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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

European Food Safety Authority
European Centre for Disease Prevention and Control

Abstract

An outbreak of invasive *Listeria monocytogenes* (*L. monocytogenes*) infections confirmed by whole-genome sequencing (WGS) and linked to frozen corn and possibly to other frozen vegetables has been ongoing in five EU Member States (Austria, Denmark, Finland, Sweden and the United Kingdom) since 2015. As of 15 June 2018, 47 cases have been reported and nine patients have died due to or with the infection (case fatality rate 19%). WGS analysis of 29 non-human *L. monocytogenes* isolates found them to be closely related to the multi-country human cluster of *L. monocytogenes* serogroup IVb, multi-locus sequence type 6 (ST6). The majority of the non-human isolates were obtained from 2017 season products: mainly frozen corn (13 samples), followed by frozen vegetable mixes including corn (8 samples), frozen spinach (1) and frozen green beans (1). Only one isolate was reported from a frozen vegetable mix produced in 2016, while three isolates were obtained from spinach products produced in 2018. In addition, two isolates were also obtained from two environmental samples collected at two different plants which were freezing and handling frozen vegetables in France and Hungary during the 2017 and the 2018 production seasons, respectively. The WGS analysis provides a strong microbiological link between the human and the non-human isolates and this is indicative of a common source related to frozen corn and other frozen vegetable mixes, including corn, persisting in the food chain. Traceability information for the contaminated products pointed to the source of contamination in a freezing plant in Hungary (company A). As *L. monocytogenes* IVb ST6 matching the outbreak strain has been isolated from frozen spinach and frozen green beans sampled at the Hungarian plant, it is possible that frozen vegetables other than corn which have been processed in this plant, could also be implicated as a vehicle of human infection. The finding of *L. monocytogenes* IVb, ST6 matching the outbreak strain in frozen corn and other frozen vegetables produced during the 2016, 2017 and 2018 production seasons at the plant of Hungarian company A suggests that this strain could be persisting in the environment of the processing plant after standard cleaning and disinfection procedures carried out during periods of no production activity and the rotation of the processed products. Moreover, the use of the contaminated production lines for several food products may represent an additional risk for potential cross-contamination of the various final products processed at the plant. The information available confirms contamination within the Hungarian processing plant, but does not yet enable identification of the exact point(s) and/or stage in production at which *L. monocytogenes* contamination has occurred. Further investigations, including thorough sampling and testing, are needed to identify the source of contamination at the Hungarian processing plant concerned. Consumption of frozen or non-frozen corn has been confirmed by eleven out of 26 patients interviewed from Denmark, Finland, Sweden and the United Kingdom. Of the other 15 cases, six consumed or possibly consumed frozen mixed vegetables, six did not know whether they had consumed corn or mixed vegetables and three cases reported not having consumed corn or mixed vegetables. Food business operators in Estonia, Finland, Poland and Sweden have withdrawn and recalled the implicated frozen corn products from the market. Since March 2018, the implicated Hungarian plant has been under increased official control and no frozen vegetable products from the 2018 production season have been distributed to the market yet. Following the positive findings from

food and environmental samples collected during the 2018 production, freezing activities at the affected Hungarian plant have been halted since June 2018. On 29 June 2018, the Hungarian Food Chain Safety Office banned the marketing of all frozen vegetable and frozen mixed vegetable products produced by the plant between August 2016 and June 2018, and ordered their immediate withdrawal and recall. This restrictive measure is likely to significantly reduce the risk of human infections and contain the outbreak. As the outbreak is still continuing or at least has been ongoing until very recently, there are indications that contaminated products may still be on the market or that contaminated products purchased before the recalls are still being consumed. Any potentially contaminated frozen vegetables (e.g. frozen corn, frozen vegetable mixes including corn, frozen spinach and frozen green beans) from the 2017 and 2016 production seasons could still represent a possible risk to consumers until completely withdrawn and recalled. This risk may exist even at a low level of contamination if the products are not properly cooked before consumption. In addition, new invasive listeriosis cases may be identified due to the long incubation period (1–70 days), the long shelf-lives of frozen corn products, and potential consumption of frozen vegetable products bought by consumers before the recalls and eaten without being properly cooked.

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

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Amendment: An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. On pages 1 and 18, the sentence (same sentence repeated on both pages): 'Of the other 15 cases, six consumed or possibly consumed frozen mixed vegetables, six did not know whether they had consumed corn or mixed vegetables and three cases reported not having consumed corn or mixed vegetables.' was replaced by the sentence: 'Of the 15 cases that did not report corn consumption, two replied that they had consumed non-frozen mixed vegetables, three cases reported no consumption of corn or mixed vegetables, six cases did not know if they consumed corn or mixed vegetables, four cases had possibly not consumed corn and one of these four had possibly consumed frozen mixed vegetables.'. On page 17, the sentence: 'Two cases from United Kingdom consumed the same brand of frozen corn from the same UK supermarket known to be supplied by Hungary' was replaced by the sentence: 'Two cases from United Kingdom consumed frozen corn from UK supermarket(s) known to be supplied by Hungary'. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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JOINT ECDC–EFSA RAPID OUTBREAK ASSESSMENT

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

3 July 2018

Conclusions

An outbreak of invasive *Listeria monocytogenes* (*L. monocytogenes*) infections confirmed by whole-genome sequencing (WGS) and linked to frozen corn and possibly to other frozen vegetables has been ongoing in five EU Member States (Austria, Denmark, Finland, Sweden and the United Kingdom) since 2015. As of 15 June 2018, 47 cases have been reported and nine patients have died due to or with the infection (case fatality rate 19%).

