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Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections probably linked to frozen corn

European Food Safety Authority
European Centre for Disease Prevention and Control

Abstract

An outbreak of invasive *Listeria monocytogenes* (*L. monocytogenes*) infections defined by whole-genome sequencing (WGS) and probably linked to frozen corn has been ongoing in five EU Member States (Austria, Denmark, Finland, Sweden and the United Kingdom) since 2015. As of 8 March 2018, 32 cases have been reported and six patients have died due to or with the infection. WGS analysis of six non-human *L. monocytogenes* isolates detected from 2016 to January 2018 in Austria, Finland, France and Sweden found these isolates closely related to the multi-country cluster of *L. monocytogenes* serogroup IVb, multi-locus sequence type 6 (ST6). The non-human isolates were detected in two different samples from mixed frozen vegetables; three samples from frozen corn, and one sample from a surface where various vegetables could have been processed. The only common food item in all non-human samples was corn. The WGS analysis provides a strong microbiological link between the human and the non-human isolates and is suggestive of a potential contaminated food source related to frozen corn persisting in the food chain at least since 2016. Traceability information for the three frozen corn samples pointed to frozen corn products packed in Poland and processed/produced in Hungary. Two additional non-human strains isolated in Austria from frozen vegetable mixes with corn as an ingredient were traced back to the same common origin in Hungary. Further investigations are needed to verify the point of contamination in the food chain. Consumption of frozen corn has been confirmed by two patients, one in Finland and one in Sweden. In addition, a Danish patient reported consumption of mixed frozen vegetables, which could have included corn. The Finnish patient confirmed consumption of frozen corn of one suspected brand, supporting an epidemiological link between the outbreak cases and frozen corn. However, no traceability and microbiological information was available for the corn consumed by the Finnish and the Swedish patients. Food business operators in Estonia, Finland, Poland and Sweden have withdrawn and recalled the implicated frozen corn products from the market. These measures are likely to significantly reduce the risk of human infections in these countries. However, new invasive listeriosis cases may be identified due to the long incubation period (1–70 days), long shelf-lives of frozen corn products and potential consumption of frozen corn bought by the customers before the recalls and eaten without being properly cooked. Furthermore, until the root source of contamination is established and control measures implemented, new cases may occur.

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Key words: *Listeria monocytogenes*, frozen corn, multi-country outbreak, multi-locus sequence type (MLST), Whole Genome Sequencing (WGS)

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Conclusions

An outbreak of invasive *Listeria monocytogenes* (*L. monocytogenes*) infections defined by whole-genome sequencing (WGS) and probably linked to frozen corn has been ongoing in five EU Member States (Austria, Denmark, Finland, Sweden and the United Kingdom) since 2015. As of 8 March 2018, 32 cases have been reported and six patients have died due to or with the infection.

WGS analysis of six non-human *L. monocytogenes* isolates detected from 2016 to January 2018 in Austria, Finland, France and Sweden found these isolates closely related to the multi-country cluster of *L. monocytogenes* serogroup IVb, multi-locus sequence type 6 (ST6). The non-human isolates were detected in two different samples from mixed frozen vegetables; three samples from frozen corn, and one sample from a surface where various vegetables could have been processed. The only common food item in all non-human samples was corn.

The WGS analysis provides a strong microbiological link between the human and the non-human isolates and is suggestive of a potential contaminated food source related to frozen corn persisting in the food chain at least since 2016. Traceability information for the three frozen corn samples pointed to frozen corn products packed in Poland and processed/produced in Hungary. Two additional non-human strains isolated in Austria from frozen vegetable mixes with corn as an ingredient were traced back to the same common origin in Hungary. Further investigations are needed to verify the point of contamination in the food chain.

Consumption of frozen corn has been confirmed by two patients, one in Finland and one in Sweden. In addition, a Danish patient reported consumption of mixed frozen vegetables, which could have included corn. The Finnish patient confirmed consumption of frozen corn of one suspected brand, supporting an epidemiological link between the outbreak cases and frozen corn. However, no traceability and microbiological information was available for the corn consumed by the Finnish and the Swedish patients.

Food business operators in Estonia, Finland, Poland and Sweden have withdrawn and recalled the implicated frozen corn products from the market. These measures are likely to significantly reduce the risk of human infections in these countries. However, new invasive listeriosis cases may be identified due to the long incubation period (1–70 days), long shelf-lives of frozen corn products and potential consumption of frozen corn bought by the customers before the recalls and eaten without being properly cooked. Furthermore, until the root source of contamination is established and control measures implemented, new cases may occur.

Options for response

In order to reduce the risk of *L. monocytogenes* infection due to potentially contaminated frozen corn, consumers should consider adequately heat treating frozen vegetable products that are not 'ready-to-eat products' before consumption.

Competent authorities should report new human cases associated with this event and the findings of public health investigations to the Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses (EPIS-FWD) and consider interviewing new and recent listeriosis cases about consumption of frozen corn and other frozen vegetables.

ECDC can support WGS analysis of human isolates from cases possibly related to this outbreak and reported in countries that are not routinely performing WGS. The European Reference Laboratory for *L. monocytogenes* (EURL for *Lm*) can support the Member States by performing WGS analysis of non-human isolates for strains possibly related to the outbreak.

ECDC and EFSA encourage the competent authorities of public health and food safety sectors in the affected EU countries and at European level to continue sharing information on the epidemiological, microbiological and environmental investigations, including tracing information, by issuing relevant notifications using the Early Warning and Response System (EWRS) and the Rapid Alert System for Food and Feed (RASFF).

EWRS is the official channel for notifying cross-border threats to humans from communicable diseases.

RASFF is the official EU system for sharing information on hazards found in food and feed, the trade of potentially contaminated batches between Member States and the tracing of such batches. RASFF notifications should be completed with information on exposure to food for related human cases, as well as traceability information on the suspected food vehicles and analytical results to support traceability investigations.

Source and date of request

ECDC request to EFSA on 29 November 2017. The European Commission request to EFSA on 16 February 2018. EFSA acceptance of the two requests on 21 February 2018.

Public health issue

This document provides an assessment of the cross-border public health risk probably associated with consumption of frozen corn that is potentially contaminated with *L. monocytogenes*. ECDC published a rapid risk assessment concerning this event on 6 December 2017 [1].

