

Investigation Report:
Factors Potentially Contributing to the
Contamination of Leafy Greens Implicated
in the Fall 2020 Outbreak of *E. coli*
O157:H7

Executive Summary

Between August and December 2020, the U.S. Food and Drug Administration (FDA) and multiple state and federal partners were involved in an outbreak investigation related to *E. coli* O157:H7 illnesses and the consumption of leafy greens. The outbreak, which caused 40 reported domestic illnesses, was linked via whole genome sequencing (WGS) and geography to outbreaks traced back to the California growing region associated with the consumption of leafy greens in 2019 and 2018. FDA, alongside state and federal partners, investigated the outbreak to identify potential contributing factors that may have led to leafy green contamination with *E. coli* O157:H7. The *E. coli* O157:H7 outbreak strain was identified in a cattle feces composite sample taken alongside a road approximately 1.3 miles upslope from a produce farm with multiple fields tied to the outbreaks by the traceback investigations. In addition, several potential contributing factors to the 2020 leafy greens outbreak were identified.

Isolates within this cluster of illnesses are part of a reoccurring strain of concern and are associated with outbreaks that have occurred in leafy greens each fall since 2017. The two most recent outbreaks associated with this strain were an outbreak in 2018 (linked to romaine lettuce from the Santa Maria growing region of California) and an outbreak in 2019 (linked to romaine lettuce from the Salinas growing region of California). Clinical isolates from cases in this 2020 outbreak appear more closely related to those from the 2019 outbreak than the 2018 outbreak. In addition, several specific food and environmental isolates that appear to be highly related to this 2020 outbreak include a fecal-soil composite sample collected by FDA in February 2020 from the Salinas growing region and two leafy green samples collected in 2019 by state partners as a part of the 2019 investigation that traced back to the Salinas growing region.

As part of this investigation, tracebacks of leafy greens consumed by ten ill individuals from eleven points of service were conducted. Although that traceback investigation was based on a relatively small number of the total cases, it was based on those cases which presented the strongest evidence via purchase card information, invoices, bills of lading, and electronic data. The traceback investigation identified the Salinas growing region of California as a geographical region of interest.

In light of this most recent finding, combined with previous outbreak investigation findings in the region, FDA has identified key trends regarding the issues of a reoccurring strain, a reoccurring region, and reoccurring issues around adjacent and nearby land use of primary importance in understanding the contamination of leafy greens by *E. coli* O157:H7 that occurred in 2020 and previous years.

FDA also recognizes the interconnection between people, animals, plants, and their shared environment when it comes to public health outcomes. As such, we strongly encourage collaboration among various groups in the broader agricultural community (i.e. livestock owners; leafy greens growers, state and federal government agencies, and academia) to address this issue.

With this collaboration, the agricultural community, alongside academic and government partners, can work to identify and implement measures to prevent contamination of leafy greens. FDA recommends that these parties participate in efforts to understand and address the challenge of successful coexistence of various types of agricultural industries to ensure food safety and protect consumers against foodborne illnesses.

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I. Outbreak Overview

Between August and December 2020, the U.S. Food and Drug Administration (FDA) and multiple local, state, and federal partners were involved in an outbreak investigation related to *E. coli* O157:H7 and the consumption of leafy greens.

On October 28, 2020, FDA, in conjunction with the U.S. Centers for Disease Control and Prevention (CDC), and state partners announced an investigation into an outbreak of *E. coli* O157:H7 infections in multiple U.S. states associated with the consumption of leafy greens. FDA and CDC's epidemiological and traceback investigations were not able to determine a specific type or source of leafy green linked to illnesses, but because the strain of *E. coli* was genetically related to a strain linked to the [fall 2019 romaine outbreak](#), teams were deployed to dozens of ranches in the Salinas Valley growing region to conduct large-scale environmental sampling.

In total, there were 40 reported illnesses across 19 states. The outbreak resulted in 20 hospitalizations, and four people developed hemolytic uremic syndrome (HUS), a type of kidney failure. No associated deaths were reported. Illness onset ranged from August 10 through October 31, 2020. The outbreak was declared over by [CDC on December 22, 2020](#).

