Review

Fresh Produce–Associated Listeriosis Outbreaks, Sources of Concern, Teachable Moments, and Insights

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ABSTRACT

Foodborne transmission of *Listeria monocytogenes* was first demonstrated through the investigation of the 1981 Maritime Provinces outbreak involving coleslaw. In the following two decades, most listeriosis outbreaks involved foods of animal origin, e.g., deli meats, hot dogs, and soft cheeses. *L. monocytogenes* serotype 4b, especially epidemic clones I, II, and Ia, were frequently implicated in these outbreaks. However, since 2008 several outbreaks have been linked to diverse types of fresh produce: sprouts, celery, cantaloupe, stone fruit, and apples. The 2011 cantaloupe-associated outbreak was one of the deadliest foodborne outbreaks in recent U.S. history. This review discusses produce-related outbreaks of listeriosis with a focus on special trends, unusual findings, and potential paradigm shifts. With the exception of sprouts, implicated produce types were novel, and outbreaks were one-time events. Several involved serotype 1/2a, and in the 2011 cantaloupe-associated outbreak, serotype 1/2b was for the first time conclusively linked to a common-source outbreak of invasive listeriosis. Also in this outbreak, for the first time multiple strains were implicated in a common-source outbreak. In 2014, deployment of whole genome sequencing as part of outbreak investigation validated this technique as a pivotal tool for outbreak detection and speedy resolution. In spite of the unusual attributes of produce-related outbreaks, in all but one of the investigated cases (the possible exception being the coleslaw outbreak) contamination was traced to the same sources as those for outbreaks associated with other vehicles (e.g., deli meats), i.e., the processing environment and equipment. The public health impact of farm-level contamination remains uncharacterized. This review highlights knowledge gaps regarding virulence and other potentially unique attributes of produce outbreak strains, the potential for novel fresh produce items to become unexpectedly implicated in outbreaks, and the key role of good control strategies in the processing environment.

Key words: *Listeria*; Outbreaks; Produce; Serotypes; Whole genome sequencing

Each year in the United States, *Listeria monocytogenes* is responsible for approximately 1,600 cases of foodborne illness (listeriosis), resulting in an estimated 1,500 hospitalizations and 260 deaths *(31)*. Although relatively uncommon, listeriosis is a severe invasive illness with high mortality and morbidity. Invasive listeriosis typically results in stillbirths or abortions; septicemia and meningitis can ultimately lead to death *(34)*. Populations at increased risk for invasive listeriosis include elderly individuals, pregnant women and their fetuses, and individuals with cancer and other immunosuppressive conditions or treatments. Case fatality rate remains high (approximately 20 to 21%) even with the use of appropriate antibiotics *(5, 34)*.

Listeriosis is most often caused by eating ready-to-eat foods contaminated with the pathogen. The source of contamination has typically been the food processing environment. *Listeria* is widely distributed in the environment and may enter a food processing facility by various routes that remain largely uncharacterized but may include

equipment, food products, and human traffic. Once introduced, *Listeria monocytogenes* can colonize the processing plant environment and equipment and can persist, sometimes for years *(14, 22)*.

The first outbreak of listeriosis that conclusively demonstrated foodborne transmission of the pathogen was in 1981 in the Maritime Provinces, Canada, and involved contaminated coleslaw *(32)*. Most subsequent outbreaks in the following two decades in Europe and North America involved processed, refrigerated ready-to-eat products of animal origin such as hot dogs, deli meats, other processed specialty meat products, seafood, and dairy products, especially soft cheeses *(35)*. Analysis of trends in listeriosis outbreaks in the United States between 1998 and 2008 revealed that outbreaks in the earlier portion of this period involved more individuals and lasted longer than the outbreaks in later years and tended to involve ready-to-eat meats. Implicated *L. monocytogenes* strains frequently were of serotype 4b. However, later outbreaks tended to involve novel vehicles such as sprouts and taco or nacho salads, and many of the implicated strains were serotype 1/2a *(4)*. Several of the outbreaks subsequent to 2008 have continued

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to involve novel vehicles (e.g., cantaloupe, celery, apples, stone fruit, ice cream), and several of the implicated strains had serotypes other than 4b *(6, 7, 15, 20, 25)*.

