Analysis (Analytical Epidemiology)
To confirm the Chain X hypothesis, a matched case-control study is conducted. The Outbreak Team drafts the questionnaire. It is decided that the state teams will conduct the interviews, and use random-digit dialing to identify age-matched controls.

**Front Page of Case-Control Interview Form**

| Interviewer __________________ | Date of Interview __________________ |

**MATCHED CASE INFORMATION:**
Name: ____________________  Age Group: ________________  Onset of Illness: ________________

**CONTROLS:**
Match controls by case’s age group and location:

- **Age Groups:** 1-9 years
  - 10-19 years
  - 20-29 years
  - equal to or greater than 30 years

- **Location:** Same area code and first 3 digits of case’s phone number

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Hello, I’m ___(interviewer name)___ calling on behalf of the California Department of Health Services. We are investigating an outbreak of diarrheal illness in Northern California. We’re trying to find the cause of this outbreak by asking questions of persons with and without this illness. I would like to ask you some questions about the restaurants you eat at. The survey should take about 5 minutes and all information that you provide will be kept confidential. Can you talk now? (If not, when would it be more convenient?)

Date ________ Time ____________

Random digit dialing is a method to select controls from similar geographic locations by using the case’s 3 digit prefix to generate a random list of the other 4 digit numbers. The interviewer cold-calls, introduces themselves and describes the purpose of the study, then asks if the individual is willing to participate. If they say yes, the person is asked if there is a household member that “matches” the age group for the case. If they say yes, then they are asked if this individual has had a diarrheal illness compatible with *E. coli* O157 since April 1. If they say “no,” they are enrolled in the study and asked a series of questions about which popular restaurant chains they ate at in the 7 day period before the matched case’s onset of illness.
The results from the case-control study are analyzed by using a 2 x 2 table, and calculating an Odds Ratio (OR). Due to the difficulty finding matched controls, only a subset of cases are included in the study.

### 2 x 2 table used during the restaurant case-control study

<table>
<thead>
<tr>
<th>Restaurant</th>
<th>Cases (ill)</th>
<th>Controls (not ill)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate at Chain X</td>
<td>15 (a)</td>
<td>11 (b)</td>
<td>26 (a+b)</td>
</tr>
<tr>
<td>Did not eat at Chain X</td>
<td>4 (c)</td>
<td>27 (d)</td>
<td>31 (c+d)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (a+c)</td>
<td>38 (b+d)</td>
<td>57</td>
</tr>
</tbody>
</table>

Odds Ratio (OR) formula = a * d/b * c

In this case the OR= \((15\times27)/(11\times4)\) = 9.2

p-value = 0.0004

In reality, the calculations are done in a statistical software program, but the formula is shown here for illustration purposes.

**Interpretation:** cases were 9.2 times as likely to have eaten at Chain X compared with controls from their same age group and geographic location. The p-value measures the strength of the association, and is usually set at 95% confidence. A p-value less than 0.05 means that the Outbreak Team is 95% certain that eating at the restaurant was associated with illness, and the finding was not due to chance alone.

Odds ratios and p-values are calculated using 2 x 2 tables for each restaurant chain on the questionnaire (data not shown). In this example, Chain X was the only restaurant that was significantly associated with cases (illnesses).

Once Chain X is implicated as the source of the outbreak, another case-control study is conducted to identify the food or ingredient that caused the illness. It is not unusual in a multi-state outbreak to conduct multiple case-control studies, especially if the suspected food is a multi-ingredient menu item (for example, salads and sandwiches).

Dr. Frank’s Team conducts a case-control study in California. His questionnaire is based on the Restaurant Chain X menu during the time of the outbreak. For the menu-specific questionnaire, Dr. Frank again matches on age group, but he selects controls by using meal companions rather than random digit dialing. Meal companions of cases that did not get ill are a convenient group for interviewing, although sometimes there is so much similarity in food exposures among cases and
meal companion controls that additional studies are needed to tease out the implicated food or ingredient.

Dr. Frank is only able to enroll 6 cases and 10 matched controls, but alfalfa sprouts are identified as significantly associated with illness (p = 0.007) as shown in the table below. Results from subsequent case-control studies at CDC also identify sprouts as significantly associated with *E. coli* O157:NM illnesses in CA, OR, WA and UT.