II. Outbreak Response Activities and On-Farm Outbreak Investigation

The epidemiologic and traceback investigation conducted by FDA, CDC, and state partners showed that leafy greens were the likely source of this outbreak. Ninety-six percent of ill persons interviewed (22/23) reported yes or maybe to eating a variety of leafy greens (21 yes, 1 maybe) in the week before their illnesses started. Compared to the 2006-2007 FoodNet population survey data, in which 71.3% of respondents reported eating leafy greens, the proportion of cases reporting eating any type of leafy greens in this outbreak was significantly higher than expected ($p < 0.001$). Of the 23 people who were asked what types of leafy greens they ate, 16 (70%) reported yes or maybe to eating spinach (11 yes, 5 maybe) and 15 (65%) reported yes or maybe to eating romaine lettuce (10 yes, 5 maybe). Most ill people reported eating a variety of leafy greens.

Food exposure information for ill consumers in the outbreak investigation was evaluated to determine points of service at which leafy greens may have been served or purchased. Based on this information, FDA, in collaboration with state and other government officials, initiated a traceback investigation of multiple types of leafy greens associated with case exposures. Traceback of leafy greens from eleven points of service associated with ten case-patients was conducted. These ten case patients are represented in the traceback by seven different legs. Six single case-patients with leafy green exposures make up six of the seven traceback legs. Four case-patients represent a sub-cluster of individuals that make up a single traceback leg. Although the traceback was based on a relatively small number of the total case-patients, it was based on those case-patients which presented the strongest evidence via purchase information, invoices, bills of lading, and electronic data. The traceback investigation identified the Salinas growing region of California as a geographical region of interest.

The 2020 outbreak strain was confirmed to match (i.e., genetically closely related) one of the fall 2019 romaine outbreak strains. As a result, attention was focused on foods commonly grown in this region. Based on whole genome sequencing (WGS) analysis, the 2019 outbreak investigation revealed that the [2019 outbreak strain](#) of *E. coli* O157:H7 was found in two different brands of fresh-cut salads containing romaine lettuce which were traced back to the Salinas growing region of California, as well as a fecal-soil composite sample taken from a cattle grate on public land less than two miles upslope from a produce farm in the Salinas growing region of California with multiple fields tied to the outbreaks by the traceback investigations.

The epidemiological, traceback, and WGS evidence obtained during the 2020 outbreak investigation informed and helped prioritize three subsequent in-depth investigations of leafy green growers in the Salinas growing region of California. The on-site investigations were conducted in collaboration with California state partners between October and December 2020.

Investigations were conducted by the FDA's Office of Regulatory Affairs (ORA) Produce Safety Network, with assistance from the ORA Human and Animal Food Division and the California Department of Food and Agriculture (CDFA). The multi-disciplinary teams had expertise in public health, produce safety, agriculture, microbiology, and environmental health. In addition to the extensive epidemiological and traceback analyses performed during the current outbreak investigation, the investigation teams' actions and reports were informed by on-site observations, environmental sampling, and inquiries directed to responsible persons on-site at farm operations.

Potential sources and routes of *E. coli* O157:H7 were assessed on numerous leafy green fields, located in the Salinas growing region of California, that may have grown leafy greens possibly contaminated with the outbreak strain. Environmental sample types collected included animal scat, biological soil amendments of animal origin (BSAAO), agricultural water and other water near fields of interest, environmental swabs of food contact and non-food contact surfaces, soil samples, debris samples, air samples, and sediment samples. Product samples of leafy greens were also collected and tested for the presence of the outbreak strain. In total, FDA conducted over 800 tests of materials collected during this investigation.

