Reoccurring *Escherichia coli* O157:H7 Strain Linked to Leafy Greens–Associated Outbreaks, 2016–2019

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Jessica Chen, a bioinformatician at CDC in Atlanta. We'll be discussing *E. coli* O157:H7 strain linked to leafy greens-associated outbreaks.

Welcome, Dr. Chen.

[Jessica Chen] Thanks for having me, Sarah.

[Sarah Gregory] What is *E. coli* O157 and how is it different from other strains of *E. coli*? Is it worse for people to get it?

[Jessica Chen] Thanks for that question. *E. coli* O157:H7 is a specific type of *E. coli* bacteria. Those letters and numbers attached to *E. coli* denotes the "serotype" of bacteria, and serotyping is a laboratory technique that we use to characterize the antigens present on the bacterium. Some *E. coli* are harmless, but O157:H7 can make a special type of toxin called Shiga toxin, and so we refer to O157 and other bacteria that produce this toxin as Shiga toxin producing *E. coli*, or STEC for short. Other *E. coli* can make Shiga toxin, but O157 is the most common in the US. And this can really result in severe infections, notably haemolytic uremic syndrome, which is a disease that impacts the kidneys. And STEC can even result in death.

[Sarah Gregory] And when was this strain first discovered?

[Jessica Chen] *E. coli* O157:H7 was first associated with an outbreak in the early 1980s. And this outbreak was investigated after reports of illness associated with undercooked hamburgers.

[Sarah Gregory] Are there growing numbers of outbreaks from it?

[Jessica Chen] *E. coli* O157:H7 is a major cause of foodborne illness, and we see approximately 63,000 cases each year. However, in terms of overall illnesses from STEC, we know that through active surveillance we have seen an increase in STEC infections in recent years, though there was a dip in reported infections during COVID.

[Sarah Gregory] You mentioned hamburgers in the 80s. Is that still the main source of contamination? Or has it broadened out to other foods?

[Jessica Chen] We do see other food sources associated with STEC infections. So ground beef was initially a major cause of outbreaks, however more recently, more recent data with STEC we see vegetable row crops (which is a designation that includes leafy greens) responsible for a growing number of infections.

[Sarah Gregory] Are some leafy greens found to have more contamination than others?

[Jessica Chen] There is some evidence of this. There was a publication that came out in 2020 that looked at leafy greens outbreaks from 2019 through 2018, and the study showed us that more outbreaks were linked to romaine lettuce than any other type of leafy green. We're not exactly sure of the reason behind this, and I think additional studies are needed to really get at this question.

[Sarah Gregory] There seems to be some thought that leafy greens are getting contaminated because they are often planted near where cattle are raised, grazed, housed...I don't know. Why are they planted so near cattle?

[Jessica Chen] So commercial leafy greens production really requires very specific environmental conditions. And so, there are specific lands that are most suitable for this type of leafy greens production and these areas...they often provide enough resources to house other agricultural practices, and this can include cattle production. So some 2022 USDA estimates indicate that there are more than three and a half million cattle and calves in Sacramento and San Juaquin Valleys, and this coincides with key areas of fresh produce production.

[Sarah Gregory] Is there a solution to this growing contamination? It’s not just this strain. Cattle running off onto vegetables seems to be an ongoing problem.

[Jessica Chen] So in response to these...there were two large leafy green outbreaks in 2018, producers in California and Arizona increased the buffer zones between cattle operations and field production. And as we continue to revisit the evidence, additional changes might be necessary on, you know, how big that buffer zone is. It's also important to note while cattle operations can be an important source of leafy green contamination, they are not the only source of contamination. For example, wildlife may play an important role, at least in terms of some contamination. So it's really a multidisciplinary problem that's going to require a lot of collaboration to continue to minimize risks.

[Sarah Gregory] What prompted this study?

[Jessica Chen] So we started this study following a large *E. coli* O157:H7 outbreak that occurred in late 2019 associated with romaine lettuce from the Salinas Valley, California. And we sought to have a look at the related isolates to this outbreak and really get a snapshot of this strain and understand its role in outbreaks over time.

[Sarah Gregory] Without getting too technical here, how did you go about determining the strain?

[Jessica Chen] So we leveraged available data from PulseNet, which is a national laboratory network, and this laboratory network allows us to compare DNA sequences from disease-causing *E. coli* bacteria from across the country. So by looking at these DNA sequences in PulseNet, we could identify the strain causing the 2019 outbreak and identify related outbreaks and cases.

[Sarah Gregory] Let's take a moment here for you to tell us a little bit more about your study.

[Jessica Chen] Thanks for this question. In this study, we examined whole-genome sequence data. So this looks at the entire genome of the bacteria, and it really affords us unprecedented resolution in studying these strains of disease-causing *E. coli*. And so, we used this data to understand what outbreaks and cases were linked over time, start to understand how this strain emerged, and identify unique genetic features associated with this strain.

[Sarah Gregory] After you did all this and looked at all of this, what were your conclusions?