WGS analysis of 29 non-human *L. monocytogenes* isolates found them to be closely related to the multi-country human cluster of *L. monocytogenes* serogroup IVb, multi-locus sequence type 6 (ST6). The majority of the non-human isolates were obtained from 2017 season products: mainly frozen corn (13 samples), followed by frozen vegetable mixes including corn (8 samples), frozen spinach (1) and frozen green beans (1). Only one isolate was reported from a frozen vegetable mix produced in 2016, while three isolates were obtained from spinach products produced in 2018. In addition, two isolates were also obtained from two environmental samples collected at two different plants which were freezing and handling frozen vegetables in France and Hungary during the 2017 and the 2018 production seasons, respectively.

The WGS analysis provides a strong microbiological link between the human and the non-human isolates and this is indicative of a common source related to frozen corn and other frozen vegetable mixes, including corn, persisting in the food chain. Traceability information for the contaminated products pointed to the source of contamination in a freezing plant in Hungary (company A). As *L. monocytogenes* IVb ST6 matching the outbreak strain has been isolated from frozen spinach and frozen green beans sampled at the Hungarian plant, it is possible that frozen vegetables other than corn which have been processed in this plant, could also be implicated as a vehicle of human infection.

The finding of *L. monocytogenes* IVb, ST6 matching the outbreak strain in frozen corn and other frozen vegetables produced during the 2016, 2017 and 2018 production seasons at the plant of Hungarian company A suggests that this strain could be persisting in the environment of the processing plant after standard cleaning and disinfection procedures carried out during periods of no production activity and the rotation of the processed products. Moreover, the use of the contaminated production lines for several food products may represent an additional risk for potential cross-contamination of the various final products processed at the plant. The information available

Errata

On 9 July 2018, the following corrections were made: p. 1 (last paragraph, last sentence), p. 18 (paragraph 5, third sentence) now read: 'Of the 15 cases that did not report corn consumption, two replied that they had consumed non-frozen mixed vegetables, three cases reported no consumption of corn or mixed vegetables, six cases did not know if they consumed corn or mixed vegetables, four cases had possibly not consumed corn and one of these four had possibly consumed frozen mixed vegetables'; p. 17 (paragraph 7, third sentence, now reads: 'Two cases from the United Kingdom consumed frozen corn from the UK supermarket(s) known to be supplied by Hungary'.

confirms contamination within the Hungarian processing plant, but does not yet enable identification of the exact point(s) and/or stage in production at which *L. monocytogenes* contamination has occurred. Further investigations, including thorough sampling and testing, are needed to identify the source of contamination at the Hungarian processing plant concerned. Consumption of frozen or non-frozen corn has been confirmed by eleven out of 26 patients interviewed from Denmark, Finland, Sweden and the United Kingdom. Of the 15 cases that did not report corn consumption, two replied that they had consumed non-frozen mixed vegetables, three cases reported no consumption of corn or mixed vegetables, six cases did not know if they consumed corn or mixed vegetables, four cases had possibly not consumed corn and one of these four had possibly consumed frozen mixed vegetables.

Food business operators in Estonia, Finland, Poland and Sweden have withdrawn and recalled the implicated frozen corn products from the market. Since March 2018, the implicated Hungarian plant has been under increased official control and no frozen vegetable products from the 2018 production season have been distributed to the market yet. Following the positive findings from food and environmental samples collected during the 2018 production, freezing activities at the affected Hungarian plant have been halted since June 2018. On 29 June 2018, the Hungarian Food Chain Safety Office banned the marketing of all frozen vegetable and frozen mixed vegetable products produced by the plant between August 2016 and June 2018, and ordered their immediate withdrawal and recall. This restrictive measure is likely to significantly reduce the risk of human infections and contain the outbreak.

As the outbreak is still continuing or at least has been ongoing until very recently, there are indications that contaminated products may still be on the market or that contaminated products purchased before the recalls are still being consumed. Any potentially contaminated frozen vegetables (e.g. frozen corn, frozen vegetable mixes including corn, frozen spinach and frozen green beans) from the 2017 and 2016 production seasons could still represent a possible risk to consumers until completely withdrawn and recalled. This risk may exist even at a low level of contamination if the products are not properly cooked before consumption. In addition, new invasive listeriosis cases may be identified due to the long incubation period (1–70 days), the long shelf-lives of frozen corn products, and potential consumption of frozen vegetable products bought by consumers before the recalls and eaten without being properly cooked.

Options for response

In order to identify the exact point(s) and/or stage of production where the contamination with *L. monocytogenes* has occurred at the plant of Hungarian company A, it is strongly recommended that thorough sampling and testing are carried out at the critical sampling sites along the production lines. This should follow EFSA's recommendations for sampling and testing at frozen vegetable processing plants to detect *L. monocytogenes* [1a]. EFSA's guidelines, intended for both competent authorities and food business operators and requested by the European Commission, focus on sampling to identify the point of microbiological contamination at plants processing frozen vegetables, fruit and herbs, in particular during outbreak investigation.