This review focuses on listeriosis outbreaks associated with fresh produce and includes new findings obtained in the course of the investigations of these outbreaks, their public health and microbiological implications, and the potential paradigm shifts in our understanding of *L. monocytogenes* as a foodborne pathogen.

EARLY PRODUCE-ASSOCIATED OUTBREAKS

Boston, United States, 1979 and Maritime Provinces, Canada, 1981. The epidemiological analysis of a listeriosis outbreak among hospitalized patients in Boston, MA, in 1979 (Table 1) implicated contaminated raw vegetables (celery, tomatoes, and lettuce); however, microbiological confirmation was not available *(18)*. Conclusive evidence for foodborne transmission of *L. monocytogenes* first emerged through the epidemiological and microbiological investigation of the 1981 Maritime Provinces (Canada) outbreak associated with coleslaw (Table 1) *(32)*. This outbreak affected 41 people (7 adult and 34 perinatal cases). The outbreak investigation revealed that the vehicle of transmission was contaminated coleslaw. The cabbage used for the coleslaw may have become contaminated via sheep manure from a flock with listeriosis, but the strains from the implicated flock had not been preserved, manure and other farm environmental samples failed to yield *L. monocytogenes*, and conclusive evidence for the source of the contamination could not be obtained through the outbreak investigation *(32)*.

The implicated *L. monocytogenes* strain was serotype 4b. Strains with the same serotype and genotype (determined with the subtyping tools available at the time) were isolated from coleslaw left in a patient's refrigerator and from the patient's blood *(2, 32)*. This outbreak constituted the first documented evidence of involvement of a major *L. monocytogenes* clonal group, designated epidemic clone (EC) I *(17, 22, 28)*.

ECs are groups of strains that have been implicated in multiple outbreaks and are genetically closely related, suggesting relatively recent evolution from a common ancestor. Subsequent to the 1981 coleslaw outbreak, numerous studies have demonstrated that ECI strains are extensively disseminated in the food supply and the environment and are responsible for a large number of common-source outbreaks and sporadic cases of listeriosis *(3, 12, 23, 29)*. The strain implicated in the outbreak among hospitalized patients in Boston in 1979 was also of serotype 4b but was a member of a different EC, designated ECIa (or ECIV), which is also frequently encountered in outbreaks and sporadic cases of listeriosis *(3, 11, 12, 23, 29)*. ECIa strains (with strain Scott A as the prototype) are genetically distinct from ECI, with separate sets of unique genetic markers. Based on multiple-locus sequence typing (MLST), ECI and ECIa correspond to two distinct clonal complexes, CC1 and CC2, respectively *(3)*.

FRESH PRODUCE–ASSOCIATED LISTERIOSIS OUTBREAKS, 2008 TO 2010

Sprouts (2008 to 2009, United States, multistate). Subsequent to the coleslaw-associated outbreak, most outbreaks in North America and Europe involved ready-to-eat meats and dairy products, especially soft cheeses, and strains of serotype 4b, frequently of ECI, ECIa, or an EC first recognized in the 1998 to 1999 hot dog–associated outbreak and designated ECII *(22, 35)*. However, in 2008 a multistate outbreak in the United States was attributed to sprouts contaminated with a strain of serotype 1/2a. This outbreak involved 20 cases leading to 16 hospitalizations (Table 1) *(9)*. No deaths or fetal losses occurred, and 20% of the affected individuals were pregnant women (Table 1) *(21)*.