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Disclaimer

ECDC issued this outbreak assessment document in accordance with Article 10 of Decision No 1082/13/EC and Article 7(1) of Regulation (EC) No 851/2004 establishing a European Centre for Disease Prevention and Control (ECDC), and with the contribution of EFSA in accordance with Article 31 of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, laying down the general principles and requirements of food law, establishing the European Food Safety Authority (EFSA) and laying down procedures in matters of food safety.

In the framework of ECDC's mandate, the specific purpose of an ECDC-EFSA outbreak assessment is to present different options on a certain matter, with their respective advantages and disadvantages. The responsibility on the choice of which option to pursue and which actions to take, including the adoption of mandatory rules or guidelines, lies exclusively with EU/EEA Member States. In its activities, ECDC strives to ensure its independence, high scientific quality, transparency and efficiency.

This report was written under the coordination of an internal response team at ECDC, with contributions from EFSA, at the behest of the European Commission based on a mandate requesting scientific assistance from EFSA in the investigation of multinational food-borne outbreaks (Ares (2013) 2576387, Mandate M-2013-0119, 7 July 2013).

All data published in this rapid outbreak assessment are correct to the best of our knowledge on 21 March 2018. Maps and figures published do not represent a statement on the part of ECDC, EFSA or its partners on the legal or border status of the countries and territories shown.

Disease background information

Listeria monocytogenes isolation in humans

Background information about listeriosis can be found in ECDC, US CDC and WHO disease fact sheets [2-4].

In the years 2012–2016, between 1 754 and 2 555 *L. monocytogenes* cases were reported annually to The European Surveillance System (TESSy) by 30 EU/EEA countries [5]. PCR serogroup IVb [6] is the most commonly reported PCR serogroup (44% of cases with available information on PCR serogroup), with between 332 and 403 notifications annually from 13 EU/EEA countries. France, Germany and the United Kingdom, accounted for 45%, 23% and 17%, respectively, of the reported serogroup IVb cases in this period. Cases of PCR serogroup IVb were more common in males (52%) and among persons over 65 years (61% of cases) in both genders. The majority (99%) of the serogroup IVb cases were of domestic origin [7].

Of 2 969 *L. monocytogenes* isolates with 'Accepted' sequencing quality reported to TESSy isolate-based surveillance, 308 (10.4%) are ST6 spanning 2009–2017. Serotype is available for 263 of these isolates, with 247 (93.9%) serotype 4b, which belongs to serogroup IVb. Pulsed Field Gel Electrophoresis (PFGE) of 'Accepted' quality is available for 65 of these ST6 isolates based on multi-locus sequence type (MLST), including 26 unique PFGE profiles. Two isolates have indistinguishable combined PFGE profiles AscI.0003-ApaI.0070 with profiles of the *L. monocytogenes* serogroup IVb, ST6 Finnish representative outbreak strain (one isolate matching with 10 allelic differences (in cgMLST Moura scheme [8]) and the other one with 20 allelic differences).

Foodborne outbreaks caused by *L. monocytogenes*

This section presents the information on foodborne outbreaks caused by *L. monocytogenes* reported to EFSA under the framework of Directive 2003/99/EC*. Based on this Directive, the reporting of serotyping information of the causative agent of the foodborne outbreaks is not mandatory and this level of details is usually not reported to EFSA. Thus, no information on foodborne outbreaks caused by *L. monocytogenes* group IVb ST6 was reported to EFSA based on Directive 2003/99/EC.

During the period 2010–2016, 63 foodborne outbreaks due to *L. monocytogenes* were reported at EU level by 15 EU/EFTA countries, involving 536 cases, 174 hospitalisations and 32 deaths. For 32 of these outbreaks, the evidence supporting the association with the suspected food vehicle was reported to be strong. Three strong-evidence outbreaks reported by Germany, Spain and Switzerland were associated with the consumption of 'vegetables and juices and other products thereof'. Specifically, one outbreak associated with the consumption of mixed salad was reported by Germany in 2013 and another outbreak associated with the consumption of pre-cut salad was reported by Switzerland in 2014. No additional information on the type of implicated vegetables and/or derived products was provided for the third outbreak reported by Spain in 2015. Overall these three outbreaks involved 37 human cases, with five deaths. The place of exposure was a 'hospital or medical care facility' in the German outbreak, 'household' in the Swiss outbreak and a 'restaurant or cafe or pub or bar or hotel or catering

* Although mandatory, not all the Member States report the complete information on foodborne outbreaks to EFSA and consequently the information summarised in this section may not be exhaustive for certain countries.

service' in the outbreak reported by Spain. In 2014, Spain also reported one *L. monocytogenes* weak-evidence outbreak associated with the consumption of 'vegetables and juices and other products thereof' (no further information on the food-vehicle was reported). Details on the number of cases for the strong-evidence outbreaks is summarised in Table 1 of the annex, by food vehicle and reporting year (Annex - Table 1).

In addition, two foodborne outbreaks caused by *L. monocytogenes* occurred in Denmark in 2016, one of which was a weak-evidence outbreak caused by *L. monocytogenes* ST6 (genetically different from the ST6 strain of the current outbreak) involving four cases with disease onset between April 2016 and July 2017, where the implicated food vehicle is still unknown [9]. An additional strong-evidence outbreak caused by *L. monocytogenes* ST6 (genetically different from the current and the previously described ST6 cluster) and associated with the consumption of cold smoked fish was reported in Denmark in 2014 [10,11].

***L. monocytogenes* isolation in food**

L. monocytogenes is widely distributed in the environment and can enter food-processing settings via incoming raw materials and the movement of personnel and equipment. *L. monocytogenes* can colonise in the form of biofilms on food-processing equipment and food-contact surfaces and can therefore persist for prolonged periods in food-processing environments. Hence, a wide range of foodstuffs can become contaminated during various stages of food production and distribution, particularly during the food-processing stage. Food business operators perform their own checks to monitor and control possible contamination by *L. monocytogenes* of the food-processing environment and the final product.

Because *L. monocytogenes* can multiply at refrigerator temperatures, listeriosis is usually associated with ingestion of contaminated milk products, meat or vegetable products that have been kept at refrigeration temperatures and eaten without being cooked properly. European monitoring data on *L. monocytogenes* in foods that are provided by Member States to EFSA mostly originate from sampling conducted under EU Regulation (EC) No 2073/2005 on microbiological criteria, which lays down the food safety criteria for *L. monocytogenes* in ready-to-eat (RTE) foods and which has been in force since 1 January 2006.

