Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015

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SUMMARY

Whole apples have not been previously implicated in outbreaks of foodborne bacterial illness. We investigated a nationwide listeriosis outbreak associated with caramel apples. We defined an outbreak-associated case as an infection with one or both of two outbreak strains of *Listeria monocytogenes* highly related by whole-genome multilocus sequence typing (wgMLST) from 1 October 2014 to 1 February 2015. Single-interviewer open-ended interviews identified the source. Outbreak-associated cases were compared with non-outbreak-associated cases and traceback and environmental investigations were performed. We identified 35 outbreak-associated cases in 12 states; 34 (97%) were hospitalized and seven (20%) died. Outbreak-associated ill persons were more likely to have eaten commercially produced, prepackaged caramel apples (odds ratio 326·7, 95% confidence interval 32·2–3314). Environmental samples from the grower's packing facility and distribution-chain whole apples yielded isolates highly related to outbreak isolates by wgMLST. This outbreak highlights the importance of minimizing produce contamination with *L. monocytogenes*. Investigators should perform single-interviewer open-ended interviews when a food is not readily identified.

Key words: Foodborne infections, food safety, infectious disease epidemiology, *Listeria*, outbreaks.

INTRODUCTION

Listeria monocytogenes is estimated to cause over 1600 illnesses and 250 deaths annually in the United States, making it a leading cause of foodborne illness death [1, 2]. Older adults, persons with immunocompromising conditions, and pregnant women and their neonates are at higher listeriosis risk than the general population [3, 4]. Rarely, otherwise healthy persons develop invasive listeriosis [5]. Most listeriosis is foodborne and sporadic; outbreaks have historically been linked to ready-to-eat meats and dairy products [5–8]. However, produce-associated listeriosis outbreaks are

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increasingly recognized [9], including those involving sprouts [10], stone fruit [11], celery [12], and cantaloupe [13]. Whole apples have not been implicated in listeriosis outbreaks, although apples have been recalled for possible *L. monocytogenes* contamination [14] and unpasteurized apple cider has been implicated as the cause of Shiga toxin-producing *Escherichia coli* and *Cryptosporidium* outbreaks [15].

On 14 November 2014, epidemiologists at the Arizona Department of Health Services and the New Mexico Department of Health notified the U.S. Centers for Disease Control and Prevention (CDC) of four listeriosis cases that occurred within the previous month, including two in otherwise-healthy children. Pulsed-field gel electrophoresis (PFGE) results were not yet available. The same day, PulseNet, the national molecular subtyping network for enteric disease surveillance, identified a listeriosis cluster with a rare PFGE pattern with cases in older adults in Minnesota (three cases) and Missouri (one case) [16, 17]. The following week, PulseNet identified a second unrelated PFGE cluster of five *L. monocytogenes* isolates, including the four listeriosis cases reported by Arizona and New Mexico. By 1 December, the patient count in the two clusters had risen to 22, including a child with an *L. monocytogenes* cerebrospinal fluid (CSF) isolate matching one cluster and a blood isolate matching the other. Given this finding and the overlap in the temporal and geographic case distribution, investigators suspected a common source could account for cases in both clusters, and the clusters were combined into a single investigation. We describe the nationwide investigation into the infection source, including the use of real-time whole genome sequencing.

METHODS

Case definition and laboratory methods

Clinical *L. monocytogenes* isolates are sent to state public health laboratories for two-enzyme PFGE subtyping [18]. Since September 2013, whole genome sequencing is performed on clinical, environmental, and food *L. monocytogenes* isolates at state laboratories, CDC, Food and Drug Administration (FDA), and the US Department of Agriculture's Food Safety and Inspection Service. CDC analyses these sequences using whole-genome multilocus sequence typing (wgMLST), which provides superior genetic resolution compared with PFGE [11, 19]. The data have been deposited with links to BioProject accession number PRJNA212117 in the National Center for Biotechnology Information BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

We defined an outbreak-associated case as a laboratory-confirmed *L. monocytogenes* infection in a US resident with an isolate cultured from a normally sterile site (e.g. blood or CSF) or from products of conception (e.g. placenta) that was highly related to other outbreak isolates by PFGE and wgMLST and collected during 1 October 2014 to 1 February 2015. Pregnancy-associated cases were defined as infection in a pregnant woman or infant (aged ⩽ 28 days). Medical records and death certificates were reviewed to assess whether listeriosis contributed to patients' deaths. PFGE data were shared with other countries via PulseNet International [20] for additional case finding.

