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An Outbreak Investigation of *Salmonella* Typhimurium Illnesses in the United States Linked to Packaged Leafy Greens Produced at a Controlled Environment Agriculture Indoor Hydroponic Operation – 2021

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Abstract

In 2021, the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and state partners investigated a multistate outbreak of *Salmonella* Typhimurium illnesses linked to packaged leafy greens from a controlled environment agriculture (CEA) operation in Illinois. Thirty-one illnesses and four hospitalizations were reported in four states, with a significant epidemiologic signal for packaged leafy greens from Farm A. A traceback investigation for leafy greens included seven points of service (POS) with food exposure data from eight ill people. Each POS was supplied leafy greens by Farm A. FDA investigators observed operations at Farm A and noted that 1) the firm did not consider their indoor hydroponic pond water as agricultural water, 2) condensate dripping from the chiller water supply line inside

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer

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 and the US Food and Drug Administration.

the building, and 3) unprotected outdoor storage of packaged soilless growth media and pallets used for finished product. FDA collected 25 product, water, and environmental samples from Farm A. The outbreak strain was recovered from a water sample collected from a stormwater drainage basin located on the property adjacent to Farm A. In addition, an isolate of *Salmonella* Liverpool was recovered from two indoor growing ponds within the same growing house, but no illnesses were linked to the isolate. Farm A voluntarily recalled all implicated products and provided their root cause analysis (RCA) and return-to-market plan to FDA. While the source and route of the contamination were not determined by the RCA, epidemiologic and traceback evidence confirmed the packaged salads consumed by ill persons were produced by Farm A. This was the first investigation of a multistate foodborne illness outbreak associated with leafy greens grown in a CEA operation. This outbreak demonstrated the need for growers using hydroponic methods to review their practices for potential sources and routes of contamination and to reduce food safety risks when identified.

Keywords

Controlled environment agriculture; Foodborne illness outbreaks; Hydroponic agriculture; Leafy greens; *Salmonella*

Hydroponic horticulture is a soilless plant production technique that uses mineral nutrients dissolved in aqueous solutions and a soilless media (e.g., rockwool, perlite, peat, sand, etc.) rather than soil used in open, terrestrial crop production operations (Khan, 2018). Controlled Environment Agriculture (CEA) is an advanced and intensive form of efficient farming practices where plants grow within a controlled environment to optimize conditions for growth and crop yield (Shamshiri et al., 2018). Examples of CEA systems may include hydroponic operations, sprout operations, or enclosed greenhouses. CEA systems can allow for greater control of the plant growing environment, including a number of factors such as temperature, the management of nutrients, light quality and duration, and atmospheric composition. Deep water culture, for example, is a type of soilless hydroponic plant cultivation technique where plant roots are constantly submerged in a plant nutrient and oxygen-rich water solution and is common in the production of leafy greens (Jones, 2016; Kaiser and Ernst, 2012; Shrestha and Dunn, 2010). Globally, the CEA market is projected to grow from $74.5 billion in 2020 to $172.2 billion in annual sales in 2025 (Market, 2021). Some research indicates consumers and growers may hold positive perceptions related to the safety and sustainability of hydroponic agriculture compared to traditional field agriculture (Despommier, 2011), which may contribute to increased utilization of hydroponic growing methods in the future. Additionally, the number of hydroponic operations is on the rise particularly in urban settings, because they can produce crops with less water and on a smaller land area over a longer production time compared to field-grown produce (Despommier, 2011). However, opportunities for contamination still exist in hydroponic growing (Dankwa et al., 2021; Shaw et al., 2016; Wang et al., 2020). Tomatoes, leafy greens, peppers, cucumbers, and herbs make up the majority of hydroponically grown produce in the United States (Research, 2021); with lettuce being one of the vegetables most commonly produced hydroponically (Nester, 2022).

Salmonella enterica is a common foodborne pathogen and has caused many multistate and international outbreaks associated with fresh produce in recent years (Carstens et al., 2019). Animals can be reservoirs for the pathogen and shed it in their waste, which may contaminate water and subsequently produce crops (Wiedemann et al., 2014). Water used for irrigating crops is one of the most important agricultural inputs and if its quality is not adequate, there may be adverse ramifications to food safety, as evidenced by foodborne illness outbreaks of the recent past linked to produce (Crowe et al., 2017; Wiedemann et al., 2014). Hydroponic systems commonly use closed irrigation systems, which are used for plant cultivation in areas with environmental hazards in which the nutrient solution is not released into the surrounding environment but recycled, and if any bacterial pathogens are introduced to these systems, they may spread and contaminate produce (Hosseinzadeh et al., 2017; Shaw et al., 2016).

In 2021, the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), state, and local partners investigated a multistate outbreak of *Salmonella* Typhimurium illnesses. We describe the epidemiologic, laboratory, and traceback investigations that identified packaged leafy greens as the source of this outbreak and the importance of CEA-specific produce safety practices in preventing foodborne infections.

Materials and methods

Outbreak Detection and Epidemiologic Investigation.