This section focuses on the reporting of *L. monocytogenes* in vegetables, which represent the suspected food category in this investigation. According to the classification used in the different reporting countries, where often vegetables are considered as belonging to the broad food category 'fruit and vegetables', information from the following food categories are included in this section: 'ready-to-eat salad', 'vegetables', 'fruit and vegetables' and 'fruits'. Table 2 of the annex summarises the information reported on *L. monocytogenes* in fruit and vegetables from 2004 to 2016. Information on both ready-to-eat (RTE) and non-RTE food is included in this section (and Table 2 of the annex). *L. monocytogenes* contaminated products were reported either qualitatively (presence or absence) or quantitatively (either ≤ 100 or > 100 cfu/g).

In 2016, 108 *L. monocytogenes* isolations were reported in fruits and vegetables (77 isolates from 'vegetables', 28 isolates from 'RTE salads' and three isolates from 'fruits and vegetables') mostly sampled at retail sites (87.0%), while only few isolations were reported from samples collected at processing plant (eight isolations) and at restaurants/catering facilities or other public services (three isolations). The sampling stage was unspecified for three isolations. The majority of *L. monocytogenes* isolations reported in 2016 were from unspecified vegetables, followed by RTE salads, and pre-cut vegetables. *L. monocytogenes* isolations in the above-listed food categories were reported by 12 Member States and one non-Member State: Austria, Belgium, Czech Republic, France, Germany, Greece, Ireland, Italy, Luxembourg, Slovakia, Spain, Sweden and Bosnia and Herzegovina.

The number of *L. monocytogenes* isolations reported between 2004 and 2016 are presented by food category in Table 2 of the annex.

Information on MLST is usually not reported in the context of monitoring zoonoses and zoonotic agents in food based on Directive 2003/99/EC. Specifically, no data on *L. monocytogenes* serogroup IVb ST6 have been reported to EFSA (this information is not mandatory and is only be reported on a voluntary basis).

Event background information

On 3 November 2017, Finland launched an urgent inquiry in EPIS FWD relating to three *L. monocytogenes* clusters, confirmed by whole genome sequencing (WGS), with cases from different parts of Finland in 2017. The largest WGS cluster was associated with *L. monocytogenes* serogroup IVb, ST6, with 14 cases detected between January 2016 and January 2018. Two patients have died due to or with the infection.

Multi-country investigations

EU/EEA outbreak case definition

ECDC and the members of the outbreak investigation team in the affected countries agreed on an European outbreak case definition to harmonise the investigation of outbreak cases and take into account the different molecular typing systems (cgMLST, wgMLST, SNP-based analysis) of surveillance across Member States.

Confirmed outbreak case

A laboratory-confirmed listeriosis patient with symptom onset on or after 1 January 2015 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

AND

- Fulfilling the additional laboratory criterion: with *L. monocytogenes* having ≤ 7 core-genome Multi-locus Sequence Typing (cgMLST) allelic differences from the outbreak isolate FI 122265 based on cgMLST analysis (assembly uploaded to EPIS UI-444 as IVb_MLST6_122265_S3_L001_R_q30w20.fasta). The cgMLST scheme is either that of Moura or Ruppitsch, or a respective scheme [8,12].

OR

- Fulfilling the additional laboratory criterion: with *L. monocytogenes* within a five SNP cluster from the outbreak isolate FI 122265 based on SNP analysis (assembly uploaded to EPIS UI-444 as IVb_MLST6_122265_S3_L001_R_q30w20.fasta).

Probable outbreak case

A laboratory-confirmed listeriosis patient with symptom onset on or after 1 January 2015 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

AND

- Fulfilling the additional laboratory criteria: with an isolate of *L. monocytogenes* serogroup IVb and with PFGE indistinguishable from the profile AscI.0003-ApaI.0070 (TESSy) (uploaded to EPIS as UI-444: BioNumerics.PFGE.AscI.0003-ApaI.00070.zip).

Exclusion criteria

Cases with travel history outside of the EU/EEA in the 30 days before disease onset.

Epidemiological and microbiological investigation of human cases

Following WGS, four Member States reported human cases with isolates closely matching the Finnish *L. monocytogenes* ST6 cluster (0 to 5 allelic differences based on cgMLST or 0 to 5 SNP difference from the representative outbreak isolate FI 122265).

Based on the European outbreak case definition, as of 6 March 2018, a multi-country foodborne outbreak has been verified in five countries, involving 32 confirmed cases and six deaths due to or with the infection (Table 1, Figure 1).

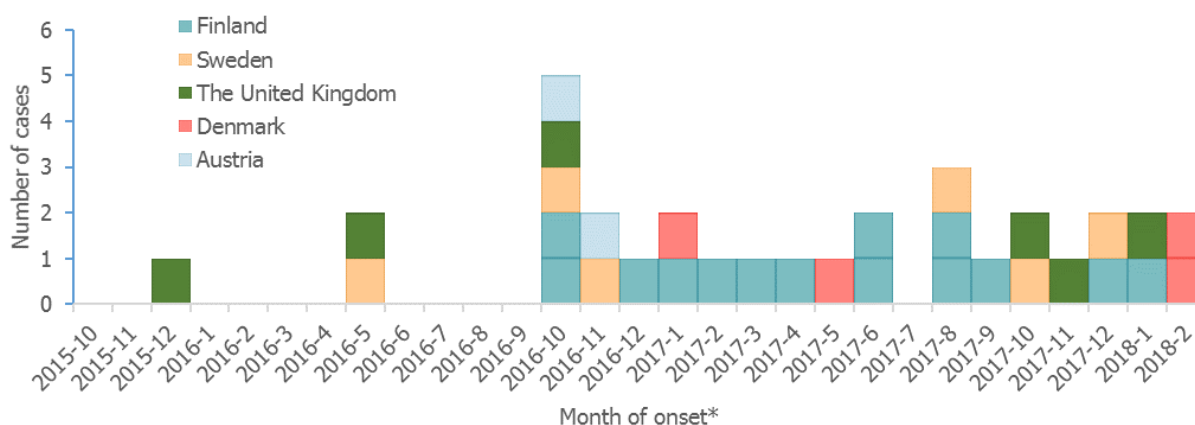
- **Austria** has reported two confirmed cases from 2016. Both cases are males, aged over 85 years at symptom onset, one case was fatal.
- **Denmark** has reported four confirmed cases with sampling dates in January 2017, May 2017 and February 2018 (two cases). Three cases are female patients and one is male. Their age ranges between 37 and 74 years. One case was fatal.
- **Finland** has reported 14 confirmed cases with sampling dates from September 2016 to January 2018. Nine cases are female patients and five are males. Their age ranges between 22 and 92 years. Two cases were fatal.
- **Sweden** has reported six confirmed cases with isolates. Five cases are female patients and one is male. Their age ranges between 70 and 94 years. Two cases were fatal.
- **United Kingdom** reported six confirmed cases between 2015 and 2018. Age range of cases is from 22 to 84 years. Four cases were male and two female.

