



GROW WITH SCIENCE.



REP Strains
CDC and FDA Perspective and STEC Investigation Learnings

May 18, 2022





Logistics

- This webinar is being recorded. Questions and comments made in the Q&A window are anonymous to the audience.
- Everyone is muted.
- Please use the Q&A window to send us questions throughout the webinar
- Adjourn (60 minutes).



Content Overview

1. CDC perspective on reoccurring, emerging, and persisting (REP) strains
2. FDA perspective on REP strains and collaborative STEC investigations



Speakers

Michael Vasser

Epidemiologist/REPs Coordinator
CDC

Allison Wellman

Epidemiologist
FDA



Reoccurring, Emerging, and Persisting (REP) Strains: A CDC Perspective

Western Growers Webinar

May 18, 2022

Michael Vasser, MPH

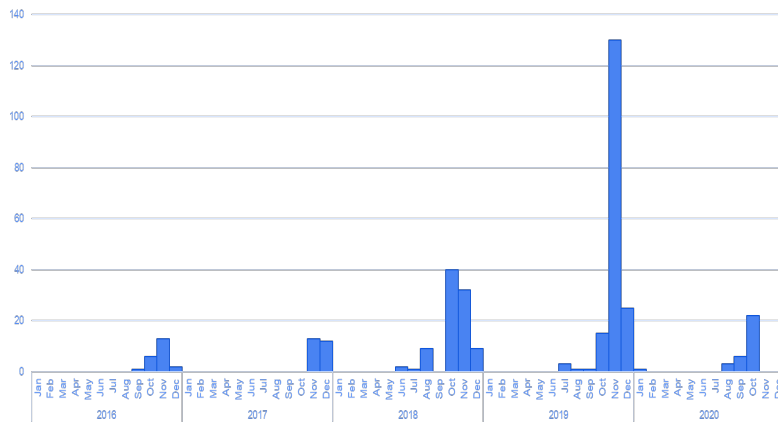
Outbreak Response and Prevention Branch, CDC

Definition and Criteria

- A REP strain is a reoccurring, emerging, or persistent set of bacteria related by whole genome sequencing that continues to cause illness over time
 - Definition kept broad on purpose
- What escalates a strain to a REP strain?
 - Cluster/Outbreaks
 - Resistance patterns
 - Non-clinical isolates
 - Genetic relatedness over time
- Allows us to characterize new sources of enteric disease and develop novel prevention approaches

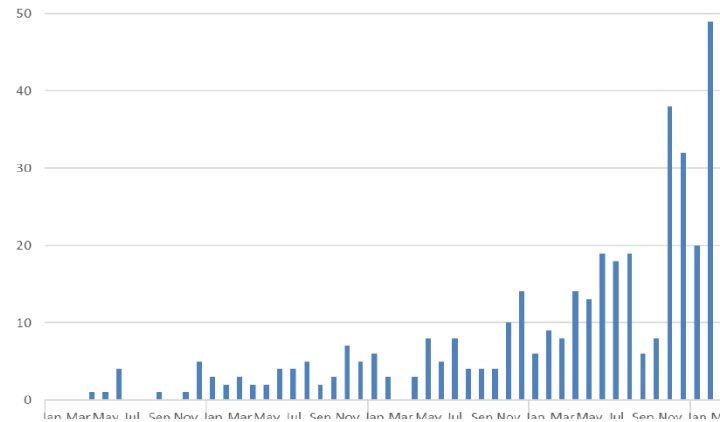
REP Strain Types

Reoccurring Strain



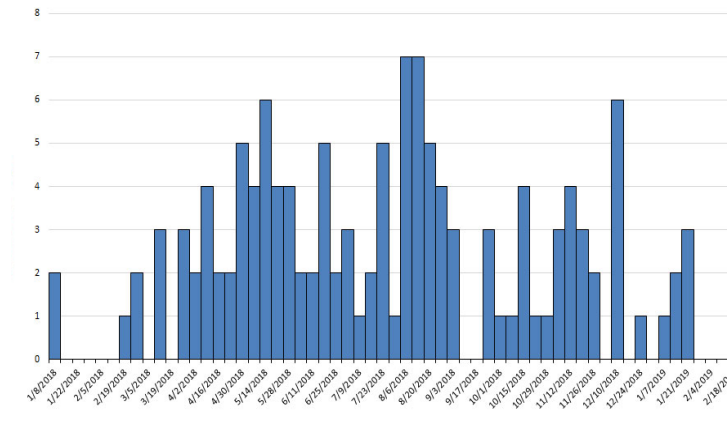
Strain that periodically causes a substantial number of illnesses, typically in outbreaks, separated by periods when it is not isolated from people or it causes very few illnesses

Emerging Strain



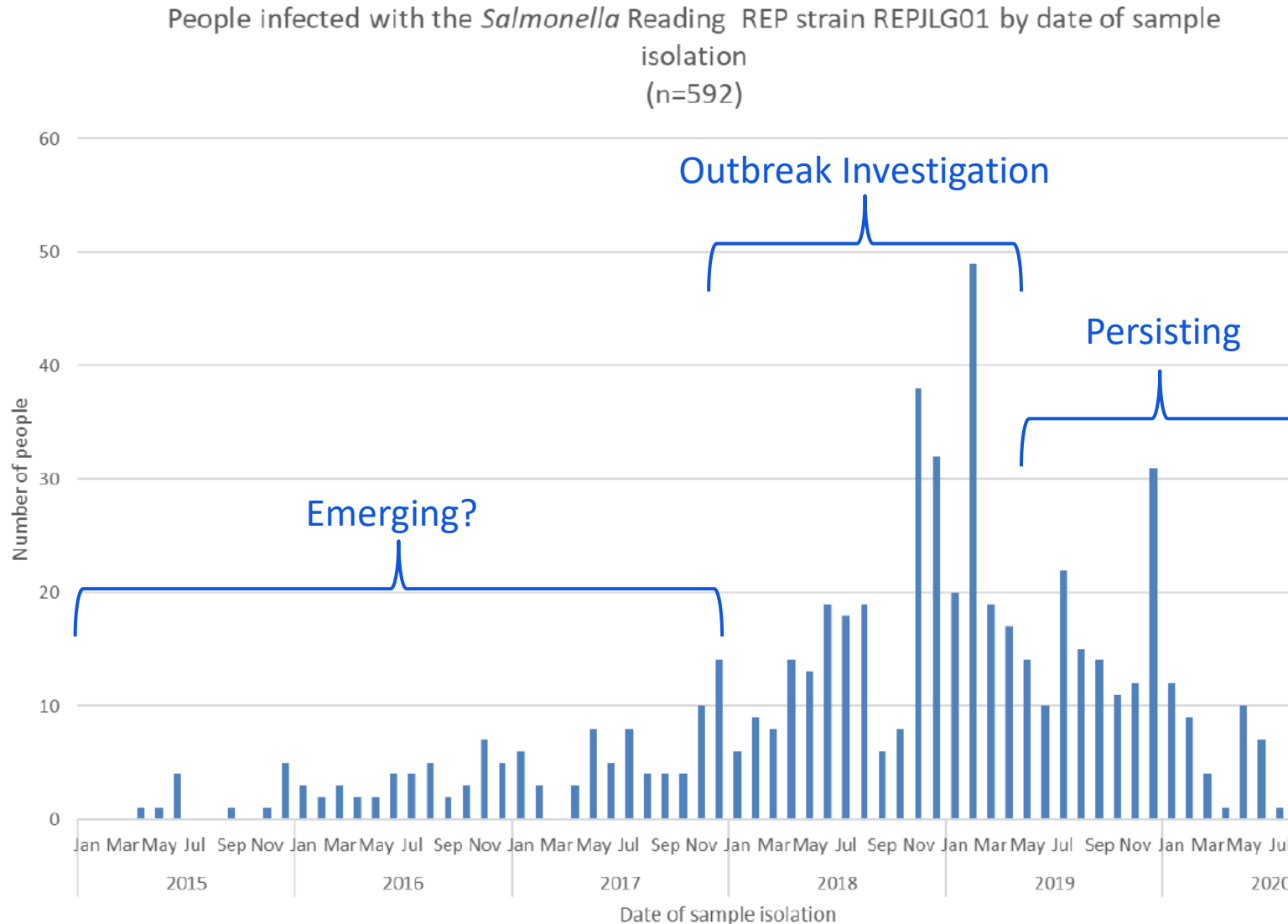
Strain that causes illnesses that have increased in frequency, or have the potential to increase in frequency, over time

Persisting Strain



Strain that causes illnesses consistently over time, although the frequency of illnesses may fluctuate

Dynamic Nature of REP Strains: *Salmonella* Reading Associated with Turkey Products



Phases of REP Strain Investigations

- 1. Monitoring:** working to understand the strain in more detail (WGS analyses, breaking down genetic and epi subclusters, working with industry partners, etc.), line lists updated on a less-regular basis
- 2. Active follow-up:** an uptick in cases leads to need for investigating specific cases, gather some early epi and traceback data as needed, notify partners of possible outbreaks down the line (if applicable), initiate conversations with states, line lists updated more frequently, etc.
- 3. Acute (outbreak) investigation:** traditional response to an outbreak, rapidly collecting information, working with regulatory agencies, line lists updated much more frequently, etc.

Current REP Strains

| REP Code | Years | Species/ serotype | No. isolates | Transmission / vehicle or population | Multidrug-resistant |
|----------|-----------|----------------------------|--------------|---|---------------------|
| REPJBG01 | 2017–2021 | Blockley | 281 | Foodborne / chicken | No |
| REPJEG01 | 2014–2020 | Enteritidis | 1136 | Foodborne, travel-associated / truffle oil puree | No |
| REPJGP01 | 2011–2021 | Kentucky | 156 | Unknown | Yes |
| REPJJP01 | 2016–2021 | Newport | 1149 | Foodborne, travel-associated / Mexican-style cheese, beef | Yes |
| REPJJP02 | 2016–2021 | Newport | 713 | Foodborne / ground beef | No |
| REPJJP03 | 2000-2021 | Newport | 1,797 | Foodborne / cucumbers / tomatoes | No |
| REPJLG01 | 2010–2021 | Reading | 1146 | Foodborne / turkey | Yes |
| REPGX601 | 2018–2020 | Typhimurium | 32 | Laboratory / ATCC strain/lettuce | No |
| REPTDK01 | 2019-2022 | Hadar | 1,541 | Zoonotic / poultry / foodborne / ground turkey | No |
| REPJFX01 | 2012-2022 | Infantis | 6,714 | Foodborne / chicken | Yes |
| REPEXH01 | 2016–2021 | O157:H7 | 862 | Foodborne, Waterborne/ romaine, leafy greens, recreational water, ground beef | Yes |
| REPEXH02 | 2016–2020 | O157:H7 | 399 | Foodborne / romaine, leafy greens | No |
| REPK1601 | 2017–2020 | <i>V. parahaemolyticus</i> | 245 | Foodborne / raw oysters | No |
| REPGX601 | 2011-2022 | <i>L. monocytogenes</i> | 74 | Foodborne / potatoes | No |

