

FOODBORNE INVESTIGATION CASE STUDY

PINING FOR A COMMON SOURCE

STUDENT MANUAL

DEVELOPED BY

New York Integrated Food
Safety Center of Excellence



Learning Objectives

After completing this case study, students will be able to:

- Explain what constitutes a foodborne illness cluster/outbreak
- Describe the steps in a foodborne illness outbreak investigation
- Identify the roles and responsibilities of the outbreak investigation team
- Understand the importance of information sharing during an outbreak investigation
- Draw and interpret an epidemic curve
- Establish and refine an outbreak case definition
- Describe the types of epidemiologic tools used during an outbreak investigation, including novel techniques
- Describe the purpose of conducting an environmental assessment and traceback investigations
- Describe the types of laboratory techniques used during an outbreak investigation
- Explain how to summarize and communicate outbreak investigation findings

This case study was developed by the New York Integrated Food Safety Center of Excellence (2021).

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<https://nyfoodsafety.cals.cornell.edu/about>

PART A: BEGINNING THE INVESTIGATION

During the first week of September, five laboratory reports of *Salmonella* are reported to Empire County in Liberty (LB) State from local laboratories.

Note: In LB State, physicians, hospitals, and laboratories are required to report confirmed/suspected *Salmonella* infections to the local health jurisdiction where the patient resides.

This number of laboratory reports of *Salmonella* is not unusual within the given time period and not considered above baseline for the county. Epidemiologists at Empire County investigate *Salmonella* cases following standard procedures. Each case is contacted to complete an initial interview which will collect information in a standard manner on exposures preceding their illness.

Note: In LB State, local health jurisdictions interview cases using a standardized *Salmonella* questionnaire and enter the clinical, laboratory, and exposure data into a secure LB State Health Department electronic surveillance system.

Once completed, questionnaires are reviewed and compared to identify any commonalities (i.e., household contacts, attended same party or event) or high-risk exposure settings (i.e., cases that work/reside in a sensitive setting such as a restaurant or healthcare facility).

Initial Report from the Laboratory

On Friday, September 16, an epidemiologist from the LB State Health Department (LBHD) Communicable Disease Program was notified that the LB State Public Health Laboratory (LBPHL) had identified 2 *Salmonella Enteritidis* isolates (from the 5 cases reported in early September) that were 0 alleles apart using Whole Genome Sequencing (WGS) in Empire County.

Note: In LB, local laboratories (hospital and commercial) are required to submit all *Salmonella* isolates to the LBPHL where confirmation, serotype determination, and WGS are completed.

Question 1: You are an epidemiologist at LBHD. What additional laboratory information would you request from the LBPHL?

The epidemiologist places an inquiry to LBPHL to request that PulseNet be searched to identify case-isolates from other states that are closely related by WGS.

Note: The state lab normally looks back 60 days to identify other related isolates, but this may vary by state or disease. SEDRIC can also be utilized.

Question 2: You are an epidemiologist at LBHD. What steps would you take next?

The epidemiologist decides as a first step to review both patient case reports in attempt to identify any commonalities or unique exposures among the 2 cases.

The epidemiologist will also conduct a quick literature search to determine what products, if any, have been implicated in other outbreaks involving *S. Enteritidis*, in general.

Question 3: What type of questionnaire do you use in your jurisdiction? What type of information is gathered?

The epidemiologist reviews the initial case reports.

- Case 1 is a 5-year-old male from Empire County who had illness onset on 8/31. Symptoms included vomiting, diarrhea and fever and the duration of illness was approximately 7 days.
- Case 2 is a 16-year-old female from Empire County who had illness onset on 9/9. Symptoms included nausea, vomiting, diarrhea and headache and the duration of illness was approximately 5 days.

Cases were not household contacts. Although both cases were children, they did not attend the same school. Neither case attended large gatherings during their incubation period.

After reviewing both patients' case reports for exposure history, a variety of commonalities were identified. Both cases had exposure to commonly consumed items (chicken, tomatoes, and eggs), dining out (different locations), and household pets. Both case households shopped at Grocery Store Chain W but at different store locations. Chain W is an extremely popular supermarket chain in the region and has multiple locations.

Late that Friday afternoon, the LBPHL verifies that there are no closely related isolates in other states and these isolates do not have any historic isolates close by.

The epidemiologist prepares a summary to send to the individuals on their investigation team to inform them of the ongoing cluster investigation and the action steps underway.

Question 4: Who might be part of the investigation team? In one sentence, describe the role of each team member.

Members of the investigation team would include:

- Laboratory Investigator: focuses on testing clinical specimens and environmental and food samples, and on analyzing and interpreting test results.
- Environmental Health Investigator: focuses on the contaminated food and/or food preparation site, water, and environmental sources.
- Epidemiology Investigator: focuses on cases, developing and testing hypotheses, and case finding to identify and describe clusters
- Public Health Nurse: focuses on patient and public health education

At LBHD, epidemiologists and public health nurses often play overlapping roles. Members of this team may also be comprised of staff from various levels of government (county/local health department, state health department, etc.)

Question 5: Would you involve all these team members right now? If not, who would you involve?

The epidemiologist shares the cluster summary with the members on their investigation team, which includes LBHD epidemiology and environmental health investigators, as well as staff at

the LBPHL. Epidemiologists/public health nurses at the local health jurisdiction of the cases (Empire County) are also provided with a cluster summary. Based on relevance (i.e., strength of food/event association), cluster information may also be shared with environmental health investigators in Empire County.

Question 6: What are your next steps?

With a sigh of relief, the epidemiologist leaves their office at 5PM - very pleased to not have to stay late and spend the entire weekend following up on this investigation. Probably nothing to worry about...!

PART B: ADDITIONAL CASES REPORTED

On Monday September 26th, LBHD is notified that the LBPHL has just identified 7 additional *S. Enteritidis* infections that are 0 alleles apart from each other and the initial two cases reported on September 16, making them closely related.

Six of the 7 new cases reside in Empire County, while the 7th case resides in a separate county in the state, Apple County.

The epidemiologist compares the interview forms for all 9 LB cases to date. Two case interviews are pending. Review of the remaining 7 case reports indicates that 6/7 cases shop at Chain W, but at various locations. Again, Chain W is a common grocery store to shop at in the location the cases are occurring. Food history exposures are extensively reviewed to identify the food items consumed by 50% or more of the cases. The epidemiologist identifies the following exposures: chicken, tomatoes, eggs, and grapes. No cases are members of the same household and none of the cases work or reside in a sensitive setting. There are no common restaurants or event exposures among cases.

