



An outbreak of human listeriosis associated with frozen sweet corn consumption: Investigations in the UK

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ABSTRACT

The use of Whole genome sequencing (WGS) identified a multi-country outbreak of human listeriosis associated with consumption of frozen sweet corn produced in Hungary. The purpose of this report was to summarise information on the cases occurring in the UK which were part of this outbreak and outline investigations on the presence of *Listeria monocytogenes* in the affected food chain. Prior to the international recall of this product in 2018, 12 UK cases of listeriosis were identified as infected by the outbreak strain between 2015 and 18. Epidemiological and microbiological investigations confirmed these cases as belonging to the outbreak. A further case occurred in 2019 and a contaminated frozen pack from one of the implicated batches of sweet corn was recovered from the patient's domestic freezer. The outbreak strain was also detected in products from a sandwich manufacturer in 2018 which added frozen sweet corn directly to sandwich fillings. The sandwich manufacturer's sweet corn was supplied by a distributor in England which obtained frozen products from the Hungarian manufacturer implicated in the outbreak. Within the distributor's premises, 208 food and environmental samples were taken: *L. monocytogenes* was detected in 44% of 70 samples of frozen sweet corn and 5% of 79 other foods. The outbreak strain was detected in the frozen sweet corn, in one other frozen food (mixed vegetables) and in the factory environment. The outbreak strain was also recovered from frozen beans on retail sale in the first four months of 2019. Five other *L. monocytogenes* strains together with two other *Listeria* species were detected in samples from the importer's premises. One of the *L. monocytogenes* strains in the importer's factory, which was distinct from the outbreak strain, was also recovered from sweet corn collected from the sandwich manufacturer, sweet corn tested in England in 2013 and 2016 and the blood of two cases of human listeriosis which occurred in England in 2014. This report shows how analysis by WGS provides evidence to understand complex food chains. This report also highlights risks for transmission of human listeriosis from frozen sweet corn and the potential for misuse of this food as a ready-to-eat product.

1. Introduction

Listeriosis is predominantly a foodborne illness caused by the bacterium *Listeria monocytogenes*. The disease causes a severe systemic infection most often affecting those over 60 years of age, the immunocompromised as well as pregnant women with her unborn or new born infant (Farber and Peterkin, 1991). Listeriosis is the most severe

foodborne infection reported in the European Union in terms of death and hospitalisation (EFSA and ECDC, 2018). Contamination of foods associated with listeriosis can be at primary production, or more frequently, from food production environments where the bacterium colonises harbourage sites for years and even decades (Ferreira et al., 2014). Following consumption of contaminated food, cases occur both sporadically or as part of outbreaks (McLauchlin et al., 2020a). The

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disease has a low attack rate and a variable (1 to 90 day) incubation period (Goulet et al., 2013; Linnan et al., 1988). Because of national and international food distribution chains, cases in outbreaks related by common food exposures can be both temporally and geographically widely distributed and specific foods are, at best, only identified in 10% of the cases (McLauchlin et al., 2020a).

The annual totals of human listeriosis reported in the UK varied between 192 and 160 cases per year between 2013 and 2017 (0.24–0.3 cases per 100,000 population; EFSA and ECDC, 2018). Public Health England (PHE) coordinates surveillance of listeriosis in England and Wales: case ascertainment is by the mandatory reporting under the Health Protection (Notification) Regulations 2010 of laboratory-diagnosed cases from clinical microbiology laboratories via an electronic system. Brief clinical details of each case are collected by hospital staff using a structured questionnaire (Public Health England, 2018a). Food preference questionnaires are administered to patients (or an immediate family member if the patient cannot complete the questionnaire because of age, morbidity or mortality) about food exposures up to 3 weeks prior to onset of illness (Public Health England, 2018b). Voluntary submission of *L. monocytogenes* cultures from clinical cases are made to the PHE national reference laboratory (Gastrointestinal Bacteria Reference Unit, GBRU). Epidemiological and microbiological data are combined, de-duplicated, and stored in a bespoke electronic database. PHE also performs testing of food, water and environmental samples in England for the presence of *Listeria* through the Food Water and Environmental Microbiology Service (FW&E).

In December 2015, whole genome sequencing (WGS) was introduced by PHE as a routine service for characterisation of all referred isolates of *L. monocytogenes* for public health purposes in England and Wales with selected isolates analysed prior to this time. Data originating from epidemiological, clinical, and food/environmental investigations are combined with the results from WGS to enable detection of associations and elucidate sources of contamination.

In October 2017, routine surveillance identified a cluster of four listeriosis cases infected by the same *L. monocytogenes* strain which occurred between 2015 and 2017 in England and Scotland. In November 2017, public health colleagues in Finland released an alert of two clusters of listeriosis cases together with associated isolate sequences. The sequences of isolates from one of the outbreaks were shown to be indistinguishable to the four UK cases previously mentioned. These observations contributed to detection of an outbreak of listeriosis occurring from at least 2015 to 2018 which involved 53 cases and ten deaths across six European countries (EFSA, 2018b,c, 2020). The source of the outbreak was traced to consumption of frozen sweet corn that was contaminated at the point of production in Hungary. The use of WGS identified the outbreak strain in multiple frozen food samples and surface swabs from a single plant in Hungary where vegetables were processed and frozen. The food chain associated with this producer was complex, and there was an international recall of products from this plant in the summer of 2018 (EFSA, 2018b). In the UK in July 2018, the Food Standards Agency and Public Health England (PHE) issued precautionary advice on cooking frozen vegetables before eating (FSA, 2018a; PHE, 2018c). Furthermore, the company distributing products from the Hungarian factory in the UK carried out precautionary recalls of frozen vegetables in July 2018 (FSA, 2018b).

The purpose of this report was to summarise information on the cases of listeriosis occurring in the UK which were identified as associated with this outbreak, outline investigations on the presence of *L. monocytogenes* in the affected food chain and discuss controls.