Epidemiological investigation

We used several epidemiological techniques in the investigation. State and local health departments interviewed patients or surrogates using the *Listeria* Initiative (LI) form, which collects clinical, laboratory, demographic, and epidemiological information [21], including questions on 44 possible food exposures during the 28 days before illness onset; five questions involve produce (cantaloupe, watermelon, honeydew melon, sprouts, fruit salad). Patients were also interviewed using CDC's National Hypothesis Generating Questionnaire (NHGQ) that includes >300 food and restaurant exposures, and, when possible, additional open-ended interviews were performed [22]. Open-ended interviews involve lengthy, semi-structured discussions with the patient or surrogate about foods consumed during the 28-day exposure window. These interviews include assessment of the patient's typical diet and foods eaten at special events or holidays. After open-ended interviewing revealed that eight patients ate commercially produced, prepackaged caramel apples, an item not listed on the LI form or NHGQ, during their incubation periods, CDC requested that states administer a supplemental questionnaire to all recent listeriosis cases, including outbreak-associated and non-outbreak-associated cases. This supplemental questionnaire included questions about caramel apples, caramel, whole apples, and pre-cut apple slices. We compared outbreak-associated case exposures with exposures reported in the 2006–2007 FoodNet Population Survey [23] and with exposures for non-outbreak-associated listeriosis cases that

occurred during the same time period. We calculated odds ratios (OR) and 95% confidence intervals (CI) for exposures of interest.

Traceback and environmental investigation

FDA and state and local health and regulatory agencies performed a traceback investigation using information reported from patients about caramel apple brands and purchase locations to identify caramel apple manufacturers. Manufacturer invoices were used to determine ingredient sources. FDA and state public health and agricultural officials sampled and cultured whole apples from the distribution chain in Minnesota, Missouri, and Nebraska and sampled caramel apple production facility environments in California and Missouri to identify *L. monocytogenes.* After traceback investigation identified an apple grower (Grower A) that supplied the involved manufacturers, California and FDA officials inspected and sampled Grower A's apple packing facility.

RESULTS

Outbreak description

We identified 35 outbreak-associated cases in 12 US states. Most infections occurred in the southwestern United States, particularly Arizona and New Mexico, and the Midwest (Fig. 1). Eleven illnesses (31%) were pregnancy-associated, including one fetal loss. Of non-pregnancy-associated cases, the median age was 62 years (range 7–90 years) and 33% were female. Three children, all otherwise healthy and aged 5–15 years, developed meningitis. Thirty-four patients (97%) were hospitalized. Listeriosis contributed to at least three of seven reported deaths (Table 1). Illness onset dates ranged from 17 October 2014 to 7 January 2015 (Fig. 2). The Public Health Agency of Canada (PHAC) identified an additional case with a patient isolate that was indistinguishable from outbreak-associated isolates by PFGE and highly related by wgMLST.

Epidemiological case investigation

No food emerged as a likely source following initial interviews with the LI form. By 10 December, ten NHGQ interviews were completed and no exposures of interest were identified; five (63%) of eight patients reported eating apples compared with 62% of population survey respondents. Therefore, more open-ended

interviews were begun. On 15 December, CDC and Texas state and local public health officials interviewed a patient's spouse; a Texas epidemiologist inquired about caramel apple consumption after noting such consumption in a previous open-ended interview. The spouse reported that the patient had eaten a caramel apples three-pack from a large retail chain. Based on this information, eight additional patients were re-contacted and all eight reported eating caramel apples. CDC deployed a supplemental questionnaire the following day.