Clinical samples from ill people were cultured for *Salmonella,* serotyped, and subtyped by whole genome sequencing (WGS) at state public health laboratories. State laboratories submit sequencing data to PulseNet, the national laboratory network for molecular subtyping for foodborne disease surveillance. On July 2, 2021, the Illinois Department of Public Health identified a cluster of four *Salmonella* Typhimurium infections with isolates that differed within 0–1 alleles by core genome multilocus sequence typing (cgMLST). On July 9, 2021, CDC initiated a multistate investigation. Subsequently, additional isolates within 0–2 allele differences by cgMLST were considered to be the outbreak strain. The FDA initiated outbreak response activities on July 12, 2021, traceback on July 13, 2021, and a farm investigation on July 14, 2021. A confirmed case was defined as an infection with the outbreak strain of *Salmonella* Typhimurium with illness onset between June 10 and August 13, 2021. State and local health officials interviewed ill people with state enteric and outbreak-specific questionnaires to gather food exposure information.

Traceback Investigation.

A traceback investigation was initiated as per standard FDA traceback practices (Irvin et al., 2021; Council to Improve Foodborne Outbreak, 2014). Information related to individuals' exposure to packaged leafy greens, including dates, receipts, and purchase documents was reviewed. Ill people selected for traceback had reported eating packaged leafy greens from a single grower, Farm A, and had confirmed dates of purchase or consumption. Packaged salad purchase and production records were collected from the retailers and distributors

along the leafy green supply chain. Information relevant to timeline construction collected from suppliers included stock rotation, delivery frequency, and shelf-life of product.

Farm Investigation.

FDA investigators visited Farm A in Illinois for an on-farm investigation on July 14, 2021, which consisted of interviews with key personnel, direct observation of Farm A's operations, records review, and sample collection for microbiological analysis. Samples of finished product, seeds, growth media, and water were collected from locations on and around the exterior of the CEA building, as well as environmental swabs of surfaces throughout the operation. The environmental swabs and indoor pond water and sediment samples were collected using the grab and Dead-end ultrafiltration (DEUF) methods (Mull and Hill, 2009, 2012).

Microbiological Investigation.

State and FDA laboratories used the FDA *Bacteriological Analytical Manual* (BAM) method to isolate *Salmonella* spp. from finished product samples collected by investigators at various points of distribution including points of service (POS) and distribution centers (Andrews et al., 2018). DEUF samples collected at Farm A were analyzed with the following modifications to the FDA BAM method: an equal volume of backflush sample was added to 2x modified Buffered Peptone water for preenrichment. Secondary enrichment cultures were incubated at 42C and subsequently plated onto Xylose lysine tergitol$_4$ agar (XLT4) and Hektoen enteric agar supplemented with 5 ug/mL novobiocin (HE+N) (Mull & Hill, 2009). Suspect colonies were screened by restreaking to XLT4 and HE+N before confirmation according to FDA BAM. Serotyping and phylogenetic analyses of WGS data (Davis et al., 2015) were conducted to characterize the isolates and compare them to clinical and historical product sample isolates (Andrews et al., 2018; Crowe et al., 2017); by comparing high-quality single nucleotide polymorphisms (hqSNP) or cgMLST; genomes were considered highly genetically related to other isolates when they were within 0–20 SNPs or 0–2 alleles, respectively. WGS data for isolates collected during the investigation were submitted to the genome sequence repository maintained by the National Center for Biotechnology Information to identify closely related isolates and then analyzed with the FDA Center for Food Safety and Applied Nutrition (CFSAN) SNP Pipeline.

Results

Outbreak Detection and Epidemiologic Investigation.

PulseNet identified a total of 31 cases from four states (Fig. 1). Illness onset dates range from June 10 to August 13, 2021 (Fig. 2). The median age of ill people was 47 (range of four months to 86 years), and 19 (61%) were female. Five ill people were hospitalized (17%), and no deaths were reported. Overall, 26 of 27 (96%) people reported consuming any leafy greens in the seven days prior to illness onset, specifically, 20 of 24 ill people reported consuming packaged leafy greens. Fourteen of 22 ill people (64%) named Farm A products.

Traceback Investigation.

A traceback investigation was initiated at seven points of service (POS) using consumer purchase data obtained from eight ill people epidemiologically associated with the consumption of leafy greens from Farm A (Fig. 3). Six of the seven POS were supplied by one distribution center. A separate distribution center supplied the seventh POS. Both distribution centers were supplied by Farm A hydroponic operation. While different Farm A leafy greens products were noted on the loyalty card data obtained, all contained one explicit variety of lettuce grown at the farm, demonstrating an additional layer of commonality that was later used for prioritization of inspectional activities and scope of the recalled products. Loyalty card data were received for a total of nine ill people, each showing Farm A purchases. One ill person's purchase history showed Farm A product purchased after the reported onset date and was excluded from the above analysis. The Farm A products purchased were confirmed to be products from Farm A's hydroponic operation. Of these eight ill people, five had a documented purchase of product between June 5 and 7, 2021. Based on the calculated likely turn-around times and the aforementioned three purchase days, the harvest window was estimated to be between May 29 and June 4, 2021.

Farm Investigation.

During the farm investigation, Farm A was found to use common commercial high-density hydroponic growing techniques to grow leafy greens, including deep water culture and floating raft production methods. Three enclosed greenhouses, with screened windows and open-air roof venting that provided exposure to the exterior environment, contained 19 indoor hydroponic ponds utilized to grow a variety of leafy greens. Hydroponic ponds were plastic-lined and filled with the municipal water that was treated onsite using sand filtration and UV light. The hydroponic ponds typically received nutrients and additional UV-treated water from a storage cistern, as needed to maintain adequate pond levels. While each pond utilizes a pump for circulation, all water within each growing pond is isolated with no recirculation of water between the 19 ponds; none of the ponds had been drained prior to the FDA farm investigation.