2. Materials and methods

2.1. Case definition

A case of listeriosis was defined as a person with an illness clinically compatible and a diagnosis of listeriosis by the isolation of

L. monocytogenes, usually from a normally sterile anatomical site.

A confirmed outbreak case was previously defined (EFSA, 2018b) and the outbreak strain was a *L. monocytogenes* serogroup 4, clonal complex (CC) 6 and sequence type (ST) 6. Sequence data was compared to isolates from cases outside the UK and involved with the outbreak of listeriosis (EFSA, 2018b,c, 2020) and shown to be a monophyletic group.

2.2. Food and environmental sample collection

Food and environmental surface samples were collected by sampling officers, usually of at least 100 g of food or sponge swab (3M Health Care, St Paul, USA) and transported in accordance with the Food Standards Agency Food Law Code of Practice (Food Standards Agency, 2017). Environmental swabs were taken from defined areas to be representative of specific factory sites (e.g. hoppers, wheels, sinks, food contact surfaces) and therefore were of variable surface areas. Microbiological examination was performed in PHE Official Control Laboratories in England (located in either London, Porton or York). A 10⁻¹ homogenate of each food sample was prepared in Buffered Peptone Water (Thermo Scientific, Oxoid Microbiological Products, Basingstoke, UK), according to ISO 6887-1:2017 (International Organisation for Standardisation, 2017a), which was used to enumerate *Listeria* species (including *L. monocytogenes*) (based on ISO 11290-2:1998/Amendment 1:2004 but with the variation that 0.5 ml of sample homogenate was inoculated onto single agar plates of Oxford and OCLA agars (Thermo Scientific, Oxoid Microbiological Products, Basingstoke, UK); International Organisation for Standardisation, 2017b). A 25 g portion of each sample was also tested for the presence of *Listeria* species using an enrichment procedure (ISO 11290-1:1996/Amendment 1:2004; International Organisation for Standardisation, 2017c). Sponge swabs were immersed in 100 ml of half-strength Fraser broth (Thermo Scientific, Oxoid Microbiological Products, Basingstoke, UK) and tested similarly to that above (International Organisation for Standardisation, 2017c). Selective agar plates (Oxford and OCLA agars) described above were used for both the swab and enrichment broths. Identification of *Listeria* isolates was performed in each of the individual laboratories as outlined in the standard methods above including the use of API *Listeria* (Bio-merieux, Basingstoke, UK) or *Listeria* ID (Neogen, Heywood, UK) identification kits.

2.3. Characterisation of *Listeria monocytogenes* isolates by WGS

Cultures of *L. monocytogenes* were sent to the PHE Gastrointestinal Bacteria Reference Unit (GBRU) for confirmation and further typing: all *L. monocytogenes* were subjected to WGS (Dallman et al., 2018; Elson et al., 2019; Nastasijevic et al., 2017). DNA from purified cultures of *L. monocytogenes* was obtained by automated extraction (QIAasympyony DSP DNA Kit) according to manufacturer instructions (Qiagen, Manchester, England). Genomic DNA was sequenced by the PHE Genomics Development and Services Unit: sample preparation was using the NexteraXT (Illumina Inc., San Diego, USA) and sequenced using Illumina HiSeq 2500 platform with 2x100bp reads (Illumina Inc., San Diego, USA). Short reads were quality trimmed using Trimmomatic removing the sequence adaptor (Bolger et al., 2014). *L. monocytogenes* identification was confirmed using kmer analysis (Painset et al., 2019) and four serotypes (1/2a, 1/2b, 1/2c, and 4) were derived from the WGS by alignment to four specific marker genes (*lmo0737*, *ORF2110*, *lmo1118*, and *ORF2819*; Doumith et al., 2004) using Bowtie2 (Langmead and Salzberg, 2012). Clonal complexes (CCs) were derived from WGS analysis: CCs were assigned using MOST (Tewolde et al., 2016) in accordance with the designation of the Institut Pasteur international MLST database for *L. monocytogenes* (<http://bigsd.db.pasteur.fr/listeria/listeria.html>). A core single nucleotide polymorphism (SNP) alignment for each clonal complex was generated using SnapperDB (Dallman et al., 2018), recombination removed using Gubbins (Croucher et al., 2015) and a seven-threshold SNP sequencing address

generated (Dallman et al., 2018). Pairwise comparisons of SNP distances were performed between cultures: *L. monocytogenes* linked within a 5 SNP single linkage cluster were considered to be part of the same point source with each culture having ≤ 5 SNPs difference with at least one other culture within that same cluster as described previously (McLauchlin et al., 2020b). A Maximum-likelihood phylogeny was derived for each clonal complex using RAxML v8.2.8 (Stamatakis, 2014) under the GTRCAT model to confirm the 5-SNP clustering were monophyletic.

2.4. Data analysis

WGS data derived from testing *L. monocytogenes* cultures from cases are added to the case register, which is compared to data derived from cultures recovered from other cases, foods or the environment. Data is maintained in a PHE curated database named the Gastro-data Warehouse (GDW). At the time of completing this analysis (July 2020), GDW contained over 5200 sequences derived from *L. monocytogenes* cultured from clinical cases of listeriosis, food and the environment, which were recovered in the UK between 2009 and 2020. Sequence reference numbers from the cultures described in this study are deposited to the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>; BioProject PRJNA475189). Sequence reference numbers appear in supporting data (publisher please provide url). Sequences were also compared in the same way as described above to those in NCBI sequence database in February 2020 (<https://www.ncbi.nlm.nih.gov/pathogens/>).

Data was extracted from the GDW onto Excel spreadsheets and combined with metadata associated with the food and environmental isolates.

3. Results

3.1. Cases associated with the outbreak and food exposures

Analysis of the national database identified 12 cases of listeriosis which occurred between December 2015 and July 2018 where the outbreak strain was recovered from clinical specimens: *L. monocytogenes* was recovered from blood in ten cases and cerebrospinal fluid (CSF) from the remaining two patients. The patients' ages were available for 11 of the cases and ranged from 1 to 86 years old (mean 58 years): apart from the one patient aged one year and one aged 22 years, all other patients were 58 years or older. Eight of the patients were male and four were female: there were no pregnant women or unborn/newly delivered infants. Nine of the cases resided in England (four in the North East, one in the North West, two in the Midlands and two in London) and three were in Scotland. One case occurred in 2015, two each in 2016 and 2017, and seven in 2018. Two of the cases died.

