

Investigation Report:

Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated in the Three Outbreaks of *E. coli* O157:H7 During the Fall of 2019

Executive Summary

In November and December 2019 there were three *E. coli* O157:H7 foodborne illness outbreaks A (167 illnesses), B (11 illnesses), and C (10 illnesses) associated with consumption of romaine lettuce or leafy greens from the Salinas Valley area of California. FDA and multiple state and federal partners investigated these three foodborne illness outbreaks to identify any contributing factors that may have led to romaine lettuce contamination with *E. coli* O157:H7 and subsequent illnesses. During the course of these investigations, it was determined that:

- 1) each of these three outbreaks was caused by distinctly different strains of *E. coli* O157:H7 as determined by whole genome sequencing (WGS) analysis;
- 2) Outbreak A strain of *E. coli* O157:H7 was found in two different brands of fresh-cut salads containing romaine lettuce in 2019;
- 3) traceback investigations of multiple illness sub-clusters and supply chain information identified a common grower with multiple ranches/fields, which supplied romaine lettuce during the timeframe of interest to multiple business entities associated with Outbreaks A, B and C; and
- 4) the Outbreak A strain of *E. coli* O157:H7 was detected in a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations.
- 5) other STEC strains, while not linked to outbreaks A,B, or C, were found in closer proximity to where romaine lettuce crops were grown, including two samples from a border area of a farm immediately next to cattle grazing land in the hills above leafy greens fields and two samples from on-farm water drainage basins.

FDA considers adjacent or nearby land use for cattle grazing as the most likely contributing factor associated with these three outbreaks. While the agency could not confirm a definitive source or route(s) of contamination of the romaine fields, the Agency considers indirect transmission of fecal material from adjacent and nearby lands from water run-off, wind, animals or vehicles to the romaine fields, or to the agricultural water sources used to grow the romaine, as possible routes of contamination. Working with our state partners, FDA is continuing, through the 2020 growing/harvest season, to conduct mission critical STEC investigations in the Salinas growing region to follow up on our findings from the 2019 outbreaks.

This document provides an overview of the investigation approach and factors that potentially contributed to the contamination of romaine lettuce with *E. coli* O157:H7 in two multistate and one single-state foodborne illness outbreaks in the fall of 2019. Of note, the number of cattle we observed on nearby lands during the 2019 investigations was far lower than the volume of what is considered a large concentrated animal feeding operation, offering a useful reminder that high-density animal operations are not the only

factor to consider. These findings reinforce our concern about the possible impacts of nearby and adjacent land use on the safety of leafy green crops and further underscore the importance of implementing appropriate risk mitigation strategies.

Contents

Executive Summary	1
I. Three Concurrent Foodborne Illness Outbreaks	4
Outbreak A	4
WGS Analysis of Outbreak A Compared to Previous Outbreaks Linked to Romaine/Leaf Lettuce Consumption	5
Outbreak B	5
Outbreak C	6
Outbreak Response Activities	6
II. Multiple Traceback Investigations	8
III. On-Farm Foodborne Illness Outbreak Investigation Methodology	8
IV. Key Investigation Observations and Findings	11
V. Summary of Factors Potentially Contributing to Contamination.....	13
VI. Recommendations.....	14
VII. Collaboration Across Agencies	15
VII. Glossary	16
IX. Relevant Links	17
X. Technical Appendix	18

I. Three Concurrent Foodborne Illness Outbreaks

Between November and December 2019, the U.S. Food and Drug Administration (FDA) and multiple state and federal partners were involved in three outbreak traceback investigations related to *E. coli* O157:H7 and the consumption of romaine lettuce or leafy greens. Although the outbreak strains themselves were genetically distinct, the traceback investigations suggested some overlap in the supply chains for each of these outbreaks.

Outbreak A

On November 20, 2019, FDA, in conjunction with the U.S. Centers for Disease Control and Prevention (CDC), state partners and Canadian officials (Public Health Agency of Canada and Canadian Food Inspection Agency) announced an investigation of an outbreak (Outbreak A) of *E. coli* O157:H7 infections in multiple U.S. states and Canadian provinces. By November 22, 2019, based on the mounting epidemiological and laboratory evidence, FDA issued a [public health advisory to help prevent further illnesses](#). While the exact growing locations and dates of harvest were uncertain early in the investigation, based on good work done by state labs in Maryland and Wisconsin, some traceability best practices adopted by industry, and the fact that the outbreak appeared to be ongoing, FDA recommended that consumers not eat any romaine lettuce harvested from the Salinas, California growing region until further notice.

In total, in the U.S. there were 167 reported illnesses in 27 states, resulting in 85 hospitalizations and 15 cases of hemolytic uremic syndrome (HUS). No deaths were reported. Illness onset ranged from September 20 through December 21, 2019. Outbreak A was declared over in the U.S. by [CDC on January 15, 2020](#).

The investigation conducted by FDA, CDC and state partners determined that consumption of contaminated romaine lettuce from the Salinas Valley growing region was the probable cause of this outbreak in the U.S. Ninety-four of the 113 (83 percent) ill people interviewed reported eating romaine lettuce in the week before they became ill. This percentage was significantly higher than results from a CDC survey of healthy people in which 47 percent reported eating romaine lettuce in the week before they were interviewed. Ill people reported eating different types of romaine lettuce in several restaurants or at home.