High-density leafy greens are grown in reusable polystyrene rafts that are mechanically seeded after being filled with a soilless growth media for germination and to support seedling growth. Seeded rafts are sprayed with municipal water and placed in a climate-controlled room for germination. After germination, rafts are moved onto the surface of hydroponic ponds for growth until harvest. Seedlings remained in hydroponic ponds throughout the growing process, which ranged from approximately 12–21 days, depending on the leafy green type, until removed for harvest. Harvesting of the leafy greens involved moving rafts from ponds to a mechanical cutting device which severed plants to separate leaves from growing rafts. Harvested leaves were placed into reusable plastic containers to precool to a lower temperature for two to three hours prior to manually packing into retail packages; leafy greens were not washed before packaging.

Farm A's raw material screening for pathogens on incoming seeds and growth media was limited; they received a letter of guarantee from the seed supplier and acknowledged the growth media supplier's certification. Incoming growth media and pallets used for the

finished packaged product were stored outdoors, as needed, and a forklift was utilized to transport the material into the CEA operation. Outdoor storage practices of the growth media and pallets did not adequately provide protection from animal intrusion, bird droppings, water runoff, or other potential sources of contamination. Soilless growth media routinely discharges from rafts during the growing process, accumulating as thick sediment deposits on the bottom of the hydroponic ponds. Farm A did not have practices in place for the removal of sediment deposits. Farm A's documentation of cleaning and sanitizing equipment, tools, and buildings was not routinely performed in accordance with their internal procedures. In addition, FDA investigators observed condensate dripping from chiller water pipelines onto leafy greens and on production line conveyors used for leafy greens.

Water in growing ponds was UV-treated prior to filling the ponds but was not routinely disinfected or otherwise treated as part of maintenance of the pond water quality afterward. Farm A reported to investigators that grab samples (~100 mL) were collected from each hydroponic pond weekly and tested for the presence of generic *Escherichia coli*. Farm A indicated pond water was treated with a hydrogen peroxide and peracetic acid solution when testing results showed the presence of *E. coli*; however, Farm A did not have a documented procedure or systematic approach to ensure adequate pond water treatment on a regular basis.

After FDA reported results from testing of the hydroponic ponds (see Microbial Results below) to Farm A, they reported draining the affected pond water, removing all soilless growth media that deposited into the ponds onto the plastic pond liner, then using a hydrogen peroxide and peracetic acid solution to power wash and scrub the plastic pond liners and the exterior of pipes used for pond water circulation. The hydroponic ponds were then refilled with water and a hydrogen peroxide and peracetic acid solution. Farm A reported that their hydroponic ponds had not been cleaned or drained subsequent to Farm A initiating their CEA operation in 2016.

Samples of raw material collected at the time of the investigation did not result in the recovery of any pathogens. However, a strain not associated with the outbreak, *S.* Liverpool, was recovered from water samples collected from two of the hydroponic ponds. Farm A indicated that leafy greens in contact with the hydroponic pond water were removed and discarded during the harvesting process and were not intended for finish product packaging. Investigators observed the mechanical harvesting process, and noted the process was inadequate to exclude harvesting leafy greens that possibly contacted water in the hydroponic pond. Without measures to ensure that leaves coming into direct contact with the hydroponic pond water were not harvested, hydroponic pond water potentially contaminated with human pathogens may have contaminated the finished packaged product.

FDA investigators collected two water samples from a stormwater drainage basin located approximately 25 feet from the CEA operation's property. One of the water samples tested positive for the outbreak strain of *Salmonella* Typhimurium (see Table 1). During the investigation, geese and geese scat were observed near the adjacent stormwater drainage

basin but FDA investigators did not observe infiltration of stormwater runoff, intrusion by animals and pests, or other specific routes of contamination surrounding Farm A's operation.

Microbiological Investigation.

In total, FDA collected 25 product, water, and environmental samples, comprised of approximately 300 subsamples, as a result of this outbreak investigation. Samples were collected from Farm A's hydroponic operation, an outdoor stormwater drainage basin adjacent to Farm A's hydroponic operation and from another firm that received one of the same seed lots during the time of interest. Samples included finished leafy greens (four), seeds (seven), soilless growth media (three), pond water (seven), environmental swabs, scat (two), and stormwater drainage basin sediment (Table 1).

Two samples of indoor pond water collected at Farm A from two different ponds yielded the same strain of *Salmonella* Liverpool, a different serovar from the outbreak strain. One of the DEUF samples, collected from a stormwater drainage basin located approximately 25 feet away on property adjacent to Farm A hydroponic operation, yielded a *Salmonella* Typhimurium isolate that was highly genetically related to the outbreak strain. Leafy greens, seeds, soilless growth media, environmental swabs, and scat samples tested negative for *Salmonella*. The lettuce product samples collected by FDA during this outbreak investigation were not from the same lots purchased by the ill people and thus not representative of the packaged salads that may have caused this outbreak.

