An outbreak of an unusual strain of \textit{Listeria monocytogenes} infection in North-East Scotland

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Summary \textit{Listeria monocytogenes} infection is an important cause of illness and hospitalization in vulnerable individuals. In the present study, we describe a community outbreak of \textit{Listeria monocytogenes} in the North-East region of Scotland, which was epidemiologically, environmentally and microbiologically linked to a local meat product and ready-to-eat product manufacturer. Infected individuals were interviewed, and an environmental investigation was conducted. Clinical and environmental samples were tested by culture, and isolates were typed by fluorescent amplified fragment length polymorphism (fAFLP). Three cases of \textit{Listeria monocytogenes} were linked geographically, had the same serotype (1/2a) and were indistinguishable by fAFLP type XII.6. The human, food and environmental isolates were of the same serotype and were indistinguishable by molecular typing.

This is the first community outbreak of \textit{L. monocytogenes} reported in Scotland since the current outbreak surveillance was established in 1996. Epidemiological and laboratory evidence indicated poor hand hygiene, unhygienic practices and cross-contamination throughout the manufacturing process of ready-to-eat foods as a
Introduction

Listeriosis is a bacterial infection caused by *Listeria monocytogenes*. The bacteria is ubiquitous in the environment and can contaminate a variety of foods. The risk of infection is higher in elderly individuals, pregnant women, neonates, and immunocompromised individuals. In these individuals, infection may lead to septicemia, meningitis, meningoencephalitis and fetal loss in pregnant women [1–7].

Human listeriosis is a problem in many European Union (EU) countries including the UK and Scotland [8]. Although listeriosis is rare, the average annual incidence of reported cases in several European countries is increasing [8]. In 2012, the reported average rate of listeriosis in the EU was 0.41/100,000 compared to 0.32 per 100,000 in 2011. This increase is thought to be due to an increase in the population of those individuals over the age of 60 years old, or perhaps an increase in the population of vulnerable individuals including people under the age of 60 years with predisposing illnesses. The rate in the UK during the same period was 0.30 and 0.26 per 100,000 [9,10].

*L. monocytogenes* is transmitted primarily through the consumption of contaminated food, such as meat, vegetables, ready-to-eat meals, unpasteurized milk and cheese, and its incubation period (IP) ranges from 1 to 70 days with a median of 8 days in invasive disease [11,12].

Current EU food safety regulations require that *L. monocytogenes* must not be present in levels exceeding 100 cfu/g during the shelf life of ready-to-eat (RTE) products and that levels may not be present in 25 g of RTE food that are able to support the growth of the organism at the time of departure from the production plant [13].

A number of outbreaks of listeriosis associated with contaminated hospital food, mainly sandwiches [14–18], and foodborne community outbreaks associated with ready-to-eat meats and poultry, unpasteurized soft cheese, milk and delicatessen have been reported [19].

Between July and November 2013, NHS Grampian’s Health Protection Team in the northeast region of Scotland was notified of three seemingly sporadic cases of listeriosis: two adults admitted to the hospital and a neonate delivered prematurely. This report describes the investigation carried out to determine the source of these three infections and control measures that were instituted to reduce the risk to the general public.

Methods

Description of cases

Case 1

In late July 2013, a 57-year-old female with a past medical history of type II diabetes and hypertension presented to the hospital with complaints of progressive worsening nausea and diffuse headaches for 2 months, and 1 day of fever, mild diarrhea, vomiting, neck stiffness and body aches. Her vital signs were normal except for pyrexia (38.6°C). Systemic examination was unremarkable. Cultures of blood samples drawn at admission revealed growth of *L. monocytogenes*, and a blood stain examination revealed gram-positive bacilli. A lumbar puncture was performed to rule out meningitis, and examination of the patient’s cerebrospinal fluid (CSF) was normal. The patient was treated with intravenous amoxicillin and gentamicin for 2 weeks. She recovered and was discharged from the hospital and remained in good clinical condition.