On November 18, 2019, the Maryland Department of Health reported that romaine lettuce from an intact sample of Ready Pac Bistro® Chicken Caesar Salad collected from the household of an ill case yielded *E. coli* O157:H7; subsequently on November 21, 2019, Maryland investigators reported that the romaine lettuce yielded the same Outbreak A strain of *E. coli* O157:H7 by whole genome sequencing (WGS). Romaine lettuce associated with this sample was determined by state and federal investigators to have been sourced from up to three growers in multiple California counties in the Salinas Valley growing region.

On December 10, 2019, Wisconsin state partners reported that an intact sample of Fresh Express Leafy Green Romaine product obtained from the household of an ill person yielded *E. coli* O157:H7; subsequently on December 13, 2019, it was reported by Wisconsin state investigators that the romaine lettuce isolates were a part of the Outbreak A strain of *E. coli* O157:H7 by WGS. Romaine lettuce associated with this sample was determined by state and federal investigators to have been sourced from up to two growers in multiple California counties in the Salinas Valley growing region.

As determined by WGS, all *E. coli* O157:H7 isolates from ill consumers in Outbreak A were closely related genetically to those previously seen in ill consumers in the U.S. and Canada in outbreaks that occurred during the fall of 2018, the fall/winter of 2017, and the fall of 2016. However, based on additional WGS analyses described below, the fall 2018 *E. coli* O157:H7 outbreak represents a different genetic sub-cluster of the same strain as compared with the 2019 *E. coli* O157:H7 outbreak that traced back to Salinas Valley. Additional research (outlined in the [2020 Leafy Green STEC Action Plan](#)) is needed to further investigate this finding. The strain of *E. coli* O157:H7 in Outbreak A was not related to strains seen in Outbreak B or Outbreak C.

WGS Analysis of Outbreak A Compared to Previous Outbreaks Linked to Romaine/Leaf Lettuce Consumption

Analysis of genetic differences (i.e., single nucleotide polymorphisms) identified from the whole-genome sequence (WGS) data of *E. coli* O157:H7 isolates that caused Outbreak A and *E. coli* O157:H7 isolates from a large 2018 *E. coli* O157:H7 outbreak associated with romaine lettuce consumption are closely related and likely share a recent common genetic source. The majority of isolates from each group, which include food, environmental, and clinical isolates, cluster together to the exclusion of isolates in the other group and isolates from the two groups are not interspersed among one another. Thus, these two groups likely represent different genetic sub-clusters of the same strain. This genetic differentiation is potentially due to the different geographic sources of contaminated romaine lettuce that caused the outbreaks, which is supported by traceback and clustering of samples collected from the environment (See Technical Appendix).

Broader analysis of WGS data of genetically similar *E. coli* O157:H7 isolates from outbreaks that occurred in 2016, 2017, and 2018 indicates that there were likely a number of recurring Shiga-toxin producing *E. coli* O157:H7 (STEC) outbreaks associated or potentially associated with consumption of leafy greens from both the Salinas growing region (i.e., the region associated with Outbreak A) and Santa Maria growing region (i.e., the region associated with the large Fall 2018 *E. coli* O157:H7 outbreak).

As noted above, isolates from a fall/winter 2017 outbreak of *E. coli* O157:H7 infections associated with romaine lettuce consumption in Canada and suspected leafy greens consumption in the United States, were closely related and phylogenetically interspersed within the *E. coli* O157:H7 isolates that caused the large outbreak of *E. coli* O157:H7 infections in 2018 associated with romaine lettuce from the Santa Maria growing region of California. Another small cluster of *E. coli* O157:H7 infections from 2016 with an unknown outbreak vehicle also had isolates interspersed among the isolates associated with the large 2018 outbreak associated with romaine lettuce from the Santa Maria growing region.

Traceback investigation data and WGS data of matching environmental and food samples also support the distinction between contamination events in the Salinas and Santa Maria growing regions. At this time with limited data available it cannot be determined whether there was a common source of the related *E. coli* O157:H7 isolates. Further in-depth analysis regarding the possible effects of growing conditions, environmental antecedents and animal (including cattle) movement (outlined in the [2020 Leafy Green STEC Action Plan](#)) is needed before more definitive conclusions can be drawn.

In addition to the three outbreaks in 2016, 2017, and 2018, isolates from two additional 2018 *E. coli* O157:H7 foodborne illness outbreaks/clusters were found to be closely related and interspersed on the phylogenetic tree within the *E. coli* O157:H7 isolates that caused Outbreak A.

Outbreak B

On November 26, 2019, King County Health Department in Washington State announced an outbreak investigation related to additional *E. coli* O157:H7 infections associated with locations of a particular restaurant chain in the state (Outbreak B). In total, there were 11 confirmed illnesses, resulting in 3 hospitalizations. There were no deaths or cases of HUS. Illness onsets ranged from November 8 through November 17, 2019. FDA announced Outbreak B was over on [January 15, 2020](#).

The investigation conducted by FDA and Washington State partners determined that leafy greens, including romaine sourced from the Salinas Valley growing region, consumed at multiple locations of a Washington State-area restaurant chain was the likely cause of Outbreak B. Ten of 11 case-patients reported eating leafy greens in salads at seven restaurant locations in Washington. Of those, nine specifically reported eating romaine lettuce. In this investigation, WGS showed that the *E. coli* O157:H7 isolates from ill consumers were closely related genetically. This strain of *E. coli* O157:H7 was not related to strains seen in Outbreak A or Outbreak C.