It is strongly recommended that the processing plant concerned is completely cleaned and disinfected, which involves dismantling and thoroughly cleaning and disinfecting all the plant equipment, as well as any additional surfaces that may represent a point of *L. monocytogenes* contamination (e.g. refrigerator system).

In order to avoid *L. monocytogenes* being introduced into a plant through workers (e.g. uniforms, shoes, personnel) it is important that appropriate hygiene measures are adopted by food business operators.

The recommendations above also apply to other companies belonging to the same commercial group as the Hungarian company A if environmental contamination with *L. monocytogenes* is detected in their plants.

In order to reduce the risk of *L. monocytogenes* infection due to the consumption of contaminated non ready-to-eat frozen vegetables, consumers should thoroughly cook these products before consumption, as it is not unusual for them to be consumed without being cooked (e.g. in salads, smoothies). The affected countries are advised to consider targeted communication options (e.g. campaigns to inform consumers to properly cook frozen vegetables originating from potentially contaminated batches at the affected plants).

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, vegetable mixes, spinach, green beans and other (frozen) vegetables.

ECDC is supporting WGS analysis of human isolates from cases possibly related to this outbreak and reported in countries that do not routinely perform WGS. The European Reference Laboratory for *L. monocytogenes* (EURL for *Lm*) is providing support to those Member States who have no WGS capacity 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 epidemiological, microbiological and environmental investigations, including tracing information, and by issuing relevant notifications using the Early Warning and Response System (EWRS) and the Rapid Alert System for Food and Feed (RASFF).

EWRS is a rapid alert system for notifying alerts at EU level in relation to serious cross-border threats to health of biological, chemical, environmental or unknown origin. The EWRS enables the Commission and the competent authorities of the Member States to be in permanent communication for the purposes of alerting, assessing public health risks and determining the measures that may be required to protect public health. National competent authorities should notify an alert in EWRS where the development or emergence of a serious cross-border threat to health fulfils the criteria listed in Article 9 of Decision 1082/2013/EU on serious cross-border threats to health.

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, traceability information on the suspected food vehicles and analytical results to support traceability investigations.

Source and date of request

On 11 April 2018, the European Commission sent a request to ECDC and EFSA to update the Rapid Outbreak Assessment published on 22 March 2018, and this request was accepted by EFSA and ECDC on 12 April 2018 [1].

Public health issue

This document provides an updated assessment of the cross-border public health risk associated with consumption of frozen corn and possibly linked to other frozen vegetables contaminated with *L. monocytogenes*. ECDC published a rapid risk assessment concerning this event on 6 December 2017 [2] and a joint ECDC-EFSA Rapid Outbreak Assessment was published on 22 March 2018 [1b].

Consulted experts

- ECDC experts (in alphabetical order): Margot Einöder-Moreno, Saara Kotila, Taina Niskanen, Ettore Severi, Johanna Takkinen, Therese Westrell.
- EFSA experts (in alphabetical order): Giusi Amore, Raquel Garcia Fierro, Ernesto Liebana Criado, Valentina Rizzi.
- European Reference Laboratory for *Listeria monocytogenes* (EURL for *Lm*): Benjamin Felix, Jean Charles Leblanc, Bertrand Lombard, Jean-François Mariet, Maroua Sayeb.
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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. Responsibility regarding the choice of option and 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 3 July 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 on listeriosis can be found in ECDC, US CDC and WHO disease fact sheets [3-5]. *L. monocytogenes* ST6 is a hypervirulent clone of *L. monocytogenes* associated with neurological forms of listeriosis [6,7]. Pregnant women, the elderly, and immunocompromised individuals are at increased risk of invasive listeriosis, which is associated with severe clinical course and potentially death.

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 [8]. PCR serogroup IVb [9] 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 [10].

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 [11]) and the other one with 20 allelic differences).

Growth of *Listeria monocytogenes* in frozen vegetables

A recent study has investigated the growth characteristics of *L. monocytogenes* inoculated onto frozen foods (including blanched, individually quick-frozen corn and individually quick-frozen green peas) and thawed by being stored at 4, 8, 12, and 20 °C [12]. The results of this study showed that thawed frozen corn and green peas supported the growth of *L. monocytogenes* at each of the storage temperatures, with the growth rate increasing with the temperature. This research demonstrated using real food samples that *L. monocytogenes* can initiate growth without a prolonged lag phase after being frozen, even at refrigeration temperature (4 °C).

The growth of *L. monocytogenes* in fresh corn and green peas was also observed in an older study [13].

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. At the time the event was reported, two patients had 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) for 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 [11,14].

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).

A second PFGE profile was described from non-human isolates matching the outbreak genomic profile. The analysis of the profile is on-going to determine the reference type.

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).