Table 1. *Listeria monocytogenes* IVb, ST6 confirmed outbreak cases by country and year, EU 2015–2018 (as of 6 March 2018)

Country	Confirmed cases (No. of deaths)				Total number of cases	Total number of deaths
	2015	2016	2017	2018		
Austria	0	2 (1)	0	0	2	1
Denmark	0	0	2	2 (1)	4	1
Finland	0	3	10 (2)	1	14	2
Sweden	0	3 (1)	3 (1)	0	6	2
United Kingdom	1	2	2	1	6	0
Total	1 (0)	10 (2)	17 (3)	4 (1)	32	6

France, Germany, Ireland, Italy, Luxembourg, the Netherlands, Norway and Portugal report no human isolates matching the Finnish outbreak strain.

Information on patients' food exposures are captured at the national level through interviews using national questionnaires. However, information on exposure to corn, the only common food item identified in all non-human isolates matching the outbreak strain, was not routinely requested in the national questionnaires used in the affected countries. The patient identified in December 2017 in Sweden was asked about and reported corn consumption. One Finnish patient, reported in December 2017, referred to consumption of corn from the brand A mentioned in RASFF 2018.0216 but the case reported in January 2018 did not. One of the two Danish patients reported in February 2018, mentioned eating frozen mixed vegetables that could possibly include corn. The latest patients identified in the United Kingdom are being re-interviewed to collect missing information on a possible corn exposure.

Figure 1. *Listeria monocytogenes* PCR serogroup IVb, ST6 confirmed outbreak cases by month of symptoms onset*, European Union 2015–2018 (n=32)

*If month of onset missing: month of sampling or month of receipt in reference laboratory

European whole genome sequencing analysis of human and non-human isolates

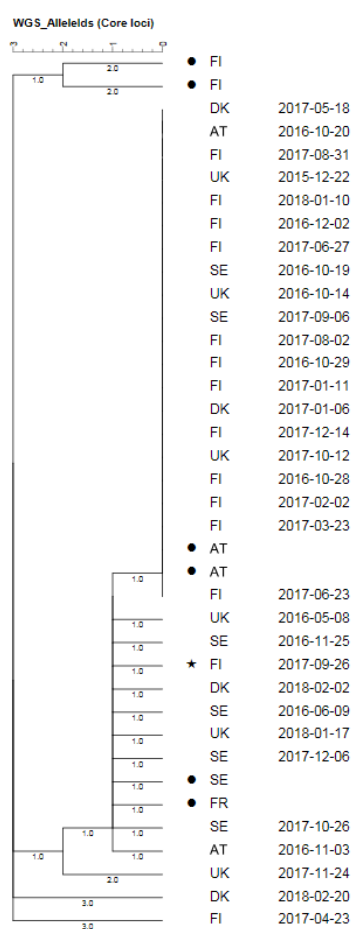
Raw sequence data from human *L. monocytogenes* isolates matching the European case definition were collected by ECDC. The EURL for *Lm* collected sequence data on non-human isolates from National Reference Centres and the NRL network of the EURL for *Lm*. WGS data analysis of human and non-human isolates was performed jointly by ECDC, the EURL for *Lm* and EFSA.

The reads were trimmed to remove adaptor and barcode sequences (added during library generation) and low-quality reads using Trimmomatic 0.36 (min. Phred score 25). They were assembled with SPAdes v.3.7.1 in BioNumerics version 7.6.2 (Applied-Maths, Sint-Martens-Latem, Belgium) including post-assembly optimisation by mapping reads back onto the assembly and keeping the consensus. cgMLST analysis was done based on the assembly using the Moura scheme [8] in BioNumerics. Isolates were retained in the analysis if at least 1 661 (95%) of the 1 748 core loci were detected, no contamination with other *Listeria* species was detected and not more than one locus with more than one allele was called. Results from this analysis are described in Table 2 below and visualised in Figure 2.

Table 2. *Listeria monocytogenes* isolates within seven allelic differences from the *L. monocytogenes* ST6 Finnish representative outbreak strain in cgMLST

Country	Number of human isolates (No. of differing cg alleles from the FI representative outbreak strain)	Number of non-human isolates (No. of differing cg alleles from the FI representative outbreak strain)
Austria	2 (1-2)	2 (1)
Denmark	4 (1-5)	-
Finland	14 (0-5)	2 (4)
France	-	1 (2)
Sweden	6 (1-2)	1 (2)
United Kingdom	6 (1-4)	-
Total	32 (0-5)	6 (1-4)

Figure 2. CgMLST-based (Moura scheme) single-linkage tree including sequences from 32 human and 6 non-human *Listeria monocytogenes* isolates from six countries, 2015–2018 (software: BioNumerics version 7.6.2)



The *L. monocytogenes* ST6 Finnish representative outbreak strain 122265 is marked with a star and non-human isolates marked with a dot. Dates used for statistics (i.e. date of sampling or date of receipt if the former was not available) included for human isolates. Isolates with ≤ 7 differing cg-alleles are considered as genetically closely related and form the basis of the confirmed case definition

The single-linkage tree including all human (n=32) and non-human (n=6) isolates (figure 2) shows that all of them are within three cg-allelic differences (and within 0-5 cg-allelic differences from the *L. monocytogenes* ST6 Finnish representative outbreak strain (122265)), indicating close genetic relatedness. The Finnish representative outbreak strain (122265) has 1 722 of the 1 748 core loci (98.5%), and 1495 of the 1 748 core loci (85.5%) were shared by all isolates – i.e. the unique loci were detected in each of the 38 isolates. The results of the WGS analysis were confirmed using the Ruppitsch cgMLST scheme [12] on Ridom SeqSphere+ 4.1.9 and the SPADES assembler on BioNumerics 7.6 (Applied-Maths, Sint-Martens-Latem, Belgium).

Information on the non-human strains included in the joint WGS analysis

One non-human strain was isolated from an environmental own-check sample collected by a food business operator in France, two strains were isolated from two different frozen vegetable mixes in Austria, one strain was isolated from a sample of frozen corn in Sweden and two strains were isolated from two samples of frozen corn in Finland. Details on the origin of the food and environmental samples from Austria, France, Sweden and Finland where these six non-human strains were isolated are reported by country in the following sections on food and environmental investigations. The only common food item in all non-human samples was corn.