Note: Typically, any exposure reported by 50% or more of the cases could be considered a possible hypothesis to explore further.

Question 7: Describe how this situation could be defined as a cluster AND an outbreak? Provide an explanation for your choice.

Question 8: Who would you notify about this growing investigation?

The epidemiologist notifies members of their investigation team, which includes LBHD epidemiology and environmental health investigators and the LBPHL. The epidemiologist also notifies LBHD Leadership staff for situational awareness, as well as epidemiologists/public health nurses and environmental health investigators at the local health jurisdiction of the cases (Empire and Apple Counties).

The epidemiologist decides it is too early in the investigation to communicate with either the public (via a press release or public notification) or the USDA/FDA as a contaminated product has not been suspected or confirmed. The epidemiologist may or may not choose to notify CDC at this time to alert them about this investigation. Because no other closely related cases (by WGS) have been identified outside of LB State, the epidemiologist decides not to notify the CDC to alert them about this ongoing investigation.

The epidemiologist shares the new case count and exposure summary with the investigation team, and classifies the cases as a cluster, not an outbreak. Although multiple closely related cases have been identified, no common associations among cases have been identified from

review and comparisons of initial case questionnaires. To classify cases as part of an outbreak, there must be a known association among the cases, like dining at the same restaurant, attending the same catered event, or eating a common food item.

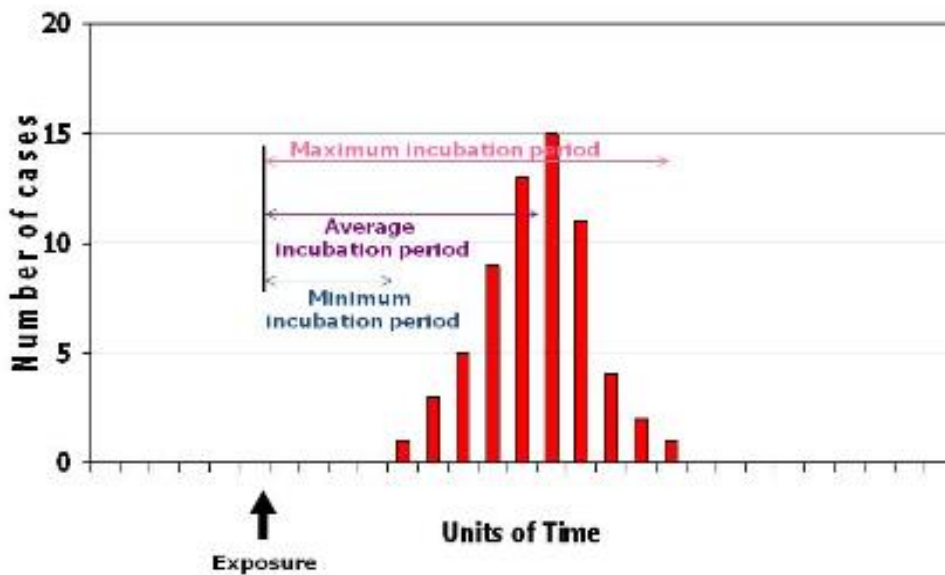
Note: A cluster is defined as: more cases than expected for a given time and location OR 2 or more cases having the same diagnosis/similar illness WITHOUT a known association/identified common exposure among cases. An outbreak is defined as: 2 or more cases having the same diagnosis/similar illness associated with a common exposure.

The epidemiologist draws an epidemic curve of the cluster hoping to shed some light about how the *Salmonella* has spread and to provide a visual representation of the cluster. The shape of the epidemic curve can reveal the outbreak's likely mode of spread.

During a point source outbreak, all or most cases occur within one incubation period. The short duration of the outbreak suggests a single "point" of exposure event. Cases rise rapidly to a peak and fall off gradually. Examples include a gastrointestinal (GI) outbreak following a wedding reception.

https://www.med.uottawa.ca/sim/data/Pub_Infectious_e.htm#epi_curves

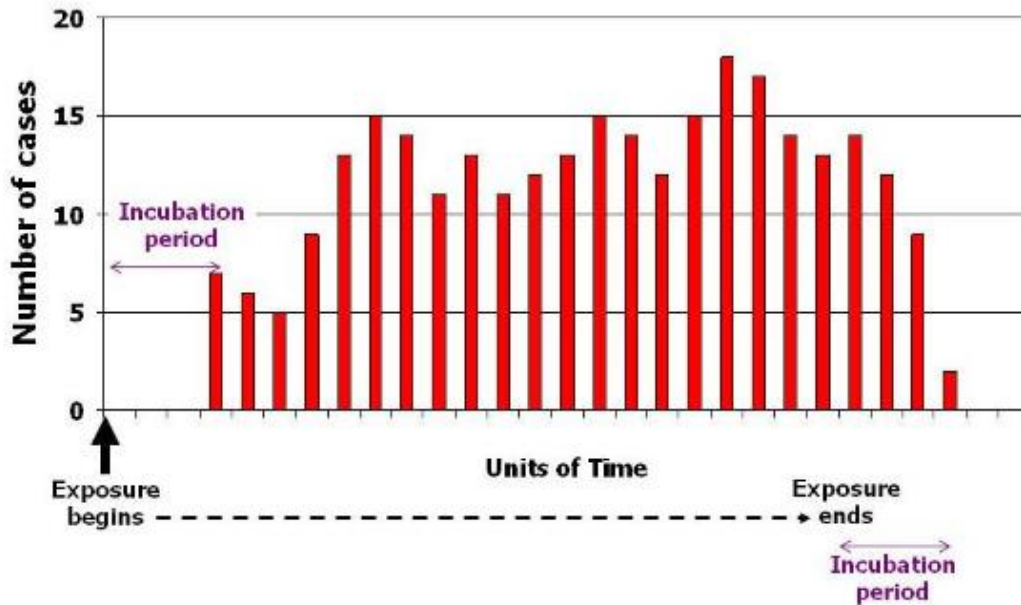
Point Source outbreak with no propagation



During a continuing common source outbreak, cases occur over a prolonged period of time, spanning multiple incubation periods. Cases are exposed to the same source, but exposure is ongoing over a period of days, weeks, or longer. The curve tends to rise gradually and may plateau. Examples include consumption of contaminated, commercially distributed dried cilantro, a shelf-stable food that is available to consumers over weeks and months.