Information on consumption of sweet corn was not available for four of the five cases in 2015 to 2017. For the remaining eight cases, five reported consumption of frozen vegetables including sweet corn, four ate fresh mixed vegetables containing sweet corn, and two reported eating canned sweet corn. Information on specific food preparation methods using sweet corn was not available except for the one year old child who consumed frozen sweet corn puréed with other vegetables: food remnants were available from the domestic environment of this child and the outbreak strain was recovered from 20 g of this food by enrichment only (<10 cfu/g).

In February 2019, the outbreak strain was recovered from the CSF of a 58-year-old male patient in London. The outbreak strain was also recovered from an opened pack of frozen sweet corn from his domestic freezer and an opened pack of cheese from his refrigerator: *L. monocytogenes* was recovered at <10 cfu/g from both foods. The pack of sweet corn was of a batch subject to the international recall in the summer of 2018: it was not possible to ascertain when either of these products were purchased.

3.2. Phylogenetic confirmation of the *L. monocytogenes* outbreak strain and comparison with isolates from outside the UK

Phylogenetic analysis confirmed that the isolates from the 13 clinical cases, plus the isolates from food collected from two of the patients' homes, plus the samples of food collected from retail and from the two manufacturers (see below) all formed a monophyletic group (Fig. 1). Sequences from the UK isolates formed a monophyletic group with isolates from clinical cases in other European countries identified as part of this outbreak as well as isolates from the Hungarian food producer. A further genetic match was identified with a human case in Australia in 2018 who also reported consuming frozen sweet corn (personal communication, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia).

At the time of writing (July 2020), no additional cases of listeriosis infected by the outbreak strain were detected in the UK after the February 2019 case.

3.3. Microbiological investigations of the food chain

In June 2018, the *L. monocytogenes* outbreak strain was detected in a chicken and sweet corn sandwich filling collected at the point of production: the sampling of this food was for routine testing purposes and was unrelated to the investigation of the outbreak. The chicken and sweet corn sandwich filling was collected from a commercial manufacturer of sandwiches (Company A). It was subsequently identified that this manufacturer received frozen sweet corn from Company B which distributed frozen vegetable products in the UK, including frozen vegetables from the Hungarian factory associated with the outbreak. Company B instituted a voluntary product recall of frozen sweet corn in the UK in July 2018 (FSA, 2018b).

Sandwiches manufactured by Company A were prepared by adding frozen sweet corn directly to sandwich fillings without any additional cooking or blanching. Following this chance detection of the outbreak strain, a total of 54 food samples and 10 environmental swabs and a cloth were collected during June and July 2018 from the premises of Company A and were tested for the presence of *Listeria* (Table 1). *L. monocytogenes* was detected in 20 (37%) of the food samples, all at <20 cfu/g. The outbreak strain was recovered from 12 sandwich fillings (all containing sweet corn), and was not recovered from any other fillings or other foods which did not contain sweet corn. A second *L. monocytogenes* strain (strain 2, see following text) was recovered from eight out of 16 samples taken from three bulk bags of frozen sweet corn. *Listeria innocua* was detected in 15 of the food samples, seven of which also contained *L. monocytogenes*. No *Listeria* spp. were recovered from any of the 10 factory swabs collected from Company A's premises.

Microbiological investigations were also performed in the premises of Company B which was located in two sites in England and which imported frozen vegetable products (including those from Hungary). This company also repackaged and distributed these products in the UK. For Site 1, 32 samples were collected between July and November 2018, and comprised 16 swabs of sites within the factory and 16 food samples. For Site 2, 171 samples were collected, 144 in June–July 2018 (115 food samples and 29 factory swabs), and a further 27 samples (18 foods and nine factory swabs) in May 2019. Amongst all 131 food samples tested in 2018, *L. monocytogenes* was detected in 32 (24%). *L. monocytogenes* was recovered from 31 (44%) of the 70 bulk or retail packs of frozen sweet corn tested, with the outbreak strain identified in 19 packs. *L. monocytogenes* was detected in only one (2%) of the 61 other frozen foods tested: this isolate, from a pack of frozen peas was distinct from the outbreak strain (Table 1). Amongst all the frozen foods contaminated with *L. monocytogenes*, the bacterium was detected at 20 cfu/g in two samples of bulk frozen sweet corn contaminated with the outbreak strain: all of the remainder were contaminated at <20 cfu/g.

L. innocua or *Listeria seeligeri* were detected in 21 of the frozen foods, 6 (9%) of the 70 samples of frozen sweet corn and 15 (25%) of the 61