In addition, WGS is being performed on the non-human strains isolated in Poland (see country specific section on food and environmental investigations) and the analyses are ongoing.

Food and environmental investigations

This section summarises country-specific information on food and environmental investigations associated with this outbreak that has been reported through RASFF (news 17-849 and alert 2018.0216), EPIS FWD (UI- 444) and directly to EFSA by national competent authorities and provided by EURL for *Lm* (up to 21 March 2018) since 29 November 2017.

Finland

On 26 January 2018, Finland notified a RASFF alert (reference: 2018.0216) on the finding of *L. monocytogenes* in two batches of frozen corn (batches A and B of the brand A) sampled at the Finnish trader/broker A. Overall, five samples of frozen corn were tested for *L. monocytogenes* (three samples from batch A and two samples from batch B) and all were contaminated. Specifically, the enumeration of *L. monocytogenes* were 140 cfu/g, 70 cfu/g and 50 cfu/g in the three samples from batch A; in the two samples from batch B the levels of *L. monocytogenes* were 80 cfu/g and 55 cfu/g.

The Finnish trader/broker A received the implicated batches A and B of frozen corn, from the Polish company C who packed the product originating from Hungary.

The Finnish trader/broker A dispatched the implicated batches A and B to a Finnish wholesaler A, who further distributed the product to the Finnish market, as well as to a retailer B in Estonia.

The Finnish trader/broker A also received two additional batches of frozen corn from the Polish Company C – batch D and batch E. On 12 March, Finland confirmed that *L. monocytogenes* was also detected in these two additional batches.

According to WGS analysis, the two *L. monocytogenes* isolates originating from the batches D and E were found to be closely related to the 32 outbreak strains using cgMLST (4 allelic differences). See the section 'European whole genome sequencing analysis of human and food isolates'.

As of 22 January, the Finnish wholesaler A voluntarily recalled all the implicated batches of frozen corn in Finland and a press release was issued.

Finland recently confirmed the consumption of corn from brand A for the most recent Finnish patient (detected in December 2017) (RASFF 2018.0216). However, the Finnish food safety authorities were not able to provide information on the batch number of the product consumed by the patient.

Sweden

On 19 February 2018, Sweden reported in RASFF (news 17-849 follow-up 8; alert 2018.0216 follow-up 6) the finding of a *L. monocytogenes* isolate from frozen corn that matched the *L. monocytogenes* ST6 outbreak strain using WGS (with 0 SNP difference). The WGS analysis also showed that the *L. monocytogenes* isolate was closely related to the 32 human outbreak strains using cgMLST (2 allelic differences). The sample was collected from an opened package of frozen corn stored in a consumer's fridge, and sent for analysis in January 2018. The consumer was not tested for listeriosis and therefore was not a confirmed listeriosis case. The Swedish authorities informed that the sample from which the isolate was detected originated from batch A of frozen corn. The consumer bought the product (batch A) at the Swedish retailer A, which received the product from the Polish company C, where the product was packed after having been processed in Hungary.

On 19 January 2018, the Swedish retailer A published a press release and the implicated batch A was recalled (follow-ups 3 and 6 - RASFF ref 2018.0216). The restrictive measures were applied to all the packages concerned. One week later, on 26 January the company expanded the recall to include batch C. As a precautionary measure, the Swedish retailer A has decided to stop distributing the product until further notice.

On 23 February, Sweden confirmed in RASFF (follow-up 10-RASFF ref 2018.0216) that the patient identified in December 2017 in Sweden reported having consumed corn. However, it was not possible to identify the brand of corn consumed. Frozen corn of different brands had been bought on several occasions from two different supermarket chains, but none of them was the retailer A.

Estonia

On 18 January 2018, the Estonian retailer B was informed by its provider, the Finnish wholesaler A, about the presence of *L. monocytogenes* in frozen corn from batches A and B (follow-up 1 -RASFF alert 2018.0216). The Estonian retailer B informed its clients about the recall of all the implicated batches, by written notice displayed in shops and via media. Part of the consignment (51 units) was sold to final consumers. Thirty-three units of frozen corn were destroyed by Estonian retailer B and not sold to final consumers. All the recalled units will be destroyed in Estonia.

Poland

Following the communication by Finland regarding the finding of *L. monocytogenes* in batches A and B of frozen corn, on 31 January 2018, the Polish company C voluntarily withdrew these batches from the recipients in Finland and Sweden (follow-up 2- RASFF ref 2018.0216). The Polish company C tested all batches of frozen raw materials originating from Hungary used for the production of batches A and B in its own laboratory and found one common batch X1 contaminated with *L. monocytogenes* at the level 150 cfu/g. The Polish company C also decided to withdraw from the market batch C, that had been produced from the same batch of frozen raw materials, as well as the additional batch D.

The three batches A, B and C were produced on 9–10 December 2017 by company C in Poland. The following batches of frozen raw materials originating from Hungary were used for the production of batches A, B and C: X1, X2, X3, X4, X5, X6, X7. Five additional batches of frozen raw materials were used for the production of batches A and B that were distributed to Finland (batches X8, X9, X10, X11, and X12), while for the production of batches A and C distributed to Sweden two additional batches were also used: batches X13 and X14.

The Polish company B provides storage service for company C (follow-up 15 - RASFF 2018.2016). The frozen corn is delivered to company B from the Hungarian company A in containers called octabins. The containers are not opened and there is no handling of the product at the Polish company B. The product - in the same container - is then delivered from company B to company C by an external transport company. Octabins are opened at company C and repacked into final single packages for the consumers.

Company C in Poland dispatched batches A and B to the Finnish trader/broker A, and batches A and C to the Swedish retailer A.

On 20 February 2018, Poland gave notification in RASFF (Ref 2018.0216, follow-up 7) of the detection of *L. monocytogenes* by the Polish company C in the three batches of frozen corn (batches A, B and C) tested in their own laboratory. Company B did not test the implicated products, as this company only provides storage service for company C and did not process the product.

The Polish competent authorities collected official samples from the two implicated batches A and C available at company C's plant and *L. monocytogenes* was detected in both batches and the enumeration results showed <40 cfu/g of *L. monocytogenes* in all samples (follow-up 17 -RASFF alert 2018.0216).