https://www.med.uottawa.ca/sim/data/Pub_Infectious_e.htm#epi_curves

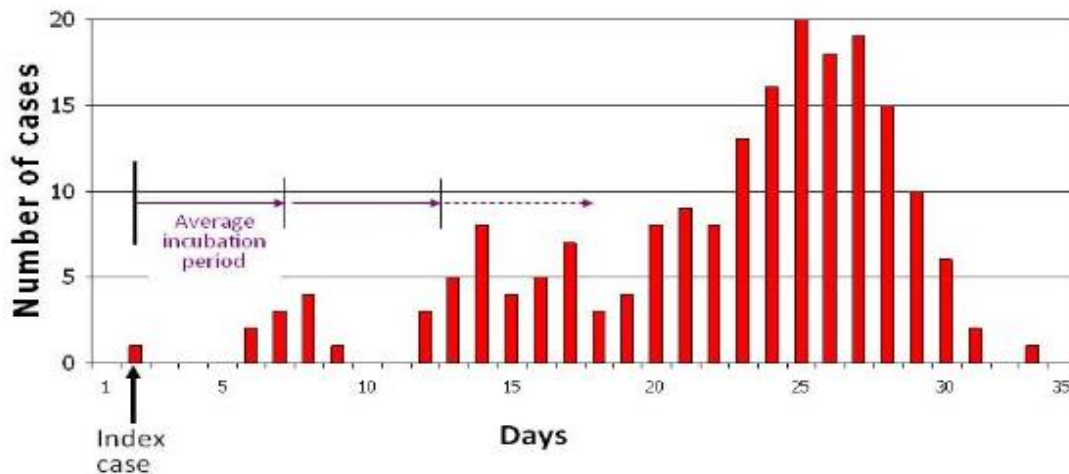
Continuing Source outbreak



A propagated or progressive source outbreak is suggestive of person-to-person transmission, whereby a single infected case (index case) infects a group of others (initial wave), who then go on to infect others (secondary wave), etc. The curve presents as a series of progressively taller peaks, with peaks typically occurring one incubation period apart.

https://www.med.uottawa.ca/sim/data/Pub_Infectious_e.htm#epi_curves

Disseminated outbreak originating from an index case with propagated spread



The epidemiologist creates a line list of illness onset dates for WGS-related *S. Enteritidis* cases.

Question 9: Draw an epidemic curve, using the information provided below. Use page 10.

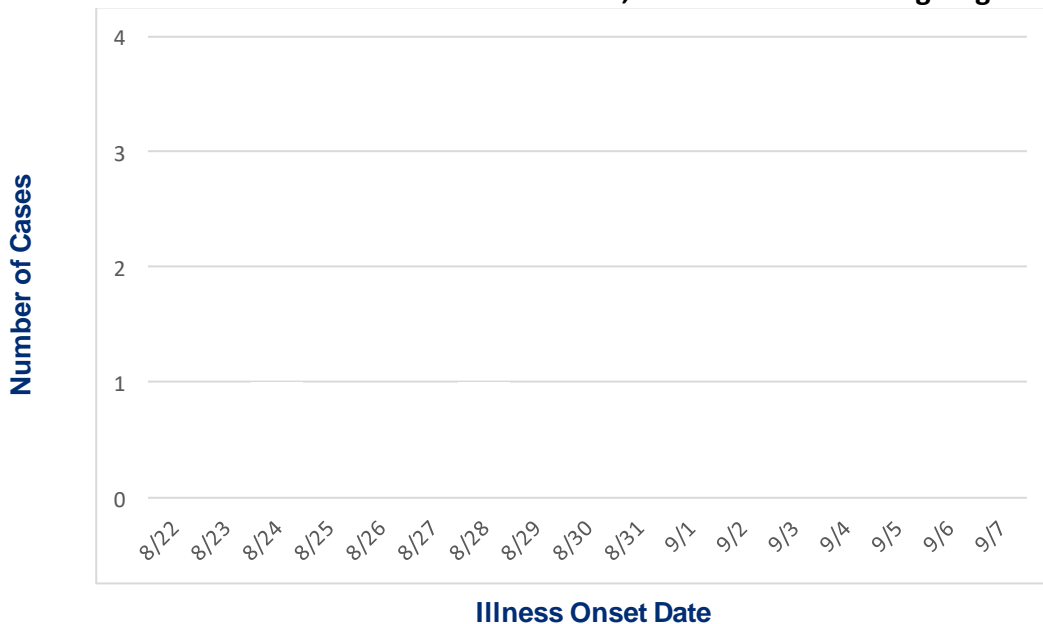
Case ID	Onset Date
1	8/24/2017
2	9/1/2017
3	8/30/2017
4	9/5/2017
5	9/5/2017
6	8/28/2017
7	9/1/2017
8	9/5/2017
9	9/3/2017

Hints for creating epidemic curves:

- The epidemic curve is a histogram showing the number of outbreak associated cases by their time of onset.
- Should include a brief, but descriptive title (including place and time)
- The x/y axis should be clearly labeled
 - The x-axis represents the date or time of illness onset among cases; the unit of time is usually 1/4 to 1/3 of median incubation period.
 - The y-axis shows the number of ill cases

Note: The typical incubation period for Salmonella is 2-3 days

Illness Onset for *S. Enteritidis* Cases, within 0 alleles during August – September 2017



Question 10: What does the epidemic curve tell us about the transmission vehicle?

This epidemic curve is most consistent with a continuous common source outbreak. Cases do not all occur within one incubation period, thus decreasing the likelihood of a point source outbreak. The epidemic curve suggests that our cases were exposed to the same source, but exposure was prolonged over a period of days, weeks, or longer (e.g., a shelf-stable food that was available to consumers over weeks and months). Cases will continue to be identified until the source of the outbreak is no longer available to the public.

The epidemiologist next decides to examine demographic characteristics of cases, such as gender, age, and race/ethnicity, for possible clues as to what exposure is causing the illness cluster. Strong demographic characteristics of a cluster may suggest a particular food vehicle because certain segments of the population are more likely to eat certain foods; in accordance with religious practices, cultural traditions, or preferences by age and/or gender. For example, if the cluster cases are predominately adult females, this could potentially suggest a produce item like leafy greens or sprouts as the source, since adult females have been documented to consume these products more than the general population.

The population of Empire County is approximately 220,000. The racial makeup of the county is 92% White, 4% Asian, 3% Black or African American, and 1% from other races. Two percent of the population is Hispanic or Latino of any race. 22.7% of the county's population is under age 18 and the median age is 40.9 years.