Celery outbreak (2010, United States, Texas). In 2010, a listeriosis outbreak with high mortality (five fatalities among the 10 documented cases) among hospitalized patients in Texas involved chicken salad made with diced celery that was contaminated with *L. monocytogenes* serotype 1/2a *(15)*. The outbreak was challenging because cases occurred over 7 months at five different hospitals. Patients were 56 to 93 years old, and all had one or more immunocompromising conditions or were receiving corticosteroids or acid inhibitors *(15)*. The production facility had structural defects, including cracks in the floors and walls, and handling and cleaning techniques were considered inadequate. All food samples that were whole or cut by hand were negative for *L. monocytogenes*. The only machine-cut food product available during the investigation was diced celery, which was positive for *L. monocytogenes*, as were numerous samples from the environment and equipment in the facility. Indistinguishable pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem-repeat analysis (MLVA) profiles were obtained from *L. monocytogenes* isolated from the diced celery, environmental samples from the facility, and clinical samples (blood and pleural fluid) and implicated food items at the hospitals *(15)*.

Both PCR and culture-based isolation methods were used in this investigation. Fewer positive results were obtained with PCR, suggesting the possibility of false-negative results and the need for method optimization if the PCR assay is to be exclusively relied upon for surveillance. The implicated chicken salad was recalled, the production company closed permanently, and no subsequent cases were detected *(15)*.

Phylogenetic analysis based on whole genome sequencing (WGS) revealed that the celery outbreak strain was not closely related to serotype 1/2a strains implicated in other listeriosis outbreaks *(36)*. Such findings suggest that the celery outbreak strain does not belong to a known EC.

FRESH PRODUCE–ASSOCIATED LISTERIOSIS OUTBREAKS, 2011 TO 2014

Cantaloupe outbreak (2011, United States, multistate). Even though a trend for smaller outbreaks was noted in the 1998 to 2008 listeriosis outbreaks trends analysis *(4)*,

TABLE 1. *Produce-related listeriosis outbreaks*

Year	Implicated food	Location	No. of cases	No. of deaths	No. hospitalized	No. of perinatal cases	Serotype (EC)[a]	Comments	Reference
1979	Raw vegetables (celery, tomatoes, lettuce)	Boston, MA	20	5[b]	20	0	4b (ECIa)	Hospitalized patients; produce epidemiologically implicated but not microbiologically confirmed	18
1981	Coleslaw (cabbage)	Maritime Provinces, Canada	41	18	41	34	4b (ECI)	First outbreak to confirm foodborne transmission of *L. monocytogenes*	32
2008–2009	Sprouts (multiple types)	Multistate	20	0	16	4	1/2a		9
2010	Diced celery	Texas	10	5[b]	10	0	1/2a	Hospitalized patients	15
2011	Whole cantaloupe	Multistate	147[c]	33[d]	143	7	1/2a (ECVII[e]), 1/2b (ECVI[f])	First time cantaloupe implicated in listeriosis outbreak; first time serotype 1/2b documented in invasive common-source listeriosis outbreak; first time multiple strains implicated in common-source outbreak	25
2014	Stone fruit (nectarines, peaches)	Multistate	2	1	2	0	4b[g]	First time stone fruit linked to human listeriosis	20
2014	Mung bean sprouts	Multistate	5	2	5	0	4b[h]		8
2014	Commercially produced, prepackaged caramel apples	Multistate	35	7[bd]	34	11	4b (ECI[i])	First time caramel apples implicated in listeriosis outbreak; two serotype 4b strains implicated; human coinfection with two strains documented	6

[a] EC, epidemic clone.
[b] Three deaths were conclusively attributed to listeriosis.
[c] Not including one case of intraocular listeriosis.
[d] Not including one fetal loss.
[e] In addition to ECVII (serotype 1/2a), this outbreak involved unrelated serotype 1/2a strains that do not appear to be members of any identified EC (*24, 25*).
[f] In addition to ECVI (serotype 1/2b), one unrelated serotype 1/2b strain was involved in this outbreak (*25*).
[g] Based on MLST and virulence locus typing data extracted from WGS, the stone fruit outbreak strain was classified as ST382 with a novel virulence type (*10*).
[h] Based on MLST and virulence locus typing data extracted from WGS, the mung bean sprout outbreak strain was classified as ST554 with a novel virulence type (*10*).
[i] Based on MLST and virulence locus typing data extracted from WGS, one of the caramel apple outbreak clusters was ECI and the other was classified as ST382 with a novel virulence type (*10*).

in summer 2011 a surprisingly large outbreak with cases in 28 states was attributed to whole cantaloupe from one farm in Colorado. With 147 cases, 33 deaths, and one fetal loss, the 2011 cantaloupe outbreak was "one for books," as described in a popular press article *(41)*, rapidly emerging as the largest listeriosis outbreak on record and the largest foodborne outbreak due to any agent in recent decades in the United States *(25)*.