WGS analysis of the *Salmonella* isolates from the two hydroponic pond samples determined their genetic relatedness to each other, clinical, and other nonhuman isolates (Fig. 4). Five *Salmonella* Liverpool isolates from the indoor pond water sample grouped into one cluster that were highly genetically related to each other (0 SNPs) and were an average of 12–17 SNPs away from one chicken, one pork, and two cattle isolates, but were not genetically related to any clinical isolates. One of the cattle isolates (four SNPs away from clinical isolates) was from a heifer slaughtered in Illinois, but the origin of the heifer was not determined. These isolates were not highly genetically related to the outbreak strain. A total of 17 isolates identified as *Salmonella* Typhimurium were recovered from the stormwater drainage basin. WGS analysis grouped all 17 isolates into one cluster and was considered highly genetically related to clinical isolates in the outbreak strain at 0–4 SNPs. (Fig. 5).

Public Health Actions.

Based on the combined evidence from states, CDC, and FDA, Farm A agreed to voluntarily recall select packaged leafy greens produced at their hydroponic operation in Illinois on July 15, 2021 (Food and Administration, 2021). Retailers in Illinois, Indiana, Iowa, and Wisconsin were notified via e-mail and instructed to remove all affected product from store shelves, and consumers were directed to discard them or return them to the place of purchase for a refund. FDA issued an Outbreak Advisory and CDC published a Food Safety Alert, notifying consumers about Farm A products associated with this recall. After FDA notified Farm A that subsamples collected from two of their ponds yielded *Salmonella* spp. isolates, farm management reported they would not distribute product from those two ponds, and they ceased planting in the ponds. As a result, on July 27, 2021, Farm A expanded the

recall to include products sold in Michigan and spinach with best-by dates through July 26, 2021. Food Safety Alerts were published for subsequent recalls. On July 29, 2021, Farm A reported they would not distribute any products from the farm until FDA pond water sample results were complete.

On August 2, 2021, Farm A provided a root cause analysis and return-to-market plan which included a test and hold policy for the recalled lettuce variety until all corrective actions were complete and at least two months passed with no positive *Salmonella* findings in ponds, product contact surfaces, or product. FDA typically performs follow-up inspections to observe corrective actions and routinely performs inspections for regulatory compliance to the Produce Safety Rule (PSR). Farm A also drained the affected ponds, removed all the sediment, and cleaned and sanitized the ponds before refilling with water and nutrients.

Public Communication.

An outbreak advisory was posted on July 15, 2021, by FDA (U.S. Food and Drug Administration, 2021) and CDC (U.S. Centers for Disease Control and Prevention, 2021), which was updated on July 16, 2021, to include advice to consumers detailing information on the recall (Food and Administration, 2021).

Discussion

Significance of outbreak.

This is the first investigation of a multistate foodborne illness outbreak linked to hydroponic-grown leafy greens grown in a domestic CEA operation. The outbreak resulted in 31 ill people and four hospitalizations across four states. Epidemiologic, microbiological, and traceback data were used to identify leafy greens grown hydroponically and packed onsite at an Illinois CEA operation that led to the outbreak of *Salmonella* Typhimurium infections. FDA and state partners conducted an extensive onsite investigation that supported the conclusions outlined below. However, the investigation did not identify the original source of the pathogen nor the route of contamination of the leafy greens. Nonetheless, investigators did recover the outbreak strain from a water sample collected from a stormwater drainage basin located on property adjacent to the Farm A hydroponic operation, as well as *Salmonella* Liverpool isolates from water collected from Farm A's two indoor ponds used to grow the leafy greens. FDA investigators also observed storage practices that could result in contamination of growth media, inadequate water management practices, and poor general sanitation practices. This evidence, in combination with the epidemiologic data linking leafy greens to the outbreak and the traceback investigation that identified the CEA operation as having supplied those leafy greens to the ill people, confirmed that the leafy greens produced at this facility were the vehicle responsible for these reported illnesses. Based on this evidence, Farm A agreed to a voluntary recall of nine select packaged leafy green products produced at their hydroponic operation. The manufacturer ultimately ceased production temporarily and conducted a root cause analysis and return-to-market plan. Farm A resumed production after implementing the return-to-market plan.

Investigational Challenges.

The FDA encountered challenges during the traceback investigation with respect to identifying implicated lots of products. Specifically, while many cases had consumer purchase dates, many also had multiple purchases, sometimes of additional brands of leafy greens. In addition, loyalty shopper card data did not include any identifying codes or lot numbers purchased by the cases. The main distribution center involved did not keep a record of the outgoing lots to individual stores despite receiving the lot information with incoming shipments from Farm A. Because the outgoing lot information is not captured beyond the distribution center, there was a three-to-seven-day uncertainty window when estimating the harvest date based on the purchase date. In addition, when using loyalty shopper card data, it is possible that the ill person did not use the card for every purchase, the ill person may not have consumed all of the products purchased, and multiple households may have used the same card.

Investigational findings and link to outbreak.

The outbreak strain of *Salmonella* Typhimurium was isolated from a stormwater drainage basin adjacent to the CEA farm, approximately 25 feet from the enclosed greenhouses. Investigators were not able to determine if the stormwater drainage basin was the source of the *Salmonella* that contaminated the leafy greens and caused the outbreak, if the bacterium was already present on the farm and was then transferred by stormwater runoff or other means to the stormwater drainage basin, or if there was another common source that independently contaminated both locations. Nonetheless, the recovery of the outbreak strain in such close proximity to the CEA operation adds to the epidemiologic and traceback evidence implicating Farm A as the source of the outbreak. While the definitive route of contamination for this outbreak remains unknown, various on-farm practices observed during FDA's investigations may have contributed to the contamination and spread of *Salmonella* on leafy greens in the facility. Based on these observations and findings relayed to them by FDA, Farm A conducted an internal root cause analysis and return-to-market plan, which identified mitigation measures intended to minimize the potential for future contamination events within the operation. The firm's root cause analysis likewise did not identify a definitive route of contamination.