Ultimately, 34 ill persons or surrogates were interviewed with the LI form, 12 with the NHGQ, eight with open-ended interviews, and 15 with the supplemental questionnaire; 16 patients were asked about caramel apple consumption but were not administered the supplemental questionnaire. Twenty-eight (90%) of 31 patients with available information consumed commercially produced, prepackaged caramel apples in the 28 days before illness onset compared with one (3%) of 36 non-outbreak-associated cases interviewed with the supplemental questionnaire during the same time period (OR 326·7, 95% CI 32–3314). The patient from Canada also reported eating caramel apples. Of three ill persons who did not report eating caramel apples, one ate apples with caramel dipping sauce, one ate a Granny Smith apple, and one ate pre-cut apple slices, the sources of which were unknown. Nine (56%) of 16 people with outbreak-associated illnesses ate whole apples not part of caramel apples compared to 20 (67%) of 30 non-outbreak-associated cases. Twenty-four outbreak-associated patients reported eating caramel apples purchased from a retail establishment, two purchased caramel apples at a fair, one received caramel apples from a food bank, and the source for one was unknown. Three patients named the caramel apple brand; various purchase locations were reported.

Traceback and environmental investigation

FDA and state regulatory agencies conducted a traceback investigation using caramel apple purchase locations for 11 outbreak-associated cases. These 11 locations received caramel apples from four manufacturers through multiple distributors. The four manufacturers did not share a common source of caramel, sticks, toppings (e.g. nuts, sprinkles), or packaging. However, all made caramel apples using Grower A's apples from California. FDA and California state officials inspected Grower A's apple packing facility
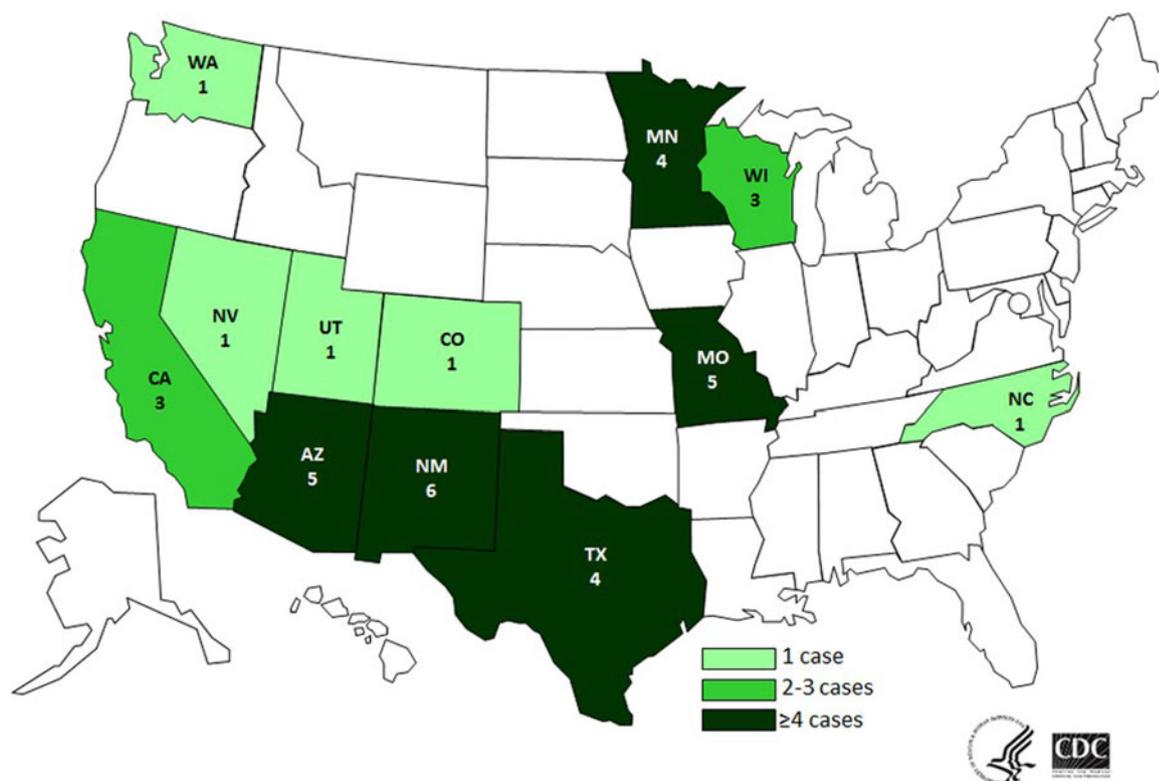
Fig. 1. Number of laboratory-confirmed cases of *Listeria monocytogenes* infection associated with caramel apples by state of patient residence, United States, 1 October 2014 to 1 February 2015 ($n = 35$).