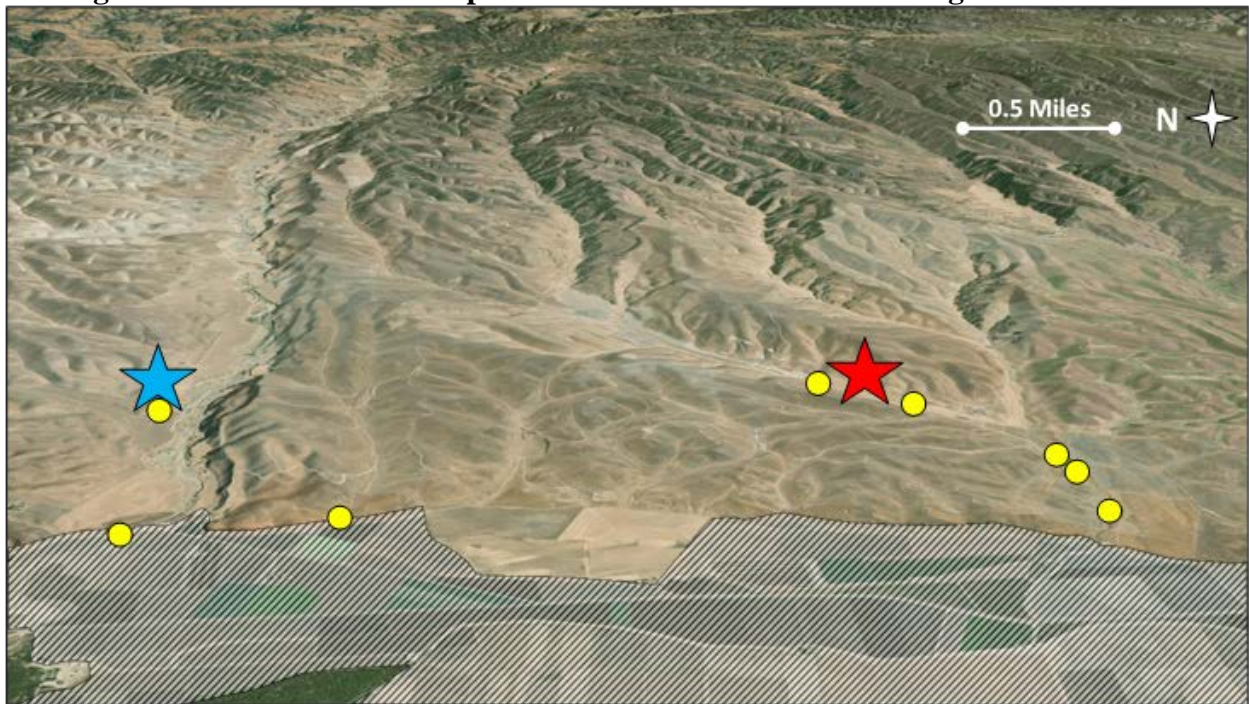
The investigation teams conducted detailed interviews with available farm personnel during investigations at locations identified by traceback. The investigation teams collected information regarding relevant food safety procedures, policies, and practices. However, because the investigations were all conducted near the end or after the growing season for Salinas had shut down, investigators were limited in their ability to observe product in the field or harvest conditions, and in many cases could not observe any activities due to the conclusion of growing and harvesting at the farms.

The scope and approach of these investigations focused on FDA-regulated entities to identify growers and firms in the leafy green supply chain that may have contributed to the outbreak. The investigation provided insights on several factors, which potentially contributed to the contamination of leafy greens grown in the Salinas growing region of California. One cattle feces composite sample taken alongside a road approximately 1.3 miles upslope from a produce farm with multiple fields tied to the outbreak by the traceback matched the outbreak strain of *E. coli*

O157:H7 by WGS.¹ In addition, several other samples tested positive for other strains of *E. coli* O157:H7 as well as other STEC serogroups (Figure 1).

Including the positive sample matching the outbreak strain of *E. coli* O157:H7, 38 tests of the over 800 conducted were reported positive for *E. coli* including two for *E. coli* O157:H7 (one matched the outbreak strain; one matched another previously identified strain). This most recent positive sample, matching the 2020 outbreak strain of *E. coli* O157:H7, was collected approximately 3 miles from a positive sample related to a 2019 outbreak of *E. coli* O157:H7 linked to the consumption of romaine lettuce. Of the 38 tests positive for *E. coli*, 36 tests were positive for STEC *E. coli*: three were soil samples, two were sediment, twenty-four were animal scat, three were environmental swabs, one was from leafy greens that did not get put into the supply chain, and three were water samples collected using high-volume dead-end ultrafiltration (Figure 1).

Figure 1. A geospatial subset of STEC positive investigation test results from 2020 outbreak investigation and outbreak strain positive from 2019 outbreak investigation.