Outbreak C

On December 9, 2019, FDA, in conjunction with CDC, state partners and Canadian officials (Public Health Agency of Canada and Canadian Food Inspection Agency) announced an investigation related to an outbreak of additional *E. coli* O157:H7 infections in multiple U.S. states and Canadian provinces (Outbreak C). In total, there were 10 reported illnesses in five states, resulting in four hospitalizations and one case of HUS. There were no deaths. Illness onsets ranged from November 5 through November 16, 2019. Outbreak C was declared over in the U.S. by the [CDC on January 15, 2020](#).

The investigation conducted by FDA, CDC and state partners determined that consumption of Fresh Express Sunflower Crisp Chopped Salad Kits (containing romaine lettuce, kale and cabbage), was the likely cause of this outbreak in the U.S. Of the 10 people with available information, all 10 (100 percent) reported eating leafy greens in the week before their illness started. The romaine in these salad kits was sourced from the Salinas Valley growing region. Eight ill people specifically reported eating or maybe eating a Fresh Express Sunflower Crisp Chopped Salad Kit. In this investigation, WGS showed that the *E. coli* O157:H7 isolates from ill consumers in the United States and Canada were closely related genetically, but there were no product isolates for comparison. This strain of *E. coli* O157:H7 was not related to the strains seen in Outbreak A or Outbreak B.

Outbreak Response Activities

Based on the mounting epidemiological and laboratory evidence in the 2019 investigation into Outbreak A, FDA issued a [public health advisory on November 22, 2019 to help prevent further illnesses](#). FDA recommended that consumers not eat any romaine lettuce harvested from the Salinas, California growing region until further notice.

Because of industry voluntary labeling of harvested romaine lettuce, which was requested by FDA following outbreaks of *E. coli* O157:H7 related to leafy greens in 2018, consumers were able to more quickly identify potentially contaminated product. However, some consumers still reported confusion in the marketplace, suggesting improved standardization of labels could increase clarity to expedite buyer and consumer response. FDA also requested that industry voluntarily withdraw product from the market and withhold distribution of romaine lettuce harvested from the Salinas growing region until the specific source of contaminated romaine lettuce could be identified and appropriate action taken or it could be determined that the outbreak was over.

Food exposure information for ill consumers in each of the three outbreak investigations was evaluated to determine points of service at which leafy greens (including romaine lettuce) may have been served or purchased. Based on this information, FDA, in collaboration with state officials, initiated three separate traceback investigations of leafy greens associated with case exposures. The epidemiological, traceback and laboratory information obtained during the three outbreak investigations informed and helped prioritize subsequent in-depth onsite investigations of three romaine lettuce growers in the Salinas Valley growing region, including specific fields of a common grower noted amongst the three traceback investigations.

During the course of the three investigations of each outbreak, the *E. coli* O157:H7 Outbreak A strain was detected in a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations. In addition, one water sample from the Salinas River also tested positive for a non-outbreak strain of *E. coli* O157:H7, though the farms investigated did not report use of river water as agricultural water. Over the course of the investigations at several farms identified in the outbreak traceback and other businesses and public access areas, FDA and public health partners also found 12 sub-samples positive for non-O157 *E. coli* STEC, unrelated to any illnesses and of varied to low virulence (i.e. did not contain all of the recognized virulence genes required to cause severe illness in humans), in samples of water, soil, compost, or manure. Nevertheless, these findings serves as indicators of potential fecal contamination. In total, over 70 samples,

representing approximately 400 sub-samples, were collected during the multiple farm investigations across multiple fields and public spaces. (see Table 1).

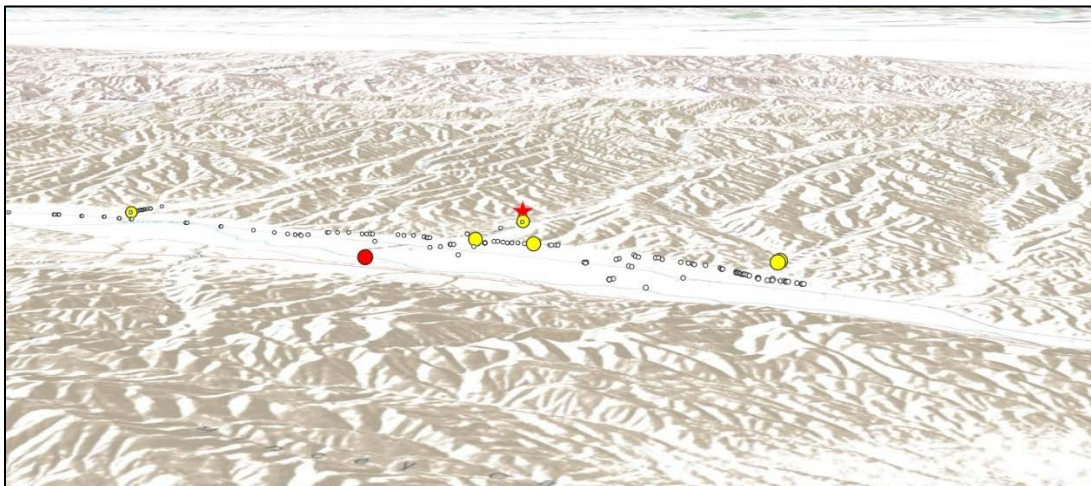
Table 1. Classification of sub-samples collected by FDA during on-farm outbreak investigations within the Salinas growing region (2019 – 2020)