The recovery of the outbreak strain in an adjacent stormwater drainage basin underscores the importance of assessing potential risks associated with adjacent and nearby land uses, even for indoor growing operations, as well as implementing measures to prevent pathogens from entering the operation. If *Salmonella* spp. are introduced into a hydroponic system, the potential for survival, growth, and spread is very high if there is no effective mitigation in place (Shaw et al., 2016). Pathogens entering the fertilizer solution tanks in hydroponic systems without a mitigation step could be expected to rapidly propagate and spread throughout the system and potentially contaminate the entire crop. Agricultural water in a hydroponic system has the potential to be in contact with growing crops over extended periods of time, and thus contamination present in the water increases the likelihood product will become contaminated and cause an outbreak.

Investigators also recovered *Salmonella* Liverpool from water collected from two indoor production ponds, both located within the farm premises. While the pathogen recovered was not the strain that caused the outbreak, its presence illustrates the importance of minimizing sources of contamination and operating and maintaining production ponds in a way that does not result in the spread of pathogens to the edible portion of the crop. When pathogens are identified through microbiological surveys, preharvest water assessments, or postharvest testing of produce, it is important to implement industry-led root cause analyses to determine how the contamination likely occurred and then implement appropriate prevention and verification measures. Additionally, while it is possible there was a common source, isolating an identical pathogen from two separate locations (i.e., two different production ponds) within the CEA operation demonstrates the potential for pathogen spread within the operation. Results from WGS analyses identified historical isolates that were highly genetically related, but not identical to the strain of *Salmonella* Liverpool collected from indoor production ponds. The genetic and geographic proximity of these isolates from the production ponds to a cattle isolate recovered from a packing plant approximately 50 miles away may suggest the contamination arose from the environmental spread, perhaps via wind or birds.

In addition to the recovery of *Salmonella* spp. isolates inside and outside the CEA operation, investigators observed practices that could contribute to the contamination or growth of pathogenic microorganisms. For example, there was limited screening to detect pathogens in raw materials, and plant growth media was stored outside in an area that did not protect it from potential sources of contamination. If pathogens were introduced to the growth media that could result in a cascade of adverse effects for the food safety integrity of the whole operation (Dankwa et al., 2020). Contaminated growth media could serve as a source of pathogens for plants during germination, which could spread to indoor production pond water or food contact surfaces that then contact edible portions of the leafy greens (Dankwa et al., 2020; Li et al., 2021). Following harvest, ineffective cooling and cold holding of the contaminated product may contribute to pathogen growth (Tarlak et al., 2020). CEA and hydroponic growing operations should thoroughly evaluate sources and routes of contamination to their products, including raw materials and agricultural inputs, adjacent land use, employee actions, and agricultural water, to limit the potential for contamination.

Food safety risk management in CEA and indoor hydroponic growing systems.

Practices utilized during CEA and indoor hydroponic growing differ in some substantial ways from the corresponding practices used in open-field growing (Holvoet et al., 2015). While some contamination risk factors are common to both indoor and outdoor agriculture, certain risk factors may be more relevant to indoor growing environments. Similar to a traditional open-field farming operation, in a closed indoor setting, irrigation water and other agricultural inputs (e.g., seeds, growth media, fertilizer nutrient solutions) can play a significant role in introducing contamination to a system. Seed contamination has historically been considered a significant risk factor in the production of hydroponically grown sprouts, and may similarly act as a source of contamination for other hydroponically grown crops (Bazaco et al., 2021). Inadequate maintenance, cleaning, and sanitizing of tools and equipment, along with employee practices, may contribute to the introduction

of pathogens to the indoor environment, as well as cause pathogens that are present to spread throughout the system and onto produce. If indoor growing operations are not fully enclosed, other risk factors may also apply. For example, indoor CEA and hydroponic operations that are not fully enclosed may encounter contamination issues arising from animal intrusion.

Because hydroponic leafy greens and other produce are usually grown in partially or fully enclosed buildings, the risk of contamination from some potential sources may be removed or reduced (e.g., contamination arising from animal intrusion, manure use, and soil contact). Research indicates some growers and consumers perceive hydroponically grown produce to be safer than produce grown outside (Despommier, 2011; Lam et al., 2020; Wang et al., 2020). This may result in too little emphasis on areas where risk remains (e.g., raw material inputs, employee actions, tools, and equipment). Accordingly, it is critical that CEA operations conduct regular assessments of potential sources and routes of contamination including the sanitary quality of the raw materials and inputs used, and the cleanliness and sanitation around their operations, as previously described by FDA (U.S. Food and Drug Administration, 2022). By nature of CEA designs and typical practices, the warm, moist environment specific to these operations can help support the ecology of bacterial communities, including microbial pathogens often implicated in foodborne illness outbreaks. Similarly, sprouts can present special concerns with respect to the proliferation of human pathogens compared to other covered produce because of the warm, moist, and nutrient-rich conditions required to produce sprouts (De Roever, 1998; National Advisory Committee on Microbiological Criteria for Foods, 1999). Due to similarities in production conditions, CEA and other hydroponic operations growing produce other than sprouts may consider voluntarily choosing to follow the PSR standards in subpart M, which applies to sprout production. The nexus between CEA and this multistate outbreak of *Salmonella* Typhimurium illnesses linked to packaged leafy greens demonstrate the importance of CEA-specific produce safety practices in preventing foodborne illness outbreaks.