This outbreak had a 22% case fatality rate, and almost all of the cases (143 of 145) were hospitalized *(25)*. Advanced age emerged as a leading risk factor, and the majority (86%) of the patients were 60 years of age or older. Of the 123 individuals with available data on underlying medical conditions, 108 (88%) had one or more potentially immunosuppressive conditions or were receiving immuno-suppressive therapy. Pregnancy-associated infections were surprisingly few and involved four pregnant women and three newborns *(25)*.

Environmental assessment of the implicated cantaloupe growing fields and packing (processing) facility revealed the outbreak-associated *L. monocytogenes* strains in samples from the facility environment and equipment and from whole cantaloupes collected from the facility's cold storage area. However, cantaloupes that had not entered the facility and soil from the fields where the melons were grown were negative for *L. monocytogenes (25, 37)*. The environmental assessment of the packing facility revealed several factors likely to have contributed to postharvest contamination of the cantaloupes. The facility had recently acquired and installed equipment previously used for another raw agricultural commodity (potatoes), and this equipment could not be easily cleaned and sanitized. The facility's floor was constructed in a way that prevented adequate cleaning and sanitizing *(37)*.

Implication of multiple *L. monocytogenes* strains of different serotypes (1/2a, 1/2b) in the 2011 cantaloupe outbreak. The 2011 cantaloupe outbreak was unusual in involving strains of different serotypes (1/2a and 1/2b) and several different PFGE profiles. Three distinct PFGE profiles were detected among the serotype 1/2a isolates, two of which were closely related, and two clearly distinct PFGE profiles were noted among isolates of serotype 1/2b *(25)*. This outbreak represents the first documented involvement of serotype 1/2b in a common-source outbreak of invasive listeriosis. Of the two different serotype 1/2b strains (based on distinct PFGE profiles) associated with the outbreak, one was encountered only once (in a clinical sample) and the other was implicated in numerous listeriosis cases (27%) and deaths (30%) and was also frequently identified among isolates from the implicated product and the environment of the packing facility *(25)*. These serotype 1/2b isolates were sequence type (ST) 5 based on MLST and virulence type (VT) 63 based on an alternative typing scheme employing virulence loci *(24)*. ST5 was one of the predominant serotype 1/2b STs previously encountered among clinical (mostly pregnancy-related) listeriosis cases in France from 1992 to 2003, with a notable increase in incidence subsequent to 1997 *(29)*, but the extent to which these isolates may have represented a common-source outbreak(s) is not known. However, ST5-VT63 was implicated in a small outbreak (two cases, a husband and wife pair) of febrile gastroenteritis in Canada in 1996 with a short incubation period (12 to 18 h) associated with imitation crab meat contaminated with the outbreak strain at high levels $(2.1 \times 10^9$ CFU/g) *(13)*. The ST5-VT63 1/2b strains from the cantaloupe outbreak were designated as members of a novel EC, ECVI *(24)*. It is intriguing that this clone was not documented in other invasive listeriosis outbreaks before the 2011 cantaloupe outbreak.