Case questionnaires are utilized to complete the chart below, detailing the demographic characteristics of the first 9 cases of the cluster.

Case	County	Age (yrs)	Gender	Race/Ethnicity
1	Empire	5	M	White, Not Hispanic or Latino
2	Empire	16	F	White, Not Hispanic or Latino
3	Apple	58	F	White, Not Hispanic or Latino
4	Empire	24	F	White, Not Hispanic or Latino
5	Empire	94	F	White, Not Hispanic or Latino
6	Empire	49	M	White, Not Hispanic or Latino
7	Empire	29	F	Asian, Not Hispanic or Latino
8	Empire	54	M	White, Not Hispanic or Latino
9	Empire	20	M	White, Not Hispanic or Latino

Question 11: Analyze the information in the table and summarize. Are there any noteworthy characteristics?

Upon analysis, the epidemiologist doesn't identify any noteworthy items. Most of the cases are white (89%) and none self-identify as Hispanic or Latino. Cases are nearly split between male and female, with females comprising 56% (5/9) of cases. Age range is 5 years to 94 years, with a median of 29 years; most cases are adults. However, since Empire and Apple Counties are neighboring counties, the epidemiologist is interested to know whether the single Apple County case travelled to Empire County during their incubation period.

To determine next steps, a conference call is held among epidemiologists and environmental health specialists from Empire County, Apple County and LBHD. The epidemiologists decide that all closely related cases would be re-interviewed with a standardized *Salmonella* National Hypothesis Generating Questionnaire (NHGQ) to obtain more comprehensive exposure information regarding food exposures, travel, water exposures, and animal exposures. This questionnaire can be found by reaching out to LBHD. Going forward, any newly identified closely related cases would be immediately interviewed with the NHGQ. Team members all agree that public notification about the illnesses is not warranted at this time.

Question 12: Do you agree or disagree about the decision not to notify the public at this time? Why?

Question 13: What are tasks the local health jurisdiction might complete? What are the tasks the counties might ask LBHD to complete?

All NHGQs will be shared with LBHD to assist with comparison analysis. LBHD will reach out to LBPHL to request prioritization of testing for *Salmonella* isolates from Empire and Apple Counties. In addition, Chain W uses loyalty/shopper cards and LBHD has interest in reviewing shopper card history data from cases.

Question 14: Describe the process by which LBHD will attain shopper card histories from cases. How and what information will be collected? What time period will you look at? What agencies will you partner with?

All cases (or their proxy) will be asked to provide a signed shopper card release for Chain W to obtain the food purchase history for the 3-month period prior to illness onset. Chain W is regulated by LB Department of Agriculture (LBDA). As such, LBHD (environmental health) and LBDA will work with Chain W to collect shopper card histories of cases with signed consent forms. Purchase histories are subsequently shared with LBHD (epidemiology) for analysis of common purchases.

Note: It is standard practice at LBHD to obtain food purchase history for the 3-month period prior to illness onset when there is no hypothesis developed for the specific source of illness. A 3-month food purchase history provides an adequate time period to review purchase data for perishable or shelf-stable food commonalities.

Chain W's corporate office was notified that LBHD is investigating a *Salmonella* outbreak where the only common exposure reported among cases was shopping at Chain W. Purchase histories were gathered more quickly after Chain W agreed to suspend their standard policy requiring a signed consent form in favor of accepting verbal consent to release purchase history. Rather than the consent process taking multiple days as forms were mailed to cardholders, signed, and mailed back to the local health jurisdiction, consent could now be obtained by health department staff over the phone during case interviews. This allowed for purchase histories to be collected and analyzed, and common exposures identified, in a much shorter time frame. This is an example of industry and public health cooperating to solve an emergent issue.

PART C: THE CLUSTER GROWS...again

On September 30, the LB State Lab reports 8 additional *S. Enteritidis* infections within 0-2 alleles of the previous cases; 6 from Empire County, 1 from Adirondack County, and 1 from Hudson County. The epidemiologist prepares a line list of the new cases, based on information collected from initial case report forms

Case ID	Onset Date	Age (years)	Sex	Grocery Store Chains	Restaurant Exposures	Tomatoes	Eggs	Leafy Greens	Grapes	Chicken	Ground Beef	Milk	Peanut Butter	Animal Exposures	Notes
26028	9/12	10	MALE	W, A	Café A, Fast Food A, Fast Food B	N	Y	N	N	Y	Y	Y	Y	Cat	Attended Adirondack County Fair
26908	9/14	16	FEMALE	W	Fast Food C	Y	N	Y	N	Y	N	Y	N	none	
27007	9/14	58	FEMALE	W	Deli A, Diner A	Y	N	Y	Y	N	Y	Y	N	Dog, Cat	Attended Empire County Fair
28060	9/17	29	MALE												Lost to follow up; no interview
28201	9/22	52	MALE	W	none	Y	Y	N	N	Y	Y	N	N	none	
30510	9/10	50	FEMALE	W, A	Café B, Fast Food A, Fast Food C	Y	N	Y	N	Y	N	N	N	Dog	
30890	9/18	46	FEMALE	P, B	Fast Food D, Deli B	Y	N	Y	Y	Y	N	N	Y	Dog	Husband also ill; onset 9/16
31532	9/5	23	FEMALE	W, B	Café C	Y	Y	Y	Y	N	N	Y	Y	Dog, Turtle	Vegetarian diet

Question 15: Analyze the data in the above table. Provide a summary of noteworthy exposures.

Analysis of the 8 cases from initial case forms indicates that one case was lost to follow-up.

Exposure commonalities among 50% or more of the 7 interviewed cases are as follows:

- 6/7: shop at Chain W (various locations), tomatoes
- 5/7: leafy greens, chicken
- 4/7: milk, contact with dogs

In addition, 1 case attended the annual County Fair in Empire County and 1 case attended the annual County Fair in Adirondack County.

LBHD notifies each local health jurisdiction of the new matches and requests the cases be interviewed with the NHGQ, and obtain verbal consent for shopper card releases from Chain W.

Question 16: What are you suspecting as a possible source? Why?

On October 3, the LBPHL notifies LBHD that ongoing lab surveillance has just identified additional *S. Enteritidis* cases from nearby states that are closely related by WGS, and within 0-2 alleles. Isolate dates range from September 9-16. Four cases reside in State X and three cases reside in State Y.

