

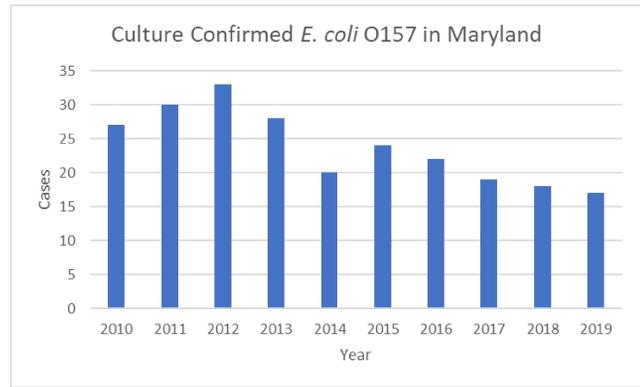
# E. coli O157 Outbreak Associated with Pre-Packaged Romaine Salad Bowls – Maryland 2019



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## Introduction

Shiga toxin-producing *E. coli* (STEC) infections are nationally notifiable. *E. coli* O157 is a type of STEC that can sometimes lead to a serious complication called hemolytic uremic syndrome (HUS). People can become infected with STEC through consumption of contaminated food or water, contact with animals, or from person-to-person contact with other ill people. In recent years, multiple *E. coli* O157 outbreaks have been associated with leafy greens, leading to nationwide recalls and market withdrawals. In Maryland, there are on average 24 confirmed cases of *E. coli* O157 per year, with additional probable cases. All reported cases of STEC in Maryland are interviewed by local health department staff with a standard Gastroenteritis Case Report Form that collects clinical, demographic, laboratory, and exposure information.



Graph 1. Culture confirmed cases of *E. coli* O157 reported to MDH from 2010-2019.

On November 6, 2019, the Maryland Department of Health (MDH) was notified of 2 STEC cases from the same county that reported eating pre-packaged chicken Caesar salad bowls purchased from Warehouse Store A prior to illness onset. Ill family members of one confirmed case also consumed the salad bowls but were not tested. On November 7, 2019, a 3<sup>rd</sup> laboratory positive case with similar exposure was identified, and a cluster investigation was initiated.

## Methods

A supplemental leafy green questionnaire was quickly developed and sent to all local health departments in Maryland along with a memo requesting prioritization of STEC investigations. An Epi-X notification was sent to health departments nationwide looking for cases with similar exposures. Shoppers card histories were requested from Warehouse Store A to identify purchase dates and product codes. Invoices and other traceback documents were collected from Warehouse Store A by the State of Maryland Rapid Response Team (SMarRRT).

Clinical isolates were culture-confirmed, sequenced, and uploaded to PulseNet by the MDH lab. An intact product sample was collected by local health department staff from a case-patient home under chain of custody (Image 1), tested using FDA's Bacteriological Analytical Manual (BAM), sequenced, and shared with federal partners. The MDH lab compared whole genome sequencing for clinical and environmental isolates to determine relatedness.

### Epidemiologic Findings:

In total, there were 8 cases of STEC associated with leafy greens in MD, 6 confirmed cases and 2 probable cases. Probable cases were symptomatic, household contacts of a confirmed case. MD cases were part of a larger, multi-state outbreak of *E. coli* O157 associated with romaine lettuce from the Salinas Valley growing region in California.

Seven of eight case-patients (87.5%) reported eating pre-packaged salad bowls purchased from Warehouse Store A prior to onset. The case-patient that denied pre-packaged salad had multiple other leafy green and salad exposures prior to onset. Seventy-five percent (6/8) of cases were females. Ages ranged from 13 to 77 years of age with a median of 18 years. Two case-patients (25%) were hospitalized, there were no cases of HUS, and no deaths. All cases were interviewed with the MDH standard GE Case Report Form, and 5 cases were also interviewed with a leafy green specific questionnaire. Membership numbers for Warehouse Store A were collected for 5 households, representing the purchase histories for 7 cases.