and collected 110 environmental samples; seven yielded *L. monocytogenes*. Six isolates were from food contact surfaces (i.e. polishing brush, drying brushes, conveyor, and inside a wooden bin) and one was from the main packing line floor drain. Although inspectors could not observe packing procedures because seasonal apple packing operations had ended, they observed multiple production line vinyl surfaces with frayed edges, exposed foam padding, and a damaged conveyor belt. FDA, California, and Minnesota officials isolated *L. monocytogenes* from 30 whole apple samples shipped by Grower A and collected at various points in the distribution chain, as well as one environmental sample from a caramel apple manufacturer.

Subtyping

Two PFGE patterns were identified in 34 patient isolates. Each PFGE pattern formed a phylogenetically distinct clade by wgMLST. Isolates within each clade were highly related to one another (<11 allele differences); a thirty-fifth patient isolate had a third PFGE pattern and grouped closely with one clade (Fig. 3). The two clades aligned with the two illness clusters initially detected by the states and PulseNet, and the clades differed by >1600 alleles. Isolates in one clade were multilocus sequence type (ST) 1 and the others were ST382; all isolates were genetic lineage 1 and serotype 4b. The sequenced *L. monocytogenes* isolates from apples, isolates from Grower A, and the environmental isolate from a caramel apple manufacturer were highly related to patient isolates within both clades by wgMLST.

Control measures

On 19 December, CDC warned consumers not to eat any commercially produced, prepackaged caramel apples until more specific information could be provided. CDC made this broad recommendation while the traceback investigation was underway and before the contamination scope was known because of listeriosis severity, the long caramel apple shelf-life, and the strong epidemiological link to caramel apple consumption. On 22 December, Grower A recalled whole apples used in caramel apple production after being notified of the epidemiological and traceback evidence. On the 24, 27, and 29 December, three caramel apple manufacturers issued product recalls. On 6 January 2015,

Table 1. *Demographic and clinical characteristics of 35 ill patients with outbreak-associated Listeria monocytogenes infections associated with caramel apples*

Characteristic	Value
Median age, years (range)	
All cases	43 (0–92)
Cases not associated with pregnancy	62 (7–92)
Female sex, *n* (%)	
All cases	15 (43)
Cases not associated with pregnancy	8 (33)
Pregnancy-associated cases, *n* (%)	
Total	11 (31)
Pregnant women	5/11 (45)
Neonates	6/11 (55)
Underlying conditions*, *n* (%)	
Cirrhosis or hepatitis	2/13 (15)
Diabetes	3/13 (23)
Heart disease (congenital and acquired)	2/13 (15)
Haemodialysis	4/13 (31)
Myelodysplastic syndrome	1/13 (8)
No underlying condition†	4/13 (31)
Hospitalizations, *n* (%)	34 (97)
Reported deaths, *n* (%)	7 (20)
Listeriosis as contributing factor	3 (9)
Fetal loss	1 (3)
L. monocytogenes source site, *n* (%)‡	
Blood	26 (72)
Cerebrospinal fluid	5 (14)
Ascitic fluid	1 (3)
Placenta	1 (3)
Other	3 (8)§

* Excludes pregnancy-associated cases. Complete information on underlying conditions was available for 13 patients.
† Includes three otherwise healthy children between the ages of 5 and 15 years and a 78-year-old female.
‡ One child had a blood isolate matching one outbreak pulsed-field gel electrophoresis (PFGE) pattern and a cerebrospinal fluid isolate matching another outbreak PFGE pattern.
§ Includes specimens from a left arm, sputum, and one site reported as other.