Collection Date	Sub-samples per Elevation Zone					
	High Elevation (Hilly Terrain; Range Land; Public Land)		Intermediate Elevation (Foothills; Produce Fields; Buffer Zones)		Lower Elevation (River Corridor; Drainage Outlets)	
	Sub-samples Collected	Positive Sub-samples	Sub-samples Collected	Positive Sub-samples	Sub-samples Collected	Positive Sub-samples
Nov. 2019	--	--	113	0	--	--
Dec. 2019	--	--	35	1 ¹	--	--
Feb. 2020	21	8 ¹ (1 ²)	222	4 ¹	15	1 ³
Total	21	8	370	5	15	1

Notes: ¹STEC Positive ²O157:H7 Positive matching Outbreak A Strain ³O157:H7 Positive

The Outbreak A strain positive sample, taken from a fecal-soil composite on public land, suggests a link with cattle as a likely source of the outbreak strain (see Figure 1). This situation is similar to the investigational finding of three outbreak strain positive samples from a cattle operation adjacent to a farm in Santa Maria that was linked to a previous outbreak, also associated with romaine lettuce consumption. However, because no romaine lettuce was present in any of the fields at the time of the Salinas investigations, samples were limited mainly to soil from fallow fields and water sources, which limit the identification of a specific route onto the contaminated produce.

Figure 1. Visualization of sub-samples collected by FDA during on-farm outbreak investigations within the Salinas growing region (2019 – 2020), viewed from the east



Notes: RED STAR is the location of the matching outbreak strain A, the RED CIRCLE is the O157:H7 sample site at the Salinas River, the YELLOW CIRCLES are sites of the other STECs, and the SMALL WHITE DOTS are a subset of the sample locations which provided negative results.

Due to persistent *E. coli* O157:H7 outbreaks associated with leafy greens from the western/southwestern U.S., particularly romaine lettuce, and because of the magnitude of this and recent associated outbreaks, working with our state partners, FDA is in the process of conducting in-depth, follow-up investigations this growing season. The investigations may further characterize how contamination might have occurred and may inform what preventive measures are needed to prevent future outbreaks. Once complete, FDA will promptly issue a report and share lessons learned, so that growers can implement preventive measures to protect consumers from contaminated produce.

II. Multiple Traceback Investigations

Traceback of romaine lettuce from 15 points of service associated with 15 case patients for Outbreak A was conducted. These points of sale included locations where products that tested positive for the outbreak strain in both Maryland and Wisconsin were purchased. During the time period of interest more than 15 growers and multiple fields in various California counties, including several in the Salinas Valley growing region and in Mexico, were identified as potentially supplying romaine lettuce. One of the identified growers that supplied romaine lettuce during the timeframe of interest in Outbreak A was in common with Outbreaks B and C.

Traceback of romaine lettuce from six points of service associated with 11 cases in Outbreak B identified up to five growers and multiple fields in various California counties in the Salinas Valley growing region as potentially supplying romaine lettuce that was consumed during this outbreak. One of the identified growers that supplied romaine lettuce during the timeframe of interest in Outbreak B was in common with the Outbreaks A and C, although different fields were reported.

Traceback of leafy green components associated with the Fresh Express salad kit of interest (e.g., romaine, kale and cabbage) from three points of service associated with a single case each for Outbreak C was conducted. During the time period of interest, more than five growers and multiple fields in various California counties in the Salinas Valley growing region were identified as potentially supplying the romaine lettuce component of these kits. One of the identified Salinas growers that supplied romaine lettuce during the timeframe of interest in Outbreak C was in common with Outbreaks A and B. Cabbage was sourced from Michigan and New York, while kale was sourced from multiple California counties including those in the Salinas Valley growing region.

Multiple, non-contiguous fields in the same region for this common grower were identified as the potential source of romaine lettuce during the timeframe of interest for each of the three outbreak investigations. At least two fields were in common among Outbreaks A and C. However, there is insufficient evidence to conclude that the common grower seen amongst the three traceback investigations was or was not the sole source of the outbreaks.

III. On-Farm Foodborne Illness Outbreak Investigation Methodology

The epidemiological, traceback, and laboratory information (e.g., WGS data) obtained during the three outbreak investigations informed and helped prioritize five subsequent in-depth investigations of romaine lettuce growers within the Salinas Valley growing region of California, including several fields implicated by the tracebacks. The multiple on-farm investigations were conducted in collaboration with CDC and CA state partners from November 2019 through February 2020.

The FDA uses a spectrum of procedures that includes facility and on-farm inspections, investigations, and follow-up investigations to identify the source of an outbreak and to gather information about potential contributing factors. This information informs agency decision-making regarding potential regulatory actions and policy.

Investigations were conducted by the FDA's Produce Safety Network and Office of Regulatory Affairs, CDC, the California Department of Food and Agriculture (CDFA), and the California Department of Public Health (CDPH). The multi-disciplinary teams had expertise in public health, including produce safety, agriculture, veterinary medicine, epidemiology, microbiology, and environmental health. In addition to the extensive epidemiological and traceback analyses performed during the three outbreak investigations, the investigation teams' actions and reports were informed by on-farm observations, environmental sampling, and inquiries directed to responsible persons onsite at farm operations.