Notes: 2020 outbreak strain match is represented by a red star; 2019 outbreak strain is represented by blue star; other samples yielding STEC positives from the 2020 outbreak investigation are represented by yellow circles. The shaded area represents produce farms and ranches. This map illustrates the samples matching the current and previous outbreak strain. Other samples yielding STEC positive samples from other non-contiguous sample sites were collected as part of this outbreak investigation (not pictured here); therefore, the number of total positive tests will not be represented in this figure.

Between October and December 2020, three on-site investigations were conducted throughout the Salinas growing region of California. The three investigations were not able to confirm the direct

¹ This cattle feces composite was collected as part of the 2020 outbreak investigation. The fecal-soil composite sample previously discussed was collected as a part of the 2019 outbreak investigation. Both are referenced in this report, but the cattle feces composite is the latest genetic match to this outbreak strain of *E. coli* O157:H7.

source or route of contamination. However, in all cases, the lands adjacent and upslope to farms and ranches of interest were noted as potential concerns. Specifically, year-round cattle grazing; other produce farming activities; and uncultivated land, which could serve as wildlife habitats during the growing and harvest season; all occurred near many of the fields investigated. Additionally, evidence of animal activity (including cattle, deer, feral pigs, rodents, coyotes, and birds) was noted on the hillsides of adjacent lands during the investigations.

Due to the timing of the three investigations, each near the end of or after the growing and harvest season had concluded, investigators were largely unable to observe product in the field or harvest conditions and in many cases could not observe any activities. This severely limited the investigators' ability to observe practices and factors that may have contributed to contamination, necessitating increased reliance on sample results during the investigations.

III. Factors Potentially Contributing to Contamination

The 2020 *E. coli* O157:H7 outbreak associated with leafy greens in the United States represents the latest in a repeated series of outbreaks associated with leafy greens originating in the Salinas growing region of California. The outbreak had serious public health consequences as well as ramifications for the produce industry. This outbreak is the third year in a row in which leafy greens have been associated with an outbreak with a closely related strain of *E. coli* O157:H7 in a similar geographic area. Since 2006 there has been a demonstrated association (e.g. outbreaks, recalls, and product testing) of leafy greens and *E. coli* O157:H7 in this region. Growers should be aware of and consider adjacent land use practices, especially as it relates to the presence of livestock, and the interface between farmland, rangeland, and other agricultural areas, and conduct appropriate risk assessments and implement risk mitigation strategies, where appropriate.

Isolates within the 2020 outbreak are part of a reoccurring strain of concern and have been associated with reoccurring outbreaks in the fall linked to leafy greens since 2017. The two most recent outbreaks associated with this strain were an outbreak in 2018 (linked to romaine from the Santa Maria growing region of California) and an outbreak in 2019 (linked to romaine from the Salinas growing region of California). Clinical isolates from cases in this 2020 outbreak appear more closely related to those from the 2019 outbreak than the 2018 outbreak. In addition, several specific food and environmental isolates that appear to be closely related to this 2020 outbreak include a fecal-soil composite sample collected by FDA in February 2020 from the Salinas growing region and two leafy green samples collected by state partners as a part of the 2019 investigation that traced back to the Salinas growing region.

Due to the timing of the investigations, each near the end of or after the growing and harvest season had concluded, investigators were largely unable to observe product in the field or harvest conditions, and in many cases could not observe any activities due to the conclusion of growing and harvesting at the farm. However, visual observations of the implicated leafy green growing fields suggested several plausible routes for contamination, including from cattle grazing on adjacent land and from animal intrusion, evidenced by the presence of signs of animal intrusion such as scat and large flocks of birds.

As a result of this investigation, FDA's leading hypothesis is that cattle are most likely the sources of outbreak strains of *E. coli* O157:H7 associated with the 2019 and 2020 leafy greens outbreaks. This hypothesis is strengthened by the finding of the matching outbreak strain in a cattle feces composite sample taken alongside a road approximately 1.3 miles upslope from a produce farm with multiple fields tied to the outbreak by the traceback investigation. The consistent recovery of outbreak strains in cattle feces demonstrates that these human pathogens are in environments which surround produce growing fields. These findings do not indicate the extent to which this and other *E. coli* O157:H7 strains persist in growing environments. It is not clear how *E. coli* is moving in the environment and causing outbreak events linked to leafy green fields.