As mentioned above, three serotype 1/2a strains were associated with this outbreak. One, of genotype ST29-VT74, was not detected among any previous outbreaks; ST29 strains appear to be generally uncommon *(29)*. Two closely related STs (ST7 and ST561, with a single-allele difference) were detected in the other two strains, which also had a single-band difference in their PFGE profiles and shared a common VT, VT56 *(24, 25)*. Although reported in the MLST database among human clinical, animal, and food isolates, primarily from outside the United States, ST7 strains had not been previously found to be implicated in invasive, common-source outbreaks; however, they were implicated in an outbreak (five cases) of febrile gastroen-teritis with short incubation period (<36 h) in Canada in the summer of 2001, involving whipping cream served at a church event *(27)*. These serotype 1/2a strains from the cantaloupe outbreak with ST7-ST561 and VT56 were designated as members of an additional novel epidemic clone, ECVII *(24)*.

Summary of unusual attributes of the 2011 canta-loupe outbreak. In addition to the large number of cases and fatalities, the 2011 cantaloupe outbreak had several unusual attributes.

(i) It involved a novel vehicle; it was the first documented listeriosis outbreak to involve whole canta-loupe. Cut melon previously had been epidemiologically implicated in sporadic listeriosis cases *(39)*, but documented transmission of *L. monocytogenes* via melons was lacking before this outbreak.

(ii) Two serotypes (1/2a and 1/2b) and five STs were involved in the outbreak. Multiple, clearly different strains had not been previously reported in a common-source listeriosis outbreak. The involvement of strains of different serotypes and genotypes in listeriosis outbreaks was reported only rarely before the 2011 cantaloupe outbreak *(30, 33, 40)* and only once in the United States (Philadelphia, 1986 to 1987) *(33)*; however, epidemiological analysis failed to provide evidence for a common source for any of these earlier multiserotype multistrain outbreaks *(30, 33, 40)*.

(iii) The genotypes of the serotype 1/2a and 1/2b strains contributing to this outbreak were not previously detected in outbreaks of invasive listeriosis.

(iv) The number of pregnancy-associated cases was low at 5% compared with other listeriosis outbreaks, in which pregnancy-associated cases accounted for 12 to 66% of all cases *(35)*.

(v) An estimated 86% of affected individuals were older than 60 years of age. Even though advanced age has been recognized as a risk factor for listeriosis *(34),* in this outbreak the number of elderly individuals was unusually high.

Two noteworthy events in the context of the 2011 cantaloupe outbreak. Two unusual and noteworthy events were identified in the context of the 2011 cantaloupe outbreak: a rare case of intraocular listeriosis and a case of listeriosis in an exotic pet.

The intraocular listeriosis case involved an 88-year-old woman with primary open-angle glaucoma in both eyes that was being controlled with medication *(19).* The woman presented to her eye doctor with acute onset of blurry vision, pain, and redness in her right eye. After initial treatment failed to resolve the symptoms, further diagnostic tests were performed, and *L. monocytogenes* was isolated from intraocular cultures taken from the eye and from blood cultures. PFGE revealed that the isolates were indistinguishable from one of the five strains associated with the 2011 cantaloupe outbreak. The patient had experienced mild gastroenteritis 2 weeks before the onset of her ocular symptoms and had eaten cantaloupe two to four times per week during the month before symptoms began. This was the first report of intraocular listeriosis associated with a foodborne outbreak *(19)* and reveals the potential for foodborne listeriosis to lead to unusual syndromes. Detection and reporting of such rare cases may be compromised during nonoutbreak periods.

The exotic pet incident involved a fatal case of listeriosis in a sugar glider (*Petaurus breviceps*) kept as pet and fed a diet that included precut cantaloupe *(26).* The pet's illness occurred during the outbreak period in 2011 in New Mexico, one of the states heavily affected by the outbreak. *L monocytogenes* isolated from the liver of the sugar glider was serotyped and genotyped to determine any association with the outbreak. The strain was of serotype 4b, which was not encountered among the outbreak isolates *(26).* However, one of the outbreak-associated serotype 1/2b strains was also encountered only once (in a human patient). The sugar glider incident is informative because it suggests that an additional strain may have been associated with the outbreak and reveals the potential role of cantaloupe in animal listeriosis cases. This case also may have intriguing One Health implications: listeriosis in companion and other animals with access to human food may signal the presence of food safety hazards to humans and vice versa, highlighting the public health importance of open communication between physicians and veterinarians *(16).*