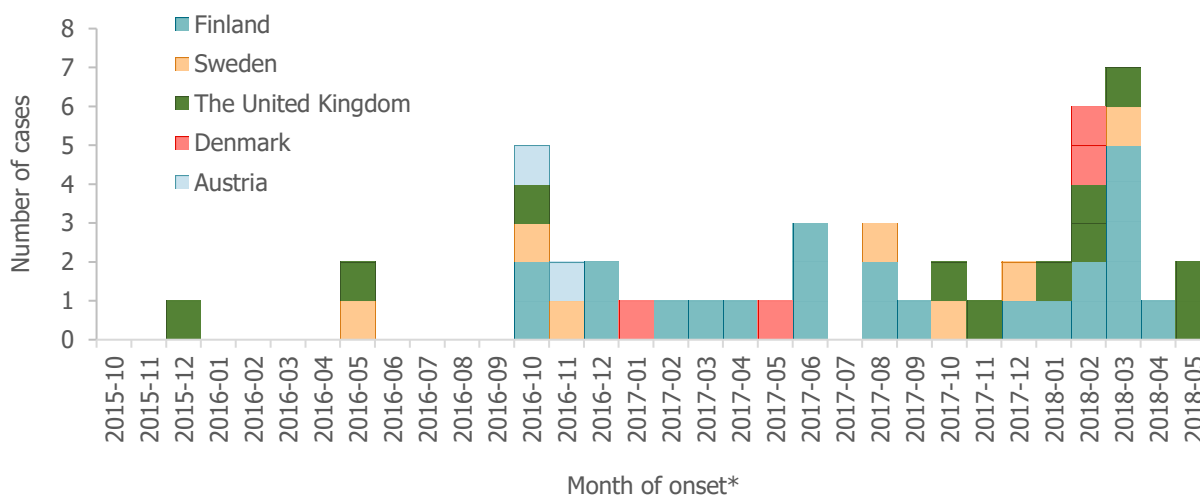
Based on the European outbreak case definition, as of 15 June 2018, a multi-country foodborne outbreak has been verified in five countries, involving 47 confirmed cases and nine deaths due to or with the infection. Cases were detected in Finland (23 cases), United Kingdom (11 cases), Sweden (7 cases), Denmark (4 cases) and Austria (2 cases) (Table 1, Figure 1). The median age of cases was 72 years (interquartile range 56–85), 26 (55%) cases were females. Information on hospitalisation was available for 16 patients, who were all hospitalised.

Table 1. *Listeria monocytogenes* IVb, ST6 confirmed outbreak cases by country and year, EU 2015–2018 (as of 15 June 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	4	10 (2)	9	23	2
Sweden	0	3 (1)	3 (1)	1 (1)	7	3
United Kingdom	1	2	2 (2)	6	11	2
Total	1 (0)	11 (2)	17 (5)	18 (2)	47	9

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

Figure 1. *Listeria monocytogenes* PCR serogroup IVb, ST6 confirmed outbreak cases by month of symptom onset*, European Union 2015–2018 (n=47)



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

Food and environmental investigations

This section summarises country-specific information on food and environmental investigations associated with this outbreak reported as of 29 June 2018 through RASFF (news 17-849 and alert 2018.0216), EPIS FWD (UI- 444) and directly to EFSA by national competent authorities or provided by EURL for *Lm* since 22 March 2018 (publication date of the first Rapid Outbreak Assessment) [1]. A short summary of the information included in the published Rapid Outbreak Assessment is provided at the beginning of each country section.

Food and environmental investigations are ongoing in the Member States concerned. A teleconference with food crisis coordinators was organised by the Commission on 20 April 2018 to discuss the increase of cases, risk management action and the investigation required. The Commission is closely following this event with Member States' competent authorities through RASFF to ensure that the appropriate risk management action is taken and that the relevant countries are promptly informed about distribution in their countries.

Finland

Overall, **seven food isolates matching the multi-country outbreak strain** (0-5 allelic differences) from the following batches of frozen corn (2017 production season) have been reported in Finland (RASFF alert 2018.0216, follow-up 35, issued on 12 April 2018; information provided by EURL for *Lm* on 19 June 2018):

- Batches A, B, D and E of frozen corn originating from Hungary
- Batch J (two isolates obtained from this batch) and K of frozen corn originating from Belgium.

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

As of 21 March 2018, Finland reported the presence of *L. monocytogenes* in two batches of frozen corn (batches A and B of the brand A) and in two additional batches of frozen corn (batch D and batch E) sampled at the premises of the Finnish trader/broker A. For the latter two batches, WGS analysis confirmed a match with the Finnish outbreak strain.

All these batches were delivered by the Polish company C who packed the product originating from Hungary. Batches A and B were then dispatched to the Finnish wholesaler A, who distributed the product on the Finnish market, as well as to a retailer B in Estonia.

The consumption of corn from brand A was confirmed for one Finnish patient but no information was provided on the batch number of the product consumed.

New information

The two *L. monocytogenes* isolates detected from batches A and B of frozen corn were closely related by WGS to the Finnish human outbreak strain (0-1 allelic differences using cgMLST).

The EURL for *Lm* has performed WGS analyses on 15 additional *L. monocytogenes* isolates obtained in Finland from official food samples collected at border control points (upon entry to Finland). Eight of these 15 isolates were from frozen corn (two batches originating from Hungary, one batch from the Netherlands and five batches from Belgium), three isolates were from frozen peas-corn-bell pepper mix (originating from Hungary), two isolates from frozen vegetables (originating from Belgium) and two isolates from frozen bell peppers (originating from Hungary). Of these isolates, three obtained from two batches of frozen corn (batches J and K originating from Belgium) matched the outbreak strain (4-5 allele differences). According to information provided to EFSA by the Finnish Competent Authority on 27 June 2018, the contaminated batches J and K were produced at the Belgian company E. No further details are currently available on the origin of the frozen corn used to produce batch J and K.