LBHD alerts all the counties with cases in LB that this is now considered a multi-state cluster. Follow-up by LBHD with States X and Y will be conducted immediately to find out what they

know about their cases (exposure histories) and to determine if there are any commonalities among the cases in the three states.

Question 17: What information would you like to know about these new closely related cases from State X and State Y?

LBHD contacts State X and State Y to collect demographic information (age, sex, race/ethnicity), clinical details (illness onset date, if hospitalized, recovery status), and all available exposure information regarding food, water, travel, and animal exposures for the closely related cases. LBHD determines that Chain W has locations in both states, but not as many as in LB State. Of utmost interest is whether the out-of-state cases shopped at Chain W, attended any fairs/festivals/events, or work(ed) in/had travel to LB.

After following up with the states, LB State Health learns that 2/3 cases from State X and all three cases from State Y shopped at Chain W, which is, based on the experience of epidemiologists in States X and Y, an uncommonly frequented chain in both states. Of note, one case travelled 1.5-2 hours to shop at the closest location of Chain W. The two cases from State X also attended a county fair in their own county. No cases from State X or Y travelled to LB State. LB State Health requests that State X and State Y obtain and share shopper card releases from Chain W from cases.

Question 18: Would you expand your investigation team to include federal partners? If yes, who would you include/not include and what information would you share at this point?

LBHD sends an email to the Foodborne Illness Outbreak team at CDC. To date, LB State has identified 17 cases of *S. Enteritidis*, within 0-2 alleles. Isolate dates range from 8/25 to 9/18. Age range is 5-94 years, with a median of 31 years. Nine of 17 cases are female (53%). Nearly all cases report race/ethnicity as white and not Hispanic. Two of 17 cases were hospitalized, and no deaths have been reported. In addition, 7 out-of-state cases have been identified with isolate dates from 9/9 to 9/16 that are closely related by WGS, within 0-2 alleles.

There is no unique association among the LB State cases, aside from the fact that almost all shop at a very common grocery store chain (Chain W) in the area (at a variety of different locations). However, the fact that most of the out-of-state cases (5/6) interviewed reported shopping at Chain W, an uncommonly frequented chain in those states, suggests that exposure to a product sold at Chain W (various locations) may be causing illness among cases.

The epidemiologist summarizes the action steps underway: re-interviewing all cases with the NHGQ, obtaining signed shopper card releases from cases (or their household proxy) to obtain their food purchase history from Chain W for the 3-month period prior to illness onset, and analyzing questionnaires and shopper card data to identify commonalities among cases.

PART D: SHOPPER CARD REVIEW

As of October 11, LBHD had received Chain W shopper card histories for 6 cases. Upon analysis, shopper card histories revealed that 3 food items were purchased among >50% of cases:

- 6/6 tomatoes
- 5/6 avocados
- 5/6 Turkish pine nuts

Question 19: List all the agencies who LBHD would contact to share these findings.

To share the findings from shopper card review, LBHD notifies all the agencies involved in the investigation including, Empire, Apple, Adirondack, and Hudson County Health Departments; State X and State Y Health Departments; and CDC. LBHD also determines that LBDA should be contacted at this point because, for the first time, exposure analysis has identified products of

note sold in facilities they regulate. On October 13, a call is held among CDC, LBHD, LBDA and all involved state health departments. Shopper card review and information analyzed from the NHGQ seems to suggest a “healthy” food signal of nuts, fruits, and vegetables. As such, the team decides it is time to deploy an outbreak questionnaire focused on produce and nut exposures. LBHD and CDC work together to design and finalize an outbreak-specific questionnaire. CDC requests that all states re-interview past cases with the outbreak-specific questionnaire and, going forward, only use the outbreak-specific questionnaire when interviewing new closely related cases. The team also decides that shopper card data be collected from new cases if they shopped at Chain W

Question 20: Would it be beneficial to contact Chain W to discuss investigation findings? Should the USDA or FDA be contacted at this time?

The team decides it would be beneficial to contact Chain W to discuss investigation findings and next steps and to notify the FDA (FDA regulates vegetables, fruits and nuts which was the leading hypothesis as source of exposure at this point in the investigation). The next day, October 14, a call was held among Chain W, LBHD, LBDA, State X and State Y Health Departments, CDC, and FDA.

Question 21: What information should be shared with Chain W? What information wouldn't you share?

LBHD provides Chain W with a national overview of the ongoing investigation including case count and descriptive epi data (person, place, time). Particularly emphasized were the current food exposures of interest, tomatoes, avocados, and Turkish pine nuts. These findings are based on the analysis of shopper card receipt data as well as information from case interviews. Identifiable information, such as names of ill persons or specifics on individual cases, was not shared on the call.

Question 22: What information would the outbreak team want to gather from Chain W?

Chain W is asked to share general information about the common products identified among cases from interviews and/or shopper card histories, including tomatoes, Turkish pine nuts, and avocados. Chain W is asked to provide invoices dating back 3 months to conduct traceback activities (source of products, brand name, shipment records) to understand the distribution pattern of tomatoes, Turkish pine nuts, and avocados. The investigation team is interested to know whether these products are used as “stealth” ingredients (used as ingredients in other products made/sold at Chain W), for example, tomatoes in pre-made salsa. Information on consumer interest in these products (from inventory records), as well as any customer illness complaints received to the store related to these products is also requested. The investigation team also inquires as to whether any of these products had the potential to be distributed to all the Chain W locations cited by cases. Chain W is cooperative and agrees to work on gathering the requested information and submitting it as soon as possible

In the meantime, investigation by the epidemiologist continues. While shopper card data suggests that most of cases purchased tomatoes, Turkish pine nuts, and avocados from Chain W, it is important to identify the statistical significance of these exposures.

Question 23: What method(s) could you use to determine statistical significance?

Some type of analytic study is appropriate to quantify the significance of the food items in question, and therefore validate 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 used. However, such a study can be extremely labor intensive and time consuming. A less resource intensive-approach would be to conduct a case-case comparison study in which exposures from the cases of interest are compared to the exposures from another group of cases to identify sources of illness or risk factors. But there is one more type of analytic study to consider, that is even less labor and time intensive - the CDC Foodborne Diseases Active Surveillance Network Population (FoodNet Pop) Survey and Binomial Probability calculation.