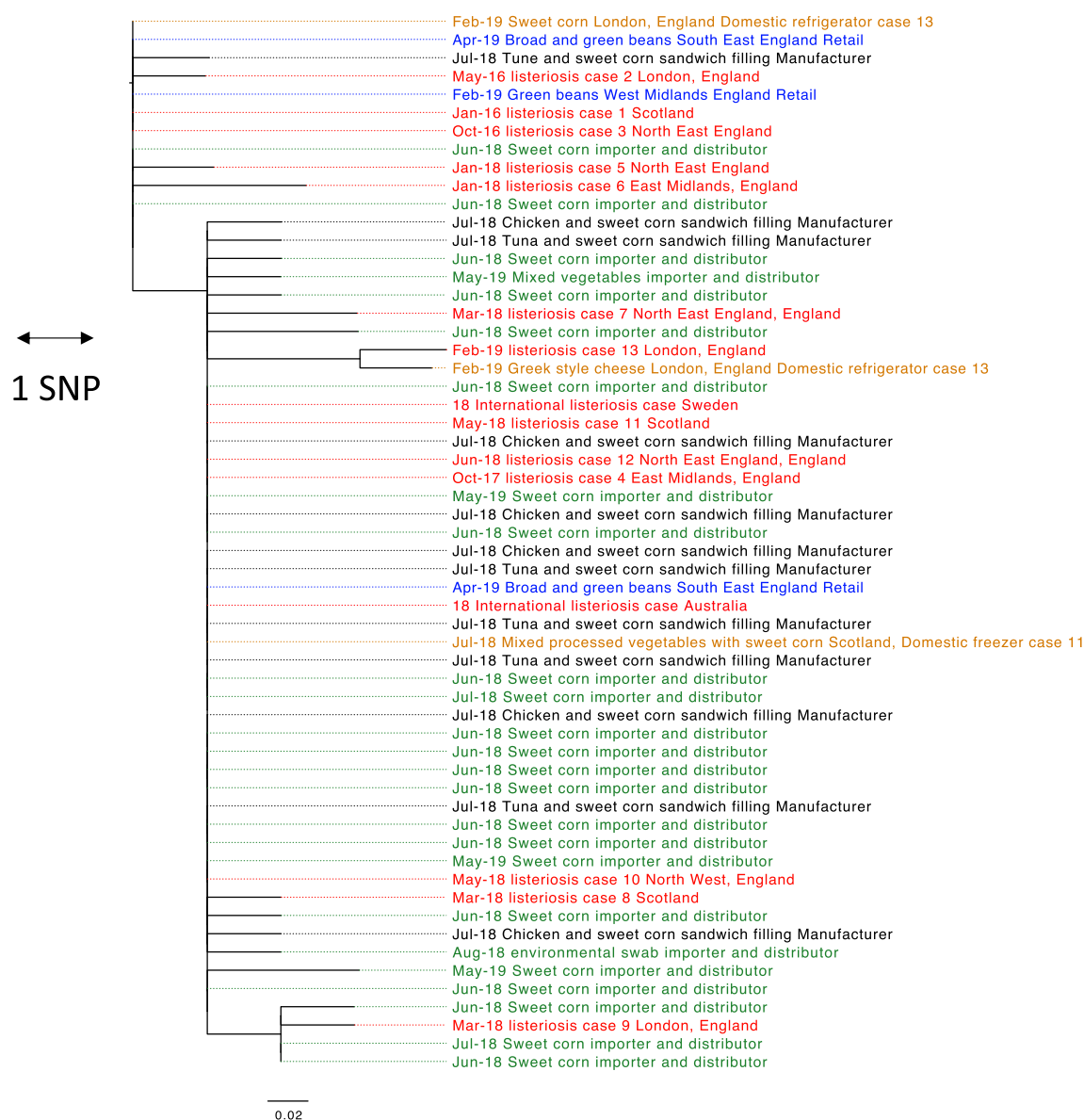


Fig. 1. Listeriosis outbreak, 2015–2019, maximum-likelihood phylogeny of *L. monocytogenes* serogroup 4, ST 6, CC 6, SNP type 1.2.2.2.2.2.%. Red = clinical cases; orange = food collected from domestic environments; green = UK importer and distributor; black = UK sandwich manufacturer; blue = UK retail. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other frozen foods tested from Company B. These two species of *Listeria* were recovered at <20 cfu/g in all instances. No food sample was found to be contaminated with more than one *Listeria* species. *L. monocytogenes* was recovered from four of the factory swabs (hopper, weigh head, wash hand basin trough and drainage gully) and *L. innocua* from a further two swabs (hopper and hopper chute), all taken in 2018. The outbreak strain was only detected in the wash basin.

Of the 18 food samples collected in May 2019 from Company B, *Listeria* were detected at <20 cfu/g in 8 mixed vegetable retail packs: *L. monocytogenes* was detected in four samples (all of which were the outbreak strain), *L. seeligeri* was recovered from a further three and *L. innocua* from one sample. Nine swabs of the factory environment were taken in 2019, of which *L. seeligeri* was recovered from one drain.

As a response to the outbreak, PHE instigated a 3-month survey of frozen fruit and vegetables on retail sale and from catering establishments in 2019 (Willis et al., 2020). The *L. monocytogenes* ST6 outbreak strain was detected in two samples (both of which contained beans) on retail sale in shops located in different parts of England in January and

April 2019. One of the products was marked as ready-to-cook, and cooking instructions were not recorded for the other. One of these products was identified as originating from Company B.

Phylogenetic analysis indicated that the *L. monocytogenes* isolates identified as the outbreak strain and recovered from food at retail, food from the sandwich manufacturer as well as food and the environment from the importer all formed a monophyletic group with the clinical isolates (Fig. 1). There were two separate isolates from the frozen broad beans (*Vicia faba*) and green beans on retail sale in April 2019 (Fig. 1).

In addition to the outbreak strain, SNP analysis of the *L. monocytogenes* recovered from foods and the environment of Company B identified a further five strains (designated strains 2–6; Table 1). Amongst the five additional *L. monocytogenes* strains recovered from Company B: strains 4, 5 and 6 were recovered on one occasion each; strain 2, eight times; and strain-3 five times. Strain 2 was recovered on 8 occasions from foods containing sweet corn from the sandwich manufacturer (Company A, Table 1).

Analysis of the GDW sequence database identified two UK clinical

Table 1Recovery of *Listeria* species from 272 food and environmental samples collected from a sandwich manufacturer and frozen vegetable distributor in 2018 and 2019.