The Polish company C provided the testing results for all the 14 batches of frozen raw materials originating from Hungary used to produce batches A, B and C. According to the enumeration results, 10 batches of frozen raw materials (X1, X2, X4, X5, X6, X7, X8, X11, X12, X14) were contaminated with *L. monocytogenes* at levels ≥ 10 cfu/g. In the remaining four batches of frozen raw materials (X3, X9, X10, X13) the levels of *L. monocytogenes* were <10 CFU/g. It is important to note that it is not possible to exclude the presence of *L. monocytogenes* on the basis of the enumeration results alone (even when <10 cfu/g). Qualitative detection results were not provided.

Furthermore, company C also detected *L. monocytogenes* (up to 70 cfu/g) in an additional batch of frozen corn (batch D) originating from different batches of frozen raw materials (batches X2, X15, X16, X17, X18, X19, X20, X21, X22, X23, X24 and X25) received from the Hungarian company A. This batch D was only distributed to the Finnish trader/broker A (follow-up 15, RASFF ref. 2018.0216).

WGS is being performed by EURL for *Lm* on the non-human strains isolated in Poland and the analyses are ongoing.

Other batches of final products were also produced from the batches of frozen raw materials listed above (batches X15 to X25) (follow-up 15; RASFF ref. 2018.0216) originating from Hungarian company A.

An additional batch E in which *L. monocytogenes* was detected by Finland, was also produced by the Polish Company C. The batches of frozen raw materials used for the production of this batch E were X2, X16, X26 and X27.

As described above, the same batches of frozen raw materials were used to produce more than one batch of frozen corn packed in the Polish company C. In particular, batch X2 originating from Hungary was used to produce all five batches of contaminated frozen corn (batches A, B, C, D and E).

The Polish company C provided information on its cleaning and disinfection procedures, as well as hygiene conditions that showed satisfactory results (follow-up 11 of RASFF). Poland also provided the results of swab samples taken from different devices at the Polish company C in November and December 2017. *L. monocytogenes* was not detected. In addition, Poland provided results for official swab samples collected by the authorities at plant C and all samples were *L. monocytogenes* negative (follow-up 17, RASFF alert 2018.0216).

Hungary

On 14 February 2018, Hungary gave notification in RASFF (RASFF alert 2018.0216, follow-up 4) that on 25 September 2017 the Hungarian company A had processed 9 100 kg of corn from the implicated batch of frozen raw materials X1. The raw materials originated from a Hungarian supplier A which delivered it on the same day that it was processed. The suspected batch X1 was delivered on 5 October 2017 and on 9 October 2017 to the Polish company B. There was no distribution of batch X1 to other countries. The delivery of the batch of frozen raw materials X16 on 9 October 2017 was also dispatched to Polish company B.

Other batches were also processed by the Hungarian company A (batches from X2 to X29). Microbiological testing was performed on four batches of frozen raw materials: batches X15, X16, X28 tested by an accredited laboratory and X29 tested during their own checks. According to enumeration results the levels of *L. monocytogenes* were <10 cfu/g.

On 22 February 2018, Hungary reported in RASFF (follow-up 9, RASFF alert 2018.2016) finding *L. monocytogenes* (1.4×10^3 cfu/g) in a sample of frozen corn from the batch X28 analysed by an official laboratory. This batch was delivered by Hungarian company A to Polish company B. According to the information provided by Hungary on 12 March 2018, this isolate was *L. monocytogenes* serogroup IIa, and therefore it is not related to the present outbreak caused by *L. monocytogenes* serogroup IVb ST6.

According to information provided by Hungary, 11 batches of frozen raw materials (X1, X2, X12, X15, X16, X21, X23, X25, X26, X27 and X28) used by Poland for the production of batches A, B, C, D and E were produced in the same growing area A of 31 hectares by the Hungarian supplier A. Additional batches of frozen raw materials (which were not used to produce batches A, B, C, D and E) were also produced in growing area A.

Additional information on the origin of non-human strains matching the multi-country cluster of *L. monocytogenes* ST6

This section presents the information available on the origin of the Austrian and the French non-human strains, which - based on the results of the joint WGS analysis performed - match the multi-country cluster of *L. monocytogenes* ST6. This information was provided by the national reference laboratories for *L. monocytogenes* and RASFF contact points in Austria and France. Information on the origin of the Finnish and Swedish food strains matching the multi-country cluster of *L. monocytogenes* ST6 are summarised in the country-specific sections above.

Details on the joint WGS analysis are presented in section 'European whole genome sequencing analysis of human and food isolates'.

Austria

On 23 February 2018, Austria reported in the RASFF news 17-849 (follow-up 11) that the two Austrian non-human strains matching the multi-country cluster of *L. monocytogenes* ST6 originated from samples of 'frozen Mexican vegetable mix' (ingredients: green beans, onion, corn, paprika, kidney beans, small corn cobs) and 'frozen classical vegetable mix' (ingredients: peas, corn and baby carrots) that tested positive for *L. monocytogenes* in 2017 and 2016 respectively. The labels on these products indicated that they were not considered ready-to-eat (RTE) and according to the preparation method on the label both vegetable mixes had to be cooked before consumption. The product 'frozen Mexican vegetable mix' brand B was produced at Belgian Company D, where the different frozen vegetables were mixed together with water, margarine, sunflower oil and herbs. During mixing the vegetables remain frozen and the additives are applied via nitrogen so that they immediately form a frozen coating around the vegetables. The deep frozen vegetables that are processed in Company D originated from a company in the same group, the Belgian company E or from other suppliers, as is the case for the corn included in the Mexican vegetable mix. There are several suppliers for different ingredients. According to the information provided by Belgium in follow-up 16 and 19 of the RASFF news 17-849, the frozen corn used in the Mexican vegetable mix originates from processing company A in Hungary. According to the information provided, Belgian company E is the legal owner of the companies belonging to the same group in Belgium (company D), Poland (company C) and Hungary (company A). As clarified by Belgium, Belgian company E is also the legal owner of other companies belonging to the same group in different countries (for example, France). However, it was clarified that most of the branches in the different countries operate independently. Specifically, Belgian company D is dependent on company E, whereas the Polish branches are run from Poland and the Hungarian company A is operationally independent.

The second positive product 'frozen classical vegetable mix' was produced and packed by company A in Hungary (follow-up 11 RASFF 17-849).