Mung bean sprouts outbreak (2014, United States, multistate). In this and other produce-related listeriosis outbreaks in 2014, WGS served as an important tool for outbreak investigation and resolution. The mung bean sprouts outbreak involved five people in two states, Illinois and Michigan; all five patients were hospitalized, and two died *(8).* A routine U.S. Food and Drug Administration inspection of the facility that prepared the sprouts and other products (12 August through 3 September 2014) yielded *L. monocytogenes* from mung bean sprouts, spent irrigation water from sprout production, and environmental swabs. PFGE and WGS of the isolates revealed that they were closely related to isolates from five listeriosis patients that became ill from June through August 2014 *(38).* The outbreak strain was of serotype 4b and ST554 (based on MLST data) *(10),* which had not been detected previously in listeriosis outbreaks (Table 1). To date, only 21 isolates with ST554 have been identified and, interestingly, these were all from the United States (S. Brisse, personal communication, 2015). Inspections of the implicated processing facility revealed numerous instances of unsanitary conditions and poor equipment maintenance. Follow-up inspections as long as 2 months later again yielded the outbreak strain from environmental samples, and the facility was eventually shut down *(38).*

The 2014 mung bean outbreak was relatively small but served to highlight (i) the usefulness of WGS as a tool for outbreak detection and investigation and (ii) the persistence of *L. monocytogenes* in the processing facility, which appears to have become persistently colonized. Persistence of the pathogen may have been facilitated by inadequate sanitation and maintenance.

Caramel apple outbreak (2014, United States, multistate). A novel food vehicle for listeriosis (and any other foodborne illness) was implicated in a multistate outbreak in fall 2014 that was associated with commercially produced, prepackaged caramel apples *(6).* This outbreak included 32 cases in 12 states and resulted in 31 hospitalizations, six deaths (at least three of which were conclusively attributed to listeriosis), and one fetal loss. One additional listeriosis case associated with this outbreak was detected in Canada (Table 1). In contrast to the cantaloupe outbreak, a substantial fraction of the cases (10 of 32 cases, 31%) were pregnancy-associated. Excluding pregnancy, the median age of affected individuals was 62 years (range, 5 to 92 years) *(6).*

Two clusters of distinct serotype 4b strains were isolated both from the patients and from the apple packing facility and identified by PFGE and WGS. Analysis of the WGS data *(10)* indicated that strains in one of the clusters belonged to ECI, which has been implicated in multiple listeriosis outbreaks *(3, 12, 22, 29),* while unrelated strains of ST382 constituted the other cluster (Table 1). WGS played a pivotal role in this outbreak investigation, with the use of this tool estimated to have shortened the duration of the outbreak by at least 1 week *(6).*

Unusual attributes of the 2014 caramel apple outbreak. In addition to caramel apples as a novel and highly unusual *L. monocytogenes* vehicle and the clear evidence for the efficacy of WGS as a tool in outbreak investigation and resolution, several other attributes of this outbreak were unusual.

(i) Affected individuals included three previously healthy children, 5 to 15 years of age. In all three children,

the *L. monocytogenes* infection manifested as meningitis. This population group was not affected in previous listeriosis outbreaks, which, in addition to pregnant women, typically involved people at extremes of age (neonates or those older than 60 years) and those with underlying immunosuppressive conditions *(22, 34, 35)*.

(ii) At least three of the patients had apparently consumed sliced or whole green apples, suggesting that the source of illness was the fruit itself and not contaminated caramel. This finding implicates green apples as a novel vehicle, but the level of *L. monocytogenes* on whole green apples would be expected to be low because of the low water activity and low nutrient concentrations on the smooth skin of the fruit. However, in one study, populations of *Listeria innocua* increased by 2 log units after 24 h at 10, 20, or 25°C on fresh-cut apple plugs of several varieties, including Granny Smith *(1)*.