In 2018, FDA published a draft guidance document for industry, entitled “Draft Guidance for Industry: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption” to help covered farms comply with the requirements of the PSR, which established science-based minimum standards for the safe growing, harvesting, packing, and holding of produce. CEA and other hydroponic growers should consider this draft guidance and evaluate their practices in light of this recent outbreak, and any other identified risk factors, to determine whether their practices and procedures are sufficient to prevent contamination of produce.

Conclusion

Epidemiologic, traceback, and laboratory evidence confirmed packaged leafy greens produced at Farm A as the vehicle of *Salmonella* Typhimurium infections among the 31 ill people in this outbreak; however, the initial source and route of the contamination were not determined. This outbreak investigation demonstrated the potential for environmental contamination in CEA hydroponic operations and the need to promote good agricultural practices that would prevent environmental contamination at hydroponic CEA growers. CEA

and other hydroponic growers should assess their operations to evaluate the adequacy of their current food safety practices and procedures, and to consider if any additional controls are necessary to prevent contamination of fresh produce grown indoors. Due to similarities in production conditions, CEA and other hydroponic operations growing produce other than sprouts may also voluntarily choose to adopt any appropriate sprout-specific standards of Subpart M of the Produce Safety Rule.

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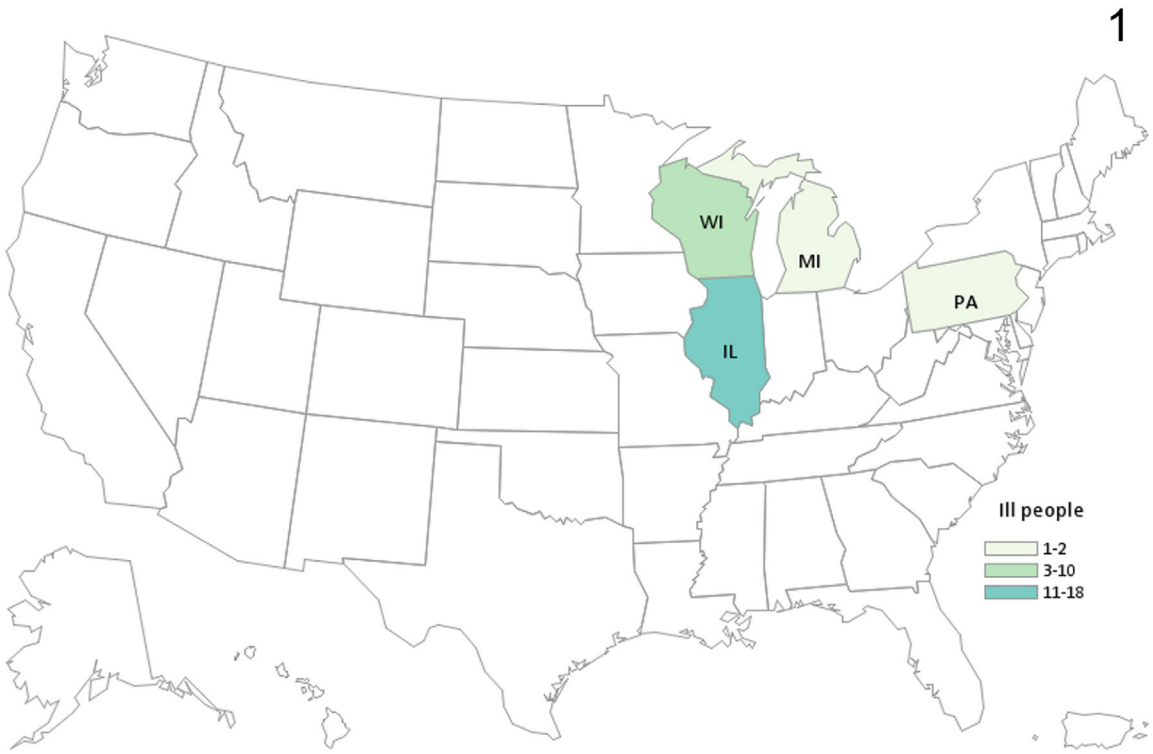

Figure 1.
Reported persons infected with the outbreak strain of *Salmonella* Typhimurium (n = 31) by the state of residence, United States, 2021.

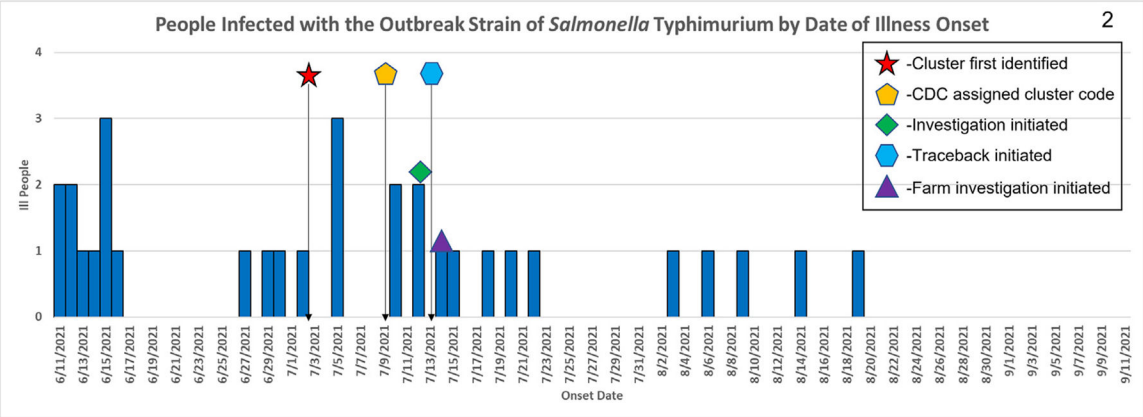

Figure 2.