On 28 February 2018, Austria also provided additional information (Follow-up 15 -RASFF ref. 17-849) from a study carried out in Austria (not an official control), in which *L. monocytogenes* was detected at low level (< 10cfu/g) in three samples of three different lots of frozen vegetable mixes originating from Belgian company E and Hungarian company A. Results of the serotyping are still pending.

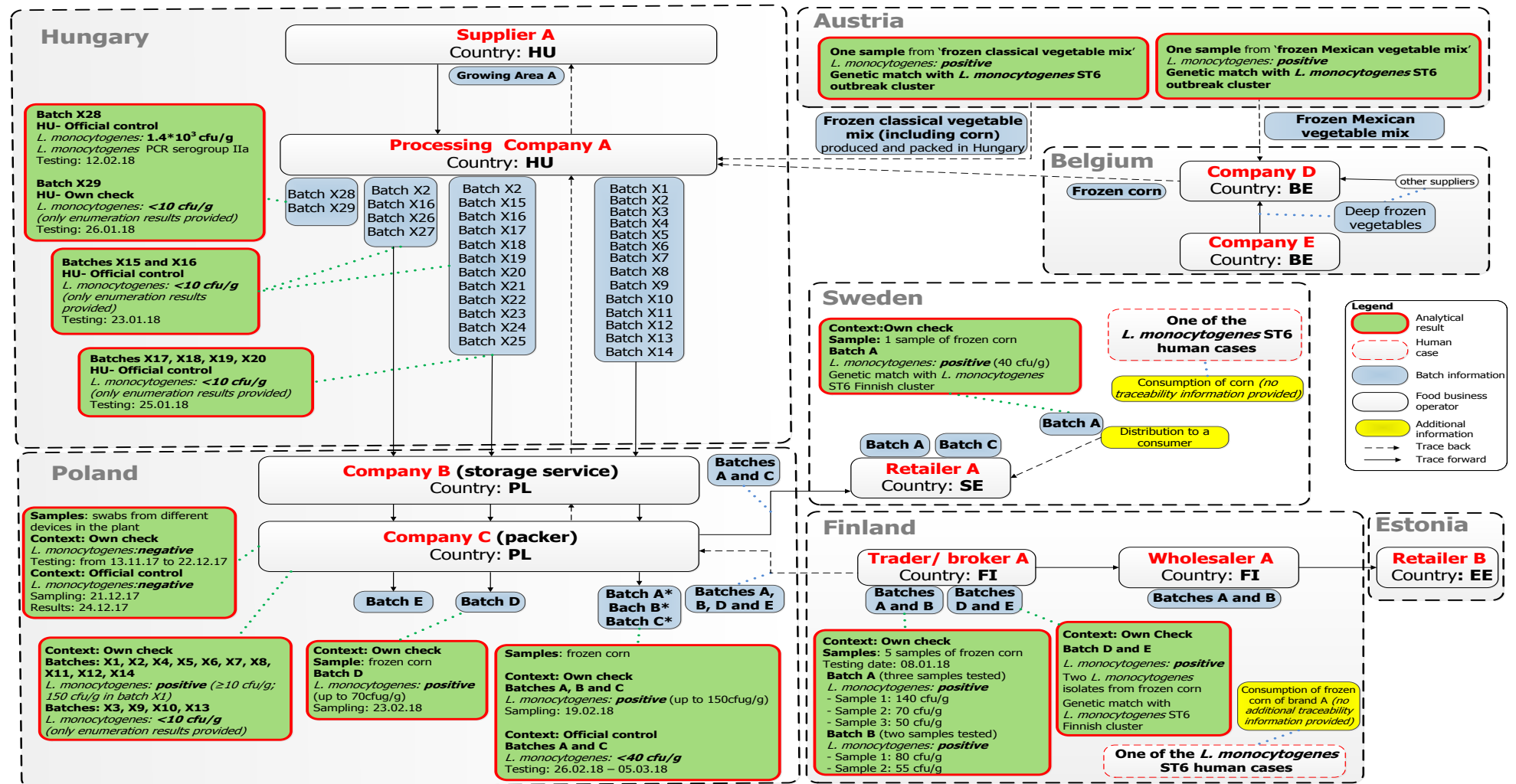
France

The French non-human strain matching the multi-country cluster of *L. monocytogenes* PCR serogroup IVb ST6 originated from an environmental sample collected at a food processing plant (company F) when carrying out its own checks in August 2017. The sample was obtained from a surface where the following products could have been processed: frozen flat-leaved parsley, soft corn grains (frozen), potato cubes, green peas (frozen). No information on the origin of the products processed by the food processing plant has been provided yet.

According to the information provided by France in follow-up 24 of the RASFF news 17-849, analysis of frozen corn sampled at French company F indicated the presence of *L. monocytogenes*. The supplier of the frozen corn is French Company G, belonging to the same group of companies mentioned for Poland (company C), Hungary (company A) and Belgium (companies D and E). Based on the information provided by the French authorities, the corn originated from France or Hungary. However, the exact origin of the contaminated frozen corn cannot be confirmed until detailed traceability information is provided.

The EURL for *Lm* has reported that the French national reference laboratory is working to collect strains from two other own control samples of frozen corn, taken by the food processing plant where the first non-human positive strain was detected at the beginning of the investigation.

Figure 3. Graphical representation of traceability and testing information available in RASFF or provided by Member States to EFSA, as of 21 March 2018



*Seven batches (X1-X7) of frozen raw materials originating from Hungary were used for the production of all batches A, B and C. Five additional batches (X8-X12) of frozen raw materials were used for the production of batches A and B that were distributed to Finland, while two additional batches (X13 and X14) were used for the production of the batches A and C distributed to Sweden.

cfu/g: colony-forming unit per gram.

AT: Austria, BE: Belgium, EE: Estonia, FI: Finland, HU: Hungary, PL: Poland, SE: Sweden

ECDC and EFSA threat assessment for the EU

An outbreak of *L. monocytogenes* serogroup IVb, ST6, is ongoing in Austria, Denmark, Finland, Sweden and the United Kingdom with 32 human cases reported since 2015. Six patients have died. Four outbreak cases have been reported in 2018. One of these patients, reported on 20 February 2018, died due to or with the infection. Thus, the outbreak is continuing, or has been ongoing until very recently. As a result, and also due to the long incubation period of invasive listeriosis, additional cases associated with this outbreak may be reported. It is also likely that the extent of this outbreak has been underestimated since the outbreak was identified through sequencing and only a subset of the EU countries routinely use this advanced technique to characterise *L. monocytogenes* isolates.