Sweden

Overall, **two food isolates matching the multi-country outbreak strain** (4-5 allelic differences) from the following batches of frozen corn (2017 production season) originating from Hungary have been reported in Sweden:

- Batches A and G of frozen corn.

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

As of 21 March 2018, Sweden reported the presence of *L. monocytogenes* in the batch A of frozen corn taken from an opened package stored in a consumer's fridge. WGS analysis confirmed a match of this isolate with the Finnish outbreak strain (4 allelic differences). The product was bought at the Swedish retailer A and was delivered by the Polish company C who packed the product originating from Hungary.

The consumption of corn was confirmed for the most recent Swedish patient but no information was provided on the brand or batch number of the product consumed.

New information

L. monocytogenes was isolated in frozen corn (batch G) sampled during own checks carried out by the Swedish retailer A (RASFF alert 2018.0216 follow-up 33, issued on 12 April 2018). WGS analysis confirmed that this isolate found in frozen corn was clustered (5 allelic differences) to the *L. monocytogenes* ST6 representative outbreak strain (RASFF alert 2018.0216, follow-up 42, issued on 23 April 2018).

Batch G of frozen corn was delivered to the Swedish retailer A on 13 March 2018 by the Polish company C that packed the product originating from the Hungarian company A (RASFF alert 2018.0216, follow-up 41, issued on 23 April 2018).

Batch G of frozen corn has been withdrawn and recalled from the Swedish retailers. Another product containing corn, peas and red peppers was also withdrawn and recalled as a precautionary measure. The latter product was also packed at the Polish company C and processed at the Hungarian company A.

Estonia

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

After the communication from the Finnish wholesaler A concerning the presence of *L. monocytogenes* in frozen corn from batches A and B, the Estonian retailer B recalled all batches of frozen corn of brand A from its clients.

New information

No update from or in relation to Estonia.

Poland

Overall, **two food isolates matching the multi-country outbreak strain** (3 allelic differences) from the following batches of frozen corn (2017 production season) originating from Hungary have been reported in Poland:

- Batches A and C of frozen corn.

Summary from the previous ROA, published on 22 March 2018

As of 21 March, the Polish company C reported finding *L. monocytogenes* in the batches A, B, C and D of frozen corn. In total 14 batches of frozen corn (initial product) used to produce these batches were tested and 10 of them were contaminated with *L. monocytogenes*. All environmental samples taken at the premises of the Polish company C in November and December 2017 were *L. monocytogenes* negative.

The initial products were delivered to the Polish company C by the Polish company B, which provides only storage services, and originated from the Hungarian company A. The Polish company C dispatched batches A, B and D to the Finnish trader/broker A, and batches A and C to the Swedish retailer A. An additional batch E was also produced by the Polish Company C and delivered to Finnish trader/broker A where it was found positive for *L. monocytogenes*.

New information

WGS analysis confirmed that the two *L. monocytogenes* IVb ST6 isolates found in batches A and C of frozen corn originating from Hungary were closely clustered (3 allelic differences) to the representative outbreak strain (information provided by the EURL for *Lm*).

In addition, *L. monocytogenes* <10 cfu/g was reported in three own-check samples from batch G of frozen corn sampled at the Polish company C. Batch G originated from the Hungarian company A, was packed in the Polish company C and then distributed to Sweden. Own-check samples from the two batches of frozen corn (initial materials) (batches X30 and X31) used for the production of batch G were also tested for enumeration and *L. monocytogenes* <10 cfu/g and 10 cfu/g was reported (RASFF news 17-849, follow-up 39, issued on 27 April 2018).

Between 24 April and 8 May 2018, one hundred official environmental samples collected at the plant of Polish company C were tested and two of them, taken from a mixer and a sealing silicone in the packaging hall, were found positive for *L. monocytogenes*. Following these analytical results, the Chief Sanitary Inspector requested the Polish plant to stop operating and conduct thorough cleaning and disinfection to eliminate *L. monocytogenes* from its environment (RASFF alert 2018.2016, fup-48; issued on 11 May 2018). According to serotyping performed by the Polish National Reference Laboratory, the two environmental isolates were type IIa and therefore not related to this outbreak. After thorough cleaning and disinfection at Polish Company C's plant, another 100 official swab environmental samples were collected and all were negative for *L. monocytogenes* (RASFF alert 2018.2016, fup-50, issued on 5 June 2018).

Between 19 and 29 May 2018, 45 samples taken from eight batches of frozen products (including vegetables and fruit) sampled at the plant of Polish company C were tested and *L. monocytogenes* was detected in five samples from frozen vegetables for frying and three samples from frozen corn. Serotyping is ongoing. The remaining samples collected from fruit (raspberry, forest fruit mix, forest fruit mix (pre-mixture), blackcurrant class A, raspberry >80% and strawberry) were negative for *L. monocytogenes* (RASFF alert 2018.2016, fup-50, issued on 5 June 2018).

Hungary

Overall, **11 non-human isolates matched the multi-country outbreak strain** (3-6 allelic differences) from the following batches of frozen products and the environment at Hungarian company A:

- five isolates from batches H, I, L, M and N of frozen vegetable mixes (containing frozen corn and peas from Hungary and baby carrots from Belgium) sampled on 5 March 2018 (2017 production season)
- One isolate from batch T of frozen spinach sampled on 27 April 2018 (2017 production season)
- One isolate from batch U of frozen green beans sampled on 27 April 2018 (2017 production season)
- Two isolates from batch V of creamy spinach puree sampled before and after freezing on 17 May 2018 (2018 production season)
- One isolate from batch Y of frozen creamy spinach puree sampled on 17 May 2018 (2018 production season)
- One isolate from an environmental sample (floor drain at the packaging area) collected on 17 May 2018.