(iii) The involvement of two markedly different *L. monocytogenes* strains in this outbreak was only the second documented involvement of multiple, unrelated strains in a common-source outbreak (the first was the 2011 cantaloupe outbreak).

(iv) Samples (blood and cerebrospinal fluid) from one patient yielded two *L. monocytogenes* strains, for the first time providing evidence of coinfection of one individual by more than one strain during a multistrain outbreak. Because clinical laboratories typically purify only one colony from a positive culture, the extent of coinfection in this and other common-source outbreaks that involve multiple strains remains undetermined.

Stone fruit outbreak (2014, United States, multistate). Stone fruit such as peaches and nectarines are extensively consumed raw but had not been implicated in human listeriosis cases before 2014. In 2014, routine testing by a stone fruit packing company identified *L. monocytogenes* strains with PFGE profiles that matched those of four clinical isolates in the PulseNet database (http://www.cdc.gov/pulsenet/) *(20)*. Application of WGS in combination with patient questionnaires suggested the involvement of contaminated peaches and nectarines in two of these four human cases, in Massachusetts and Minnesota, respectively, during summer 2014. The strains from these patients closely matched (<10 allele differences of >5,800 loci analyzed following WGS) the strains isolated from whole stone fruit during routine testing *(20)*. However, a larger number (47 to 69) of allele differences between the stone fruit isolates and the two additional clinical isolates suggested that the latter were not part of the outbreak and represented apparently sporadic cases, even though their PFGE profiles were indistinguishable from those of the outbreak strains *(20)*. The use of WGS to differentiate between sporadic and common-source outbreak isolates with indistinguishable PFGE profiles can greatly facilitate the delineation of the outbreak-associated population. Even though WGS was employed in the 2014 stone fruit outbreak to differentiate the isolates among a small number of putative cases, this technique should also be valuable for identifying widely disseminated strains with high baseline prevalence.

The stone fruit outbreak strain had serotype 4b and was classified as ST382 (Table 1), which also was encountered in some of the isolates from the caramel apple outbreak *(10)*. Further analysis is needed to determine whether these strains constitute a novel EC.

Complex public health implications of the 2014 stone fruit outbreak and further support for use of WGS as tool in outbreak investigations. The stone fruit outbreak highlights the potential of *L. monocytogenes* to cause illness via foods that would be considered unlikely as vehicles for this pathogen. The small number of cases (two) in this outbreak, especially considering that illness was caused by products that are consumed extensively and without further treatment, suggests that human illness due to consumption of contaminated stone fruit is rare. This low incidence may reflect low levels of *L. monocytogenes* on the whole fruit or relatively low virulence of the strains; the finding of only four human isolates with the same PFGE profiles in the PulseNet database suggests that overall this strain type is highly uncommon in human listeriosis cases. The public health implications are complex because the potential for illness clearly exists, and the extent to which stone fruit may contribute to other small listeriosis outbreaks or to sporadic cases remains undetermined. The outbreak did not lead to public health advisories for pregnant women or elderly individuals to avoid whole stone fruit *(20)*. Nonetheless, whole stone fruit may be a commodity of concern for individuals that may be unusually vulnerable to infection by low levels of this pathogen.

CONCLUSIONS AND EMERGING QUERIES

Common themes: novel vehicles, domestically grown produce, multistate outbreaks. Novel vehicles were involved in the majority of the outbreaks reviewed here. With the exception of sprouts, which were implicated in two outbreaks, and celery, which was epidemiologically implicated in one outbreak (Boston 1979) and confirmed in another (Texas 2010), other outbreaks have involved vehicles that were novel and considered unlikely for transmission of *L. monocytogenes*. All documented produce-related outbreaks since 2008 were in the United States, and with the exception of the 2010 celery outbreak, they were all multistate. They also all involved domestically grown produce. The tendency for multistate outbreaks and implication of domestic produce has been recognized by others *(21)*. Even though commercial dissemination of produce from specific regions to other areas in the United States can readily account for the multistate nature of the events, the absence of outbreaks via imported produce during this period (2008 to present) is more difficult to explain, as is the lack of reported produce-related outbreaks in other countries.