REP Strain Case Definitions

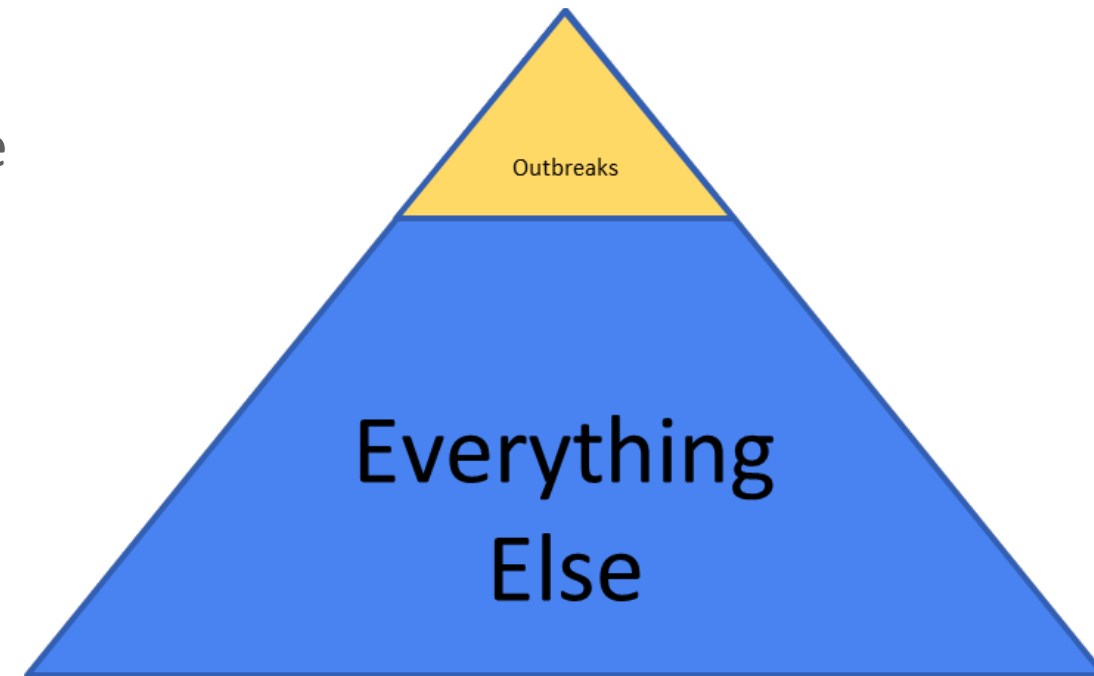
- Formal case definition developed like any other outbreak
- Genetic relatedness threshold (may permit more genetic diversity than outbreak case definitions)
 - Other genetic markers could be used (resistance, virulence, etc.)
- Timing of illnesses (often a much longer timeframe than cluster detection)
- REP strains are defined by human isolates, but related non-clinical isolates can provide critical clues about potential vehicles or reservoirs

REP Strain Communication

- Approaches considered when communicating about REP strains publicly, outside of MMWR or peer-reviewed publications:
 - Future public-facing web page that lists all active REP strains
 - Future, investigation-specific, communications tools that would permit web postings about REP strain investigations
 - Existing communication tools, such as Food Safety Alerts or Investigation Notices, provided the circumstances surrounding the REP strain are consistent with current outbreak communications framework

Potential Importance of REPs in Reducing Disease Incidence

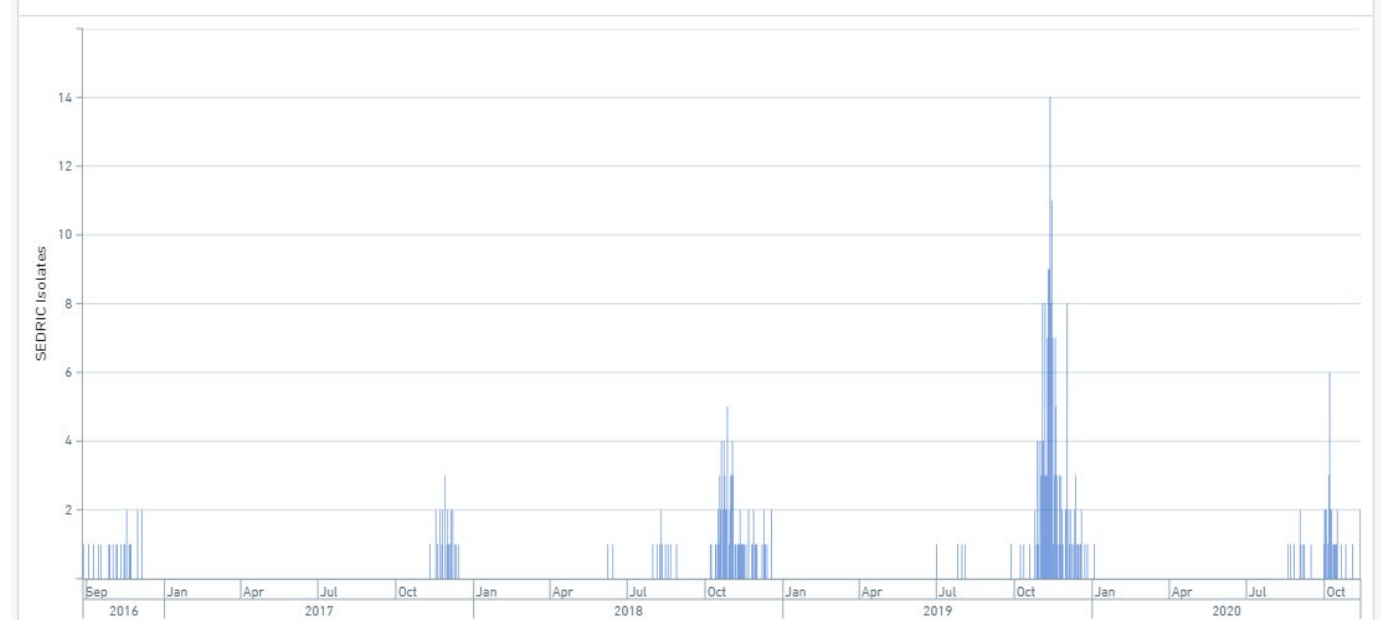
- Most illnesses reported through PulseNet are not linked to a source
 - ~90% of isolates don't have a cluster code and only a fraction of those that do are "solved"
- Driving down incidence of enteric and other foodborne pathogens requires better understanding of seemingly "sporadic" illness
- REP strains could represent a larger fraction of illnesses than traditional outbreaks



REPEXH02 Designation: Santa Maria/Salinas (SMS)

REPEXH02

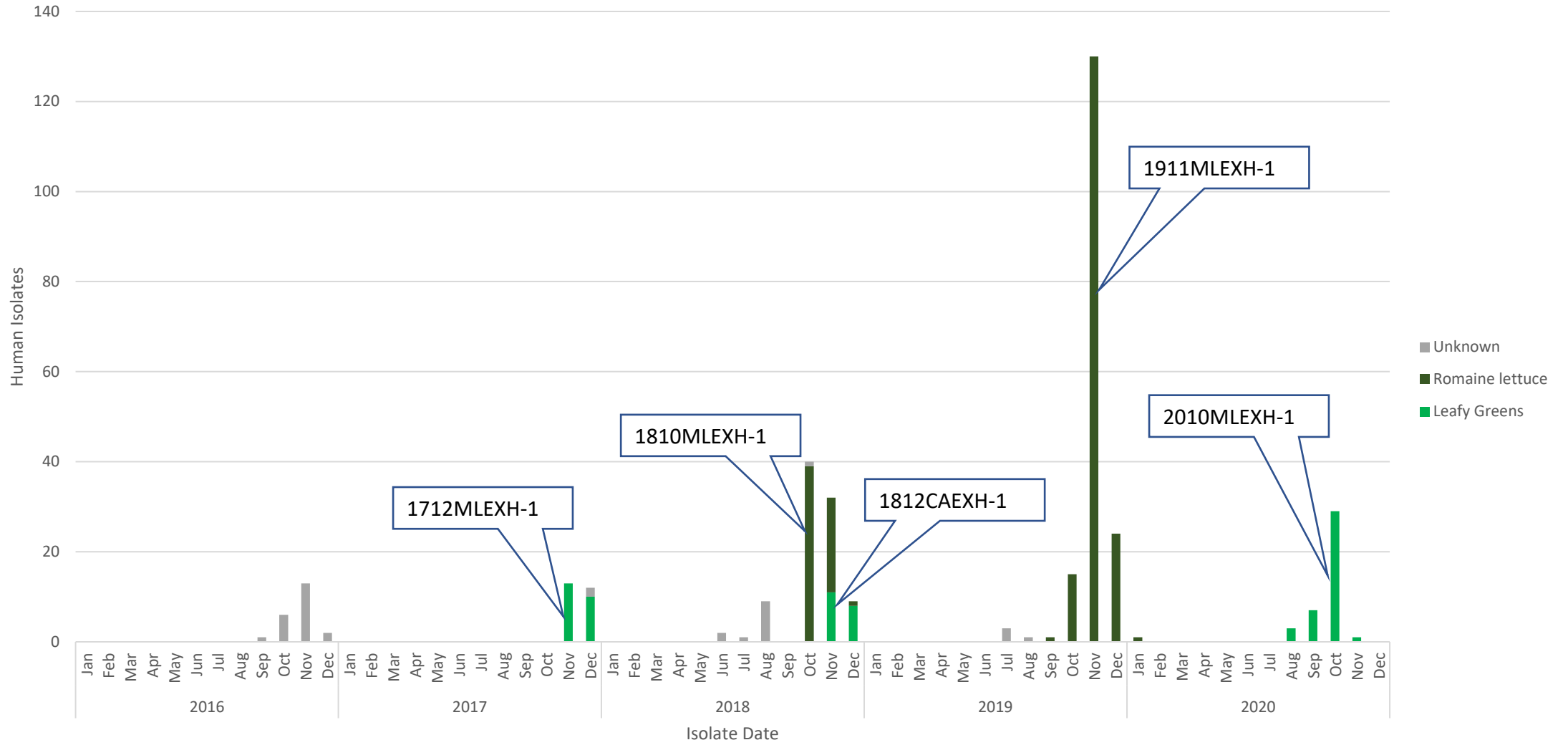
- Reoccurring strain identified in 2019 that caused repeated outbreaks each fall (~400 isolates total)
- REP strain is genetically less diverse (0-8 allele differences), but isolates fall into 2 subgroups
- Consistent linkage to leafy greens grown in California when a source is identified
- A single clinical case was identified in 2021