Case 2

An 86-year-old male on long-term steroids was admitted to the hospital in late October 2013 with complaints of fever, vomiting, diarrhea and poor fluid intake. His symptoms were reported to have begun 5 days prior to admission. Upon admission, the patient’s physical examination was notable only for pyrexia of 40°C. Gram-positive bacilli were identified by blood Gram staining, and a blood culture grew *L. monocytogenes*. Stool culture was negative. The patient was treated intravenously (IV) with antibiotics (amoxicillin and gentamicin)
and fluids. He fully recovered following treatment and was discharged.

Case 3
A baby that was delivered prematurely at 35 weeks of gestational age in the hospital in mid-November 2013 had a small intraventricular hemorrhage (IVH) Grade 1 and respiratory distress syndrome (RDS). *L. monocytogenes* was isolated from a culture of blood samples taken at birth. The baby’s mother was asymptomatic prior to the onset of labor and denied any history of poor health during pregnancy, with the exception of morning sickness and a backache that persisted throughout the pregnancy. Cultures of blood samples taken from the mother were negative for *L. monocytogenes*. The baby was treated with amoxicillin and gentamicin by IV, fully recovered and was discharged.

Epidemiological and environmental investigation
In accordance with guidelines for the management of public health incidents in Scotland [20], an incident management team (IMT) meeting was convened by the Consultant in Public Health Medicine on November 15, 2013. A detailed review of the questionnaires containing the details of the food histories for the preceding 30 days regarding potential exposure for cases 1 and 2 and the mother of case 3 was requested by the IMT and was undertaken by the Health Protection Team. This review identified a geographic link and two food premises (Food Businesses A and B) that were possibly linked to all three cases. The possibility that this was an outbreak was considered following the notification of the second case of listeriosis and the finding that both cases lived in the same geographical area. In addition, the occurrence of the three cases within a period of 4 months was unusual, with only one case on average typically reported annually in Grampian. The infections were thought to be community acquired rather than hospital acquired because all three cases were diagnosed within 48h of admission and they had no previous hospital admission in the 30 days prior to the current admission. In light of this information, further environmental investigation of the identified food premises and retail shops where the patients had bought prepared foods, including ready-made meals, meat products, sandwiches and cooked meats, was undertaken.

Environmental Health Officers (EHOs) visited the food premises that were linked to the first two cases to inspect the premises, examine records of previous inspections, review food processes, investigate food hygiene and environmental cleaning routines and staff hygiene and cleanliness, and take further environmental and food samples. Control measures were simultaneously implemented to prevent new cases while the investigation to determine the source of contamination and infection was ongoing.

Laboratory investigation
Clinical bacterial isolates of *L. monocytogenes* obtained from preliminary blood cultures of all three patients were sent to the Public Health England (PHE) Foodborne Pathogens Reference Services in London for serotyping. Molecular typing was performed by FAFLP using the methodology described by Roussel and colleagues [21]. Environmental and food samples collected during the investigation of the outbreak were sent to the local food scientific laboratory in Aberdeen, Scotland, for culture. *L. monocytogenes* was detected and enumerated in environmental and food samples according to ISO 11290-1:1996 [22] and ISO 11290-2:1998 [23], respectively. The isolates of *L. monocytogenes* obtained from food and environmental samples were then sent to the National Foodborne Pathogen Reference Laboratory for serotyping and molecular typing.

Results
Epidemiological and environmental results
All three of the cases lived in the same geographical area. Further review of the food histories of all three cases during the 30 days preceding admission indicated that they ate a large amount of ready-to-eat (RTE) foods that are commonly associated with *Listeria monocytogenes*, including ready-made meals, cold meats, and steak pies. These food products were purchased from a variety of local food businesses and retailers in the same geographical area. During the incubation period, the first two patients bought ready-to-eat pre-packed food (not the same food product) originating from the same local food producer (Food Business A). There was no reliable link between the mother of the third case and Food Business A. However, the investigation revealed that all three cases had consumed RTE foods that had been prepared in a local food production premises (Food Business B), although these had been purchased from different food retail outlets.
All of the businesses supplying food to local retail food outlets and shops identified by the three cases were further examined to establish if there were any commonalities. Following this investigation, a number of high risk food items supplied by Food Businesses A and B to local food retailers and other food establishments were identified. Large supermarket chains were not investigated because there was no history of purchase of any high risk food from these supermarkets by any of the cases. EHOs inspected over 17 local retail food businesses.