The epidemiologist decides that the quickest and most efficient way to determine whether any of the food items are significant is to use the FoodNet Pop Survey (2006-2007) and Binomial Probability calculation (<https://www.cdc.gov/foodnet/PDFs/FNExpAtl03022011.pdf>) and (<http://www.oregon.gov/oha/ph/DiseasesConditions/CommunicableDisease/Outbreaks/Gastroenteritis/Pages/Outbreak-Investigation-Tools.aspx#binomial>). The FoodNet Pop survey provides background consumption rates of food, based on geographic location. The binomial model compares case consumption rates against estimated background consumption rates. Alternatively, you could consider using CDC's FoodNet Fast's Population Survey Tool to determine background consumption rates of food; data is derived from FoodNet's 2018–2019 Population Survey (<https://www.cdc.gov/foodnet/foodnet-fast.html>).

PART E: BINOMIAL ANALYSIS

To assess the statistical significance of the food items of interest, the epidemiologist will perform binomial analysis of tomatoes, avocados, and Turkish pine nuts. To date, there are 17 outbreak-related cases in LB, and the analyses will only include these cases.

1. Analysis for tomatoes

First the model is run for tomatoes. Information about fresh tomato exposure was available for 14 of 17 cases, either from shopper card data or interviews. Shopper card data confirmed that **6/6** cases bought fresh tomatoes from various Chain W locations. Of the remaining 11 cases, 8 were interviewed using the NHGQ, with **4/8** reporting tomato consumption. In total, 10 of 14 (**71%**) cases were exposed to fresh tomatoes.

Using the FoodNet Pop Survey, the epidemiologist determines the estimated background consumption rate of store-bought fresh tomatoes in LB State is 57%.

Fresh Vegetables Consumed in the Past 7 days, by FoodNet Site																		
Exposure	California N=564		Colorado N=904		Connecticut N=915		Georgia N=931		Liberty N=933		Maryland N=929		Minnesota N=928		New Mexico N=904		Total N=7,008	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Any store-bought tomatoes	365	64.7	581	64.3	583	63.7	535	57.5	532	57.0	530	57.1	458	49.4	532	57.0	4116	58.7

Next, we enter this estimated background consumption rate (57%) into the binomial model on the next page, with a sample size of 14. This worksheet calculates cumulative binomial probabilities and can be used to estimate how likely it is that one would get the observed number of cases reporting an exposure given a known or estimated background exposure rate. This may help one assess the potential utility of a case-control study.

Note: There are only 2 variables, which should be entered into the yellow boxes (far left): the sample size (number of cases with fresh tomato exposure data) and the background exposure rate. Binomial probabilities for the number of cases with the observed exposure are obtained from the green column (far right).

	<i># cases with exposure</i>	<i>% cases with exposure</i>	<i>Cumulative Probability</i>
	14	100%	0.0004
sample size	13	93%	0.0044
14	12	86%	0.0242
	11	79%	0.0839
	10	71%	0.2078
background rate	9	64%	0.3948
57.0%	8	57%	0.6063
	7	50%	0.7887
	6	43%	0.9090
	5	36%	0.9696
	4	29%	0.9924
	3	21%	0.9987
	2	14%	0.9999
	1	7%	1.0000
	0	0%	1.0000

Question 24: 10 of 14 (71%) cases reported tomato exposure (by way of interview and/or shopper card receipt data), but is that a suspiciously high proportion? Interpret the results of the binomial survey for tomatoes.

The epidemiologist plugs in the sample size [14] and the background rate of tomato consumption in LB (57%) and determines that the probability of getting 10 tomato eaters in a sample of 14 independent cases due to chance alone is 0.2078 (21%). Typically, a chance of 0.05 (5%) or less is considered statistically significant and would require additional follow-up. With a 21% chance, tomatoes are unlikely the exposure of interest that caused the outbreak. The epidemiologist decides to next run the model for avocado exposure to see how that compares.

2. Analysis for avocados

Information about avocado exposure was available for 14 of 17 cases either from shopper card data or interviews. Shoppercard data confirmed that **5/6** cases purchased avocados from various Chain W locations. Of the remaining 11 cases, 8 have been interviewed with the NHGQ, with **3/8** reporting avocado consumption. In total, 8 of 14 (**57%**) of cases were exposed to avocados.

Using the FoodNet Pop Survey, we find the estimated background consumption rate of avocados in LB State is 9.3%.

Fresh Vegetables Consumed in the Past 7 days, by FoodNet Site																		
	California N=564		Colorado N=904		Connecticut N=915		Georgia N=931		Liberty N=933		Maryland N=929		Minnesota N=928		New Mexico N=904		Total N=7,008	
Exposure	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Avocado (or guacamole)	306	54.3	414	45.8	167	18.3	146	15.7	87	9.3	163	17.5	149	16.1	470	52.0	1902	27.1

Question 25: Enter sample size and the estimated background consumption rate of avocados in LB into the chart below.

	<i># cases with exposure</i>	<i>% cases with exposure</i>	<i>Cumulative Probability</i>
	14	100%	0.0000
sample size	13	93%	0.0000
14	12	86%	0.0000
	11	79%	0.0000
	10	71%	0.0000
background rate	9	64%	0.0000
9.3%	8	57%	0.0000
	7	50%	0.0001
	6	43%	0.0010
	5	36%	0.0068
	4	29%	0.0350
	3	21%	0.1351
	2	14%	0.3790
	1	7%	0.7450
	0	0%	1.0000

Question 26: Eight of 14 (57%) cases reported avocado exposure (by way of interview and/or shopper card receipt data) from various Chain W locations, but is that a suspiciously high proportion? Based on the number of cases with exposure, interpret the results of the binomial survey for avocados.

The epidemiologist plugs in the sample size [14] and the background rate of avocado consumption in LB (9.3%) and determines that the probability of getting 8 or more avocado eaters in a sample of 14 independent cases due to chance alone is 0.0 (0%). Could avocados be the cause of this outbreak? More information will need to be gathered to investigate this hypothesis. But first, the epidemiologist will run the final model for pine nut exposure to see how that compares.

3. Analysis for Turkish pine nuts

Information about Turkish pine nuts was available for 6 of 17 cases from shopper card data. Shopper card data confirmed that 5/6 cases bought Turkish pine nuts from various Chain W locations. Cases were not asked about their exposure to Turkish pine nuts at initial or NHGQ interviews. The epidemiologist searches the FoodNet Pop survey, but Turkish pine nut exposure is not listed in the survey. Rather than forgo the calculation altogether, the epidemiologist decides to use peanut consumption as a proxy for Turkish pine nut consumption. The true background consumption rate of Turkish pine nuts is likely lower, but better to be conservative and err on the high side when using background consumption estimates in this manner. The estimated background consumption rate of peanuts in LB is 32.5%.