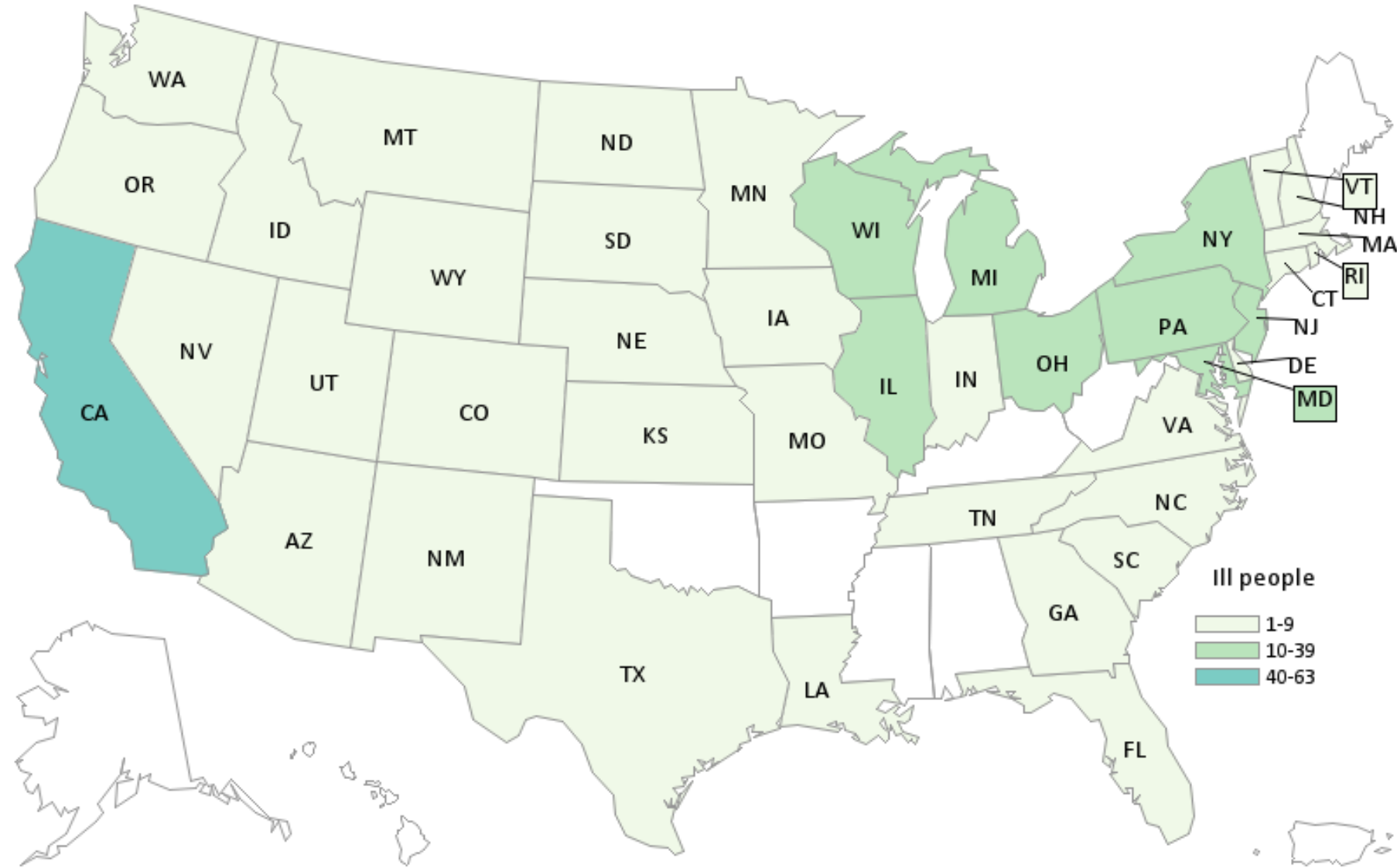
| Year | Outbreak Details |
|---------------|--|
| 2016 outbreak | <ul style="list-style-type: none">• 20 U.S. cases• Vehicle: unknown |
| 2017 outbreak | <ul style="list-style-type: none">• 25 U.S. cases• 42 Canada cases• Vehicle: leafy greens (suspected) |
| 2018 outbreak | <ul style="list-style-type: none">• 62 U.S. cases• 29 Canada cases• Vehicle: romaine lettuce (Central Coast, CA)• Outbreak strain found in ag water reservoir |
| 2019 outbreak | <ul style="list-style-type: none">• 167 U.S. cases• 4 Canada cases• Vehicle: romaine lettuce (Salinas, CA)• Outbreak strain found in unopened romaine bags |
| 2020 outbreak | <ul style="list-style-type: none">• 40 U.S. cases• Vehicle: leafy greens• Outbreak strain found in cattle feces |

People infected with the REPEXH02 strain of *Escherichia coli* O157:H7 by date of sample isolation (n=355)

*Outbreaks with suspected or confirmed vehicles are labelled (n=314)

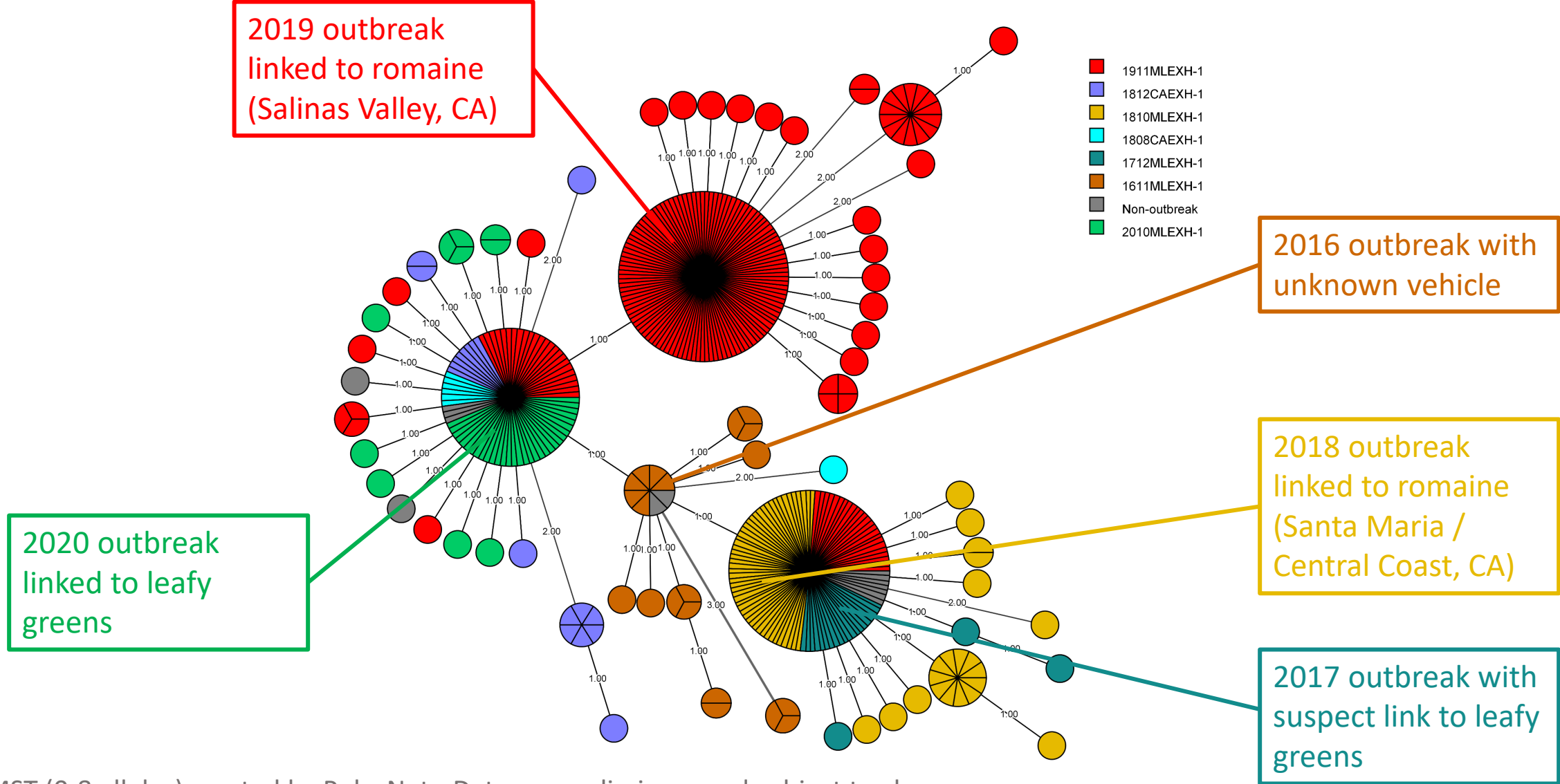


People infected with the REPEXH02 strain of *Escherichia coli* O157:H7,
by state of residence, between January 1, 2016 and March 17, 2021 (n=354)



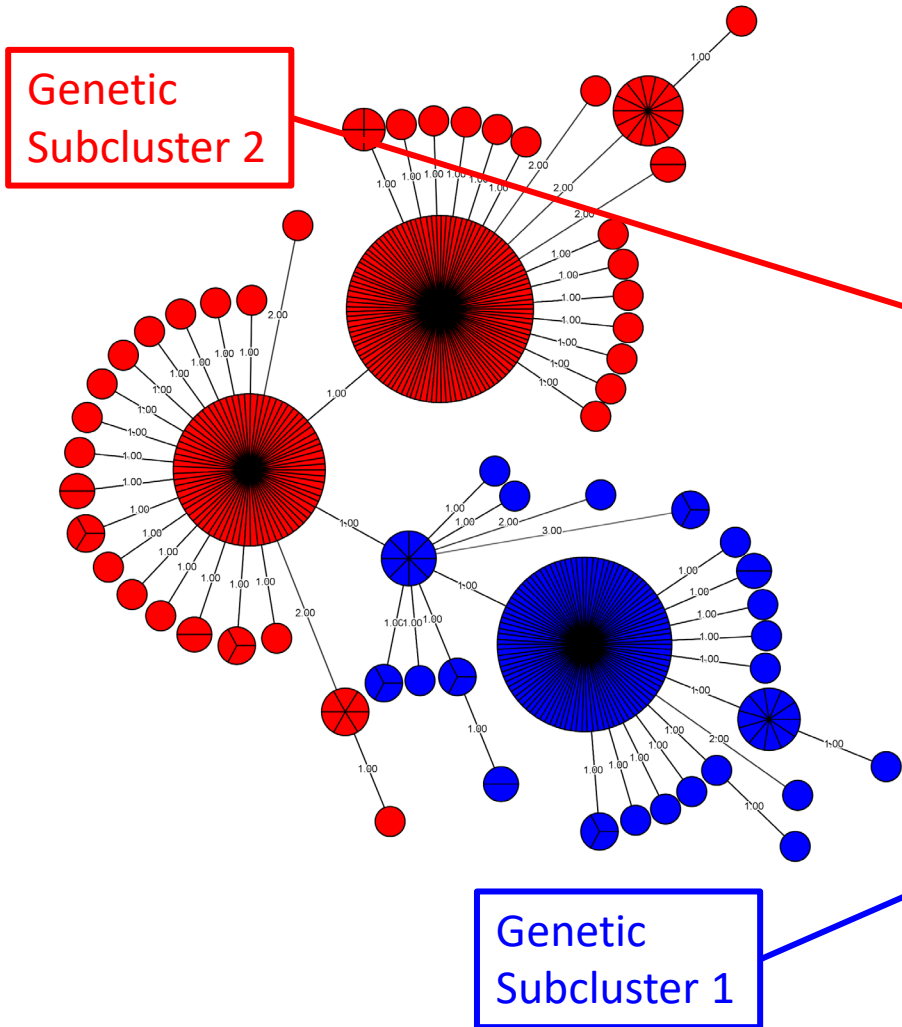
cgMLST Minimum Spanning Tree of REPEXH02 Strain by Outbreak