When possible, environmental swabbing and food sampling were undertaken at the identified food businesses that had supplied food to the retail shops where food had been purchased by the three patients.

For all three cases, there were no significant travel histories or contact with animals within the incubation period.

**Food Business A**

Environmental health records indicated that during a previous routine sampling in 2012, *L. monocytogenes* had been isolated from a sample of vacuum-packed cooked meat supplied by Food Business A to a local retailer.

During a subsequent inspection of Food Business A, no significant contraventions of food laws were found; however, best practice advice was given with respect to hygienic practices and control of cross contamination, which had been put into practice prior to the cluster of cases in 2013.

In December 2013, *L. monocytogenes* was isolated from samples of ham salad and cooked gammon. However, all environmental surface swabs were found to be negative for *L. monocytogenes*.

**Food Business B**

Food Business B supplied over 80 retail and catering businesses within the region. The food included ready-to-eat pre-packed meals, meat products, sandwiches, soups and desserts. Previous records indicated that during routine inspection of the business in July 2013, *L. monocytogenes* \( (4.7 \times 10^3 \text{ CFU/g}) \) serotype 4 was isolated from a chicken and ham sandwich produced by this food business. In December 2013, as part of the outbreak investigation, Food Business B was inspected and food sampling and environmental swabbing were conducted. Sandwiches and salads were made in an enclosed area with a barrier called the 'high care area' with dedicated staff. However, once the sandwiches and salads were made, this area was then used for bakery goods and puddings, thus increasing the likelihood of cross-contamination. The salads and sandwiches contained various salad foods and condiments. Chilled cooked ham and frozen cooked and diced chicken were bought from a national supplier. A meat slicing machine was identified during the inspection, which was used only to cut cooked ham used in the sandwiches. Although it was recently purchased, it was second hand. The owner of the business informed the EHOs that the machine was cleaned daily, but food debris was visible when it was inspected.

**Microbiological analysis and strain typing**

The human isolates were identified as serogroup 1/2a and were of the same molecular fAFLP type: XII.6. The isolates were indistinguishable by molecular typing, thus supporting a common source.

Two subtypes (serotype 4 fAFLP 133a, serotype 1/2 a fAFLP XII.6) of *L. monocytogenes* were isolated from the sandwiches and from the food production environment of Food Business B during the outbreak.

*L. monocytogenes* serotype 4 fAFLP 133a was isolated from a ham salad \(<10 \text{ CFU/g detected in } 25 \text{ g}\) produced by Food Business A.

*L. monocytogenes* serotype 1/2a of the same molecular fAFLP type XII.6 was isolated from samples taken from a slicing machine cutting blade at Food Business B and from a chicken salad sandwich \((80 \text{ CFU/g detected in } 25 \text{ g})\) produced by Food Business B that was supplied to a retail business.

The reference laboratory reported that the XII.6 fAFLP type obtained from the human cases and food and environmental samples taken from Food Business B was unique to this outbreak and had previously not been isolated from any human isolates in the UK. However, this type had been isolated from an environmental sample obtained in the West Midlands (in 2011), thus supporting the hypothesis that the mostly likely common source of listeria was 'Food Business B'.

**Control measures and interventions**

During the outbreak investigation, environmental health officers visited several business linked to the cases to inspect the operations and to offer advice. Advice was given on food hygiene, environment cleaning, and separation of preparation areas for raw food and ready-to-eat meals. Additionally, staff training was conducted, including instruction on hand hygiene and the use of appropriate personal protective equipment (PPE).

Specifically, Food Business A received comprehensive advice from environmental health officers
on deep cleaning of the preparation area for ready-to-eat foods, and further staff training was undertaken.

The meat slicer in Food Business B was dismantled and disinfected. The owner voluntarily discontinued producing food containing cooked ham, thus removing the need for the cutting machine. The owner of Food Business B was advised by environmental health officers to conduct a deep cleaning of the premises, incorporating a specific disinfectant to kill Listeria. The health officers also advised the owner to change the layout of the factory to reduce the possibility of cross contamination of pre-packed ready-to-eat meals by separating the 'high care area,' where sandwiches are prepared, from other parts of the factory and using dedicated staff in this area.

