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COMMENTARY
Listeria monocytogenes cross-contamination of cheese: risk throughout the food supply chain

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Listeria monocytogenes has been the most common microbial cause of cheese-related recalls in both the United States and Canada in recent years [1, 2]. Although much attention is often paid to raw milk cheese [3], the majority of recent recalls and cheese-related outbreaks of listeriosis in the United States have been linked to fresh-soft, soft-ripened, and semi-soft cheeses produced from pasteurized milk [4–7]. Because *L. monocytogenes* is inactivated in milk by pasteurization, these outbreaks highlight the significant risk of post-pasteurization cross-contamination of cheese from equipment and the environment. Two recent articles in this journal detailing a 2012 listeriosis outbreak in the United States attributed to ricotta salata cheese produced in Italy [8, 9] highlight the complicated risk posed by cross-contamination of foods with *L. monocytogenes* and the challenges these contamination scenarios present in subsequent illness and outbreak investigations. They also demonstrate the critical role of epidemiological investigation coupled with coordinated molecular subtyping and surveillance in the recognition and investigation of complex foodborne outbreaks.

With the growth of the speciality and artisan cheese industry in the United States there is increasing opportunity for the sale of an array of cheeses and cheese types from domestic and international producers [10]. This includes cheeses that are cut-to-order or cut-and-wrapped at distribution or retail as well as sales at local farmer's markets and through the Internet. Increased handling presents additional cross-contaminations risks as well as difficulties in traceability as a single contaminated cheese wheel can be recut several times in several locations before reaching the consumer. For example, an intact cheese may first be cut in halves or quarters by a large cheese converter at a single establishment. Cut wheels from this firm can then be recut any number of times at the wholesaler or distributor level and then again at retail or in a restaurant. Not only does this exponentially increase the number of affected units at each point, it increases the chances for cross-contamination of additional products that are cut using the same equipment (i.e. gloves, knives, and cutting boards). This cross-contamination can span geographical regions and can occur sporadically over a prolonged period of time as the contaminated products move through the supply chain.

Previous foodborne outbreaks linked to the consumption of cheese on restaurant cheeseboards [11] or in-store taste testing at retail [12] identify an additional layer of difficulty with traceback as consumers may not recall items sampled while shopping or dining as easily. The risk of cross-contamination may also increase as the product moves further down the supply chain as operations become more complex beyond cheese (i.e. delicatessens and speciality food stores). The involvement of additional products considered risk items for *L. monocytogenes* contamination (e.g. delicatessen meats) may further complicate epidemiological investigations. Moreover, the ability to control

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cross-contamination, verify suppliers, and provide valuable traceability data may concurrently decrease as the supply chain lengthens. As cheeses are recut and repackaged along the supply chain, varying formats for lot identification can become increasingly difficult for traceability especially for businesses with diverse inventory from numerous suppliers (e.g. small speciality food retailer). This is especially true when companies down the chain have less food safety-related resources or expertise.

While controlling *L. monocytogenes* in primary processing environments is paramount and has historically been the focal point, *L. monocytogenes* cross-contamination downstream cannot be overlooked from both prevention and traceback standpoints. In the 2008 outbreak of listeriosis linked to cheese at retail in Quebec, Canada, investigators isolated the outbreak strain from cheese and/or environmental samples across 22 retail stores [13]. Cross-contamination was suspected and was supported by the isolation of *L. monocytogenes* (including, but not limited to, the outbreak strain) from knives, cutting boards, and counters, among other sites within the retail environment. Nearly half of the case patients had consumed cheese made by a single manufacturer or bought cheese from retailers selling products from that manufacturer. Investigations isolated the outbreak strain from environmental sites throughout the manufacturer's facility as well as different batches of cheese. Inadequate preventive controls and sanitation at primary production served as a source of initial product contamination followed by secondary cross-contamination of countless other products at retail outlets downstream due to inadequate sanitation practices across the board. This resulted in one of the largest food recalls ever carried out in the province of Quebec. Given the unknown extent of cross-contamination, 364 retailers were also required to dispose of all cheese that had been opened on site. According to a press release the Quebec government was admittedly ill-prepared to handle this outbreak. In addition, it was noted that the provincial guide to cleaning and sanitation in food establishments for retailers failed to consider the specific risk of cross-contamination between two ready-to-eat (RTE) products [14]. The US Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Best Practices Guidance for Controlling *L. monocytogenes* in Retail Delicatessens [15] recommends cleaning and sanitizing surfaces between RTE items when using the same equipment to cut RTE products. Unfortunately, this is difficult in practice especially during busy operations when many will instead rely on a time-based cleaning and sanitation schedule. Although this certainly applies to grocery store meat slicers, it also applies to speciality cut-to-order shops selling cheese varieties that require more time, attention, and speciality knives to prepare for customers. In this case the same knife and board can be used for several similar cheeses before they are properly cleaned and sanitized.