Grower A expanded the recall to include all Gala and Granny Smith apples produced in the 2014 season following environmental sampling results, and CDC narrowed its warning to Grower A's apples.

DISCUSSION

Commercially produced, prepackaged caramel apples are a newly described listeriosis vehicle. We describe a complex, nationwide foodborne listeriosis investigation in which epidemiological, microbiological, and traceback evidence conclusively demonstrated that commercially produced, prepackaged caramel apples were the source and that the whole apples came from a single grower. This was the largest US listeriosis outbreak since one linked to cantaloupe in 2011 [13]. Caramel apple consumption was significantly higher in outbreak-associated patients than in patients with contemporaneous non-outbreak-associated listeriosis. Traceback investigations converged on a single apple grower in California, and samples from the apple packing facility, one caramel apple production facility, and apples in the distribution chain yielded *L. monocytogenes* isolates highly related by wgMLST to patient isolates in both outbreak clades. The relatively large size and scope of this outbreak suggests that a critical failure at the facility or an *L. monocytogenes* introduction occurred, allowing for contamination of whole apples used to make caramel apples. The grower's packing process was the likely contamination source given that *L. monocytogenes* was detected on the packing line and exposed porous surfaces were present.

Three otherwise healthy children developed meningitis. By comparison, only 0·6% of the >5000 human *L. monocytogenes* isolates from the *Listeria* Initiative were from patients in this age range. Compared with the cantaloupe-associated outbreak [13, 24], this outbreak had a lower median age (43 *vs.* 78 years) and higher percentage of pregnancy-associated cases (37% *vs.* 4%). Age differences in consumption patterns and food preferences or differences in serotype might explain these findings. Although not tested, patients might have been exposed to a relatively high *L. monocytogenes* dose or these *L. monocytogenes* strains might have been more virulent than others.