Food type	Total tested	Number of samples with detection of:		
		<i>Listeria</i> species (not <i>L. monocytogenes</i>)	<i>L. monocytogenes</i>	<i>L. monocytogenes</i> strain
Sandwich manufacturer (Company A). 64 samples collected during June and July 2018				
Tuna and sweet corn sandwich fillings	6	0	6	6 outbreak strain (4, ST6)
Chicken and sweet corn sandwich fillings	6	1 ^a	6	6 outbreak strain (4, ST6)
Other sandwich fillings not containing sweet corn	23	0	0	
Cooked chicken	2	0	0	
Bulk frozen sweet corn	16	14 ^a	8	8 strain-2 (1/2a, ST 101)
Frozen spring onions	1	0	0	
Factory swabs and cloth	10	0	0	
Distribution company (Company B). 176 samples collected during June–November 2018				
Frozen sweet corn, bulk packs	38	4 ^b	12	7 outbreak strain (4, ST6) 5 strain-2 (1/2a, 101)
Frozen sweet corn, retail packs	32	2 ^b	19	12 outbreak strain (4, ST6) 2 strain-2 (1/2a, ST101) 5 strain-3 (1/2a, ST 431)
Frozen peas, retail packs	34	1 ^c	1	1 strain-4 (1/2a, ST431)
Frozen mixed vegetables or cauliflower, retail packs	21	13 ^b	0	
Frozen spinach	1	0	0	
Frozen rice	5	1 ^c	0	
Swabs from factory	45	2 ^a	4	1 outbreak strain (4, ST6) 1 strain-2 (1/2a, ST 101) 1 strain-5 (1/2a, ST8) 1 strain-6 (4, ST4)
Distribution company (Company B). 32 samples collected during May 2019				
Frozen mixed vegetables, retail packs	12	4 ^b	4	4 outbreak strain (4, ST6)
Frozen peas or carrots, retail packs	6	0	0	
Swabs from factory	9	1 ^c	0	

^a *L. innocua*.^b *L. seeligeri* and *L. innocua*.^c *L. seeligeri*.

isolates within the same 5 SNP strain-2 cluster and no other matches to strains 3–6. Phylogenetic analysis confirmed that the 17 *L. monocytogenes* Strain 2 cultures isolated in 2019 from frozen sweet corn and an environmental swab from Company A and B, as well as two isolates from food in 2013 and 2016 and two isolates from clinical cases in 2014 (briefly described below) formed a monophyletic group (Fig. 2). The two *L. monocytogenes* clinical isolates identified as part of the strain-2 cluster were isolated from blood cultures collected from two cases which occurred in England in June and November 2014, one was a 69-year-old male, the other a 33-year-old pregnant female: there was no information available on food exposures for either of these patients. There were two additional *L. monocytogenes* isolates from other foods which were identified as being within the same 5 SNP strain-2 cluster, the foods being: a tuna and sweet corn sandwich sampled from a café in 2016 and a sample of sweet corn tested by a commercial laboratory in 2013. Analysis of the international NCBI sequence database did not identify any other *L. monocytogenes* being within the same 5 SNPs cluster as any of the five strains identified from Company B although there were 4 *L. monocytogenes* sequences identified within 8 and 20 SNPs. These were one environmental isolate from Ireland from 2011 and 20 SNPs away from strain 3; one environmental isolate from Ireland from 2011 that was 8 SNPs away from strain 4; and one food isolate from Austria in 2012 and one clinical isolate from Spain in 2011 that were 8 and 19 SNPs away respectively from strain 5.

Phylogenetic analysis also confirmed that the five *L. monocytogenes* strain 3 cultures (serovar 1/2a, ST 431) isolated in 2019 from the frozen sweet corn distributor's factory were a monophyletic group (results not shown).

4. Discussion

Frozen vegetables are not generally regarded as ready-to-eat foods (EFSA, 2020; Willis et al., 2020) and this report further highlights problems with *L. monocytogenes* in frozen sweet corn. We report here on

UK investigations (as well as a case in Australia) which were part of a multi-country human listeriosis outbreak associated with the consumption of contaminated frozen sweet corn and provide data in addition to that already available (EFSA, 2018a,b). Previous investigations of human foodborne listeriosis have concentrated on ready-to-eat foods which support the growth of *L. monocytogenes* (Farber and Peterkin, 1991). This outbreak provides further information on food vehicles in which the bacterium is unlikely to grow, and demonstrates that frozen sweet corn may be of greater risk as a food vehicle for listeriosis than other frozen vegetables. A survey of frozen fruit and vegetables collected at retail and catering in England during 2019 (Willis et al., 2020), show that, amongst the frozen vegetables, sweet corn was more frequently contaminated by *L. monocytogenes* than most other frozen vegetable types. The data described in this report also showed that sweet corn was more frequently contaminated than other frozen vegetable collected at the distribution company. Furthermore, sweet corn had been used as a ready-to-eat food ingredient. There is also circumstantial evidence consistent with two further cases of listeriosis associated with frozen sweet corn (*L. monocytogenes* strain 2) presented here: similar evidence is reported elsewhere for linkage between sweet corn to cases in England and Denmark (Willis et al., 2020). Frozen sweet corn was associated with a listeriosis outbreak in the United States between 2013 and 2015 involving nine cases, all of whom were hospitalised, three of whom died (CDC, 2016). Epidemiological and microbiological evidence indicated that the source was frozen vegetables from a single producer and WGS showed that the *L. monocytogenes* isolated from frozen sweet corn was indistinguishable from isolates from eight of the human cases; a culture from frozen peas from the same manufacturer was distinct from the outbreak strain but indistinguishable from an isolate from a further human case. Other food vehicles reported elsewhere in which *L. monocytogenes* is unable to grow include an outbreak associated with frozen ice-cream (Pouillot et al., 2016) and a single sporadic case associated with dried alfalfa leaves (Farber et al., 1990). These incidents highlight the need to reconsider food vehicles for human listeriosis and



Fig. 2. Maximum-likelihood phylogeney of *L. monocytogenes* strain-2, serovar 1/2a, ST 101, SNP type 2.7.7.7.7.7.%. Red = UK clinical case, green = UK importer and distributor; black = UK sandwich manufacturer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ensure appropriate controls are implemented in the food chain, including for non-ready-to-eat foods and food ingredients which can be added to ready-to-eat foods, particularly where *L. monocytogenes* has an opportunity to grow. The potential for *L. monocytogenes* to grow in foods containing sweet corn was highlighted both by the European Food Safety Authority (EFSA, 2020) and by an outbreak of more than 1500 cases of listeriosis presenting with febrile gastroenteritis which occurred in Italy in 1997 (Aureli et al., 2000). This outbreak was associated with consumption of a sweet corn and tuna salad which was prepared from canned products. In the 1997 outbreak, the products used to prepare the salad were contamination from the environment where the salad was produced after opening the cans. This is in contrast to the frozen sweet corn outbreak described here where contamination occurred at the point of freezing in Hungary (EFSA, 2018a,b). The potential for growth of *L. monocytogenes* in sweet corn kernels was previously identified where this bacterium was shown to grow to $>10^6$ cfu/g after 10 h at 25 °C in an inoculation experiment (Aureli et al., 2000).

