Lessons to be Learned from an Outbreak of Foodborne Listeriosis, Austria 2009–2010

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ABSTRACT

From June 2009 until February 2010, an outbreak of invasive listeriosis affected 25 persons in Austria, 8 in Germany and 1 in the Czech Republic. The source of this outbreak with 8 fatalities was initially identified based solely on epidemiological findings. Grocery receipts of purchases from 7 patients were collected prospectively during 3 weeks after discharge from hospital and compared for matches. This generated a hypothesis tested by a case control study, which revealed Austrian ‘Quargel’ cheese as the source of infection. At least two patients had consumed the contaminated product after it was withdrawn from the market. Risk communication with the public is of utmost importance, especially if hard-to-reach elderly are involved. Of 63 food samples of the product analyzed officially, 20 were positive for L. monocytogenes and 10 samples harbored ≥100 CFU/g (maximum 92,000 CFU/g). A leftover specimen, stored in a patient’s refrigerator, yielded 2,100,000 CFU/g of L. monocytogenes indistinguishable from the patient’s isolate. L. monocytogenes was first documented in the plant in a sample of smear fluid in May 2009, which temporarily coincided with major construction work. European legislation requires ‘Quargel’ not to exceed 100 CFU/g when products are placed on the market during their shelf life. A routine sample from a market had already shown 400 CFU/g in July 2009. We feel that a general zero tolerance policy would not have provided additional safety in this outbreak situation.

INTRODUCTION

Listeriosis is a rare but serious infection caused by Listeria monocytogenes. This organism can be found throughout the environment, in soil, vegetation, and animals. The main route of transmission is believed to be through consumption of contaminated food (1, 22, 29). However, infection can also, albeit very rarely, be transmitted directly from infected animals to humans (1). In neonatal infections, L. monocytogenes can be transmitted from mother to child in utero or during passage through the infected birth canal. There are also rare reports of nosocomial transmission attributed to contaminated material or patient-to-patient transmission via healthcare workers (1). The bacterium is particularly successful in causing foodborne disease, because it survives food processing technologies that rely on acidic conditions or high salt concentrations and, unlike many pathogens, can continue to multiply slowly at low temperatures, allowing growth to occur even in properly refrigerated foods (1).

In recent years, an increasing rate of listeriosis has been reported in several
European countries (6, 9, 13, 14, 17, 19, 20, 28). These increases primarily reflect a higher rate of bacteremic listeriosis in those > 65 years of age and are not otherwise correlated with geography, gender, ethnicity, socioeconomic factors or infectious serotypes (9, 17). At present, the available pathogenesis and subtyping data generally fail to provide adequate insight about the virulence of field isolates and the likelihood that a given strain will cause illness (18). In Austria, the introduction of routine subtyping of human \( L.\) monocytogenes isolates in 2008 has shown that up to 25% of clinical isolates belong to clusters, indicating the possibility of foodborne outbreaks. The source of infection of outbreaks of listeriosis often remains undetected (10, 30).

On 14 August 2009, the Austrian Reference Centre for Listeria in Vienna (Austria) noticed a cluster of human isolates of \( L.\) monocytogenes serotype 1/2a with a new pulsed-field gel electrophoresis (PFGE) pattern (clone 1) (Fig. 1). Subtyping was performed according to the “Standardized PulseNet Protocol for Molecular Subtyping of \( L.\) monocytogenes by pulsed-field gel electrophoresis (PFGE)” (15). Finally, fourteen cases (12 Austrian and two German) with this new clone 1, including five with fatal outcome (two of them German), were identified, with onset of disease ranging from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of this incident affecting Austria, Germany and the Czech Republic and discuss the lessons to be learned from this outbreak of foodborne listeriosis.

**OUTBREAK**

In August 2009, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna (Austria) noticed a cluster of human isolates of \( L.\) monocytogenes serotype 1/2a with a new pulsed-field gel electrophoresis (PFGE) pattern (clone 1) (Fig. 1). Subtyping was performed according to the “Standardized PulseNet Protocol for Molecular Subtyping of \( L.\) monocytogenes by pulsed-field gel electrophoresis (PFGE)” (15). Finally, fourteen cases (12 Austrian and two German) with this new clone 1, including five with fatal outcome (two of them German), were identified, with onset of disease ranging from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of infection. Approximately 16 tons of 'Quargel' were produced per week by the manufacturer. Fifty-three percent of the product was exported to the German market and small amounts to the Czech Republic and Slovakia. This cheese is made of curdled milk, which ripens for one day at 28°C after addition of starter cultures and for another two days at 14°C after being sprayed with \( Brevibacterium\) linens. The shelf life after packing and marketing is two months. The product was voluntarily withdrawn from the Austrian, German, Slovakian and Czech markets on 23 January 2010. An environmental \( L.\) monocytogenes 1/2a isolate from the production plant, from a gully (drainage pit) sample collected in December 2009, became available on 19 January 2010 and proved indistinguishable from the outbreak strain by genotyping. Microbiological investigations also confirmed the presence of this new strain (clone 1) in 'Quargel' samples taken at the factory in 2010: Two of 64 isolates available for testing (44 isolates cultured from cheese produced in 2010 and provided by the manufacturer, 20 isolates cultured from samples officially gained during outbreak investigation) showed the new PFGE pattern associated with the outbreak.