Reported persons infected with the outbreak strain of *Salmonella* Typhimurium (n = 31) by date of illness onset, United States, 2021. Some illness onset dates have been estimated from other reported information

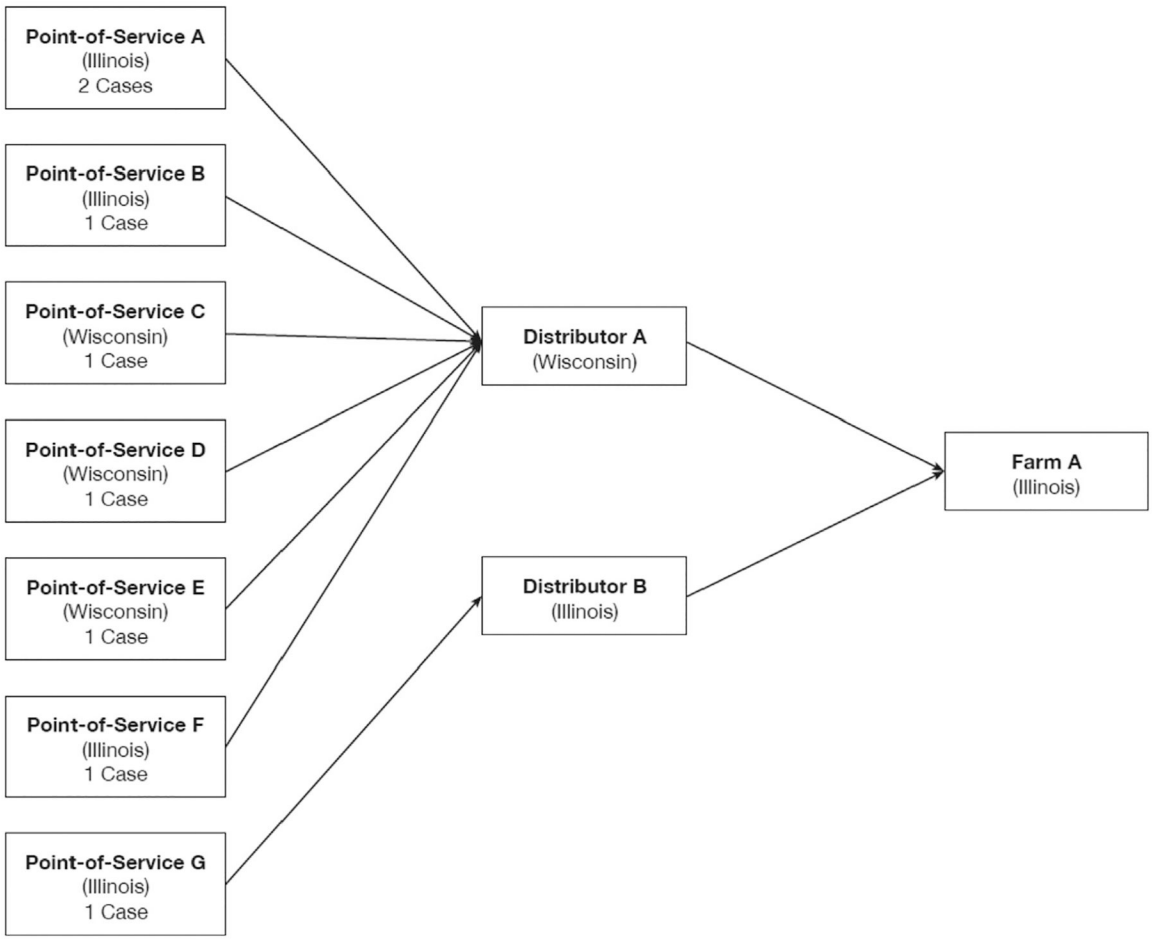

Figure 3.

Traceback diagram for multistate outbreak of *Salmonella* Typhimurium Illnesses in the United States linked to packaged leafy greens produced at a controlled environment agriculture indoor hydroponic operation in 2021. Purchases of implicated products are traced from the point of service, through the distribution chain, to distributors. Product originated from the farm that is denoted on the right side of the diagram.

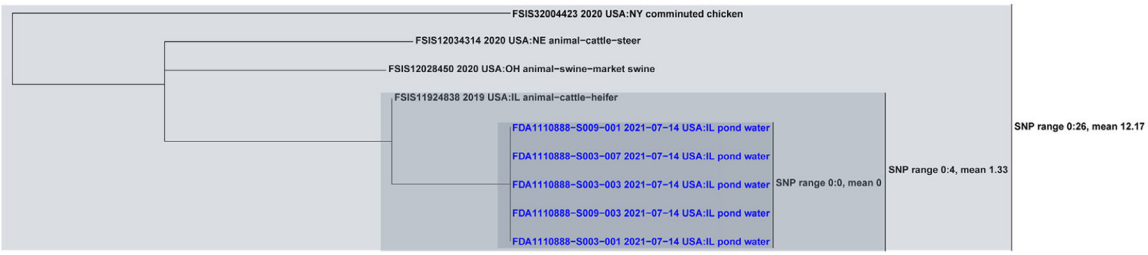

Figure 4.