The scope and approach of these investigations focused on FDA-regulated entities to identify growers and firms in the romaine lettuce supply chain that may have contributed to the three outbreaks. The information collected from these investigations can and should be used to reduce the potential for another outbreak associated with leafy greens or other fresh produce commodities.

The investigation teams used the FDA Food Safety Modernization Act (FSMA) Produce Safety Rule and Good Agricultural Practices (GAPs) as the basis for assessing potential contributing factors that could have led to contamination of romaine lettuce on farms identified in the traceback. Areas of focus on these growers included but were not limited to: agricultural water; equipment, tools, and sanitation; biological soil amendments; growing and harvesting practices; animal intrusion; adjacent land use; and employee health and hygiene practices.

Potential sources and routes of *E. coli* O157:H7 were assessed on numerous leafy greens farms in the Salinas Valley growing region of California that potentially shipped romaine lettuce possibly contaminated with the outbreak strains. Environmental sample types collected included soil, animal feces, biological soil amendments, and agricultural water (including ground water and surface water).

The investigation teams conducted detailed interviews with farm personnel during investigations at locations identified and implicated by the traceback investigations. The investigation teams collected information regarding relevant food safety procedures, policies, and practices. However, because the investigations were all conducted after the growing and harvesting season had concluded for romaine in the Salinas Valley growing region, all fields sampled were fallow, therefore limiting the information collected around harvesting activities or personnel. Additionally, many of the growers were not involved in harvest activities and directed questions about harvest methods and procedures, harvest equipment and storage to the shipper or handling firms overseeing harvest. Contamination of leafy greens has been identified in previous outbreak investigations to most likely occur in the farm environment; however, contamination occurring in the farm environment may be amplified by harvest crews and/or fresh-cut produce manufacturing/processing if appropriate preventive controls are not in place.

The investigation teams made numerous visits to romaine lettuce growers in the Salinas Valley growing region and conducted additional sampling on nearby lands in follow-up investigations. As described above, a fecal-soil composite sub-sample taken from a cattle grate (see Figure 2) on public land less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations, was found to contain *E. coli* O157:H7 with the same rare genetic fingerprint (detected by WGS) as Outbreak strain A, suggesting that a potential contributing factor has been the proximity of cattle—a persistent reservoir of *E. coli* O157:H7 and other STECs¹—to the produce fields identified in traceback investigations.

¹ See CDC *E. coli* Home Page, available at <https://www.cdc.gov/ecoli/general/index.html>.

Figure 2. Cattle Grate where a fecal-soil composite sub-sample was collected that matched Outbreak Strain A.



Source: FDA investigation team.

Also, one water sample from the Salinas River tested positive for pathogenic *E. coli* O157:H7, though farms investigated did not report use of river water as agricultural water. Finally, other non-O157 *E. coli* STEC, unrelated to any illnesses, were detected in soil, compost, and manure samples taken near where product was harvested and where cattle are known to graze, including two samples from a border area of a farm immediately next to cattle grazing land in the hills above leafy greens fields (see Figure 3) and two samples from on-farm water drainage basins.

Figure 3. Proximity of cattle grazing land in hills above leafy green fields.



Note: photograph not of a field directly tied to the investigated outbreaks, but intended to show suboptimal proximity of cattle and leafy greens operations.

Source: FDA investigation team.

IV. Key Investigation Observations and Findings

The three traceback investigations conducted in 2019 indicated that contaminated romaine lettuce may have been sourced from multiple growers as not all traceback legs led to the common grower noted amongst the three outbreak investigations. Although *E. coli* O157:H7 matching Outbreak strain A was detected in a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations, this single positive test result does not explain the source nor precise route(s) of contamination, but it provides important insights into a potential contributing factor.

Although one of the identified growers that supplied romaine lettuce during the timeframe of interest in Outbreak A was in common with Outbreaks B and C, multiple, non-contiguous fields in the same region

for this common grower were identified as a potential source of romaine lettuce during the timeframe of interest for each of the three outbreak investigations. At least two fields were in common among Outbreaks A and C. However, there is insufficient evidence to conclude that the common grower seen amongst the three traceback investigations was or was not the sole a source of the outbreaks.

Investigators observed animal grazing and feeding operations of various sizes on lands adjacent to and nearby growing fields identified during the traceback investigations, although investigators did not identify routes of contamination from these potential pathogen sources to romaine lettuce.

One of the three investigated growers was adjacent to active cattle rangeland with an 800-foot buffer zone (enforced only during the growing season) between the cattle grazing and growing areas. Testing of nearby public land (less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigation) did return a positive sample which matched, via WGS, Outbreak strain A. This positive result was pulled from a sub-sample which the on-farm investigators described as visibly containing soil and manure. One hypothesis is the pathogen is persisting in an animal reservoir which is potentially re-introducing it into the environment near growing areas of romaine lettuce.

Investigation teams also noted evidence of wild animal activity, including evidence of rodents and large flocks of birds near fields of interest.