(n=398 Human and Environmental Isolates)



Note: MST (0-8 alleles) created by PulseNet - Data are preliminary and subject to change

Food and Environmental Samples Yielding the Strain



Salinas / Genetic Sub-Cluster 2

- 2019: unopened pre-packaged salads containing romaine from Salinas
- 2020: cattle feces

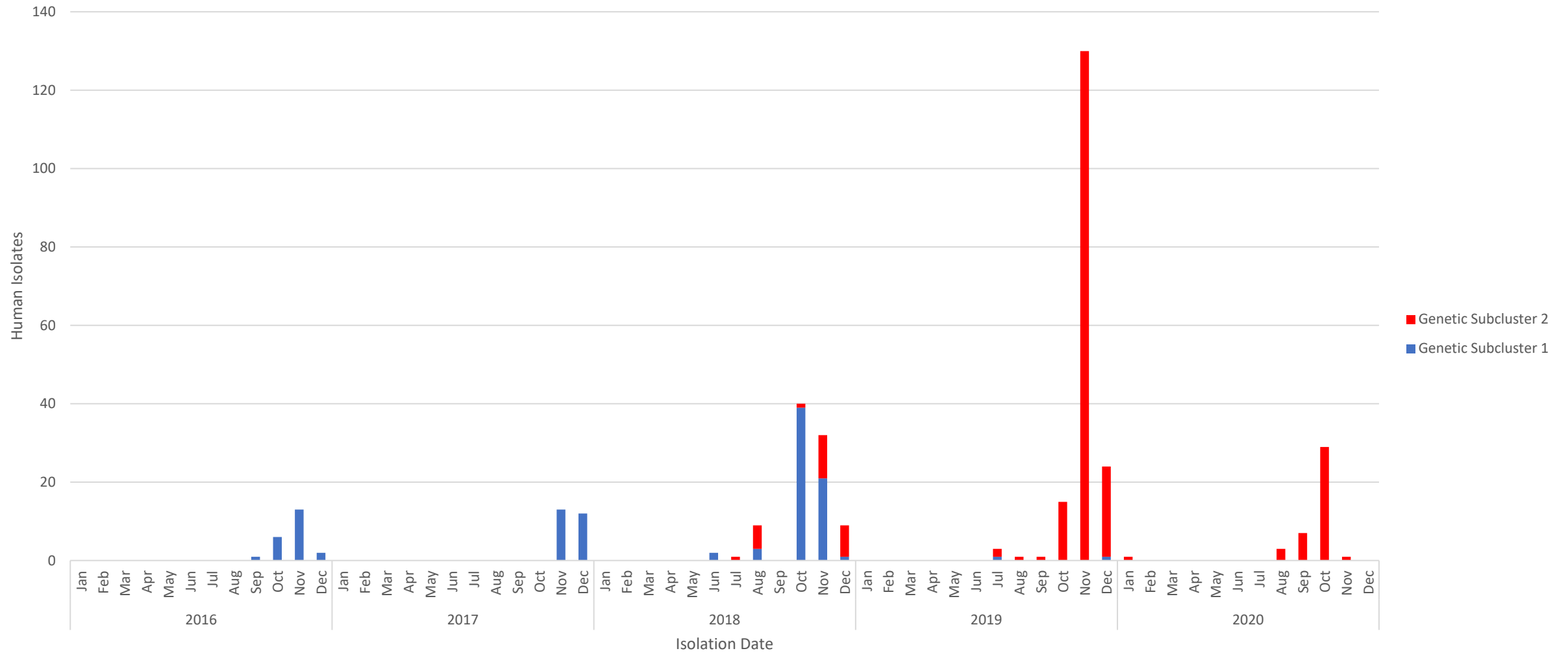
Santa Maria / Genetic Sub-Cluster 1

- 2018: irrigation reservoir sediment
- 2019: cattle trough sediment/water and cattle feces

California Growing Regions



People infected with the REPEXH02 strain of *Escherichia coli* O157:H7 by date of sample isolation (n=355)



| Outbreak | Allele range* | Human isolates | Non-Human Isolates | Total Isolates | Genetic subcluster | Vehicle (Traceback location) | Vehicle Status |
|---------------------------|---------------|----------------|--|----------------|---|--------------------------------------|----------------|
| 1611MLEXH-1 | (0-5) | 20 | | 20 | Genetic subcluster 1 | Unknown | |
| 1712MLEXH-1 | (0-3) | 23 | | 23 | Genetic subcluster 1 | Leafy greens | Suspected |
| 1808CAEXH-1 | (0-3) | 7 | | 7 | Genetic subcluster 2 (6), Genetic subcluster 1 (1)** | Unknown | |
| 1810MLEXH-1 | (0-4) | 62 | 9 (sediment from irrigation reservoir from Santa Maria, CA) | 71 | Genetic subcluster 1 | Romaine lettuce (Santa Maria, CA) | Confirmed |
| 1812CAEXH-1 | (0-5) | 19 | | 19 | Genetic subcluster 2 | Leafy greens | Suspected |
| 1911MLEXH-1 | (0-7) | 171 | 31 (5 romaine, 3 salad packs, 9 cattle trough sediment, 2 cattle trough water, 12 cattle feces from Santa Maria, CA) | 202 | Genetic subcluster 2 (187), Genetic subcluster 1 (24)*** | Romaine lettuce (Salinas Valley, CA) | Confirmed |
| 2010MLEXH-1 | (0-2) | 40 | 2 (cow patties from Salinas Valley, CA) | 42 | Genetic subcluster 2 | Leafy greens (Salinas Valley, CA) | Confirmed |
| Non-Outbreak/ Sporadic | (0-3) | 14 | | 14 | Genetic subcluster 1 (9), Genetic subcluster 2 (5) | | |

*Allele range is based on cgMLST. The allele range for the entire strain is 0-8.

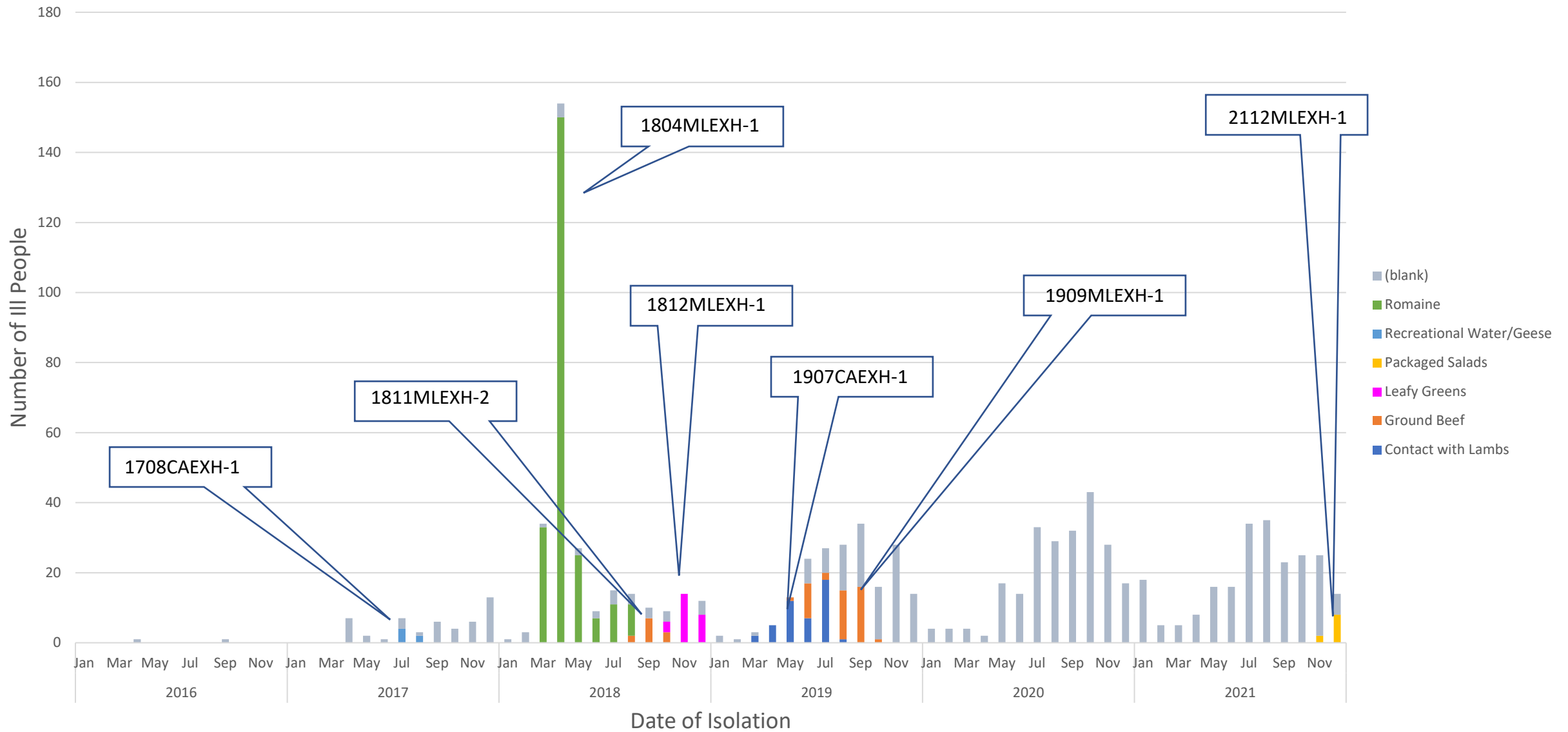
**The 1 isolate that is part of the “Genetic subcluster 1” is a clinical case from WI, while all other isolates in the outbreak were from CA.

***23 of the 24 isolates that make up “Genetic subcluster 1” were the cattle and trough isolates. Of note, these environmental samples were taken at farms in Santa Maria, CA. There was one clinical isolate from this outbreak that was part of “Genetic subcluster 1” as well. All food isolates that were sampled, and 170/171 clinical isolates are part of “Genetic subcluster 2.” The implicated romaine from the epidemiological evidence collected on cases, as well as the leafy green samples themselves, traced back to Salinas Valley, CA for this investigation.