Phylogenetic analysis of whole genome sequence data from five *Salmonella* Liverpool isolates. Whole genome sequence data for five isolates (blue text) collected during the investigation into an outbreak of *Salmonella* Typhimurium were submitted to the genome sequence repository maintained by the National Center for Biotechnology Information to identify closely related isolates (black text). Sequence data were then analyzed with the CFSAN SNP Pipeline.

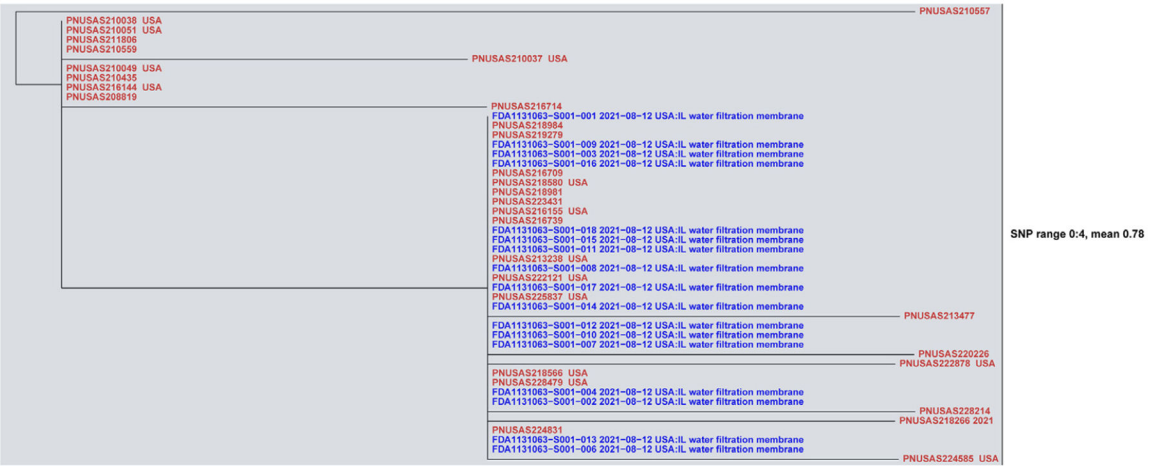

Figure 5.

Phylogenetic analysis of whole genome sequence data from 17 *Salmonella* Typhimurium isolates. Whole genome sequence data for 17 isolates (blue text) collected during the investigation into an outbreak of *Salmonella* Typhimurium were submitted to the genome sequence repository maintained by the National Center for Biotechnology Information to identify closely related isolates (red text). Sequence data were then analyzed with the CFSAN SNP Pipeline.

Table 1

Summary of FDA product and environmental samples collected from the Controlled Environment Agriculture indoor hydroponic operation and laboratory results

Sample #	Date Collected	Sample Type	Description	Analytical Result
1110888	7/14/2021	Water	Ponds 1, 6, 7, 8, 10	*Salmonella* Liverpool
				Ponds 6 & 10 (House 3)
1110890	7/14/2021	Product	Packaged Lettuce	Negative
1110891	7/14/2021	Product	Packaged Lettuce	Negative
1110892	7/14/2021	Product	Packaged Lettuce	Negative
1110893	7/14/2021	Product	Packaged Lettuce	Negative
1084031	7/16/2021	Environmental Swabs	Zones 1, 2 & 3	Negative
1110889	7/15/2021	Seeds	Lettuce seeds – lot used during May-June production	Negative
1131060	7/20/2021	Scat	Outside a building	Sample analysis terminated
1110894	7/28/2021	Water	Ponds 11, 12, 13, 14, 15, 16, 17, 18, 19	Negative
1110895	7/28/2021	Water	Ponds 2, 3, 4, 5	Negative
1110896	7/28/2021	Water	Pond 9 & Cistern	Negative
1152422	7/29/2021	Media	Vermiculite and peat mix	Negative
1152423	7/29/2021	Media	Palmetto brand vermiculite, fine-medium	Negative
1152424	7/29/2021	Media	Berger brand custom blend special mix peat growth media	Negative
1167952	8/3/2021	Seeds	Lettuce seeds	Negative
1167953	8/3/2021	Seeds	Lettuce seeds	Negative
1167954	8/3/2021	Seeds	Spinach seeds	Negative
1167955	8/3/2021	Seeds	Spinach seeds	Negative
1167956	8/3/2021	Seeds	Beet Seeds	Negative
1167957	8/3/2021	Seeds	Spinach seeds	Negative
1168914	8/11/2021	Seeds	Lettuce seeds from Maryland Farm	Negative
1131061	8/10/2021	DEUF	Ponds 7, 8, 12, 14	Negative
1131062	8/12/2021	Scat		Negative
1131063	8/12/2021	DEUF	Stormwater Drainage Basin	*Salmonella* Typhimurium – WGS highly genetically related to clinical isolates
1131064	8/12/2021	Sediment	Outdoor Pond	Negative