Related cases of listeriosis can be temporally and geographically widely distributed (McLauchlin et al., 2020a). Listeriosis outbreaks associated with frozen foods (including frozen vegetables) will present greater difficulties for investigation than those associated with many other food types since these can be stored for several years both at distribution, retail (Willis et al., 2020) and in domestic freezers. Hence cases can be even more widely distributed than those linked to other food types with shorter shelf lives. The data presented here

demonstrates that there was continued exposures to consumers in the UK from the *L. monocytogenes* strain associated with the 2015–18 outbreak in products within this food chain after the international recall in the summer of 2018: fortunately, no further listeriosis cases associated with this outbreak were detected in the UK after a case of meningitis in London in February 2019 up to the point of writing in July 2020.

The 13 cases identified as part of this outbreak showed a similar pattern to listeriosis cases reported through national surveillance (Scobie et al., 2019) in that the affected patients were most frequently over 60 years of age and male, and most frequently presented with bacteraemia. The two patients aged one year old and 22 years old are unusual for listeriosis patients in England (Scobie et al., 2019). It was only possible to obtain generic information on sweet corn consumption, and not on specific food preparation methods used by the patients except for the one year old child. There are difficulties in obtaining specific exposure information for sweet corn which can be added to many different dishes and if consumed in a catering setting, an individual is unlikely to be aware if a frozen or canned sweet corn products have been used. Recall of consuming canned sweet corn by the two patients in the outbreak described here will be an incidental finding since this product undergoes a heat treatment and was therefore unrelated to this outbreak, however this will reflect a generic exposure for consuming sweet corn. Infection in the one-year-old child reflects the consumption of puréed frozen sweet corn with other vegetables and illustrates the potential for foodborne exposure to *L. monocytogenes* from non-ready-to-

eat foods. Raw vegetables are increasingly used in a variety of products, including smoothies (González-Tejedor et al., 2018; Willis et al., 2020) and there is a need for risk communication to both producers and consumers to control this exposure.

The benefits of collecting and testing food samples from patients' domestic environments in listeriosis outbreaks occurring in England and Wales has previously been reviewed (McLauchlin et al., 2020a). For the UK cases in this outbreak, the implicated strain was recovered from food samples collected from the domestic environments of two patients (the one-year-old child and the adult with meningitis in 2019). Although cross-contamination may take place between foods in domestic environments (as is likely to have occurred in the 2019 case where the outbreak strain was recovered from an opened pack of sweet corn and from cheese) this approach should be considered for routine investigations of listeriosis patients.

In this investigation, results from unrelated microbiological testing of products from a sandwich manufacturer identified this business as part of the affected food chain without conventional product tracing. We have previously noted that the most common method to initially identify specific foods associated with incidents of human foodborne listeriosis in the community in England and Wales was where isolates recovered from unrelated food testing were recognised as indistinguishable to those from cases (McLauchlin et al., 2020a). Furthermore, microbiological testing confirmed failures of food safety management systems, sometimes several years before onset of infection of cases of listeriosis (Elson et al., 2019; McLauchlin et al., 2020a). Although there was no evidence of cases resulting from exposure to contaminated sandwiches for the UK listeriosis cases which were part of the 2015–18 sweet corn outbreak, sweet corn is frequently added to a range sandwich fillings (as well as other foods such as salads) and identifying this as a discrete exposure may be difficult. Furthermore, the contamination of sandwiches by Company A by both the outbreak strain and a second strain represents an avoidable risk which could have contributed to infection, with increased risk of infection if growth of the bacterium occurred within these sandwiches. Following these observations, additional controls were implemented by Company A which included a further heating step applied to the frozen sweet corn prior to addition to the sandwich filling.

The very high discrimination of WGS to characterise *L. monocytogenes* not only provides evidence to support epidemiological data linking patients together in an outbreak, but also provides plausible hypotheses linking parts of food chains. The multi-country reports relating to this sweet corn outbreak identified the food chain associated with the outbreak as very complex (EFSA, 2018b,c). The investigations described here show how analysis by WGS provides evidence to understand a complex food chain. Firstly, this analysis identified groups of isolates from independently sampled foods to link a frozen food distributor and a sandwich manufacturer prior to more traditional trace-back and trace-forward approaches. Secondly, WGS allowed the identification of the outbreak strain from the environment of the frozen food distributor's factory amongst six distinct *L. monocytogenes* strains together with *L. innocua* and *L. seeligeri*, which demonstrates the complexity of heterogeneous populations in a food production environment. Despite the multiple strains contaminating these products, it is unclear why only some strains are associated with human disease and the genetic and physiological basis for this should be the subject of future studies. Thirdly, a second unrelated *L. monocytogenes* (strain 2) was detected in samples from two companies' products in 2018, sweet corn tested up to five years previously and from two English cases of listeriosis in 2014. This final observation illustrates how analysis of historical isolates can implicate a food chain and provides a plausible hypothesis for public health investigations as well as reflecting both the long shelf life of frozen products and colonisation of harbourage sites within food factories (Ferreira et al., 2014). Analysis of WGS data therefore allows the recognition of *L. monocytogenes* strains (including the outbreak strains) amongst *Listeria* populations within complex food

chains over long periods of time. Combining and providing access to data from more traditionally gathered epidemiological, microbiological and food chain information with that from WGS will considerably enhance the effectiveness of all those involved both with public health responses to cases of listeriosis and control of microbiological hazards in the food chain.