Investigators also considered whether the application of contaminated agricultural water may have contributed to the spread of pathogenic *E. coli* O157:H7. Each of the investigated growers had a procedure in place to treat water from an open source with sanitizer before used as agricultural water during the growth of produce; however, investigators were unable to determine the effectiveness of treatment that occurred prior to their onsite visits. Although none of the growers interviewed indicated using Salinas River water on their crops, the positive test results of pathogenic *E. coli* O157:H7 drawn from ultra-filter samples from the Salinas River emphasize the need to better understand the ecology, survival and movement of human pathogens in the Salinas Valley growing region. Such longitudinal research approach would aid in the understanding of human pathogen reservoirs, how they may contaminate produce and what preventive measures may be implemented to prevent produce STEC contamination and resulting foodborne illnesses.

Additionally, information collected by the investigation teams indicates that some growers directly applied agricultural water from a ground water source to their romaine lettuce crop during germination and then irrigated by above-ground drip irrigation tape until harvest. Some growers irrigated by overhead sprinklers from initial planting up until harvest. Investigation teams were unable to investigate the sanitary quality of the water used to contact romaine lettuce at harvest, during postharvest handling, and to wash/rinse harvest equipment food contact surfaces as those activities were handled by contracted entities and in each instance was not taking place at the time of the investigations.

Each of the growers from the Salinas Valley growing region grew their romaine under contract and did not conduct the harvesting and packing of the lettuce. Instead, the growers were responsible for the product up until the point of harvest, caring for the product in the ground, and a separate company, with its own equipment and personnel, conducted all harvesting, packing, and transporting. For these investigations, harvest companies were not available for inspection or interviews because the Salinas harvest season had concluded and the contract harvesters had moved to another region at the time of these investigations. This further limited the scope of the on-farm investigations, as aspects related to harvest and post-harvest (e.g., activities, personnel qualifications and training, health and hygiene, agricultural water, equipment tools and sanitation) could not be adequately examined at the time of these on-farm investigations. Because we were unable to evaluate harvesting practices, FDA cannot rule out an environmental source that is independent of grower-specific practices.

The first illnesses in these outbreaks occurred in early September 2019, and therefore, the pathogen might have been present in these growing locations for some months or they may have been repeatedly introduced into the fields from an unknown source. Because fields at the time of this investigation were fallow, sample collections were limited in scope. Working with our state partners, FDA is continuing, through the 2020 growing/harvest season, to conduct mission critical STEC investigations in the Salinas growing region to follow up on our findings from the 2019 outbreaks. These assignments are intended to help FDA and the growing community identify potential routes of contamination and possible harborage sites for pathogenic STEC within the region, with an overall goal of preventing future contamination and potentially identifying contaminated product before harvest.

V. Summary of Factors Potentially Contributing to Contamination

E. coli O157:H7 matching Outbreak strain A was detected in a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations in the Salinas Valley growing region of California.

FDA has identified the following factors and findings as those that may have contributed to the contamination of romaine lettuce from the Salinas Valley growing region of California with the *E. coli* O157:H7 strains that caused these outbreaks:

- For the Salinas Valley growing region farms, animal grazing or feeding operations of all sizes appear to be the most likely sources of outbreak strains of *E. coli* O157:H7, as cattle are a persistent reservoir of *E. coli* O157:H7 and other STECs. This is especially true when cattle are adjacent to and are at higher elevations than produce fields.
- Although the route of transmission from cattle to the produce is unknown, plausible ways in which implicated romaine lettuce may have become contaminated include:
 - Run-off from these nearby lands,
 - Direct transmission from animal operations to romaine lettuce growing fields by wind, animals or farming activities such as vehicles, harvest crews, or harvest equipment;
 - Application of agricultural water contaminated with fecal material from animal operations regardless of size.
- One of the identified growers that supplied romaine lettuce during the timeframe of interest in Outbreak A was in common with Outbreaks B and C. However, there is insufficient evidence to conclude that the common grower seen amongst the three traceback investigations was or was not the sole a source of the outbreaks.
- Outbreak strain A was detected in a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from multiple fields of the common grower noted across the three outbreak investigations.
- Based on these three outbreaks and the history of outbreaks traced back to the Salinas growing region over the last 10 years, fall (Sept. – Nov.) appears to be the highest risk season for contamination of romaine from this growing region.
- FDA notes evidence of wild animal activity including rodents and birds near the examined fields, which warrants consideration as a possible source(s) or route of the human pathogen found in the contaminated product. Each of the investigated growers had a procedure in place to treat water from an open source with sanitizer before used as agricultural water during the growth of produce; however, investigators were unable to determine the effectiveness of treatment that occurred prior to their onsite visits.

- Because harvest was not conducted by the investigated farms and harvesting was not occurring during the time of the investigations, FDA was unable to determine if post-harvest water was safe and adequate for its intended use. Additionally, the investigations were all confined to the farms and public lands, which did not look at packers and/or processors.
- No evidence was found to confirm an obvious route(s) for on-farm contamination or from adjacent or nearby lands partly because the fields were not in production making it impossible to observe and very difficult to reconstruct typical routes of transmission (e.g., irrigation, crews, equipment, crop protection sprays). Except for public lands, FDA did not have access to the adjacent and nearby lands to conduct sampling to support its investigations.
- Other explanations regarding how the produce was contaminated with the *E. coli* O157:H7 outbreak strains aside from the potential contributing factors identified in this report are possible.