Both premises were regularly inspected, first monthly and then every 6 months, and environmental samples were obtained when appropriate.

Food Business B moved to a new production premises in 2014 with consultation with the Environmental Health Service with respect to the layout and construction design of the facilities and equipment provisions. Prior to commencing production operations, extensive environmental surface swabbing was undertaken, and all results proved to be negative for Listeria species.

In May 2014, approximately 5 months after notification of the last case, the outbreak was officially declared to be over by the IMT, as no further cases linked to this outbreak had been reported to the health protection team.

Discussion

In the present study, we describe the first community-based foodborne outbreak of L. monocytogenes in North East Scotland. Our investigation demonstrated that although the three cases were deemed sporadic cases over a 4-month period, they were indeed geographically linked, had the same serotype and were indistinguishable by molecular typing. This AFLP type is rare and has only been isolated in an environmental sample in the UK. All three cases were also linked to a common community food source (Food Business B) but not a common food item.

The incubation period for listeriosis can range from a few days to several weeks, which makes it difficult to differentiate hospital-acquired from community-acquired listeriosis. However, the cases in our outbreak were admitted to the hospital with symptoms that were consistent with infection caused by L. monocytogenes. Blood samples taken within 24 h of hospital admission were positive for L. monocytogenes, indicating that this was community-acquired listeriosis and therefore a community-based outbreak.

As expected, all of the cases in this outbreak had predisposing conditions. This is similar to other reported outbreaks and cases of listeriosis [7]. Two were adults with underlying medical conditions, and the third was a premature baby. The adults developed listeriosis following an episode of febrile gastroenteritis, suggesting that the bacteremia was secondary to the ingestion of a contaminated food product. The third case was a premature baby diagnosed within 48 h of delivery, suggesting that this infection was acquired in utero by transplacental transmission. Although the mother of the baby denied any history of illness, it is possible that maternal listeriosis during pregnancy may have been the cause of the premature delivery [6]. The above findings, although expected, make a compelling case for the need to educate the general public and high risk groups in particular about the risk of listeriosis. Healthcare providers including geriatricians and obstetricians could play a significant role in educating particularly vulnerable patients about these risks.

The three cases in this outbreak were reported over a long time period. Given the long incubation period, this is not surprising and highlights the possibility of such outbreaks going unnoticed and unreported. Nevertheless, it makes a compelling case for the need to thoroughly investigate every sporadic case of listeria, particularly in relation to identifying the possible source of the contamination to reduce ongoing risk to the public. It is also important for local health protection units to have a working knowledge of their geographical area.

Community-based outbreaks of listeriosis have been linked to contamination of food items during production, but it is quite difficult to identify a source or establish links to specific retail establishments [19]. Our investigation showed that the most likely vehicles of transmission of L. monocytogenes in Food Business B were the slicing machine blade, cross contamination due to poor food hygiene practices and inadequate cleaning of the food processing environment. Slicing machines and sliced meats have been previously documented as potential sources and sites for L. monocytogenes [24].

Inadequate cleaning of food preparation surfaces and food processing equipment is a recognized problem for food industries, mainly because of the ability of L. monocytogenes to form biofilms that reduce the efficiency of cleaning and disinfection...
procedures [25]. In our study, a large effort was put forth to identify all retail outlets that were supplied by the implicated food business. Appropriate cleaning advice was then provided to all of the identified outlets to reduce any ongoing risk of infection with *L. monocytogenes*.

Our investigation has one important limitation. Information and recall bias is possible given the long exposure period and the 30-day food history. The three cases were linked to a local food business that produced and supplied RTE food; however, a common food link was not established.

**Conclusion**

This is the first community-based foodborne outbreak in Scotland since the current outbreak surveillance methods were established in 1996. In our view, the investigation of the outbreak demonstrates a number of good practices, including the comprehensive review of food histories of all three cases, prompt serotyping and molecular analysis of both human and environmental isolates of listeria by the reference laboratory, and a close working relationship with Environmental Health colleagues. We recommend a tightening of food safety regulations in relation to RTE food products and provision of information and advice about listeria, specifically to high-risk and vulnerable groups.

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**Competing interests**

None declared.

**Ethical approval**

Not required.

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