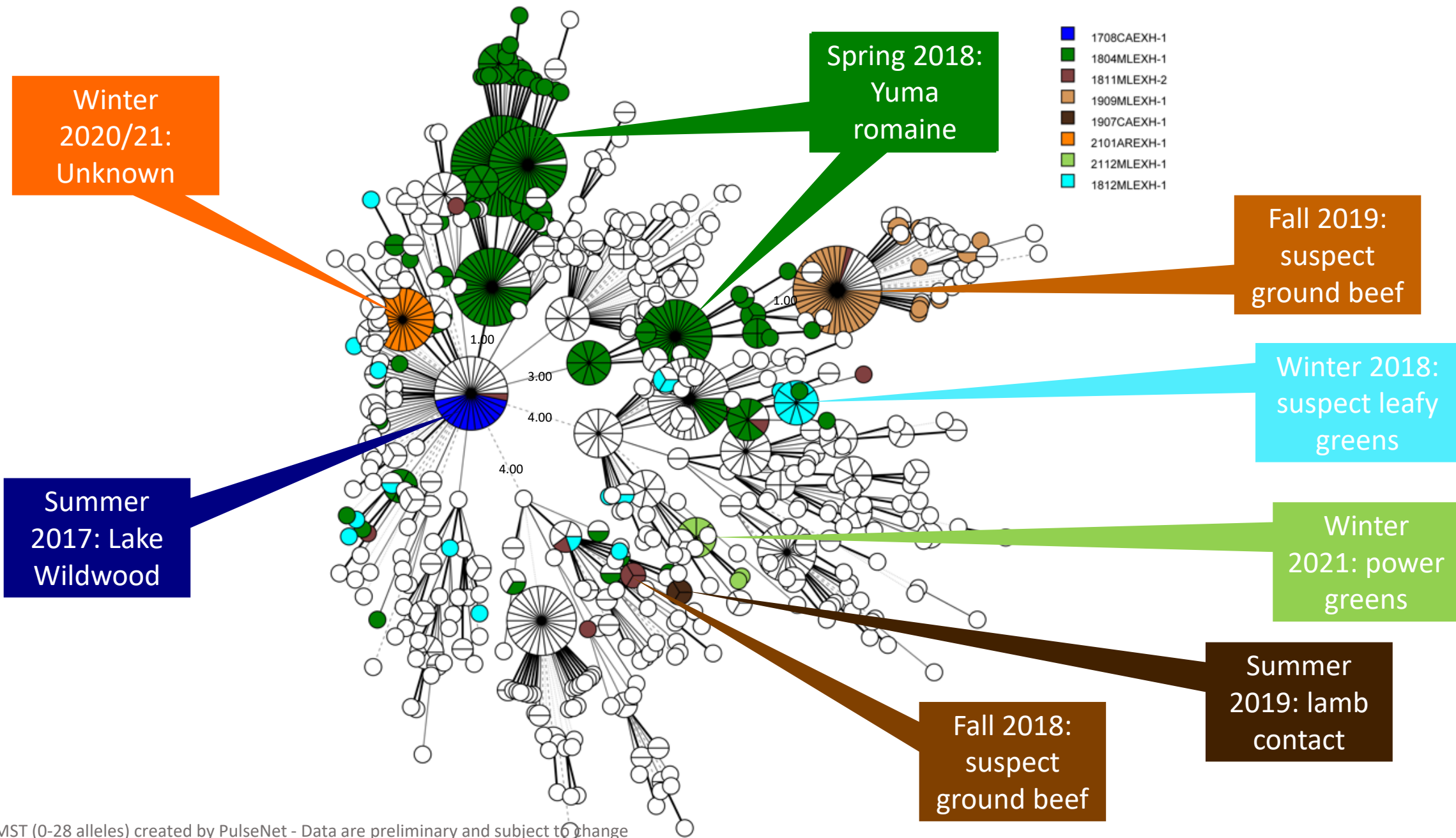
REPEXH01 Designation: CAZ

- REPEXH01 is linked to large 2018 romaine outbreak
 - Relatively diverse genetically (0-30 allele differences by cgMLST)
 - Currently divided into 5 major genetic clades and further divided into genetic subclusters
 - Continuing work to determine what to infer about vehicle or source when REPEXH01 illnesses and clusters occur
- REPEXH01 is considered a “persistent” strain rather than a “reoccurring” strain
 - Cases are reported year-round
 - No currently identified genetic clade appears to reoccur consistently
- Many illnesses that make up the strain are not part of a cluster investigation
 - The source was not determined for most of the clusters/outbreaks that were investigated
- Multiple vehicles have been suspected or confirmed in outbreak investigations of this REP strain (leafy greens, ground beef, contact with lambs, recreational water/geese)
 - No currently identified genetic clade within the strain is associated with one vehicle
 - The three outbreaks associated or possibly associated with leafy greens did not all occur at the same time of year and differed genetically

People infected with the REPEXH01 strain of Escherichia coli O157:57 by date of sample isolation (n=999) within confirmed and suspected outbreaks



cgMLST Minimum Spanning Trees of REPEXH01 strain by Outbreak (confirmed or suspected vehicles; n=1052)



Note: MST (0-28 alleles) created by PulseNet - Data are preliminary and subject to change

REPEXH01 vs. REPEXH02

- REPEXH01 and REPEXH02 are qualitatively different

| | REPEXH01 | REPEXH02 |
|---------------------|------------------------------|-----------------------------|
| REP Type | Persistent | Reoccurring |
| Genetic Diversity | Within 30 allele differences | Within 8 allele differences |
| Genetic Subclusters | 27 | 2 |
| Vehicle Linkage | Various | Leafy Greens |
| Strain Boundaries | “Fuzzy” | Definitive/concrete |

Additional Comments or Questions?

Michael Vasser, MPH – OXL8@cdc.gov

Epidemiologist and REPs Coordinator

Outbreak Response and Prevention Branch

Division of Foodborne, Waterborne and Environmental Diseases

For more information, contact CDC

1-800-CDC-INFO (232-4636)

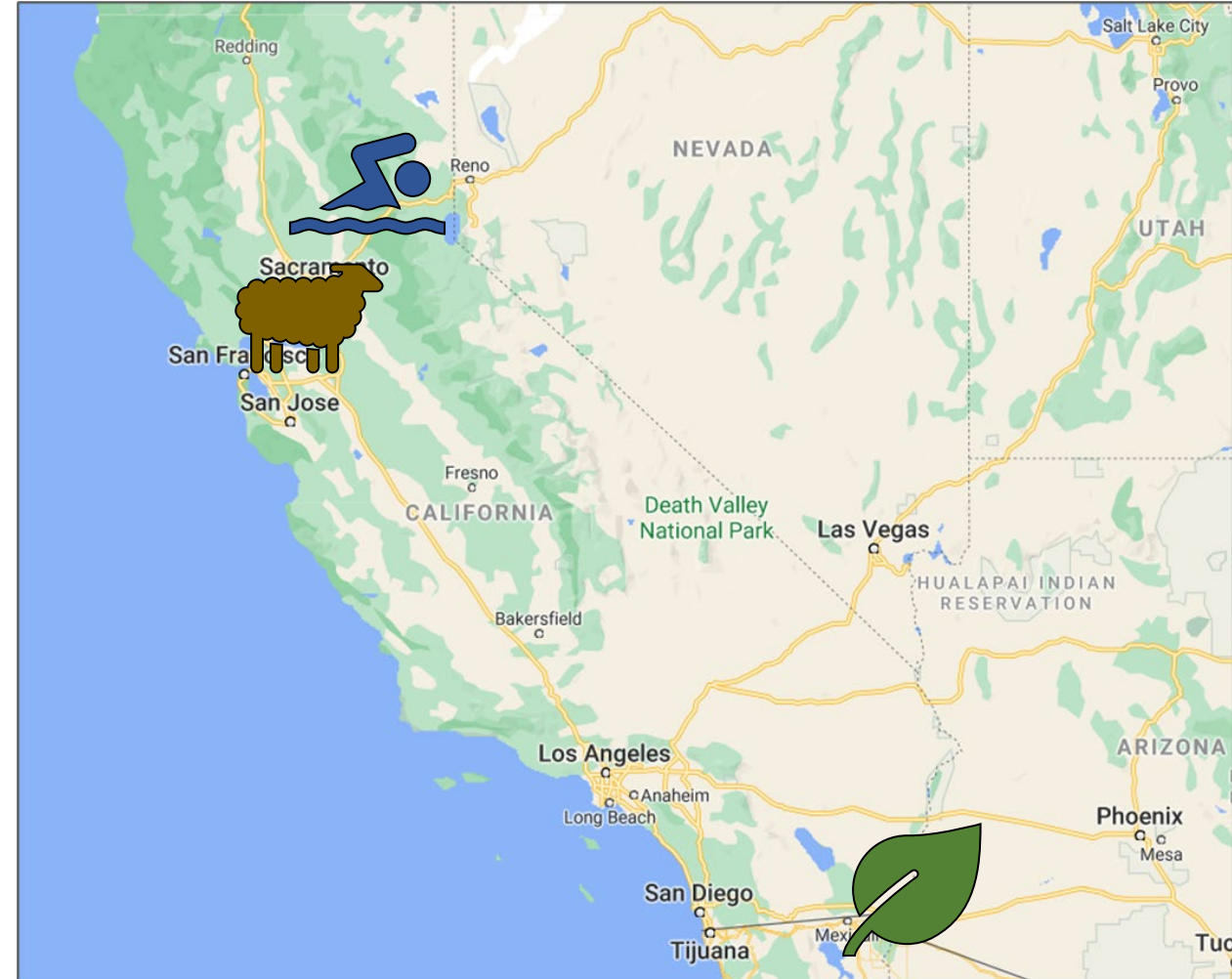
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.



Source Location of Outbreaks Linked to a Confirmed Vehicle

- 2017 Lake Wildwood (recreational water) outbreak
- Small 2019 outbreak linked to contact with lambs at a horse arena
- 2018 outbreak linked to romaine from the Yuma growing region
- Beef isolates from 2 states also identified during 3 different unsolved cluster investigations, but the source of the cattle was unknown



REP Strains: FDA Perspective and Collaborative STEC Investigations

Allison Wellman, MPH, Epidemiologist

Coordinated Outbreak Response and Evaluation (CORE) Network, US Food and Drug Administration

REP Strains Webinar for Western Growers, May 18, 2022

Overview

- CORE Background and FDA's Role in Outbreak Investigations
- Heightened Investigation Pathway for REP strains
- REP strain STEC investigations linked to leafy greens
- Environmental reservoirs for REPEXH01 and REPEXH02
- Ongoing prevention efforts