Fresh Vegetables Consumed in the Past 7 days, by FoodNet Site																		
Exposure	California N=564		Colorado N=904		Connecticut N=915		Georgia N=931		Liberty N=933		Maryland N=929		Minnesota N=928		New Mexico N=904		Total N=7,008	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Peanuts (loose or in shell)	210	37.2	357	39.5	328	35.8	347	37.3	303	32.5	318	34.2	413	44.5	333	32.5	2609	37.2

Question 27: Enter sample size and the estimated background consumption rate of peanuts in LB into the chart below.

	<i># cases with exposure</i>	<i>% cases with exposure</i>	<i>Cumulative Probability</i>
	6	100%	0.0012
sample size	5	83%	0.0159
6	4	67%	0.0921
	3	50%	0.3033
	2	33%	0.6322
background rate	1	17%	0.9054
32.5%	0	0%	1.0000

Question 28: Five of 6 (83%) cases purchased Turkish pine nuts at various Chain W locations, but is that a suspiciously high proportion? Interpret the results of the binomial survey for peanuts (as a proxy for Turkish pine nuts).

The epidemiologist plugs in the sample size [6] and the background rate of peanut (as a proxy for Turkish pine nut) consumption in LB (32.5%) and determines that the probability of getting 5 or more peanut eaters in a sample of 6 independent cases due to chance alone is 0.0159 (1.5%). Could Turkish pine nuts be the cause of this outbreak? More information will need to be gathered to investigate this hypothesis.

Question 29: Based on FoodNet Pop Survey/binomial analysis results, do you have a food exposure of interest?

Based on FoodNet Pop Survey/binomial analysis results it is determined that consumption of tomatoes is not a statistically significant exposure. Consumption of avocados and Turkish pine nuts, however, are a significant exposure related to illness, as the frequency of these exposures was above the expected baseline consumption rate of the general population.

PART F: WORKING WITH INDUSTRY & PRODUCT SAMPLING

On October 16, a call is held among LBHD, LBPHL, LBDA, and Chain W. On a call with Chain W two days prior, LBHD had asked Chain W to gather consumer and traceback information about tomatoes, Turkish pine nuts, and avocados. LBHD now informs Chain W that they are most interested to hear about this information for avocados and Turkish pine nuts. These are not necessarily the source of illness, but epidemiologic analysis has identified these products as statistically significant commonalities. The team needs more information to continue the investigation. Chain W performed analysis of shopper card histories and reviewed distribution patterns for tomatoes, avocados and Turkish pine nuts received at store locations. Per Chain W,

produce items received at their store locations (including products like tomatoes and avocados) were distributed not only to Chain W establishments, but to other commercial grocery establishments/chains around the state. In contrast, Chain W identified a unique distribution pattern regarding Turkish pine nuts. Chain W received Turkish pine nuts in bulk exclusively from Distributor T. Furthermore, it was feasible that shipments of the same lot of Turkish pine nuts were distributed to all the Chain W locations reported by cases. This is noteworthy to the investigation team since illness was only seen among persons who reported to shop at Chain W and no other grocery store establishments/chains. At the end of the call, it is decided that the collection of product samples for testing and analysis would be beneficial to the investigation.

Question 30: From what locations should you collect samples?

On October 17, LBDA collected bulk Turkish pine nuts for testing at their laboratory from a single Chain W establishment which had been reported as a common shopping location for eight cases. They also collected bulk Turkish pine nuts for testing from two additional Chain W locations cited by cases. LBDA also investigated whether any products prepared and sold at Chain W used Turkish pine nuts as an ingredient. Invoices for Turkish pine nuts were collected by LBDA and provided to the FDA. Since multiple states are involved in this investigation, traceback efforts for the Turkish pine nuts will be coordinated by the FDA.

To further sampling efforts, the epidemiology team is asked to review outbreak questionnaires to identify any cases who reported Turkish pine nut consumption and acknowledged availability of product leftovers at their home. Availability of product leftovers was asked for all exposures on the outbreak questionnaire. Review of interviews by the epidemiologist identified multiple product leftovers available for testing:

- 2 cases from Empire County had homemade pesto made with bulk Turkish pine nuts purchased at 2 separate Chain W locations (Location A, Location B)
- 1 case from Hudson County had whole bulk Turkish pine nuts purchased from a third Chain W location (Location C)

LBHD, in coordination with the FDA, contact the involved local health jurisdictions serving Empire and Hudson counties to arrange for the collection of product leftovers from cases' homes for testing at the LBPHL. All food samples submitted to the LBPHL are tested for the presence of *Salmonella* by culture and molecular methods.

On October 18, a conference call is held with LBHD, LBDA, CDC, FDA, other involved state health departments, and Chain W. There are now 30 closely related cases from 6 states associated with this cluster. Illness onset dates range from 8/24 through 10/2. All cases live in states with

Chain W locations. Shopper card histories were received from 23 of 30 (77%) cases; 13/23 (57%) cases indicated that bulk Turkish pine nuts had been purchased from Chain W sometime during the 3 months prior to illness onset. Additionally, 17/26 (65%) cases who completed the outbreak-specific questionnaire indicated exposure to bulk Turkish pine nuts purchased at Chain W.

Question 31: Not all purchase histories and/or questionnaires of ill cases indicated exposure to Turkish pine nuts. What may be some explanations as to why?

A variety of explanations could support why not all purchase histories indicated exposure to the product, including: the case bought Turkish pine nuts without using a shopper card; someone else purchased the Turkish pine nuts for the case; case used someone else's shopper card to purchase the Turkish pine nuts; exposure to pine nuts may have occurred outside the home and unknown to the case; pine nuts could have been used as a stealth ingredient in a meal; or the case may have poor exposure recall. When questioned by the outbreak team, Chain W reported that <1% of their customers purchased Turkish pine nuts. It was also learned that various foods prepared and sold at Chain W stores contained bulk Turkish pine nuts, which may not have been known or mentioned by cases during interview. Shopper card histories were not previously analyzed for these prepared products. LBHD is willing to conduct further analysis, if requested.

Based on the information relayed on the call, Chain W decides to voluntarily cease sales of both bulk Turkish pine nuts and premade products that contain Turkish pine nuts from their stores, pending further investigation.

Question 32: Do you think this represents an outbreak or a cluster? Provide an explanation for your choice.