USDA-FSIS guidance also recommends that retailers slice product at the time it is requested by consumers and not to pre-slice all at once in the morning [15]. In the aforementioned situations, one could argue that cutting individual units ahead of time with dedicated tools would better reduce the risk of cross-contamination. In fact, precutting is often recommended or required for many producers selling at farmer's markets. Cut-to-order speciality and artisan cheese is also not a common offering at many grocery stores so these products are either pre-cut and packaged units from a distributor or wholesaler or cut and repackaged at the grocery store all at once before loading a display. Although details are not provided, the isolation of the ricotta salata outbreak strain from cut and repackaged cheeses manufactured from both pasteurized and unpasteurized milk suggest inadequate segregation and preventive controls at cutting or cross-contamination at the case patient's home [8].

The key to the initial investigation in the ricotta salata case, possibly informed by the Quebec outbreak, was the hypothesis that an intact, contaminated cheese (cheese X) could have cross-contaminated other types or brands of cheese [8]. In cases where products are repackaged along the supply chain, varying formats for lot identification can increasingly complicate traceability as previously mentioned. In these cases, inventory data can be a valuable tool for identifying potential sources. In the ricotta salata case, it was commonalities in inventory data from firms in areas where patients purchased cheese and firms that distributed cheeses contaminated with the outbreak strain during the investigation period that identified a common distributor (distributor C). A review of cutting records at distributor C identified Italian-imported Frescolina Marte brand ricotta salata as the only common cheese at cutting stations used for two other non-intact pasteurized milk cheeses that yielded the outbreak strain. Frescolina Marte brand ricotta salata, also appeared on inventories at grocery chain A where the index patient purchased cut pieces of contaminated cheese other than ricotta salata including

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those made from pasteurized and unpasteurized milk. Cultures from intact wheels collected at distributor C and from a US importer yielded the outbreak strains thereby establishing Frescolina Marte brand ricotta salata imported from Italy as the hypothesized cheese X. In addition to this brand, the outbreak strain was isolated from open samples, but not intact wheels, of four other cheeses cut and repackaged at distributor C or the grocery chain A location providing evidence of cross-contamination in several geographically distinct locations and in both retail and distribution settings.

Tracing this outbreak back to an initial source in Italy also proved difficult due to added complexities of the production end of the supply chain [9]. Five different supplier plants provided semi-finished cheeses intended for export to a finishing plant (plant A) where additional special applications and packaging operations were performed. During the initial investigation following reports of the US outbreak linked to Frescolina Marte brand ricotta salata, 179 (23·6%) ricotta salata samples tested positive for *L. monocytogenes* including cheese from each of the plants supplying semi-finished cheese to plant A [9]. In total, 14 isolates from ricotta salata samples produced in plant A using semi-finished cheese supplied from plants B, C, or F matched the clinical isolates from the outbreak. In contrast, *L. monocytogenes* was only found in two (1·1%) out of 183 environmental samples collected, including one each in two of the four supplier plants (B and D) and neither matched the outbreak strain. The outbreak strain was isolated from a cheese produced in plant F but no corresponding environmental samples were available for analysis. Given the small number of samples collected at plant B, it is possible that the number of samples, or the sites selected, were insufficient to identify all contaminated site(s) including those which harboured the outbreak strain. It is also possible that the number of isolates subtyped from the two positive environmental samples was insufficient to identify all possible subtypes within each sample [16] considering the number of subtypes isolated from cheeses produced at these facilities. Given the time-frame and the inability to isolate *L. monocytogenes* from 79 environmental sites at plant A, it is also possible that the outbreak strain was transient and no longer present by the time the investigation reached the facility. In contrast to plant A, supplier plants failed to control *L. monocytogenes* contamination despite the active investigation. During a follow-up sampling 8 months after the initial sampling, both food contact and non-food contact surfaces were positive for *L. monocytogenes* in plants D (2/30, 6·7%) and E (4/29, 13·8%). Although these isolates matched others from ricotta salata samples, none were similar to the outbreak strain. Based on this evidence the authors suggest a contamination scenario in which the persistence of a strain in environmental niches within plant D possibly spread to plant A and subsequently to cheeses from other supplier plants. This was supported by the isolation of strains from plant D that matched isolates found in finished cheeses processed in plant A but produced by other suppliers.

This contamination scenario is important because the concept of an 'affineur', or finisher of cheeses produced at multiple locations, is gaining popularity in the US cheese industry. With the implementation of the Food Safety Modernization Act (FSMA) many of the larger affineurs in the United States, as well as their suppliers, are required to maintain robust food safety plans that work to mitigate the risk of cross-contamination within and between facilities [17]. Facilities accepting cheese products in from outside processors must also establish controls to reduce the risk of introducing *L. monocytogenes* into the plant including records and verification of effective environmental monitoring and control at supplier facilities. The FSMA Final Rule on Foreign Supplier Verification Programmes for Importers of Food for Humans and Animals that requires importers verify that food coming into the United States has been produced in a manner that meets applicable US safety standards is expected reduce the number of similar cases by catching issues such as those described here earlier [18].