IV. Key Findings and Recommendations

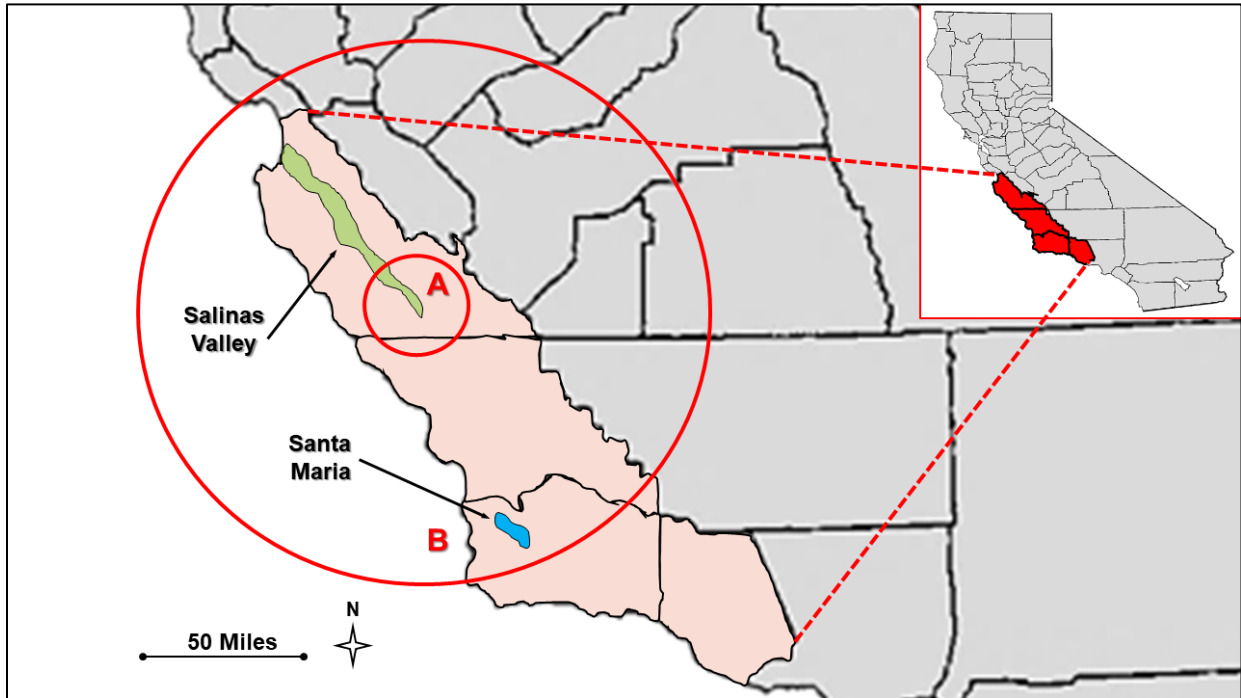
A cattle feces composite sample taken alongside a road approximately 1.3 miles upslope from a produce farm with multiple fields tied to the outbreak by the traceback investigation matched the 2020 outbreak strain of *E. coli* O157:H7. This most recent positive sample was collected approximately 3 miles from a positive fecal-soil sample related to a 2019 outbreak of *E. coli* O157:H7 linked to the consumption of romaine lettuce (Figure 1).

This reoccurring pathogenic *E. coli* strain has now been associated with numerous foodborne illness outbreaks linked to leafy greens consumption and therefore appears to be a reasonably foreseeable hazard in the California Central Coast leafy greens growing region and specifically in the South Monterey County area of the Salinas Valley. In light of this most recent finding, combined with previous outbreak investigation findings in the region, FDA has identified key trends as those of primary importance in understanding the contamination of *E. coli* O157:H7 that occurred in 2020 and previous years:

Reoccurring Region: Between November 2018 and December 2020, the FDA, state, and federal partners engaged in multiple traceback and field investigations related to *E. coli* O157:H7 foodborne illness outbreaks associated with consumption of romaine lettuce or leafy greens grown in California Central Coast growing regions (i.e., south Salinas Valley and Santa Maria Valley, illustrated in Figure 2 as area A and B, respectively).