L. monocytogenes ST6 is a hypervirulent clone of *L. monocytogenes* associated with neurological forms of listeriosis [13,14]. Pregnant women, the elderly, and immunocompromised individuals are at increased risk of invasive listeriosis, which is associated with severe clinical course and potentially death.

WGS analysis confirmed that all 32 human *L. monocytogenes* isolates have 0–5 allelic differences from the Finnish representative outbreak isolate FI 122265 based on cgMLST. In addition, six non-human strains were found to be closely related to the 32 human outbreak strains using cgMLST (1–4 allelic differences): two strains from Finland, one strain from Sweden, one strain from France and two strains from Austria. The three non-human strains from Finland and Sweden were isolated from three batches of frozen corn packed at company C in Poland, processed by a Hungarian company A and produced by a supplier A in Hungary. The two non-human strains identified in Austria were isolated from two different frozen vegetable mixes containing corn. The corn used in both frozen vegetable mixes originated from the Hungarian company A. The French non-human strain was isolated from an environmental sample collected from a food processing plant where different vegetable products (including soft-corn grains) had been processed. No detailed information was available on the origin of the products processed by the French food processing plant. The finding of *L. monocytogenes* in frozen food products is explained by the fact that the bacterium survives in freezing environments and can grow at low temperatures (4°C) [15].

Finland confirmed that one Finnish patient (identified in December 2017) consumed frozen corn from brand A. However, the information on the batch number of the consumed product was not available.

The Swedish patient identified in December 2017 also reported having consumed corn. However, it was not possible to identify the origin of the corn consumed by the case.

Information on corn consumption was not routinely requested during patient interviews in the affected countries and only four patients were interviewed about corn consumption. However, no traceability and microbiological information was available on the corn consumed by the Finnish and Swedish patients. It is worth noticing that the frozen corn products were not considered by the producer to be 'ready-to-eat' food and the consumers may have eaten the products without having cooked them properly.

The occurrence of human cases since 2015 and the finding of positive non-human isolates since 2016 suggests that the common source of contamination has been active for two to three years. However, it is not yet clear at which stage in the food production chain the contamination of frozen corn occurs. Further investigation is needed to verify this.

The recalls and withdrawals of suspected and contaminated batches from the market reduces the likelihood of new infections. However, the prolonged duration of the contamination source over two years indicates that new cases may occur until the point of contamination is verified and control measures subsequently implemented.

According to the information provided by Hungary, the most recent positive finding of *L. monocytogenes* in a sample of frozen corn in Hungarian company A was a strain of serogroup IIa which is different to the outbreak strain.

Finally, the epidemiological and molecular investigations have excluded a possible link between this outbreak and the *L. monocytogenes* ST6 outbreak in South Africa [16].

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Annex

Table 1. Strong-evidence food-borne outbreaks due to *L. monocytogenes* reported to EFSA under the framework of Directive 2003/99/EC, EU/EFTA countries, 2010–2016^(a)

Year Food category	Number of outbreaks	Number of cases	Number of hospitalisations	Number of deaths
Year 2010				
Fish and fish products	2	21	8	1
Other foods	1	4	4	1
Other or mixed red meat and products thereof	1	10	10	2
Year 2011				
Bakery products	1	2	2	0
Cheese	1	11	11	4
Mixed food	1	3	3	0
Pig meat and products thereof	1	9	0	0
Year 2012				
Bakery products	1	14	14	1
Bovine meat and products thereof	1	4	4	2
Mixed food	1	6	6	2
Other or mixed red meat and products thereof	1	20	20	3
Year 2013				
Cheese	1	2	0	0
Crustaceans, shellfish, molluscs and products thereof	3	10	8	2
Fish and fish products	1	3	3	1
Meat and meat products	1	34	0	0
Pig meat and products thereof	1	2	0	0
Vegetables and juices and other products thereof	1	3	3	1
Year 2014				
Buffet meals	1	4	4	0
Mixed food	1	2	2	0
Other or mixed red meat and products thereof	1	4	0	0
Vegetables and juices and other products thereof	1	31	0	4
Year 2015				
Buffet meals	1	13	1	0
Mixed food	3	175	6	0
Pig meat and products thereof	1	12	12	2
Vegetables and juices and other products thereof	1	3	1	0
Year 2016				
Meat and meat products	2	17	13	1
Total	32	419	135	27

(a): Although mandatory, not all Member States report the complete information on foodborne outbreaks to EFSA and consequently the information summarised in this table may not be exhaustive for certain countries.

Table 2. Isolations of *L. monocytogenes from fruit and vegetables reported to EFSA under the framework of Directive 2003/99/EC, EU/EFTA countries, 2004–2016**

Source	N. of positive units, 2004-2010	N. of positive units, 2011	N. of positive units, 2012	N. of positive units, 2013	N. of positive units, 2014	N. of positive units, 2015	N. of positive units, 2016	Total positive units, 2004-2016
Fruits								
Pre-cut (total)		3	6		2	2		13
<i>Pre-cut frozen</i>						1		1
<i>Pre-cut ready-to-eat</i>		2	6		2	1		11
<i>Unspecified</i>		1						
Products					4	14		18
Unspecified	5	12	2	2	2	1		24
Total isolations in "fruits"	5	15	8	2	8	17		55
Fruits and vegetables								
Pre-cut (total)		19				1	2	22
<i>Pre-cut ready-to-eat</i>		3				1	2	6
<i>Unspecified</i>		16						16
Products					40		1	41
Unspecified	35	1	16					52
Total isolations in "Fruits and vegetables"	35	20	16		40	1	3	115
Vegetables								
Pre-cut (total)	11	60	12	40	56	10	7	250
<i>Pre-cut frozen</i>						5		10
<i>Pre-cut ready-to-eat</i>	10	60	12	36	55	5	5	230
<i>Unspecified</i>	1			4	1		2	10
Non pre-cut			2			2	6	20
Leaves							2	2
Products	19		2	2	1	1	2	29
<i>Cooked</i>			2	2				6
<i>Unspecified</i>	19				1	1	2	23
Unspecified	69	27	25	35	6	33	60	256
<i>Frozen</i>	16							16
Total isolations in "vegetables"	99	87	41	77	63	46	77	490
Ready-to-eat salads								
Ready-to-eat salads	41	49	27	221	48	57	28	471

* *L. monocytogenes* isolations from either detection and enumeration methods are included. Information on both ready-to-eat (RTE) and non-RTE food is included, regardless of the sampling stage.