The risk of *L. monocytogenes* cross-contamination during further processing and the challenges it presents applies to all products that are further processed through the supply chain. For example, contamination at retail may be responsible for as much as 83% of human listeriosis cases associated with consumption of RTE delicatessen meats [19]. However, since there have been no major outbreaks of listeriosis linked to cross-contamination of delicatessen meats during processing at retail grocery stores it has been suggested that cross-contamination and any resulting infections occur sporadically and not frequently enough to immediately suggest a common source.

Despite their complexity, environmental sampling throughout the supply chain together with improved genotyping approaches and related databases as well

as thorough exposure histories are needed to help to resolve these and similar cases more rapidly and with greater confidence. Prior to 1996, CDC and state public health agencies relied on epidemiological reports, food exposure data, and in some cases phenotypic information to assess the occurrence of foodborne illness clusters. Relying on this information did not give very specific information about the organism and was based on extremely tedious and slow, paper-based reporting systems. Consequently, outbreaks that occurred over a wide geographical area were very difficult to detect until large numbers of cases had already occurred. Changes in computing technology and molecular biology markedly changed in the mid-1990s to quicken the pace at which case-report data were available for analysis as well as including molecular subtyping techniques to more specifically track genetic variants with a given organism or serotype. For the past 20 years, the CDC PulseNet network of public health laboratories has used a highly standardized protocol to routinely analyse pulsed-field gel electrophoresis (PFGE) molecular fingerprinting patterns from foodborne disease surveillance to assess the occurrence of outbreak disease clusters and shown a significant impact on preventing major foodborne pathogens such as *L. monocytogenes, Salmonella*, and Shiga-toxin-producing *E. coli* (STEC) [20]. This network also includes international partners around the world (http://www.pulsenetinternational.org/). Since 2013, a new technology known as whole genome sequencing (WGS) has been implemented alongside PFGE to enhance strain discrimination such that the entire nucleotide sequence of the strain's genome is available for comparison. This begun first as a partnership between the US Food and Drug Administration (FDA), National Institutes of Health (NIH) National Center for Biotechnology Information (NCBI) and several state public health laboratories known collectively as GenomeTRAKR [21]. The programme was initially focused solely on food and environmental foodborne bacterial pathogens but quickly expanded to include CDC PulseNet and all *L. monocytogenes* began undergoing real-time WGS in late 2013. One of the many benefits of WGS over PFGE is that it will be much easier to share outbreak cluster databases worldwide with international public health partners, but interpretation of illness source associations will still rely on connections between cases and their food exposure histories.

The accuracy of WGS allows for much greater certainty when trying to solve relatively small outbreaks and has been used to link cases and products involved in outbreaks that have since passed including the most recent outbreaks linked to soft cheese [22]. The 2014 outbreak associated with soft cheeses produced by Roos Foods was the first time WGS was used to match environmental and cheese samples with the CDC's human clinical isolates. These data were then used to support the suspension of food production at the facility to minimize the outbreak. Cross-contamination concerns led to the recall of several products manufactured or repacked by Roos Foods and marketed under several brands [4]. Although some products were repackaged at the grocery store, cross-contamination at retail was not identified. Later that year WGS of isolates from cheese collected during routine inspection and from environmental samples collected during the follow-up investigation were found to be highly related to strains associated with five illnesses across four states. Although limited information was available about the specific cheese products consumed by ill persons, the WGS data, together with the cheese consumption history of the patients, suggested that cases were likely related to soft cheeses produced by Oasis Brands Inc. [7]. Without the accuracy of WGS this outbreak may have otherwise gone unrecognized. Another recent outbreak linked to soft cheeses distributed by Karoun Dairies was first identified in August 2015 after investigators noted an increase in a rare PFGE fingerprint reported to PulseNet. WGS identified four other PFGE fingerprints that were closely related genetically to the first including isolates collected more than 5 years ago. Illnesses associated with those PFGE fingerprints were added to the investigation and epidemiological information was then able to identify soft cheese as well as a particular brand. WGS also showed that two environmental samples collected at a manufacturer, as well as five additional *L. monocytogenes* isolates collected in 2010 from the same facility, were closely related genetically to isolates from ill people [6].

The ability to more accurately identify small clusters of illnesses and match clinical strains with those collected from food and environmental samples will connect illnesses to foods and food production environments that may not have otherwise been suspected. This is especially important as food products are increasingly shipped around the world and the tools for tracking foodborne illness are at the same time becoming available to international public health partners for analysis on a global scale. As demonstrated

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by these recent investigations discussed, everyone involved from primary production through point of sale will need to be even more vigilant in the monitoring and control of *L. monocytogenes* and emphasize practices to reduce cross-contamination.

DECLARATION OF INTEREST

None.

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