This outbreak provides further information on *L. monocytogenes* in the frozen vegetable food chain. This bacterium is widespread in the environment and therefore raw products are likely to be contaminated and act as a route for the organism to be introduced into factory sites (EFSA, 2020). During processing and prior to freezing, vegetables are frequently blanched, either with water or steam (EFSA, 2018c, 2020). The blanching inactivates plant enzymes to reduce spoilage during storage and preserves colour, flavour and nutritional value (Ceylan et al., 2017). The blanching step is not designed or controlled as a microbial inactivation but will inevitably reduce the overall microbiota. After freezing, the presence of *L. monocytogenes* indicates either survival of contamination from the raw product through the blanching process or contamination from factory sites and machinery during freezing (EFSA, 2020). Manufacturers of frozen vegetables should control the quality of their raw materials as well as operate stringent cleaning and sanitation to reduce contamination as much as possible from factory sites including from blast freezers (EFSA, 2020). EU food legislation (European Commission, 2005) contains microbiological criteria for *L. monocytogenes* in ready-to-eat foods with a limit of 100 cfu/g during their shelf life. However frozen sweet corn (as well as many other frozen vegetables) are not generally intended as ready-to-eat foods and therefore these criteria do not apply. The European Food Safety Authority (EFSA, 2020) considered public health risks from frozen fruit and vegetables and although no microbiological criteria were recommended, they did comment that the occurrence of relatively low levels of *L. monocytogenes* at the end of the production process would be compatible with the limit of 100 CFU/g at the moment of consumption following ideal storage by the consumer of 24 h at 5 °C. However, considering reasonably foreseeable conditions of use by the consumers of 48 h at 12 °C, *L. monocytogenes* levels need to be considerably lower, even below the detection sensitivity of the current available standard analytical procedure/methods for those products that best support pathogen growth.

Frozen vegetables (including sweet corn) represent a hazard both as a source of cross-contamination in food production environments and through the potential for misuse as ready-to-eat food ingredients. This is in contrast to canned sweet corn which includes a heat inactivation treatment and does result in a ready-to-eat product. HACCP-based food safety management systems of manufacturers of frozen vegetable must identify these hazards and implement prerequisite programs to reduce or eliminate contamination and include additional controls to reduce risks to acceptable levels. Monitoring for the presence of *L. monocytogenes* (as well as *Listeria* spp.) both within food and the manufacturing environment should be a component for the verification of the effectiveness of the HACCP-based food safety management systems (EFSA, 2020). There is also a role for food regulators risk management (as well as for public health authorities) to control *L. monocytogenes* in frozen vegetables. Advice from the European Food Safety Authority (EFSA, 2018c, 2020) on public health risks as well as sampling and environmental monitoring in processing plants will help to implement better HACCP-based food safety management systems and, it is hoped, preventing further outbreaks. Consumers should also be advised on the appropriate purchase, storage and cooking of frozen vegetables. Risk communication was done in England as part of the outbreak control in the summer of 2018 (FSA, 2018a; PHE, 2018c); however, there is a continuing need to reiterate this message. Manufacturers and retailers should clearly mark packaging with the need for cooking: in the survey performed in early 2019, 77% of 673 frozen vegetables were described as not ready-to-eat and the intended use described on the packaging advised cooking or blanching (Willis et al., 2020).

Declaration of competing interest

None.

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Appendix A. Supplementary data