[Jessica Chen] So we saw this strain associated with six different outbreaks during the study period, and then a seventh outbreak immediately following the conclusion of our analysis. We determined that the strain emerged in late 2015 and quickly diversified into distinct genomic groups or what we call clades, which were associated with distinct growing regions in California.

So one of these clades was associated with the Santa Maria region of California, and it had interesting genomic features—there was extra DNA in isolates belonging to this clade in the form of two circular plasmids. And we do not yet understand the role of this extra DNA, but it's something that we noted and highlighted in our study. We also found in this clade associated with Santa Maria, California that there was a previously reported mutation in a gene that might increase tolerance to arsenic, which is a heavy metal that sometimes is found in the environment, and this might lead to increased fitness in this specific region. And then we identified another clade that was associated with the Salinas Valley, California that did not have these extra genomic features.

[Sarah Gregory] Was there anything that surprised you?

[Jessica Chen] So interestingly, cases of this strain have not been observed in the last few years. The last outbreak was in 2020, and after that, only a single isolate of this bacteria has been uploaded to PulseNet (that's our molecular surveillance network). I think it's really interesting how quickly this strain diversified into two distinct genomic groupings, and we don't yet know how this strain appeared in leafy greens or these two growing regions, or all the factors around why we don't see this particular strain of STEC anymore.

[Sarah Gregory] What does this study provide to public health?

[Jessica Chen] So this study really hits at the dynamics of enteric bacteria. We typically think of enteric bacteria occurring as part of an isolated outbreak or causing, perhaps, sporadic cases. But this study really highlights the role of reoccurring, emerging, or persisting strains of enteric bacteria—which we call in our paper REP strains—and these can cause recurring outbreaks and illnesses over longer periods of time. So we're not just looking at things in isolation; we're looking at things over a longer period of time. This study also provides a really nice analysis framework for how to approach these so-called REP strains from a genomic standpoint and gives us an idea of how to really study these in the future and in getting additional insights from the genome.

[Sarah Gregory] Okay. So apparently, this strain is emerging and there's some stuff that we don't know about. What future research is needed?

[Jessica Chen] We're really just beginning to understand these so-called REP strains. They're not unique to *E. coli* and not even leafy greens, so these REP strains are found in other pathogens and other sources. My team and other teams at CDC are studying these strains in order to gain additional insight on them, and to understand different factors that may contribute to their emergence and persistence in the food supply.

[Sarah Gregory] This may be hard to answer, but do we know if people get infected more at home or in restaurants?

[Jessica Chen] So we don't yet have that determined for this strain. However, other leafy greens outbreaks have been tied to both exposure at home and in a restaurant setting. So an analysis of these leafy green outbreaks from 1973 to 2012 in the United States indicated that most outbreaks associated with a single location were attributed to food prepared in a restaurant. However, more recent data is needed to look at this in more recent years since that study concluded in 2012.

[Sarah Gregory] How can people protect themselves from getting sick from eating these contaminated greens? Leafy greens are so healthy for people nutritionally, but I find myself shying away from them anymore. Is there a better strategy than not eating them?

[Jessica Chen] Yeah, thanks for this question. Like you said, leafy greens are a really important part of a healthy diet, but sometimes they can be contaminated with household germs. So unless your leafy greens say that they are ready to eat or triple washed, we do recommend rinsing your leafy greens under water before eating. Never use a bleach solution or other disinfectant to wash produce. When you're in the store, look for leafy greens that aren't bruised or damaged, and be sure when you get home to store that produce separately from raw meat or eggs or other things that could become cross-contaminated with. In your home, to minimize cross contamination, use separate cutting boards and utensils and just practice good food safety hygiene. We have a lot of tips on the CDC webpage to keep yourself safe in the home.

[Sarah Gregory] Dr. Chen, tell us about your job at CDC and how you became interested in this topic.

[Jessica Chen] Thanks so much for this question. So I'm a bioinformatician, and probably that's not a job title that's super familiar to people. So bioinformaticians use their knowledge of biology and computer science to analyze complex data sets of genomic data, so this study is a great example of the types of analyses that a bioinformatician can do. So bioinformaticians can study problems from, say, human cancer genomics all the way to foodborne outbreaks. So it's not just kind of been the microbial angle that we looked at in this study, but there's a lot of other disciplines that can use a bioinformatician's help. I'm super interested overall in applying my skills as a bioinformatician to start to understand the why behind certain strains of bacteria cause outbreaks, and I find these REP strains to be a really fascinating example and look forward to continued analysis on this front.

[Sarah Gregory] Well, you're right. Even working at CDC all these years, that's not a job category or title I've really interviewed anyone from before, so...

[Jessica Chen] Yeah, I might be the first one.

[Sarah Gregory] Yeah, it's so interesting. Well, thank you so much for taking the time to talk with me today, Dr. Chen.

[Jessica Chen] Oh, thanks for having me. I really appreciate it.

[Sarah Gregory] And thanks for joining me out there. You can read the September 2023 article, Reoccurring *E. coli* O157:H7 Strain Linked to Leafy Greens–Associated Outbreaks, 2016–2019, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

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