Use of wgMLST assisted this investigation by showing that patient isolates within each PFGE-defined cluster were highly related and thus more likely to have a common source. Of note, isolates were not highly related between the two clusters. Subsequently, wgMLST also demonstrated that apple and environmental isolates were highly related to patient isolates. Since September 2013, wgMLST has transformed US listeriosis investigations, helping detect more illness clusters and solve more outbreaks [25]. This investigation also highlighted the importance of 'shoe-leather' epidemiology. After standard forms (LI and NHGQ) failed to identify a likely source, time-intensive open-ended interviews and an iterative interviewing approach were used; an astute epidemiologist who recalled a previous patient's exposure asked specifically about caramel apples in a
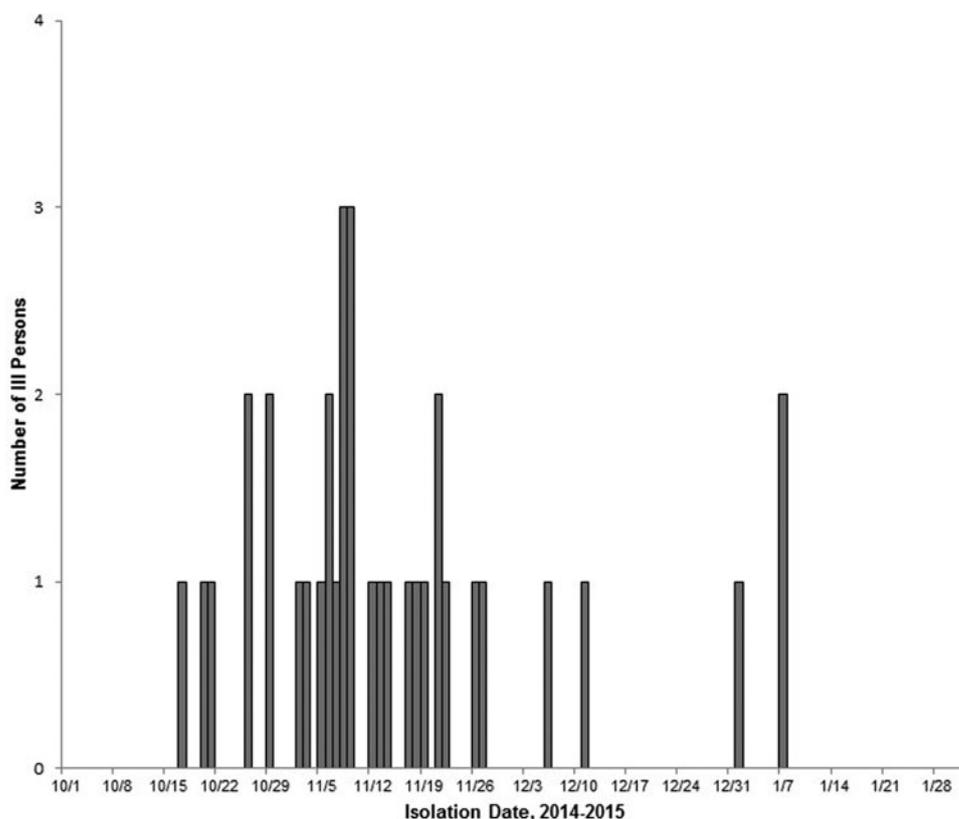
Fig. 2. Dates of *Listeria monocytogenes* isolation for 35 patients involved in outbreak associated with caramel apples, United States, 1 October 2014 to 1 February 2015.

subsequent interview. Involving the same investigator(s) in several interviews is the key feature that makes this interviewing technique succeed when other methods fail to identify a source [26].

This incident represents the sixth reported US listeriosis outbreak linked to produce since 2008 [9–13]. Whether the emergence of produce as a listeriosis vehicle represents a true increase in produce-associated illnesses or improvements in outbreak detection and investigation is unclear. Although produce is now a well-established listeriosis source, and the first foodborne listeriosis outbreak identified was linked to produce [27], caramel apples were an unexpected food. Neither apples, given their acidity, nor caramel, given its low water activity, were considered likely to support *L. monocytogenes* growth. However, a subsequent inoculation study showed substantial *L. monocytogenes* growth (>3 $\log_{10}$ increase within 3 days) on caramel apples with sticks at 25 °C [28]. Less growth was observed for caramel apples with sticks at 7 °C, and no growth at that temperature was seen in caramel-covered apples without inserted sticks. The authors hypothesized that stick insertion

into an apple allows juice to enter the space between the skin and caramel, providing a favourable environment for *L. monocytogenes* proliferation, particularly at room temperature. We did not collect information on whether patients purchased caramel apples from store shelves or refrigerators or how they stored them at home. At least one grocery store chain involved in the outbreak subsequently stopped selling unrefrigerated caramel apples [29]. Given that other acidic fruit (e.g. peaches, nectarines) were implicated in a small listeriosis outbreak in the summer of 2014, whole apples could be considered a possible *L. monocytogenes* vehicle [11]. Two research findings support this conclusion. First, commercial brush washing of apples immersed in an *Escherichia coli* solution with sanitizing agents did not reduce *E. coli* populations, likely because the relatively inaccessible stem and calyx areas are harbourage sites [30]. Second, if a pathogen is present, apple wounds or bruises can allow fungal growth that neutralizes apple flesh pH and facilitates bacterial growth [31].