Working with our state partners, FDA is continuing, through the 2020 growing/harvest season, to conduct mission critical STEC investigations in the Salinas growing region to follow up on our findings from the 2019 outbreaks. These assignments are intended to help FDA and the growing community identify potential routes of contamination and possible harborage sites for pathogenic STEC within the region, with an overall goal of preventing future contamination and potentially identifying contaminated product before harvest.

VI. Recommendations

These three 2019 STEC outbreaks associated with romaine lettuce in the United States follow closely behind one additional multistate outbreak in 2019 associated with leafy greens and two STEC outbreaks in 2018 associated with romaine. These outbreaks have had serious public health consequences as well as ramifications for the produce industry. The findings of the 2019 investigations, together with the findings from earlier leafy greens outbreaks dating back to 2013, suggest that a potential contributing factor has been the proximity of cattle—a persistent reservoir of *E. coli* O157:H7 and other STECs—to the produce fields identified in traceback investigations. Implementation of appropriate science and risk-based preventive measures to reduce the potential for contamination of leafy greens from farm to fork is the most effective and practicable means to assure the safety of produce.

In light of the findings of these investigations, FDA recommends that growers of leafy greens:

- Emphasize/Redouble efforts around Prevention
 - Assess growing operations to ensure implementation of appropriate science- and risk-based preventive measures, including applicable provisions of the FSMA Produce Safety Rule and GAPs.
 - Adjacent Land Use
 - Assess and mitigate risks associated with adjacent and nearby land uses, including grazing lands and animal operations regardless of size
 - Prevent contamination from uphill adjacent cattle grazing lands, such as by produce farms increasing buffer zones if fields are adjacent to cattle grazing lands (based on assessment); and adding physical barriers such as berms, diversion ditches and vegetative strips
 - Agricultural Water
 - Ensure that all agricultural water is safe and of adequate sanitary quality for its intended use

- Assess and mitigate risks related to land uses near or adjacent to agricultural water sources that may contaminate agricultural water
 - Ensure that any agricultural water treatment is validated, verified, and in accordance with all applicable Federal, State, Local, and other regulations
- Improve Traceability
 - Increase digitization, interoperability and standardization of traceability records, which would expedite traceback and prevent further illnesses.
 - Broader, more consistent implementation of voluntary source labeling on packaging or point of sale signs, or by other means to help consumers and retailers more readily identify product during an outbreak or recall.
- Improve Root Cause Analysis
 - Perform a root cause analysis when a foodborne pathogen is identified in the growing environment, in agricultural inputs (e.g., agricultural water or soil amendments), in raw agricultural commodities or in fresh-cut ready-to-eat produce.

Food safety is a shared responsibility that involves food producers, distributors, manufacturers, retailers, and regulators. FDA is committed to working with these stakeholders to advance work in three areas: prevention, response, and addressing knowledge gaps under the [2020 Leafy Greens STEC Action Plan](#).

VII. Collaboration Across Agencies

Throughout the course of this investigation FDA staff worked closely with multiple other agencies and departments. The coordination of state and federal agencies responding to this foodborne outbreak resulted in an efficient operation and a significant number of samples being collected and evaluated.

CDPH staff completed a significant volume of work in response to the 2019 *E. coli* outbreak linked to romaine lettuce, including but not limited to, participating in multiple farm investigations with partners from CDFA, CDC, and FDA, and completed a supplementary investigation focused on the collection and testing of irrigation water at a single farm location. All water samples collected from this location were negative for pathogenic *E. coli*.

CDC conducted the water evaluation and sampling in 2019 during one of the FDA-led farm investigations in the Salinas growing region. Along with the on-site assessment, CDC collected and tested agriculture well water samples, as well as field drainage water and sediment samples. CDC brought expertise in the environmental sources, fate, and transport of waterborne pathogens like *E. coli* O157:H7, as well as water expertise in engineering and water systems. CDC also coordinated the epidemiologic portions of the multistate outbreak investigations referred to above, including working closely with state and local officials in numerous states to compile and analyze food history data and obtain initial traceback information from points of service and sale.

CDFA deployed several field resources and provided administrative support to conduct follow-up investigations. CDFA field investigative efforts were conducted by environmental scientists to aid in on-farm assessments. The field staff conducted on-site follow up at fertilizer facilities; this included sampling the facility products and reviewing their pathogen reduction process. CDFA brings scientific expertise as well as field and livestock production knowledge.

VII. Glossary

Farm – an operation under one management in one general (but not necessarily contiguous) physical location devoted to the growing of crops, the harvesting of crops, or any combination of these activities.

Field – a contiguous set of land devoted to the growing and/or harvesting of crops, a subset of a farm.

Follow-up investigation – a second investigation, typically as a follow-up to a positive sample.

Grower – the legal business owner of an entire farming operation.

Inspection – routinely conducted of regulated entities to determine compliance with applicable laws and regulations.

Investigation – an inspection as the results of an outbreak and trace back investigation that implicated the farm and/or related operations.

Ranch – a single plot of land devoted to the growing and/or harvesting of crops, a subset of a field.

Range Land – native and naturalized pasture, forest and woodlands that support a cover of vegetation useful for grazing or browsing by wildlife and/or livestock.

Sample – a specific test for pathogens in a specific location or of specific materials, comprised of numerous sub-samples.