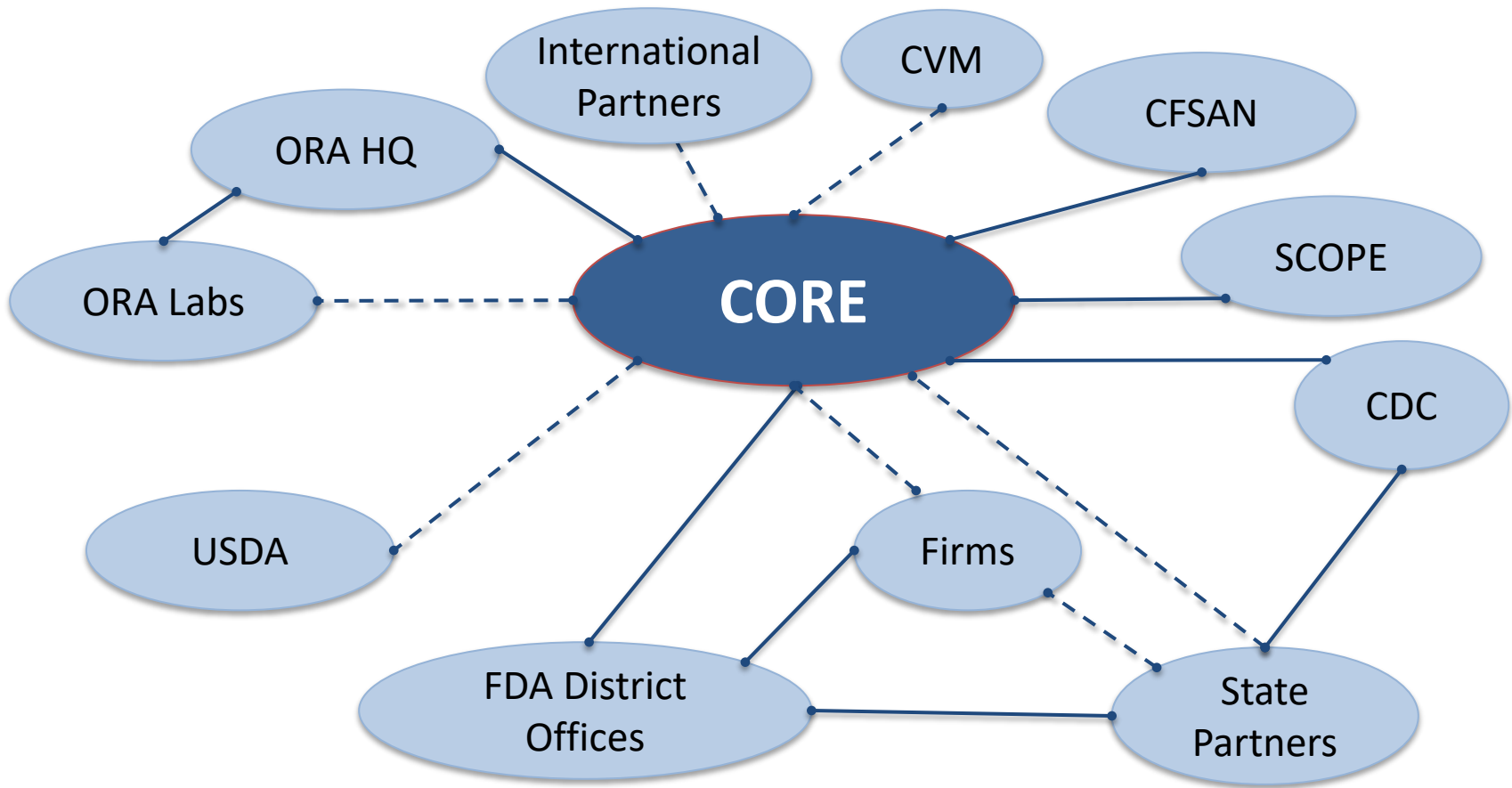
What is CORE?

- FDA's Coordinated Outbreak Response and Evaluation Network
- Coordinate Surveillance, Response, Communications and Prevention activities related to incidents of human illness linked to FDA-regulated human food, cosmetics, and dietary supplements
- Designed to streamline and strengthen FDA's efforts to:
 - Detect
 - Investigate
 - Respond
 - Communicate
 - Evaluate
 - Apply Lessons Learned

CORE Functional Areas

- Signals and Surveillance
- Response
- Outbreak Evaluation
- Outbreak Analytics
- Communications

CORE Network



Solid lines represent lines of communication that occur for nearly every CORE outbreak; dotted lines are established for specific situations.

Role of FDA in Foodborne Outbreak Investigations



Traceback of
suspected foods to
their source

Food and
environmental
testing

Communications –
Public, internal,
interagency,
congressional

Product and
regulatory actions

On-site
investigations at
farms or production
facilities

Regulations and
guidance to prevent
outbreaks

FDA Perspectives on REP Strains



- A REP strain is a reoccurring, emerging, or persistent set of bacteria related by whole genome sequencing that continues to cause illness over time
- FDA's Approaches to Persisting and Reoccurring Strains may differ (compared to non-REP outbreaks) in the following ways:
 - Earlier traceback activities
 - More widespread product and environmental sampling
 - Integration of inspectional/investigational data in the broader geographic context and across years/investigations
 - Broader/earlier public communications

REPEXH02: Fighting a Reoccurring Strain with a Heightened Investigation Pathway



- CDC and state/local partners evaluate food exposure information for cases of a particular REP strain using previously created focused questionnaires
 - Looking for produce being grown/harvested in regions previously linked to this strain (due to potential environmental contamination)
- FDA may initiate traceback activities at an earlier stage (may involve tracing a single case's exposure)
- Earlier outreach to firms to determine supplier information and growing areas

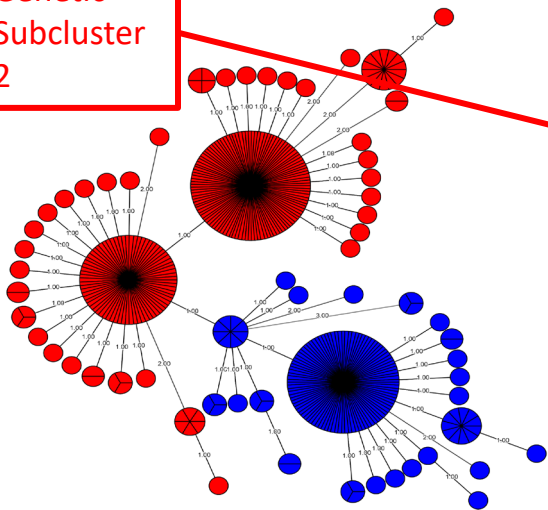
Heightened Investigation Pathway (cont.):

- Agencies consider earlier public messaging about an investigation
- Early outreach to industry (like trade organizations)
- Potential for earlier farm investigations (or follow up investigations), at locations where the strain has been identified in the environment previously and new locations identified by early traceback activity

REPEXH02: Food and Environmental Samples Yielding the Strain



Genetic Subcluster 2



Salinas / Genetic Sub-Cluster 2

- 2019: unopened pre-packaged salads containing romaine from Salinas
- 2020: cattle feces

Genetic Subcluster 1

Santa Maria / Genetic Sub-Cluster 1

- 2018: irrigation reservoir sediment
- 2019: cattle trough sediment/water and cattle feces