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

As of 21 March 2018, the Hungarian company A reported having tested several batches of frozen raw materials that were processed at the plant. Based on the enumeration method, all batches had results for *L. monocytogenes* < 10 cfu/g, with the exception of one batch (batch X28) where *L. monocytogenes* serogroup IIa (not related to the present outbreak) was found at levels of 1.4×10^3 cfu/g. In total, 11 batches of frozen corn (initial product) (X1, X2, X12, X15, X16, X21, X23, X25, X26, X27 and X28) used in Poland for the production of batches A, B, C, D and E were produced in 2017 in the same growing area A of 31 hectares by the Hungarian supplier A and processed at Hungarian company A.

New information

The main company supplying vegetables to the Hungarian company A was the Hungarian supplier A, which ceased operations at the end of 2017. At that time, company A had already concluded contracts for cultivation and supply of corn (supersweet, normal sweet) and beans with another Hungarian company (supplier B) of the same group as supplier A (RASFF ref 2018.0216, follow-up 40, issues on 23 April 2018).

The Hungarian company A is a freezing company that produces individually quick-frozen (IQF) vegetables such as peas, yellow- and green beans, spotted beans, corn (sweet, supersweet), root vegetables and leafy vegetables (spinach, sorrel, parsley leaf). Company A's plant freezes only vegetables. The production at Hungarian company A's plant is linked to the agricultural season. This company deals mainly with the production of quick-frozen vegetables during the respective seasons, and with the packaging of quick-frozen vegetables and fruits outside of the seasons. At this processing plant there are three production lines: production of IQF peas, corn, beans and root vegetables; production of peas and corn with Frigoscandia freezer and production of frozen leafy vegetables with contact freezer. The same production lines are used for several vegetables; cleaning and disinfection are carried out during the conversion of the production lines (RASFF alert 2018.0216, fup-49). No fruit is frozen and no prepared meals are processed in the factory. In addition to freezing, handling activities (e.g. storing and packing) are also undertaken at this plant. Packaging of frozen fruit (frozen by other companies) and vegetable mixes is carried out in a separate area of the plant. Some of the frozen vegetables included in the vegetable mixes are frozen by other companies (e.g. baby carrots from the Belgian company E) (RASFF alert 2018.0216, fup-57).

Information related to production season 2016

During August–September 2016, Hungarian company A's plant produced the batch F of frozen corn using four batches of frozen corn (initial products). This batch was delivered on 5 January 2017 to the French company G, which distributed it to French company F (RASFF news 17-849, fup-42 and fup-46; issued on 14 and 24 May 2018). The testing results of batch F are described in the country section 'France'.

Information related to production season 2017

On 5 March 2018, official samples were taken from several frozen products (from 2017 production season) at the plant of the Hungarian company A and *L. monocytogenes* IVb ST6 matching the outbreak strain (3-4 allelic differences) was detected in five batches of 'frozen classical vegetable mix' (RASFF news 17-849, follow-up 29, issued on 13 April 2018; WGS results provided by EURL for *Lm* on 19 June 2018):

- Batches H, I and L: contaminated with *L. monocytogenes* at level of 60 cfu/g.
- Batch M: contaminated with *L. monocytogenes* at level of 50 cfu/g.
- Batch N: contaminated with *L. monocytogenes* at level of 30 cfu/g.

These five batches (H, I, L, M, N) and two additional batches of "frozen classical vegetable mixes" (batches O and P) processed at the plant of Hungarian company A, were distributed to the Austrian retail chain C. The seven batches of 'frozen classical vegetable mixes' comprised three ingredients: peas (6-9 mm), baby carrots and corn. The peas and corn originated from Hungary, while the baby carrots were supplied by the Belgian company E to the Hungarian processing company A. (RASFF news 17-849; follow-up 46, issued on 24 May 2018).

There was a six-month seasonal period during which freezing activity was interrupted at the plant of Hungarian company A between November 2017 and May 2018.

In order to verify if *L. monocytogenes* IVb was still present after the period of seasonal inactivity and following cleaning and disinfection at the plant, the Hungarian competent authorities carried out two official samplings at Hungarian company A's plant on 27 April and 17 May 2018. In particular, on 27 April (during the non-production period) several frozen vegetables from the 2017 production season were sampled from the cold store (room temperature -18 °C) of the plant, and *L. monocytogenes* was found in spinach, green beans, cubed carrots, corn, peas and zucchini.

Two isolates of *L. monocytogenes* IVb ST6 matching the outbreak strain were isolated from:

- Batch T of natural frozen spinach (final product from consumer packaging). This final product had undergone the following processing stages before sampling: blanching, cutting and freezing (by plate freezing).
- Batch U of quick frozen green beans (semi-finished product for further packaging). This semi-finished product had undergone the following processing before sampling: blanching and IQF.

The isolates from the other positive samples were *L. monocytogenes* type IIa and therefore not related to the outbreak strain.

Information related to production season 2018

The first vegetable processed at company A's plant in 2018 was spinach, followed by IQF green peas, while corn was planned to be processed from the end of summer onwards.