Sub-sample – one single test for pathogens, many sub-samples may be taken out of one sample, as pathogens are not evenly distributed on soil, in water, or in fecal materials.

IX. Relevant Links

[FDA Statement on the Salinas-linked romaine lettuce *E. coli* O157:H7 outbreak and status update on investigation](#)

[Statement from FDA Deputy Commissioner for Food Policy and Response Frank Yiannis](#)

[FDA In Brief: FDA warns consumers not to eat romaine lettuce grown in Salinas, California](#)

[FDA Outbreak Investigation of *E. coli*: Romaine \(November 2019\)](#)

[FDA Outbreak Investigation of *E. coli*: Salad Mix \(December 2019\)](#)

[FDA 2020 Leafy Green STEC Action Plan](#)

[FDA Environmental Assessment of Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated in a Multi-State Outbreak of *E. coli* O157:H7](#)

[CDC Outbreak of *E. coli* Infections Linked to Romaine Lettuce](#)

[CDC Outbreak of *E. coli* Infections Linked to Fresh Express Sunflower Crisp Chopped Salad Kits](#)

[Washington State: Shiga toxin-producing *E. coli* O157:H7 \(STEC\) outbreak associated with 7 Evergreens restaurants](#)

[Maryland: Maryland Department of Health investigating *E. coli* cluster](#)

[Wisconsin: Outbreak of *E. coli* Infections Linked to Romaine Lettuce](#)

[Canada: Public Health Notice – United States outbreak of *E. coli* infections linked to romaine lettuce with implications for Canadians](#)

[Canada: Public Health Notice: Outbreak of *E. coli* infections linked to Fresh Express brand Sunflower Crisp Chopped Salad Kits](#)

[Investigation Summary: Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated in the Fall 2018 Multi-State Outbreak of *E. coli* O157:H7](#)

[Environmental Assessment of Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated in a Multi-State Outbreak of *E. coli* O157:H7](#)

NCBI links to the WGS information related to outbreak [A](#), [B](#), and [C](#)

[About the Produce Safety Network](#)

[About the CORE Network](#)

[About the Whole Genome Sequencing \(WGS\) Program](#)

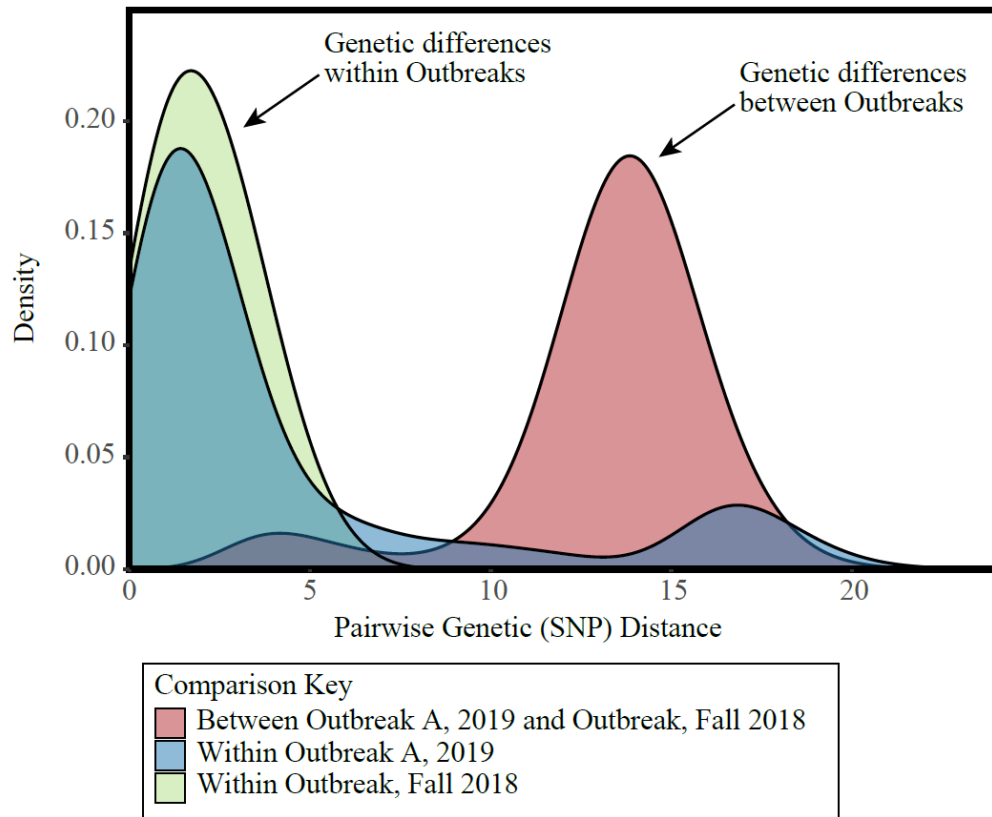
[FSMA Produce Safety Rule](#)

[FSMA Preventive Controls for Human Foods Rule](#)

[FDA Bad Bug Book](#)

X. Technical Appendix

Pairwise genetic differences within and between the two large E. coli outbreaks



Source: FDA whole-genome sequence analysis. The Figure illustrates that isolates within each outbreak are more genetically similar (i.e., fewer genetic (SNP) differences) to one another than they are to isolates from the other outbreak.