California Growing Regions



Note: Data are preliminary and subject to change

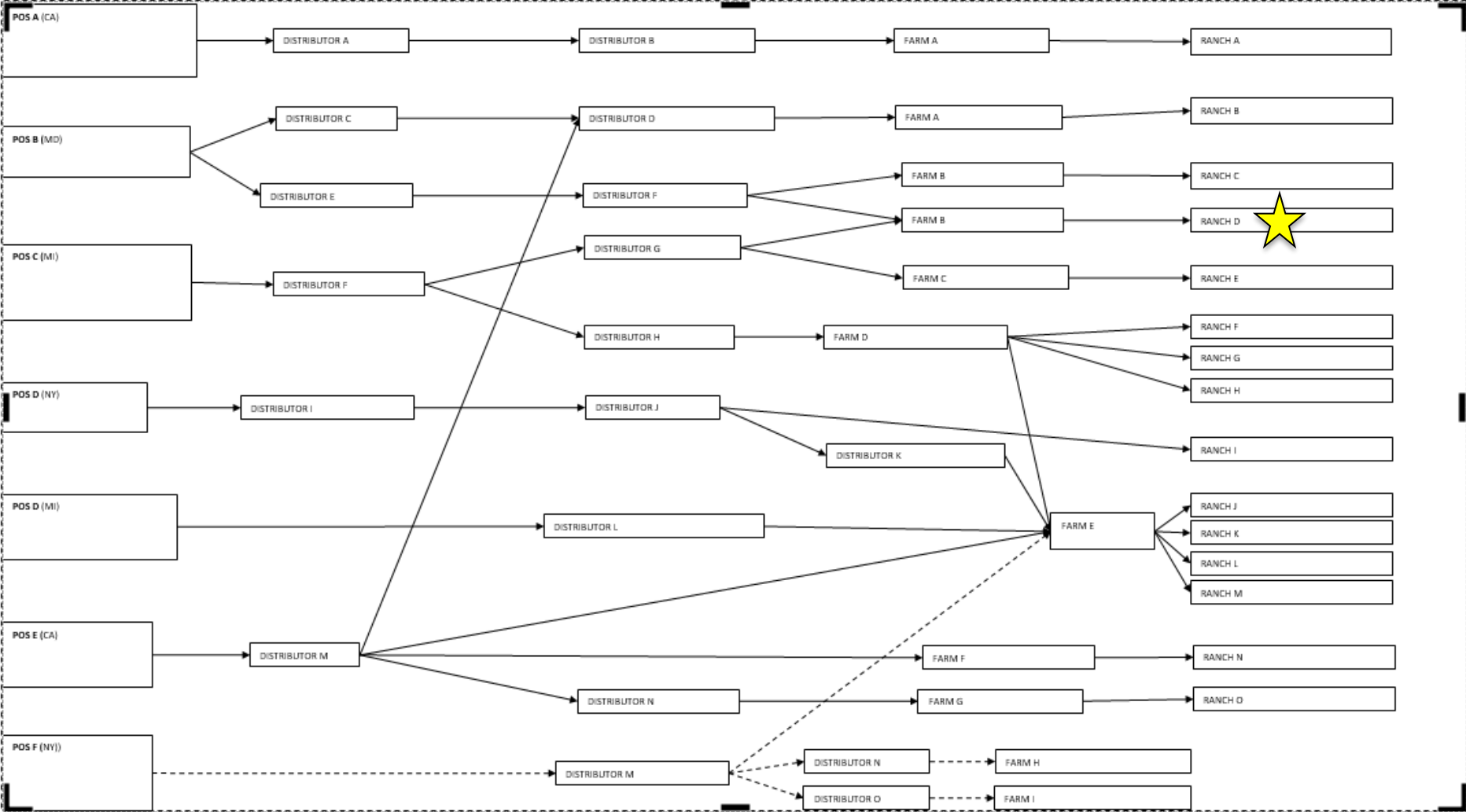
REPEXH02



Fall 2018 Outbreak: Traceback Investigation

E. coli O157/Romaine/Nov 2018
REDACTED TRACEBACK DIAGRAM – Romaine Lettuce

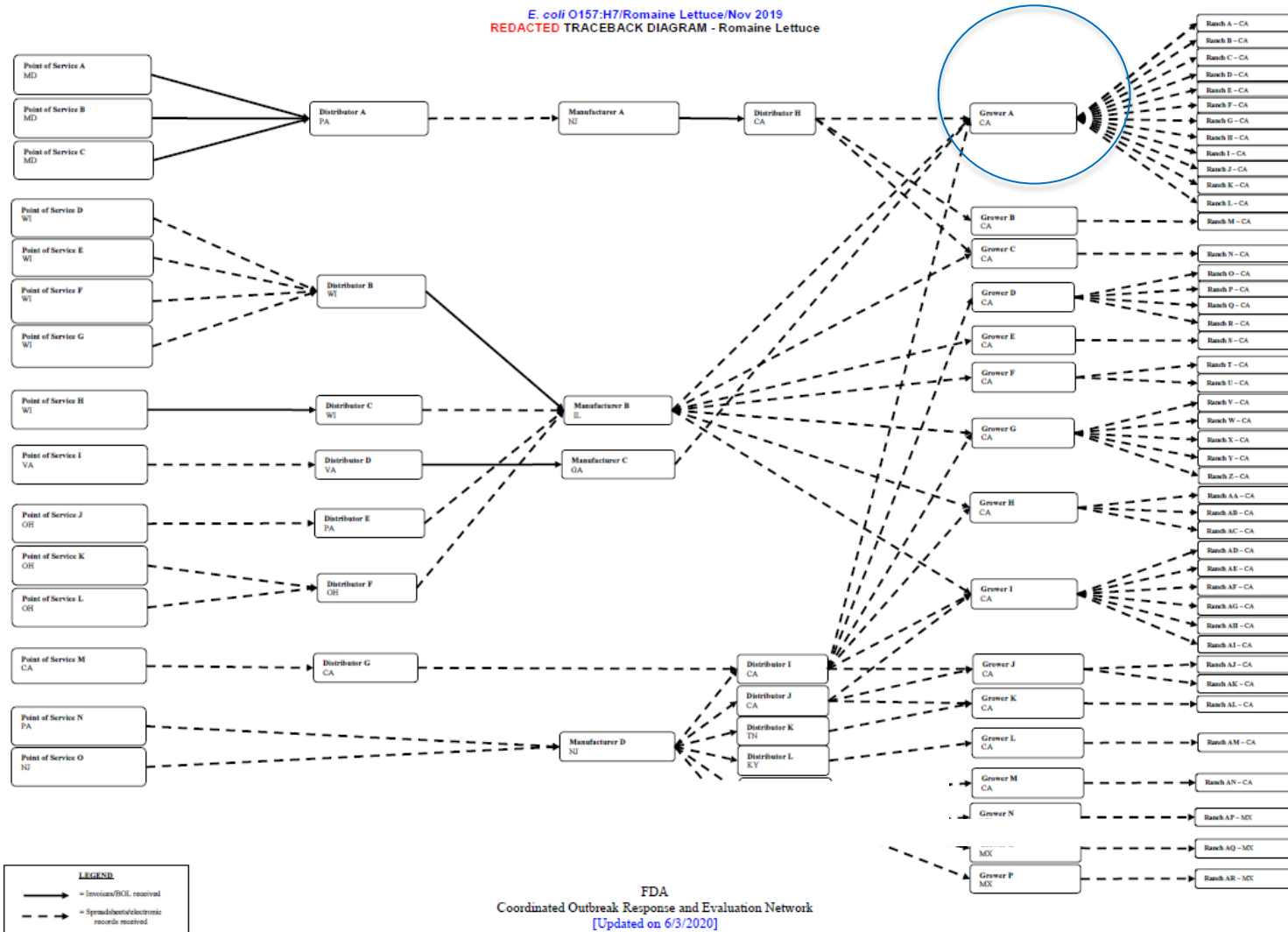
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REPEXH02

Fall 2019 Outbreak: Traceback investigation

E. coli O157:H7/Romaine Lettuce/Nov 2019
 REDACTED TRACEBACK DIAGRAM - Romaine Lettuce

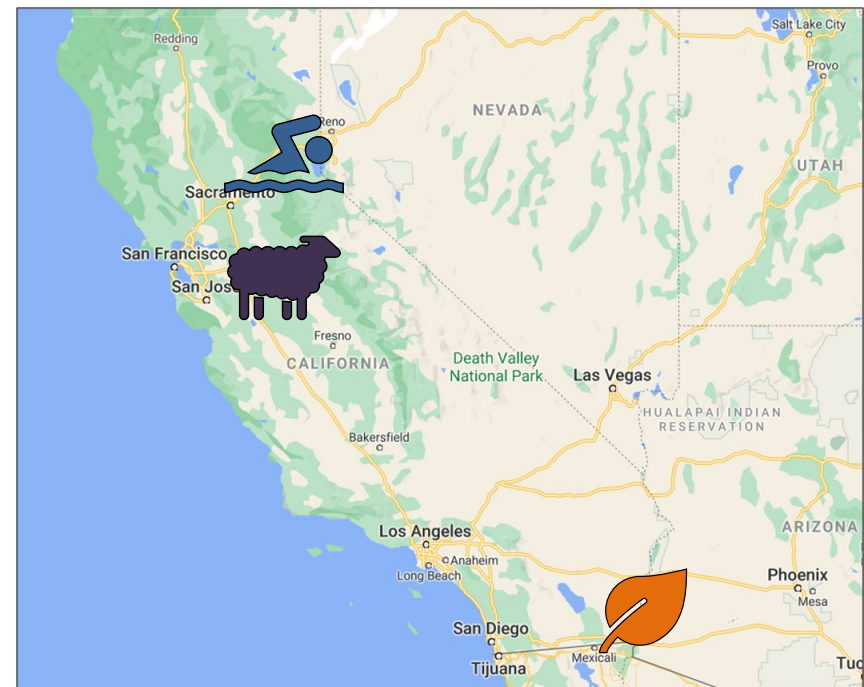


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REPEXH01: Source Location of Outbreaks Linked to a Confirmed Vehicle



- 2017 Lake Wildwood (recreational water) outbreak
- Small 2019 outbreak linked to contact with lambs at a horse arena
- 2018 outbreak linked to romaine from the Yuma growing region
- Beef isolates from 2 states also identified during 3 different unsolved cluster investigations, but the source of the cattle was unknown





Speed of FDA Response for REPEXH01 varies depending on subclade

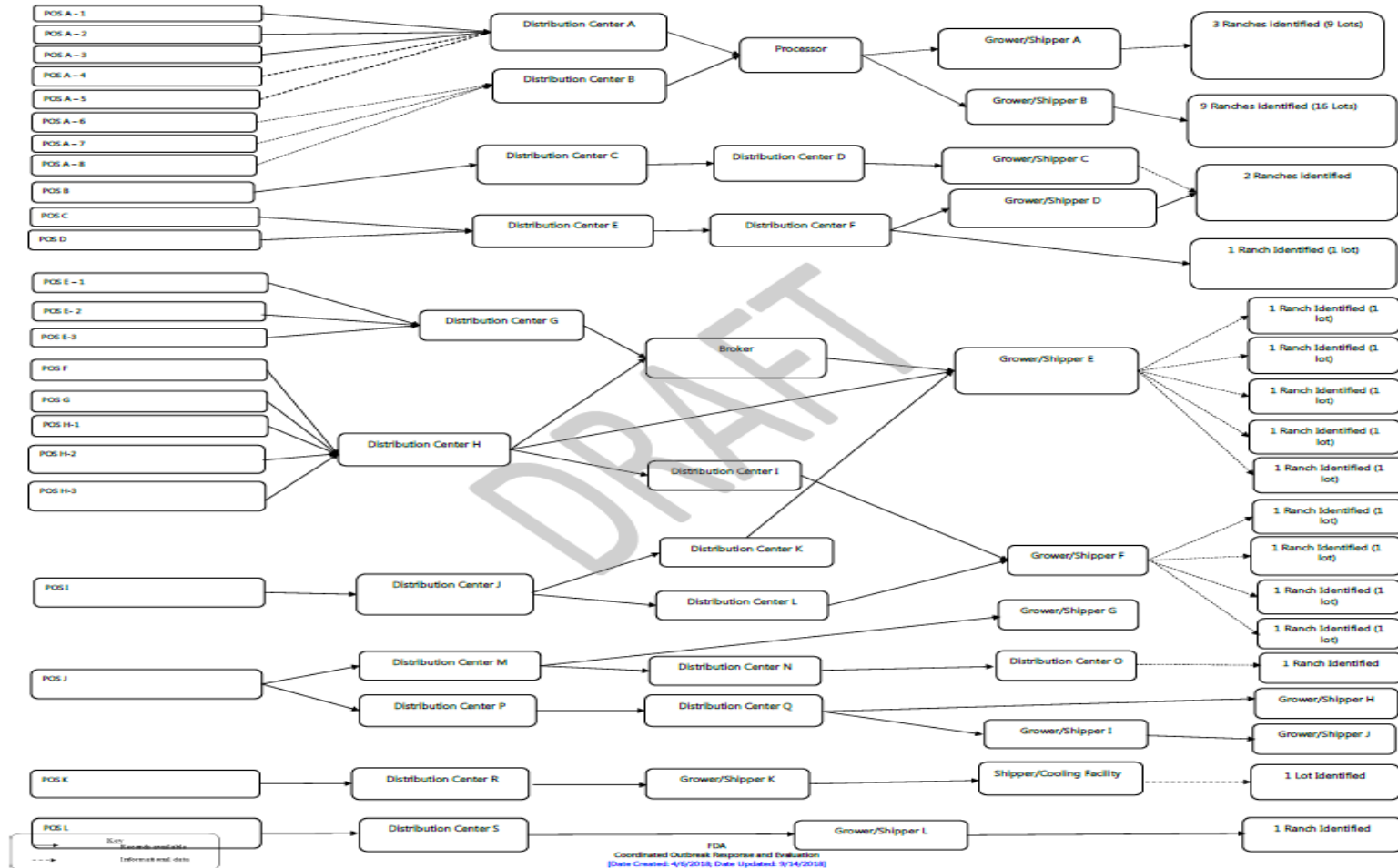
- Aggressive evaluation of single traceback candidates for cases falling in subclades 4 and 5 (main clades of 1804MLEXH-1)
 - May occur prior to identification of a cluster by PulseNet or completion of a cluster epidemiologic investigation
- For clusters in other subclades, FDA response activities contingent on findings from epidemiologic investigation

REPEXH01



Spring 2018 Outbreak Traceback Investigation

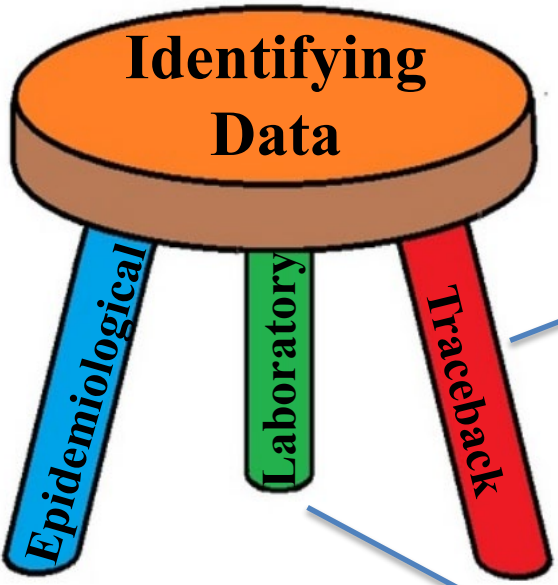
E. coli O157:H7 – Romaine
Multi-state Outbreak April 2018
DRAFT-Master Redacted Traceback Diagram
DRAFT - This report should not be further distributed beyond its intended FDA audience without express permission from FDA



Confirming an Outbreak Vehicle based on Lines of Evidence



Gold Standard



Beginning at points of sale reported by cases, product is traced back through the supply chain. Convergence at a *specific point of origin (ranch or processing facility)* confirms the vehicle and point of contamination.