On 17 May 2018, several environmental and food samples were collected at the plant of Hungarian company A during different phases of the spinach production (2018 production season). Overall, the following environmental, water and food samples were taken:

- Four environmental samples were tested: three swab samples from bands (sorter band, blanching band and band leading to grinding machine) and one swab sample from floor drain at the packaging area, the latter being the only contaminated with *L. monocytogenes* IVb ST6 matching the outbreak strain with WGS. This positive environmental sample was taken from the floor drain near the filling machine, at the point where the creamy spinach puree was filled into consumer packaging, during the production.
- Two samples of water (pre-cooled water and incoming ice water) were also tested and found negative.
- In addition, five spinach samples from the same batch (batch V) were collected before and during the different processing stages, from fresh leafy spinach (initial product) to creamy spinach puree (final product): 1) fresh leafy spinach at reception (before processing); 2) spinach after washing; 3) spinach at grinder (after blanching and cooling); 4) creamy spinach puree after closing the package (after grinding and creaming, before freezing); 5) frozen creamy spinach puree (the creamy spinach puree is frozen after packaging, using contact freezer). *L. monocytogenes* serogroup IVb was detected only in the last three samples. WGS was performed only for two of the three isolates obtained in the positive samples from the batch V, both isolates matched the outbreak strain (Table 2). It is important to note that the grinding stage (where the first positive sample was taken) occurs after blanching (at 96°C for 110 seconds) and cooling (max 7 °C) of the spinach.
- Furthermore, another two different batches of creamy spinach puree (batches W and Y) were sampled and found positive for *L. monocytogenes*, and the isolate from batch Y matched the outbreak strain. Creamy spinach puree is not a ready-to-eat product. The following cooking instructions are supplied on the packaging: boil for one minute in a cooking pot or cook for 14 minutes in microwave oven at 600W.

WGS was performed for four non-human isolates (isolated from samples 04, 09, 10 and 12, see Table 2) and confirmed the match with the outbreak strain (3–6 allelic differences). Details on the type of products, the sampling location and the corresponding processing stages, as well as the testing results, are presented in Table 2.

Table 2. *L. monocytogenes* testing results of non-human official samples collected during the different phases of the 2018 spinach production at Hungarian company A's plant (sampling date 17 May 2018)

Sample ID	Sample type	Sampling location and/or processing stage	Batch code	Test results		
				Detection	Enumeration (cfu/g)	WGS*
Environmental samples						
01	swab	Sorter band during operation	NA	Negative	-	
02	swab	Blanching band during operation	NA	Negative	-	
03	swab	Band leading to grinding machine during operation	NA	Negative	-	
04	Swab of floor drain	Packaging area during operation	NA	Positive	-	match
05a	Pre-cooled water		NA	Negative	-	
05b	Incoming ice water		NA	Negative	-	
Food samples						
06	Fresh leafy spinach		V	Negative	-	
07	Sample of spinach	After washing	V	Negative	-	
08	Sample of spinach	Grinder	V	Positive	<10 cfu/g	NA**
09	Not frozen creamy spinach puree	After closing of the package	V	Positive	<10 cfu/g	match
10	Frozen creamy spinach puree	After freezing	V	Positive	10 cfu/g	match
11	Frozen creamy spinach puree	Final product	W	Positive	Up to 30 cfu/g	NA**
12	Frozen creamy spinach puree	Final product	Y	Positive	<10 cfu/g	match

* WGS match with the multi-country outbreak strain (cgMLST <7 allelic differences).

** Please note that WGS was not performed for one of the three isolates obtained from the positive samples of batch V, or from the contaminated batch W. Thus, the WGS results for two positive samples (IDs 08 and 11) are not available (NA).

Distribution and control measures

The frozen corn and frozen vegetable mixes produced at the plant of Hungarian company A have been distributed to other plants belonging to the same Company group A in other EU Member States (Belgium, United Kingdom, Germany, France and Poland). For further information on the branches of company group A in the different countries, see the section for Belgium). The final products have been also distributed to the following Member States: Romania, Italy, Slovenia, Slovakia, Germany, Finland, Czech Republic, Croatia and Austria. Frozen vegetable mixes were delivered only to the Austrian retailer C (RASFF 17-849, fup 42). No detailed documentation has been provided on the distributed products.

The following measures have been implemented by Hungary (RASFF news 2018.0216, follow-up 30, issued on 28 March 2018; RASFF ref 2018.0216, follow-up 36, issued on 12 April 2018; RASFF 17-849, follow-up 42, issued on 14 May 2018; most recent information provided to EFSA by email on 29 June 2018):

- At production level (Hungarian company A) (taken and ongoing):
 - complete revision of the HACCP system at the plant;
 - continuous cleaning and disinfection of the equipment;
 - product labelled with clear instructions on the need to heat treat;
 - review of the water supply system;
 - revision of the microbiological control plan (increase in the numbers of samples and sampling points);
 - new cleaning and disinfection plan developed;
 - measures taken to correct and eliminate the risk of contamination: built a 'double firewall' security measure for all products, implying that both semi-finished and end-user products can be released for marketing, used or delivered only after accredited laboratory test results;
 - the Hungarian plant is under increased official control since March 2018 and in accordance with the measures (ordered by the competent authority and implemented voluntarily by the food business operator), no product from the 2018 production season has been marketed yet;

- based on the laboratory results of the environmental and food samples collected at different phases of the spinach and green pea production in May 2018, the Hungarian competent authority recently decided to stop freezing activity at the affected plant.
- At distribution level:
 - On 29 June 2018, the Hungarian Food Chain Safety Office banned the marketing of all frozen vegetables and frozen mixed vegetable products produced by the Hungarian plant concerned between August 2016 and June 2018, and ordered their immediate withdrawal and recall.
- At retail level:
 - Clearly visible posters were placed next to/on the shelves of the products concerned with instructions concerning the safe use of the products.
- At consumer level (public warning):
 - Official press release
 - Communication through web and social media (Twitter, Facebook, official website, other media).