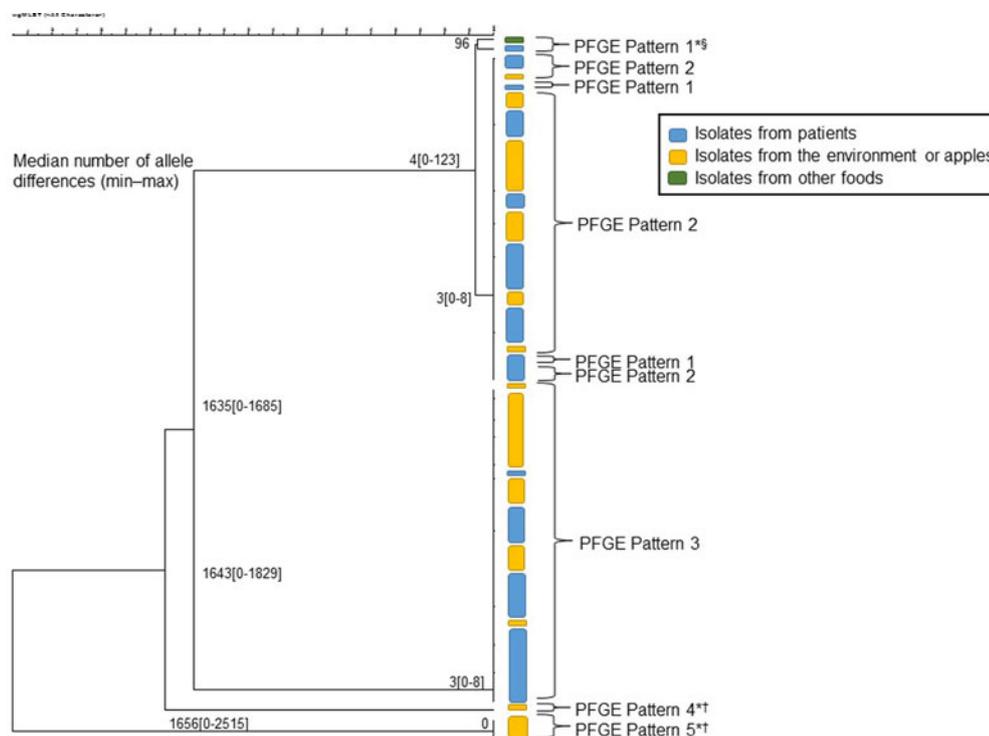Consumption of whole apples alone was not significantly associated with illness. Although apples from

Fig. 3. Dendrogram of whole-genome multilocus sequence typing (wgMLST) results of human, environmental, and produce isolates that match the outbreak strains (*n* = 50 isolates). * Isolates not directly related to the outbreak. § Isolates that are indistinguishable from the outbreak PFGE patterns but excluded from the outbreak via wgMLST. † Isolates from an unrelated caramel apple production facility that are not highly related to outbreak-associated isolates. Not all apple isolates underwent wgMLST.

the grower's packing facility bound for caramel apple production and retail yielded *L. monocytogenes,* there were no differences in apple processing or collection between those intended for caramel apples and those intended for retail. Three outbreak-associated patients who denied eating caramel apples reported eating whole or pre-sliced apples. However, ~60% of the US population report eating apples during a given week [23] and it was not possible to determine the origin of the apples eaten by these patients, so it is not known whether they came from the California grower or if they were the source of the patients' illnesses.

This outbreak highlights gaps in the understanding of microbiological risks associated with tree fruit production, the importance of preventive measures to minimize contamination of ready-to-eat produce items with *L. monocytogenes.* FDA's Produce Safety Rule under the U.S. Food Safety Modernization Act could help reduce the listeriosis risk from fruit by requiring that non-exempt producers inspect, maintain, clean, and sanitize all food contact surfaces and take other preventive actions [32]. Caramel apple manufacturers should consider package labels

recommending product refrigeration, and retailers and consumers should consider refrigerating prepackaged caramel apples and follow use-by dates to maintain quality. For future public health investigations, listeriosis questionnaires should include more produce and seasonal or holiday items questions, including caramel apples, and investigators should perform single-interviewer open-ended interviews when a food vehicle is not readily identified.

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DECLARATION OF INTEREST

None.

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