The 62 remaining food isolates showed a different PFGE pattern that had not been seen previously in Austrian isolates either (clone 2) (Fig. 1). It was indistinguishable from the pattern of a human isolate from a listeriosis patient who was hospitalized at the time and who claimed to have eaten 'Quargel' cheese. Only two of 46 human \( L.\) monocytogenes isolates documented at the Austrian Reference Centre in 2009 yielded this PFGE-pattern, both coming from patients with a food history positive for 'Quargel'. Ultimately, this second outbreak clone of \( L.\) monocytogenes serotype 1/2a accounted for 13 Austrian cases (two with fatal outcome), six German cases (one death), and one Czech case; onset of disease ranged from December 2009 until the end of February 2010. The epidemic curve shows all cases associated with the two different outbreak clones by onset of illness (Fig. 2).

In total, the outbreak involved 34 cases of invasive listeriosis, 25 of which originated from seven of nine Austrian provinces. Four of these patients presented with meningitis, two with clone 1 and two with clone 2. A further eight patients were from four of 16 German federal states, and one patient was from the Czech Republic. Eight of the 34 cases in this outbreak had a fatal outcome. The median age of the
cases was 72 years (range: 57–89 years), and 26 patients were male. There were no materno-neonatal cases. Underlying diseases were not different from those generally described for patients with listeriosis (1). In Austria, all but one case can be explained by consumption of the contaminated product before it was withdrawn from the market on 23 January; one patient who was hospitalized for meningitis on 26 February 2010 had eaten the cheese (purchased before withdrawal from the market) on February 13. A leftover specimen, stored in the patient’s refrigerator and sampled on 3 March, yielded 2,100,000 CFU/g of *L. monocytogenes*. Despite high media coverage, the patient and his wife were not aware of the product recall. In Germany, at least one person with fatal outcome ate the incriminated cheese after the product was withdrawn from the market (5).

A total of 63 food samples of the ‘Quargel’ cheese products were microbiologically analyzed. Twenty samples were found positive for *L. monocytogenes*. Ten of the 20 samples yielded less than 100 CFU/g, and ten samples harbored more than 100 CFU/g: 160 CFU/g, 170 CFU/g, 240 CFU/g, 1,500 CFU/g, 4,500 CFU/g, 6,050 CFU/g, 18,000 CFU/g, 18,000 CFU/g, 30,000 CFU/g, and 92,000 CFU/g, respectively.

The source of this outbreak was initially identified based solely on epidemiological findings. We collected grocery receipts of purchases made by seven patients in December 2009, after their discharge from hospital, and compared them for matches. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed profiles other than that of the outbreak clone 1. Patients were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009. The overall response rate was 83.3% in the case group (ten of 12 possible cases at the time) and 72.2% in the control group (24 of 33 possible controls at the time, i.e., listeriosis patients with isolates that showed profiles other than that of the outbreak clone 1). Consumption of the ‘Quargel’ cheese was identified as the only significant risk factor highly associated with the illness in question. Nine of the ten patients with clone 1 had consumed the product; the tenth provided no answer concerning this food item. Of 22 controls (none with clone 2), all but two denied having eaten this specific cheese; the remaining two provided no answer concerning this food item. The computed odds ratio was 76.6 (95% confidence interval (CI): 9.3–infinity; *P* value < 0.001).

**DISCUSSION**

Listeriosis is a foodborne illness of major public health concern because of the severity of its consequences (infections of the central nervous system, septicemia, and abortion), the high case-fatality ratio (20–23% of cases), and the long incubation time (1). Although exposure to *L. monocytogenes* is common, listeriosis is rare, and any outbreak should therefore be used to gain new knowledge on prevention of this disease. Despite the existence of technical literature on methods for outbreak investigations, the situation-specific nature of outbreaks makes it impossible for a gold standard to exist for investigations of clusters of foodborne illness (16). There are no pre-specified formulae to dictate the path that an outbreak investigation is supposed to take (16). Nevertheless, in the absence of controlled human experiments, these outbreaks provide a unique opportunity to gain new scientific knowledge on foodborne illness.

The outbreak described shows impressively that the waning of an outbreak (i.e., disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. The shift to a different outbreak clone in December 2009/January 2010 was probably caused by the change to a new commercial yeast-ripening culture (ACL2; Cargill, Minneapolis, MN) used in the cheese factory in late November 2009.

**FIGURE 2.** Outbreak cases of listeriosis by onset of illness and final outcome, Austria, Germany and the Czech Republic, 2009–2010 (n = 34)
because of a shortage of the original culture FAA1 (Cargill).