Key role of postharvest contamination in *L. monocytogenes* transmission. The investigated outbreaks revealed postharvest contamination of the produce through the contaminated environment and equipment at the packing

facility. In this regard, route of contamination is similar to that typically seen in outbreaks involving other vehicles such as meat and dairy products. In spite of the high likelihood for *L. monocytogenes* to be present in soil and water, field-level contamination has not been documented in produce-related listeriosis outbreaks, which leads to questions related to the impact of the source of contamination on likelihood for involvement in human disease. For instance, do certain *L. monocytogenes* strains involved in postharvest contamination have unusually high virulence? Does postharvest growth of *L. monocytogenes* on the produce increase virulence? Do alterations of the fresh produce microbiota through processing predispose the produce to contamination by *L. monocytogenes* strains resident in the processing environment, including strains that may have unusually high virulence?

Multistrain outbreaks and documentation of human coinfection. The involvement of multiple strains in common-source outbreaks was firmly established for the first time in two of the produce-related outbreaks (cantaloupe and caramel apples). The extent of human coinfection by more than one outbreak-associated strain remains unclear, although in at least one outbreak (associated with caramel apples) coinfection of one person with two strains was documented.

Serogroup 1/2 strains were implicated in several outbreaks with novel confirmation of serotype 1/2b in a common-source outbreak of invasive listeriosis. Since 2008, several documented produce-related outbreaks have involved strains of serogroup 1/2, especially serotype 1/2a; however, serotype 4b strains were implicated in all three produce-related outbreaks in 2014. The first documented involvement of serotype 1/2b strains in a common-source outbreak of invasive listeriosis occurred in the 2011 cantaloupe outbreak. This outbreak also led to discovery of novel ECs, specifically ECVI (serotype 1/2b) and ECVII (serotype 1/2a) *(24)*.

Implications of strain types and epidemiological findings. Strains that have previously made limited contributions to invasive listeriosis emerged as major contributors in the largest listeriosis outbreak on record, i.e., the 2011 cantaloupe outbreak. The future disease burden contribution of strains from other recent produce-related outbreaks remains to be determined. Nonetheless, the findings from the cantaloupe outbreak highlight major knowledge gaps and lead to questions concerning mechanisms underlying the involvement of certain strains in human listeriosis, including the potential roles of the food vehicle and of human susceptibility status. Novel approaches are needed to learn more from the epidemiological findings. What can we learn from the fact that most individuals in the cantaloupe listeriosis outbreak were of advanced age, but pregnancy-associated cases were underrepresented? Why did seemingly healthy children outside of the known age risk groups acquire invasive listeriosis in the caramel apple outbreak? Are certain strains such as those in

the stone fruit outbreak of low virulence or are they primarily encountered on vehicles that do not promote growth and thus only rarely contribute to human illness?

Future research directions. The current epidemiology of the produce-related outbreaks suggests that several outbreak-produce combinations have been one-time events; no coleslaw-associated outbreak has occurred since 1981, and no cantaloupe-associated outbreak has occurred since 2011. In spite of the justifiably heightened awareness by the public and by regulatory agencies of the potential for contracting listeriosis through produce, produce-related outbreaks remain uncommon in the United States and elsewhere and lag in frequency behind listeriosis outbreaks due to products such as soft cheeses *(5)*. Produce-related sporadic listeriosis cases and small outbreaks may become more commonly recognized with enhanced surveillance and epidemiological investigation. Nonetheless, produce-related common-source outbreaks may reflect "perfect storm" outcomes from the convergence of a number of otherwise uncommon or unusual factors at the pathogen-food-host interface. Combinatorial approaches integrating data and insights from WGS, metagenomic analysis, ecological assessments, epidemiological investigations, and improved tools for assessment of pathogen virulence and host susceptibility will be increasingly utilized to address these knowledge gaps. These approaches will further the utility of these outbreaks as "teachable moments" that will eventually provide new information and contribute to development of new and improved interventions.

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