Reoccurring Strain: The *E. coli* O157:H7 isolated from clinical samples (i.e. from ill persons) and environmental samples associated with these outbreaks are closely related as determined by whole genome sequencing. Because this *E. coli* O157:H7 strain has been associated with numerous foodborne illness outbreaks from multiple years that have all been associated with leafy greens grown in the California Central Coast growing regions, FDA and CDC consider them a reoccurring *E. coli* O157:H7 strain of continuing or ongoing concern. This reoccurring *E. coli* O157:H7 strain from foodborne illnesses in 2018, 2019, and 2020 linked to leafy greens consumption is also related by whole genome sequencing to an *E. coli* O157:H7 strain isolated from clinical samples (i.e. from ill persons) in 2016 and 2017.

Figure 2. Map of regions where current and past leafy green outbreak contamination has occurred.



Notes: Pink shading indicates counties within the Northern and Southern coastal production districts. Region A is indicating south Monterey County, a portion of the Salinas Valley growing region, which is highlighted in green. Region B is indicating the larger production district, including the entire Salinas Valley Growing region and Santa Maria. Santa Maria is highlighted in blue to the south of the Salinas Valley and is located within Santa Barbara County California.

Reoccurring Issues Around Adjacent and Nearby Land Use: All three 2020 on-site investigations noted that many of the farms with leafy green ranches/fields identified by traceback investigation had adjacent or nearby cattle grazing and/or adjacent uncultivated lands (which could serve as wildlife habitat during the production season). Also, evidence of animal activity (both cattle and wildlife) was noted on hillsides of adjacent or nearby lands during the investigations. These recent investigation findings and previous foodborne illness outbreak investigation findings dating back to 2013 suggest that a likely contributing factor for pathogenic *E. coli* contamination of leafy greens has been the proximity of cattle. Cattle have been repeatedly demonstrated to be a persistent source of pathogenic *E. coli* including *E. coli* O157:H7.

Due to the reoccurring nature of this pathogen in the south Monterey County area of the Salinas Valley and Santa Maria Valley growing regions in Santa Barbara County, FDA has concerns about the persistence of this strain of pathogenic *E. coli* O157:H7, which appears to be a reasonably foreseeable hazard in this area. We note that farms covered by the FSMA Produce Safety Rule are required to implement science- and risk-based preventive measures of that rule. Therefore, FDA recommends that, in the south Monterey County area of the Salinas Valley and Santa Maria Valley growing regions, the agricultural community, work to identify where this reoccurring strain of pathogenic *E. coli* is persisting and the likely routes of leafy greens contamination with STECs.

FDA acknowledges that one entity alone cannot fully address this public health issue of reoccurring pathogenic *E. coli* outbreaks associated with leafy greens consumption. FDA also recognizes the connection between people, animals, plants, and their shared environment as they are intertwined when it comes to public health outcomes. As such, we are strongly encouraging collaboration among various groups in the broader agricultural community (i.e. livestock owners and leafy greens growers, state and federal government agencies, and academia) to address this issue. With this collaboration, the agricultural community, alongside academic and government partners, can work to identify and implement measures to prevent contamination of leafy greens. FDA recommends that these parties to engage and participate in efforts to understand and address the challenge of successful coexistence of various types of agricultural industries to ensure food safety and protect consumers against foodborne illnesses. FDA acknowledges the locally led, locally convened ongoing efforts by produce growers, livestock owners, other agricultural industries (including the Monterey County Farm Bureau) and CDFA. This work should continue and broaden as both short-term and long-term solutions need to be pursued with urgency.

FDA recommends that growers of leafy greens in the **California Central Coast Growing Region (encompassing the Salinas Valley and Santa Maria growing regions)**:

- Consider this particular strain of *E. coli* O157:H7 a reasonably foreseeable hazard. Under the Produce Safety Rule (21 CFR 112.112 and 112.113), farms must:
 - take all measures reasonably necessary to identify, and not harvest, covered produce that is reasonably likely to be contaminated with a known or reasonably foreseeable hazard; and
 - handle harvested covered produce during covered activities in a manner that protects against contamination with known or reasonably foreseeable hazards.
- When pathogens are identified through microbiological surveys, pre-harvest or post-harvest testing of leafy greens, we recommend growers implement industry-led root cause analyses to determine how the contamination likely occurred and then implement appropriate prevention and verification measures.
- Actively engage in the locally-led, locally-convened effort called California Agricultural Neighbors (CAN) workgroup that is being led by the CDFA and Monterey County Farm Bureau to identify what actions can be taken to reduce the risk of STEC contamination of leafy greens in this specific growing region.
- Actively seek participation in the California Longitudinal Study (CALs) to better understand the ecology of human pathogens in the growing region. This multi-year effort is intended to provide information on how human pathogens survive in the broader agricultural environment and how they move throughout the environment, leading to potential produce contamination prior to or at harvest. The results from this collaboration are intended to lead to improved practices to prevent or mitigate food safety risks, and ultimately help enhance the safety of produce grown in the region.
- Actively encourage participation by adjacent and nearby livestock owners and other agricultural producers in the region to participate in CALs as well.

Considering the above, FDA recommends that **all producers of leafy greens**:

- Be aware of and consider risk that may be posed by adjacent land use, especially as it relates to the presence of livestock and the interface between farmland, rangeland, and other agricultural areas.

- Emphasize 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 good agricultural practices.
- Improve traceability
 - Increase digitization, interoperability and standardization of traceability records along the entire leafy green farm to store continuum, which would expedite traceback and prevent further illnesses. This is important at not only the grower level, but critical for shippers, manufacturers, and retailers as well, to improve overall traceability throughout the supply chain.
- Improve industry-led provenance labeling.
 - After the 2018 outbreak of *E. coli* associated with romaine lettuce, growers led a change in labeling to identify growing region on packaged romaine lettuce. While better food traceability back to farm is the ultimate goal, as an interim measure, provenance-labeling has been helpful in identifying during traceback investigations where potentially contaminated romaine was grown. Thus, industry should consider adoption of improved (with more detailed specificity beyond the current region designations) and expanded (to leafy greens products beyond just romaine lettuce) labeling to further protect consumers in the event of a product recall and minimize the amount of discarded product.
- Conduct 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 to identify how this contamination occurred.

Food safety is a shared responsibility, and FDA remains committed to providing technical assistance to the agricultural community in the Salinas Valley as they work to address this significant public health issue. Continued outbreaks of this reoccurring strain of pathogenic *E. coli* associated with leafy greens consumption impose a significant public health burden, reduce consumers' confidence in the food supply, and necessitate more collaboration by growers, livestock owners and the broader agricultural community to improve response efforts and protect public health.

FDA is calling upon livestock owners to consider implementing monitoring programs for human pathogenic *E. coli*, which could be used by the owners to assess food safety risk and potentially implement controls at the operations. We encourage livestock owners to share the information gained through such programs with leafy greens growers. FDA believes this type of information sharing may better enable the leafy green industry to characterize hazards in or near their growing operations and implement necessary measures to limit contamination from this pathogen.

Due to the reoccurrence of issues related to leafy greens grown in this geographical area, FDA plans to release an update to the 2020 Leafy Green STEC Action Plan (LGAP). Since the initial publication in March 2020 and outreach around the LGAP, FDA has made significant progress by enhancing prevention strategies, improving response activities by FDA and other entities, and identifying and addressing the knowledge gaps that exist around STEC contamination of leafy greens. The agency will proceed in a well-reasoned, scientific, evidence-based manner to ensure

these insights are utilized to enhance the safety of leafy greens and protect consumers. The plan has been updated and focused for 2021 and is informed by our work and knowledge gained over the past year. This updated 2021 plan is also a renewed commitment to advancing actions from the plan that we were unable to fully execute in 2020, either due to the pandemic or because they were actions that were never intended to be completed within a single year's timeframe.

V. Relevant Links

[FDA Outbreak Investigation of E. coli - Leafy Greens \(December 2020\)](#)

[CDC Outbreak of E. coli Infections Linked to Leafy Greens](#)

[NCBI link to WGS information for a representative isolate](#)

[Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated in the Three Outbreaks of E. coli O157:H7 During the Fall of 2019](#)

[FDA Announces New Sampling Plan for Romaine Grown in the Yuma, Arizona Growing Region](#)

[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](#)