Partial Results from the Menu-Specific Case-Control Study

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Matched Odds Ratio (OR)</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken salad</td>
<td>5 (83)</td>
<td>10 (100)</td>
<td>*</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Lettuce</td>
<td>2 (33)</td>
<td>5 (50)</td>
<td>0.56</td>
<td>0.04-5.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>3 (50)</td>
<td>9 (90)</td>
<td>0.30</td>
<td>0.005-4.3</td>
<td>0.85</td>
</tr>
<tr>
<td>Cucumber</td>
<td>3 (60)</td>
<td>6 (60)</td>
<td>*</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Alfalfa sprouts</strong></td>
<td>6 (100)</td>
<td>0 (0)</td>
<td>∞</td>
<td>1.92-∞</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Pasta salad</td>
<td>2 (33)</td>
<td>0 (0)</td>
<td>*</td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>Salad bar</td>
<td>4 (67)</td>
<td>3 (30)</td>
<td>4.5</td>
<td>0.28-216.4</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Indicates Mantel-Haenszel matched odds ratio could not be calculated due to zero cells; fischer's exact limits for the conditional Maximum likelihood estimate (MLE) of the OR calculated with EXACT.

**SCENE 4**
**Week of May 8 – Notification of the Public and Media**

Dr. Barbara at the CDC contacts Maria, Food Safety Officer and her management at Chain X to discuss findings from the epidemiologic investigation. The Outbreak Team works with Mary and Maria from Restaurant Chain X to compose the press release. The release is an opportunity to remind the public about the known risks associated with eating raw sprouts, especially among vulnerable populations including children, the elderly, and persons with compromised immune systems.

**SCENE 5**
**Week of May 15 - Conducting a Traceback Investigation**

**Step 8. Additional Environmental Studies**

Because this was a multi-state outbreak, and sprouts are an FDA-regulated food, Dr. Barbara schedules a conference call with FDA headquarters to discuss the outbreak findings. Dr. Frank and
the other state epidemiologists join the call along with managers from their state Food and Drug and FDA District offices. At this point, a decision must be made regarding whether or not a traceback investigation of the sprouts will be conducted. The decision is based primarily on the strength of the epidemiologic association, and the availability of records necessary for the traceback.

**Step 9. Implement Control and Prevention Measures**

Ultimately, the alfalfa sprouts used on sandwiches and salads at Restaurant Chain X were implicated as the source of the outbreak. A traceback investigation by FDA revealed that shipments of fresh sprouts to the implicated restaurants in the four western states were from a single supplier in California. A voluntary recall of sprouts produced by this company was issued.

**Step 10. Communicate Findings**

Upon completion of the outbreak investigation, the states write a Final Report describing the findings, conclusions, and recommendations. Depending on the scope of the outbreak, CDC may also post a summary of the finding on their website, and publish a summary and editorial comment in the *Morbidity and Mortality Weekly Report*. If the outbreak was especially noteworthy, the Team may write a manuscript for submission to a peer-reviewed scientific journal.
Michele Jay-Russell is currently a Project Director and Researcher at the Western Center for Food Safety at the University of California, Davis. Her research interests are in pre-harvest food safety and the interface between animal agriculture, wildlife, and the environment. Prior to joining the university, Dr. Jay-Russell was a Research Scientist with the Food and Drug Laboratory Branch at the California Department of Public Health. In this capacity, she was involved in many environmental and laboratory foodborne disease outbreak investigations including the 2006 farm investigation following the *E. coli* O157:H7 contamination of fresh, bagged baby spinach. She has also served as California's State Public Health Veterinarian and Chief Epidemiologist for the Sacramento County Department of Health and Human Services. She has published and presented on numerous epidemiological investigations and surveillance programs in public health and food safety. In 2006-2007, she was awarded the California Department of Public Health Superior Accomplishment Award, the FDA Leveraging/Collaboration Award, and the International Association of Food Protection Innovation award. Dr. Jay-Russell received her Doctor of Veterinary Medicine and Masters of Preventive Veterinary Medicine in 1992; she completed her PhD in Microbiology from the University of California, Davis in 2011. Dr. Jay-Russell was board certified with the American College of Veterinary Preventive Medicine in 1997.