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References

- Aureli, P., Fiorucci, G.C., Caroli, D., Marchiaro, G., Novara, O., Leone, L., Salmaso, S., 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. *N. Engl. J. Med.* 342, 1236–1241.
- Bolger, A.M., Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120.
- CDC (Centers for Disease Control and Prevention), 2016. Multistate outbreak of listeriosis linked to frozen vegetables (final update). Available at: <https://www.cdc.gov/listeria/outbreaks/frozen-vegetables-05-16/index.html>. (Accessed October 2020).
- Ceylan, E., Ceylan, E., McMahon, W., Garren, D.M., 2017. Thermal inactivation of *Listeria monocytogenes* and *Salmonella* during water and steam blanching of vegetables. *J. Food Prot.* 80, 1550–1556.
- Croucher, N.J., Page, A.J., Connor, T.R., Delaney, A.J., Keane, J.A., Bentley, S.D., Parkhill, J., Harris, S.R., 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using gubbins. *Nucleic Acids Res.* 43, e15.
- Dallman, T., Ashton, P., Schafer, U., Jironkin, A., Painset, A., Shaaban, S., Hartman, H., Myers, R., Underwood, A., Jenkins, C., Grant, K., 2018. SnapperDB: a database solution for routine sequencing analysis of bacterial isolates. *Bioinformatics* 34, 3028–3029.
- Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42, 3819–3822.
- EFSA (European Food Safety Authority Panel on Biological Hazards), 2020. Scientific opinion on the public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing. *EFSA J.* 18 (4), 6092, 2020. 102 pp. Available from: <https://doi.org/10.2903/j.efsa.2020.6092>. (Accessed October 2020).
- EFSA (European Food Safety Authority), 2018a. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. Available from: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1448>. (Accessed October 2020).
- EFSA (European Food Safety Authority), 2018b. *Listeria monocytogenes*: Update on Foodborne Outbreak, June 2018. Available from: <https://www.efsa.europa.eu/en/press/news/180703>. (Accessed October 2020).
- EFSA (European Food Safety Authority), 2018c. Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of frozen vegetables aiming at detecting *Listeria monocytogenes*. Available from: <https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2018.EN-1445>. (Accessed October 2020).
- EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2017. <https://doi.org/10.2903/j.efsa.2018.5500>. Accessed October 2020.
- Elson, R., Awofisayo-Okuyelu, A., Greener, T., Swift, C., Painset, A., Amar, C.F.L., Newton, A., Aird, H., Swindlehurst, M., Elviss, N., Foster, K., Dallman, T.J., Ruggles, R., Grant, K., 2019. Utility of whole genome sequencing to describe the persistence and evolution of *Listeria monocytogenes* strains within crabmeat processing environments linked to two outbreaks of listeriosis. *J. Food Prot.* 82, 30–38.
- European Commission, 2005. Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Available at: <http://eur-lex.europa.eu/leg-al-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=en>. (Accessed October 2020).
- Farber, J.M., Peterkin, P.I., 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55, 476–511.
- Farber, J.M., Carter, A.O., Varughese, P.V., Ashton, F.E., Ewan, E.P., 1990. Listeriosis traced to the consumption of alfalfa tablets and soft cheese. *N. Engl. J. Med.* 322, 338.
- Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M.J., 2014. *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *J. Food Prot.* 77, 150–170.
- Food Standards Agency, 2017. Food Law Code of Practice. FSA, London. Available at: <https://www.food.gov.uk/about-us/food-and-feed-codes-of-practice>. (Accessed October 2020).
- Food Standards Agency, 2018a. Precautionary advice on cooking frozen vegetables following Europe-wide listeriosis outbreak, 3 July 2018. Available from: <https://www.food.gov.uk/news-alerts/news/precautionary-advice-on-cooking-frozen-vegetables-following-europe-wide-listeriosis-outbreak>. (Accessed October 2020).
- Food Standards Agency, 2018b. Greenyard Frozen UK Ltd recalls various frozen vegetable products due to possible contamination with *Listeria monocytogenes*. 5th July 2018. Available from: <https://www.food.gov.uk/news-alerts/alert/fsa-prin-35-2018>. (Accessed October 2020).
- González-Tejedor, G.A., Garre, A., Esnoz, A., Artés-Hernández, F., Fernández, P.S., 2018. Effect of storage conditions in the response of *Listeria monocytogenes* in a fresh purple vegetable smoothie compared with an acidified TSB medium. *Food Microbiol.* 72, 98–105.
- Goulet, V., King, L.A., Vaillant, V., de Valk, H., 2013. What is the incubation period for listeriosis? *BMC Infect. Dis.* 13, 11.
- International Organization for Standardization, 2017a. ISO 6887-1:2017 Microbiology of the food chain — preparation of test samples, initial suspension and decimal dilutions for microbiological examination — part 1: general rules for the preparation of the initial suspension and decimal dilutions. Available from: <https://www.iso.org/standard/63335.html>. (Accessed October 2020).
- International Organization for Standardization, 2017b. ISO 11290-2:2017. Microbiology of the food chain — horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — part 2: enumeration method. Available from: <https://www.iso.org/standard/60314.html>. (Accessed October 2020).
- International Organization for Standardization, 2017c. ISO 11290-1:2017. Microbiology of the food chain — horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — part 1: detection method. <https://www.iso.org/standard/60313.html>. (Accessed October 2020).
- Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359.
- Linnan, M.J., Mascola, L., Lou, X.D., Goulet, V., May, S., Salminen, C., Hird, D.W., Yonekura, M.L., Hayes, P., Weaver, P., Audurier, A., Plikaytis, M.S., 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319, 823–828.
- McLauchlin, J., Grant, K.A., Amar, C.F.L., 2020a. Human foodborne listeriosis in England and Wales, 1981 to 2015. *Epidemiol. Infect.* e54, 1–14.
- McLauchlin, J., Aird, H., Amar, C., Barker, C., Dallman, T., Elviss, N., Jorgensen, F., Willis, C., 2020b. *Listeria monocytogenes* in cooked chicken: detection of an outbreak in the UK (2016–2017) and analysis of *L. monocytogenes* from unrelated monitoring of foods (2013–2017). *J. Food Protect.* 83, 2041–2052, 2020, published ahead of print.
- Nastasic, I., Milanov, D., Velebit, B., Djordjevic, V., Swift, C., Painset, A., Lakicevic, B., 2017. Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome Sequencing as a food safety management tool: a proof of concept. *Int. J. Food Microbiol.* 257, 157–164.
- Painset, A., Björkman, J.T., Kiil, K., Guiller, L., Mariet, J.F., Félix, B., Amar, C., Rotariu, O., Roussel, S., Perez-Reche, F., Brisse, S., Moura, A., Lecuit, M., Forbes, K., Strachan, N., Grant, K., Møller-Nielsen, E., Dallman, T.J., 2019. LiSEQ - whole-genome sequencing of a cross-sectional survey of *Listeria monocytogenes* in ready-to-eat foods and human clinical cases in Europe. *Microb. Genomics* 5, e000257.
- Pouillot, R., Klontz, K.C., Chen, Y., Burall, L.S., Macarasin, D., Doyle, M., Bally, K.M., Strain, E., Datta, A.R., Hammack, T.S., Van Doren, J.M., 2016. Infectious dose of *Listeria monocytogenes* in outbreak linked to ice cream, United States, 2015. *Emerg. Infect. Dis.* 22, 2113–2119.
- Public Health England, 2018a. Listeria: enhanced surveillance questionnaire for microbiologists, updated August 2018. Available at: <https://www.gov.uk/government/publications/listeria-enhanced-surveillance-questionnaire-for-microbiologists>. (Accessed October 2020).
- Public Health England, 2018b. Listeria: enhanced surveillance questionnaire, updated February 2018. Available at: <https://www.gov.uk/government/publications/listeria-enhanced-surveillance-questionnaire>. (Accessed October 2020).
- Public Health England, 2018c. Listeriosis cases linked to frozen sweetcorn: advice on cooking frozen vegetables following Europe-wide outbreak. Available from: <https://www.gov.uk/government/news/listeriosis-cases-linked-to-frozen-sweetcorn>. (Accessed October 2020).

- Scobie, A., Kanagarajah, S., Harris, R.J., Byrne, L., Amar, C., Grant, K., Godbole, G., 2019. Mortality risk factors for listeriosis - a 10 year review of non-pregnancy associated cases in England 2006–2015. *J. Inf.* 78, 208–214.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.
- Tewolde, R., Dallman, T., Schaefer, U., Sheppard, C.L., Ashton, P., Pichon, B., Ellington, M., Swift, C., Green, J., Underwood, A., 2016. MOST: a modified MLST typing tool based on short read sequencing. *PeerJ.* 4, e2308.
- Willis, C., McLauchlin, J., Aird, H., Amar, C., Barker, C., Dallman, T., Elviss, N., Lai, S., Sadler-Reeves, L., 2020. Occurrence of *Listeria* and *Escherichia coli* in frozen fruit and vegetables collected from retail and catering premises in England, 2018–2019. *Int. J. Food Microbiol.* 332, 108849.