Illness onsets ranged from October 25, 2019, to December 4, 2019. Seven (87.5%) cases reported an onset during the same one week period of October 25-21, 2019. All 7 of these cases reported exposure to the same brand of pre-packaged salad from Warehouse Store A. The case without exposure to pre-packaged salad bowls had the latest onset, and was included in the outbreak because it was closely related to the outbreak strain by WGS.



Image 1. Intact chicken Caesar salad bowl collected under chain of custody from case-patient home (a) (b). Best by, USDA establishment code, and growing region from product (c).

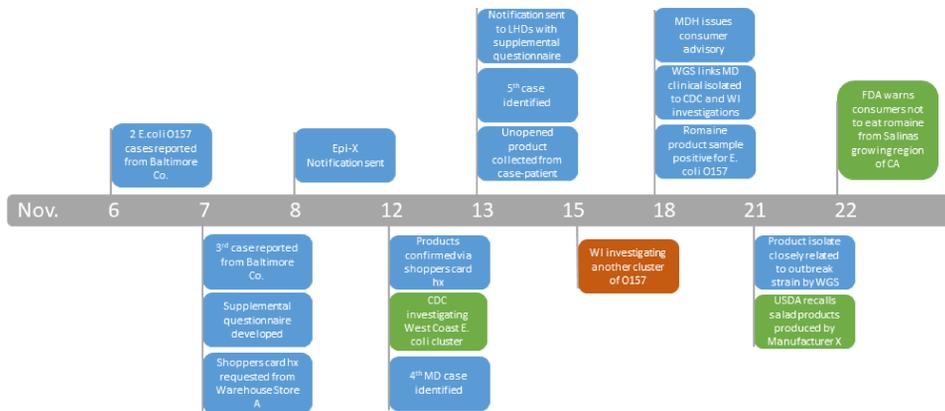


Figure 1. Timeline of MD outbreak investigation

## Results and Discussion

### Environmental Assessment Findings:

Warehouse Store A purchase histories were compared for 5 households. All 5 histories included purchases of Santa Fe Salad Bowls, and/or Caesar Salad Bowls. SMarRRT used the UPC codes to confirm that the products available at all stores reported by cases were in fact the same product. Both the Santa Fe Salad Bowls, and Caesar Salad Bowls came from the same firm, Manufacturer X, is regulated by both the FDA and USDA. Product information, including ingredient lists, and suppliers were obtained from Manufacturer X, and shared with federal partners (FDA, USDA), for further traceback. The romaine lettuce used in both types of salad bowls was highly suspect, based on historic outbreaks, and epidemiologic data. FDA continued traceback efforts to identify a single source of contamination of the romaine product.

A Maryland case-patient had an intact Chicken Caesar Salad Bowl remaining. SMarRRT conducted just-in-time training with local health department staff on chain-of-custody sample collection. The product was collected from case-patient's home and submitted to the MDH Lab for *E. coli* testing. Product pictures (Image 1), including lot codes, expiration dates, and establishment codes were collected and used for traceback.

### Laboratory Findings:

Clinical specimens from the 6 confirmed cases were received at MDH for confirmation and analysis. All 6 were identified by culture as *E. coli* O157. Shiga toxins were confirmed by EIA, Shiga toxin gene (*stx*) types were all *stx2a* by PCR, and WGS was performed. The MDH lab compared the sequences locally, and all were very closely related to each other. When compared to the PulseNet national database, they were closely related to isolates in another multi-state cluster being investigated by CDC.

The unopened Chicken Caesar Salad Bowl collected from a case-patient's home was purchased at the same time as another salad bowl consumed prior to the illness onset. The salad bowl consisted of 4 different components: romaine lettuce, Caesar salad dressing, chicken, and cheese. The components were tested separately to better identify the potential vehicle, except for chicken and cheese which could not be separately tested. On November 18, 2019, the romaine lettuce tested positive for *E. coli* O157. The other components were negative. The isolate from the romaine was sequenced by the MDH lab, and compared to clinical isolates in Maryland, and nationally (Fig. 2). The product sample was very closely related (0-1 alleles, 0-1 SNPs) to clinical isolates included in the outbreak.