The investigation team determines that this cluster of illness now constitutes an outbreak, most likely related to Turkish pine nut exposure from Chain W. Although there are no positive product laboratory results to date, the epidemiology and environmental health investigations

identify sufficient evidence to classify this cluster as an outbreak. In collaboration with CDC, the team agrees upon a multi-state outbreak case definition.

Question 33: Develop a case definition for this outbreak.

Note: A case definition is a standard set of criteria used to classify ill people as being cases associated with an outbreak. Criteria include clinical findings (signs/symptoms and/or laboratory results) and is restricted by time, place, and person.

For this outbreak, a case was defined as a person with *S. Enteritidis* infection, with illness onset date after August 20th, 2017, with closely related isolates by WGS, within 0-2 alleles.

Question 34: Should we have included exposure to Chain W or Turkish pine nuts in the case definition? Why, or why not?

Never include the suspect source of the outbreak in your case definition. Instead, possible exposures/vehicles that caused the disease should be described as part of your hypotheses. Hypotheses for this outbreak will include exposure to Chain W and/or Turkish pine nuts.

Admittedly, the outbreak investigation team does not know anything about how pine nuts are harvested. The team would like to understand how contamination of pine nuts may occur and if their hypothesis is plausible. So, they did a little research...

The team learned that Turkish pine nuts are harvested by shaking pine nuts out of pinecones. The process occurs by:

- Picking the pinecones from the tree
- Placing the pinecones into a burlap sack
- Placing the bag of pinecones in the sun for 3-4 days to dry out and subsequently, open and release their seeds
- Turning bag over every day to evenly dry
- Checking the bag to determine if the pinecones are dry and open; and if not, allowing them to dry for another two days

- Once the pinecones are open, holding the bag closed and shaking it to dislodge pine nuts from the pinecones
- Picking the pine nuts out of the bag and placing them onto a screen
- Shaking the screen to gently clean the pine nuts
- Removing the pine nut shells by breaking them off with your fingers

Question 35: How might contamination to Turkish pine nuts occur during the harvesting process?

During the harvesting process, contamination to Turkish pine nuts may occur if the pinecones fall on the ground and pick up contamination from the soil or, when left drying in the sun in a bag, are accessed and contaminated by animals. If pine nuts are handled by ill persons during the harvesting process, they may contaminate them with their hands or through the use of contaminated bags/screens.

PART G: LABORATORY RESULTS & ENVIRONMENTAL TRACEBACK

On October 26th, the investigation team was notified by LBPHL that the 3 food samples (2 homemade pestos, 1 bulk pine nuts) collected from three separate case-patient homes contained *S. Enteritidis*, with outbreak strains closely related to the human isolates, within 0-2 alleles. State X Health Department also reported leftover pine nuts collected from a case's home and pine nuts collected from a Chain W store in their state were both closely related to the human isolates, within 0-2 alleles. LBDA laboratory did not isolate *S. Enteritidis* from whole pine nut samples collected from the 3 Chain W locations.

LBDA coordinates with Chain W to review invoices for bulk Turkish pine nuts received at Chain W locations. LBDA confirms that bulk Turkish pine nuts were purchased from Distributor T, located in LB. Distributor T was the only distributor supplying Turkish pine nuts to Chain W. All information was provided to the FDA. The FDA and LBDA collect Turkish pine nut samples from Distributor T for testing. Additionally, FDA conducts a traceback study, linking the Turkish pine nuts to a single out-of-state importer, Importer V. FDA visits Importer V and collects pine nut samples for testing.

Question 36: Based on the epidemiological, environmental health and laboratory information received to date, what public health response actions would be appropriate to take at this point?

On October 27th, CDC issues a public health notification on their website about the outbreak. Chain W recalls approximately 5,000 pounds of potentially *Salmonella*-contaminated, bulk Turkish pine nuts sold between July 1st and October 18th from their stores. Affected State and local health jurisdictions issue press releases for local media to access. Education at pine nut harvest locations is provided to prevent similar outbreaks in the future.

On November 9, FDA reports that *S. Enteritidis* outbreak strain was isolated from Turkish pine nut samples collected from two warehouses (Distributor T and a Distributor T customer) and Turkish pine nut samples collected from Importer V.

PART H: OUTBREAK SUMMARY

In the aftermath of the outbreak, the investigation team prepares a final report on the investigation.

Question 37: What are the key components of a final outbreak report?

The final report included the following key components: Context/background; Investigation methods; Investigation findings/results; Discussion/conclusion; Recommendations; and Key investigators.

At final report, this outbreak of *S. Enteritidis* consisted of 53 cases from 6 states. Illness onset dates ranged from August 20 to December 29, 2017. The median age of patients was 43 years (range: <1-94 years); 64% were female. Two hospitalized cases and no deaths were reported. Case interviews and shopper card records identified 35/40 (87.5%) cases were exposed to Turkish pine nuts from Chain W. Traceback studies identified a single out-of-state importer as

the source of Turkish pine nuts to Chain W. The outbreak strain was isolated from Turkish pine nuts, and products containing Turkish pine nuts, collected from case homes, retail locations, and warehouses. In total, over 21,000 pounds of Turkish pine nuts were voluntarily recalled from Distributor T. Several lines of evidence associated the cases with exposure to *S. Enteritidis* contaminated bulk Turkish pine nuts from a single importer:

1. The outbreaks in all 6 states were clustered in time
2. All case isolates (in all states) were in the range of 0-2 alleles, making them closely related by WGS.
3. Turkish pine nuts sold in bulk from Chain W was found to be a common exposure among all investigations
4. *S. Enteritidis* isolates from leftover food products collected from cases' homes in 4 different states, retail samples of bulk Turkish pine nuts collected from Chain W in 2 different states, samples from Distributor T and samples from Importer V were in the range of 0-2 alleles, making them closely related by WGS and matching to the outbreak strain

This case study was adapted from an actual *Salmonella* investigation initiated by the New York State Department of Health in 2011, heralding a multistate outbreak. It was the first *Salmonella* outbreak in the U.S. associated with pine nuts

Obtaining shopper card information and engaging industry proved essential throughout this cluster investigation. While investigating the source of this outbreak, collection of Chain W shopper card purchases allowed investigators to narrow down the list of potential exposures and develop an outbreak specific questionnaire which eventually led to a hypothesis that exposure to Turkish pine nuts was associated with illness.

Product recalls likely prevented additional illnesses. As evidenced in this outbreak investigation, early identification of patient exposures can assist in determining a common source of infection so that effective control efforts can be implemented.