In this outbreak, the producer and the local food inspection authorities were not aware of the legal European requirement for zero tolerance in ‘Quargel’ when the plant facility was inspected. Therefore, failure of the producer’s HACCP system and possibly inadequate training of local food inspectors have to be blamed for this foodborne outbreak that finally cost eight lives. Laboratory documents provided to the justice department by an employee of the cheese production plant (who reported himself in February 2010) revealed that microbiological tests on ‘Quargel’ delivered to Germany had already shown presence of 400 CFU/g in July 2009.

In Austria, the role of environmental testing in a possible HACCP regulatory framework and its potential to lower public health risk is still a matter of debate. Several sources in the literature question the role of testing as an HACCP verification tool, comparing it to “looking for the proverbial needle in the haystack” (8). Others contend that environmental testing is essential to ensure that the processing facility is not causing incidental contamination that could easily go undetected in finished product tests (26). In the described outbreak, an L. monocytogenes isolate from an environmental sample was the first available strain confirming the epidemiological finding of the outbreak source. This could be seen as an argument in favor of mandatory environmental Listeria spp. testing in production of soft cheeses like ‘Quargel’.

The investigation was not able to elucidate decisively when and how L. monocytogenes entered the plant. Soil on workers’ shoes and clothing, contaminated raw material and human carriers are among a multitude of possibilities (27). Schoder et al. (23) hypothesize that L. monocytogenes was introduced into the plant during major construction work. From 23 February 2009 until 27 May 2009, a ripening room in direct proximity to the central production facility was remodeled, which temporarily coincides with the first documented detection of L. monocytogenes in a sample of smear fluid on 12 May 2009, allegedly after years of no microbiological proof of L. monocytogenes. Sample testing from the recall campaign (performed on behalf of the incriminated company after January 27, 2010) revealed that all 16 batches of the smear-ripened cheese tested were positive for L. monocytogenes, a finding that is compatible with ripening smear as the causative vehicle of spread inside the facility (23). “The lots were tested after delivery [to the laboratory], at the end of shelf life (that is, up to 50 days post production), and to mimic a worst case scenario, at time points exceeding the shelf life unless the sensorial properties remain unchanged. Each lot was positive qualitatively at each time point and, with exceeding times of storage, L. monocytogenes multiplied dramatically. The highest value found was 2.8 × 10^9 colony forming units (CFU) per gram of cheese” (23). Investigations after closure of production revealed the outbreak clone 2 even in ground beetles (Family Carabidae) collected from insect traps in the production facility (24). Carry over of the bacteria via beetles is just one of many possible ways the Listeria could have been introduced during major construction work. In May 2010, the producer decided to close down the incriminated cheese production facility permanently.

This outbreak underlines the considerable potential of molecular subtyping of human L. monocytogenes isolates as a tool to recognize clusters and also emphasizes the immense importance of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Further improvements in pathogen identification techniques, especially in inter-laboratory exchange of typing data, may increase the chances that foodborne illnesses will be linked to specific food products and firms in the near future (29).

Industrial food production combined with international marketing of food, the low proportion of a population affected after consuming food contaminated by L. monocytogenes, and the fact that the food evidence has usually already been eaten generally hinder epidemiological outbreak investigation with traditional concepts (7). Furthermore, the long incubation period of up to 70 days makes it difficult to generate a hypothesis on the food source of a listeriosis outbreak. To our knowledge, this was the first outbreak in which grocery bills were collected prospectively — after patients’ discharge from hospital — and successfully used to reveal the food consumption patterns of listeriosis patients, indicating sour milk curd cheese ‘Quargel’ as a possible source for this outbreak, to be tested by analytical epidemiology.

Spriggs and Isaac (25) identified outbreaks of foodborne illness as the major force for change in regulations and markets. In this outbreak situation, the time span between epidemiological elucidation of the outbreak source (January 15, 2010) and the “voluntary” product recall (January 23, 2010) was criticized as inappropriately long. At this time, Austrian law required microbiological proof before any public health action could be taken on an unsafe food product. As a direct consequence of this outbreak, on 21 April 2010, Austria amended its Food Safety and Consumer Protection Act, allowing health authorities in future to order market withdrawals and to recall products even without microbiological proof of their being unsafe.

Recalling the food and advising people not to eat the contaminated product controlled this outbreak. At least two cases were due to consumption of the contaminated product after it was withdrawn from the market. Risk communication with the public is of utmost importance, especially if the outbreak involves hard-to-reach elderly, a risk group that is known to be reluctant to dispose of food even after it has exceeded the “best before” date. The case of the Austrian patient with meningitis who still had a leftover specimen of the causative food in his refrigerator also underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. Some sporadic cases may be unrecognized common-source outbreaks (21). A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness.

The United States has a zero tolerance policy for L. monocytogenes in ready-to-eat foods, but in Europe, there is some tolerance for it in certain foods (21). COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005
REFERENCES