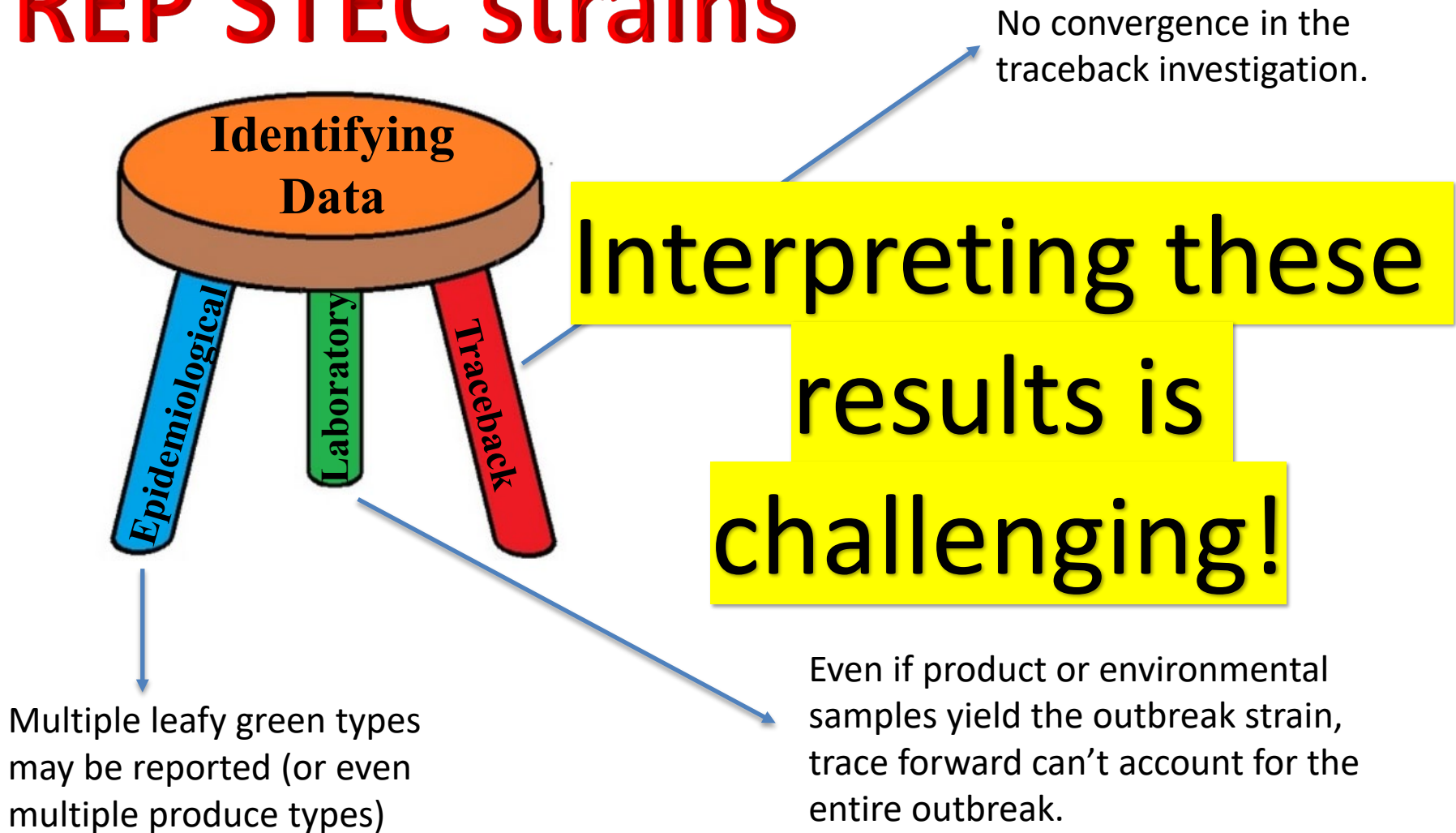
Interviews of case patients identify a common food product.

Sampling of the identified food yields the outbreak strain.

Confirming an Outbreak Vehicle based on Lines of Evidence

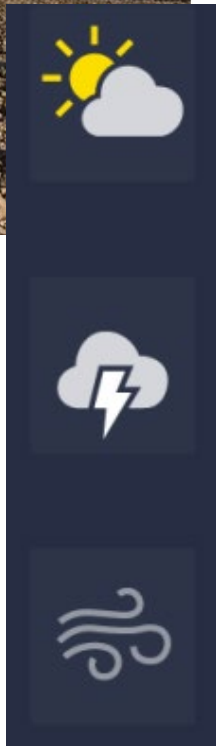
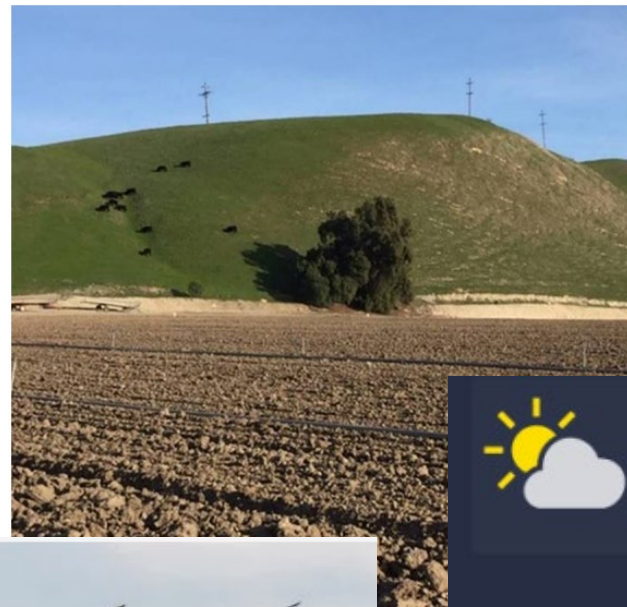


REP STEC strains



Factors Potentially Contributing to Contamination

- Cattle on adjacent land
- Animal intrusion and wildlife
- Agricultural water
- Weather patterns



Impact of Environmental contamination on Traceback Investigations

- Lack of convergence at a particular farm or ranch for STEC REP outbreak investigations and shift in public health messaging
 - Outbreaks may be due to contaminated products from multiple ranches/farms
 - Geographic convergence



Shift in Public Messaging and Product Actions for REP strains

- Public advisories focused on broad geographic areas
 - Once we reach a certain level of confidence that an outbreak is caused by leafy greens, we have relied on public communications and recalls as our primary methods for protecting public health
 - Compared to historical outbreak investigations, recent REP investigations have resulted in broader consumer warnings
 - Broader voluntary product action (withdrawing romaine from market, withholding distribution for certain CA growing regions)
- Importance of product labeling with harvesting information to help scope product actions

Impact of the broad Romaine Advisory



Romaine lettuce advisory leads to tons of waste, and new labels

By ERIKA MAHONEYKAZU • DEC 12, 2018

- Share
- Tweet
- Email



A sign posted at Hannaford Supermarket in North Conway advises customers that all romaine lettuce products have been removed from the shelves due to a federal health advisory. (DAYMOND STEER PHOTO)



First look: the new romaine labels

November 30, 2018 - Produce with Pamela

- Print
- Email
- Facebook
- Twitter
- +



Church Brothers shared a photo of its new romaine lettuce labels showing harvest date and location. View the post at https://www.instagram.com/p/Bqu2_yvFyQ2/



Romaine lettuce has been pulled from Food Lion shelves in Durham, North Carolina. Ann Taylor

November 1, 2018: FDA's Notice to Leafy Greens Industry



November 1, 2018

Mark Killian, Director, Arizona Department of Agriculture
Teresa Lopez, Arizona Leafy Greens Marketing Agreement
Arizona Department of Agriculture
1688 West Adams St
Phoenix, AZ 85007

Karen Ross, Secretary, California Department of Food and Agriculture
Scott Horsfall, California Leafy Greens Marketing Agreement
California Department of Food and Agriculture
1220 N Street
Sacramento, California 95814

Dear Colleagues:

The Food and Drug Administration (FDA) has released its Environmental Assessment (EA) report concerning the serious outbreak of *E. coli* O157:H7 infections associated with consumption of romaine lettuce that occurred earlier this year. The findings of this outbreak and their implications make it clear that FDA's relationships with its state partners and the leafy greens industry are more important than ever. The findings also show that our approaches to prevent leafy greens contamination must change to protect public health.

We each have an important role in making that happen. It is industry's role to ensure that the foods they bring to market are safe for consumers to eat. Therefore, we urge all segments of the leafy greens industry to review their operations and make all necessary changes.



← Home / Food / Recalls, Outbreaks & Emergencies / Outbreaks of Foodborne Illness / Environmental Assessment of Factors Potentially Contributing to the Contamination of Romaine Lettuce Implicated

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

Share Tweet LinkedIn Email Print

November 1, 2018

This document provides an overview of factors that potentially contributed to the contamination of romaine lettuce with *E. coli* O157:H7 that was implicated in a 2018 multi-state foodborne illness outbreak.

I. Background

Outbreaks of Foodborne Illness

Environmental Assessments from Foodborne Illness or Contamination Events

About the CORE Network

<https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigated-multistate-outbreak-e-coli-o157h7-infections-linked-romaine-lettuce-yuma-growing>

“A key element that would assist tracing efforts during an outbreak is the ability to identify specific farms or ranches that contribute to production lots...Without the ability to identify the growing region or specific suppliers of suspected shipments, public messaging by FDA and other public health partners during recalls or outbreaks is of necessity broad, possibly implicating farms and growing regions that are not responsible for the contamination”



What's Next with REPEXH01 and REPEXH02

- Continuous evaluation of REPEXH01 and REPEXH02 isolates, with particular emphasis on seasonal transition times
 - Working with leafy greens industry to enhance food safety practices – Leafy Green STEC Action Plan
- Tailoring FDA outbreak investigation processes for REP strains (not a one-size-fits-all approach)



Ongoing Prevention Activities

- For leafy greens in general, many ongoing activities to enhance prevention and response activities related to the safety of leafy greens, and addressing knowledge gaps
- Prioritized sampling activities
- Leafy Greens STEC Action Plan
- Food Safety Modernization Act - Produce Safety Rule, Traceability requirements
- Partnering w/ industry, consumer groups, retailers, Ag community, government agencies

And More Broadly...

What's Next with REPs in General



- Application of REP framework to other pathogen/commodity pairs
 - Salmonella in various produce commodities
 - Salmonella in Beef & Cheese
 - Listeria
 - Harborage in a facility vs. a persisting strain
- Interagency Collaboration to identify new REPs

Acknowledgements



- CDC/National Center for Emerging and Zoonotic Infectious Diseases
 - Outbreak Response & Prevention Branch and Enteric Diseases Laboratory Branch/Division of Foodborne, Waterborne and Environmental Diseases
- FDA/Center for Food Safety and Applied Nutrition
 - Division of Microbiology/Office of Regulatory Science
 - Office of Analytics and Outreach
 - Office of Food Safety, Division of Produce Safety
 - Coordinated Outbreak Response and Evaluation (CORE) Network
- All other investigation partners





Q&A





Closing Remarks

Leading Industry's Response and Engagement

1. WG will continue its engagement with CDC/FDA regarding the current CDC/FDA REP framework
 - Including considerations for applying the REP framework to different pathogens and commodities
2. WG is developing genomic tools/services (including considerations for use of WGS)



Closing Remarks

Contact us

If you are interested in learning more or working with us on these efforts, contact the WG Science team at wgscience@wga.com or reach out directly to:

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Thank you!



GROW WITH SCIENCE.



REP Strains
CDC and FDA Perspective and STEC Investigation Learnings

May 18, 2022