### Consumer Alert & Recalls:

On November 18, 2019 MDH issued a consumer advisory urging Marylanders not to consume specific lots of pre-packaged salad bowls produced by Manufacturer X, corresponding with the positive product sample. By this time, the product was past the "Best By" date of October 31, 2019, but there was still concern that product might be in consumer's refrigerators. On November 21, 2019, USDA issued a recall for pre-packaged salad bowls from Manufacturer X produced during the same time period as the positive product sample. On November 22, FDA warned consumers not to eat romaine from the Salinas Growing Region.

## Conclusion

Romaine lettuce has increasingly been associated with large-scale outbreaks of *E. coli* O157, but pinpointing a specific growing region, let alone individual farm or ranch is extremely challenging. Collaboration with LHD to obtain brand information and shoppers card histories quickly provided an early indicator that romaine was a suspect vehicle and allowed MDH to start traceback. The product sample collected from a case-patient home included best by date and growing region which narrowed down the source of contamination and directed the FDA traceback and farm investigations. Epidemiologic, laboratory, and traceback data ultimately linked this outbreak to romaine lettuce from the Salinas Valley growing region of California. FoodNet surveillance and RRT infrastructure at MDH directly contributed to the speed at which we went from initial case report to actionable public health consumer alert issued by MDH, a USDA recall notice, and FDA's advisory not to consume romaine lettuce from the Salinas Valley growing region. These actions prevented further illness and stopped the outbreak. The multi-state outbreak of which this MD outbreak is part of included 172 culture confirmed cases of *E. coli* O157 from November 2019 to January 2020. There is still much to learn about these romaine-associated *E. coli* O157 outbreaks. Environmental exposures, such as proximity to feed lots, irrigation water contamination, and winds may all play a part. Traceability of romaine through the food system aids in these types of investigation, but a more robust system is still needed.

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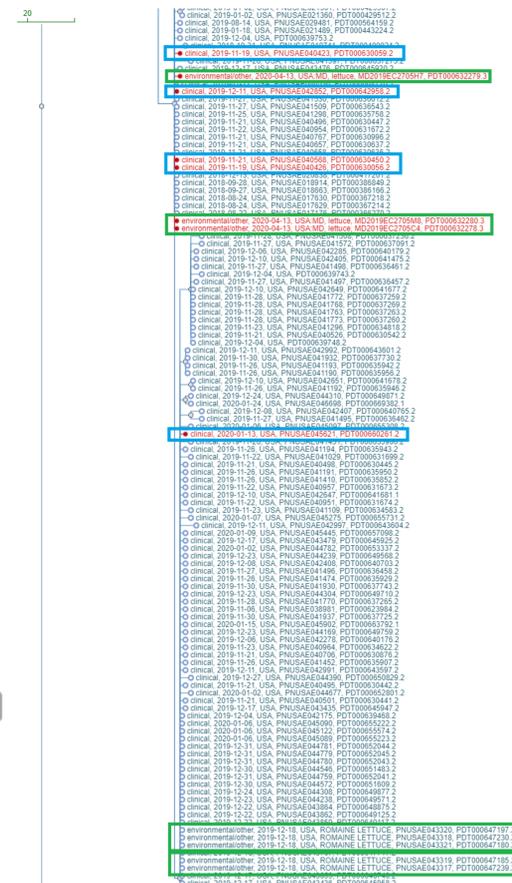


Figure 2. Partial WGS dendrogram from NCBI of multi-state outbreak. All isolates within 0-2 snps. MD isolates in red. Blue boxes are clinical isolates, green isolates are romaine samples (MD and WI product samples).