Austria

Overall, **four food isolates matching the multi-country outbreak strain** (3-4 allelic differences) from the following batches of frozen vegetable mixes (all from 2017 production season, but one from 2016) have been reported by Austria:

- batch Q and S of 'frozen Mexican vegetable mixes'
- batches O and P of 'frozen classical vegetable mix'.

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

As of 21 March 2018, Austria reported two food isolates matching the multi-country cluster of *L. monocytogenes* ST6:

- One isolate was detected in 2016 from the product 'frozen classical vegetable mix' (batch O), produced and packed by company A in Hungary. Ingredients comprised: peas and corn (both originated from Hungary) and baby carrots (supplied by the Belgian company E (RASFF news 17-849; follow-ups 14, 15, 20 and 46; see also specific section for Hungary).
- The other isolate was detected in 2017 from a sample of 'frozen Mexican vegetable mix' (batch Q) brand B produced at the Belgian Company D. The frozen corn included in the vegetable mix originated from the Hungarian processing company A (RASFF news. 17-849; follow-ups 14, 15 and 20).

New information

Two additional *L. monocytogenes* isolates matching the multi-country cluster of *L. monocytogenes* ST6 were reported in the framework of a study carried out in Austria from the following batches of frozen vegetable mixes (RASFF news 17-849, follow-ups 33):

- Batch P of 'frozen classical vegetable mix', comprising the same ingredients as batch O, originating from Hungarian company A.
- Batch S of 'frozen Mexican vegetable mix' originating from Belgian company E. *L. monocytogenes* IVb was detected at low levels (< 10 cfu/g). The ingredients used in this batch of 'frozen Mexican vegetable mixes' included: corn, red kidney beans, onion, red and yellow paprika, spices, etc. The frozen corn used in this vegetable mix originated from processing company A in Hungary, while the beans originated from Belgian company E (RASFF news 17-849; follow-up 44). The following two sets of preparation instructions are reported as appearing on the label of this 'frozen Mexican vegetable mix': Heat for 7–9 minutes in a skillet wok or 9 minutes at 600W in the microwave oven (RASFF news 17-849; follow-up 44, issued on....)

L. monocytogenes IVb was reported in an additional batch of 'frozen Mexican vegetable mix' (batch R) with the same ingredients, origin and label indications as batch S. However, according to the WGS results, the *L. monocytogenes* IVb isolate obtained from batch R was not genetically related to the outbreak strain.

In addition, *L. monocytogenes* serotype IIa, not related to this outbreak, was also detected in batches P and R.

Belgium

Overall, information on **two food isolates matching the multi-country outbreak strain** (1-2 allelic differences) in frozen corn originating from the Hungarian company A (2017 production season) was provided by Belgium.

The Belgian company E is the legal owner of the companies belonging to the same group and located in different countries: Belgium (company D), Poland (company C), Hungary (company A) and France (company G). It is also the

legal owner of other companies belonging to the same group in different countries (for example, two branches in the United Kingdom). However, most of the branches in the different countries operate independently. Specifically, Belgian company D is dependent on company E, whereas the Polish and the French branches are run from their respective countries, and the Hungarian company A is operationally independent (RASFF news 17-849, fup 25).

Analyses were carried out by the Belgian company D on frozen corn (initial products). Two samples of frozen corn originating from the Hungarian company A were tested and found to be contaminated with *L. monocytogenes* IVb, ST6 and clustered closely (1-2 allelic differences) to the representative outbreak strain. In one of the two samples (taken from an already packaged product, batch Z1) the result for *L. monocytogenes* was <10 cfu/g, while the other sample (taken from a raw material that was repackaged, batch Z2) had a level of contamination of 80 cfu/g.

In parallel, the Belgian authorities took four official samples of the Hungarian corn used in batches Q, R and S of the 'frozen Mexican vegetable mixes'. These four samples were found to be contaminated with *L. monocytogenes* 1/2b at a level of <100 cfu/g (RASFF news 17-849, fup58).

At present, no action has been taken by the Belgian authorities.

France

Overall, one environmental isolate matching the multi-country outbreak strain (4 allelic differences) has been reported by France.

Summary from the previous Rapid Outbreak Assessment, published on 22 March 2018

As of 21 March 2018, France had reported one non-human isolate matching the multi-country cluster of *L. monocytogenes* serogroup IVb ST6 originating from an environmental sample collected at a food processing plant (company F) during own checks in August 2017 in an area where the following products could have been processed: frozen flat-leaved parsley, soft corn grains (frozen), potato cubes, green peas (frozen) (RASFF ref.17-849, follow-up 24, issued on 21 March 2018).

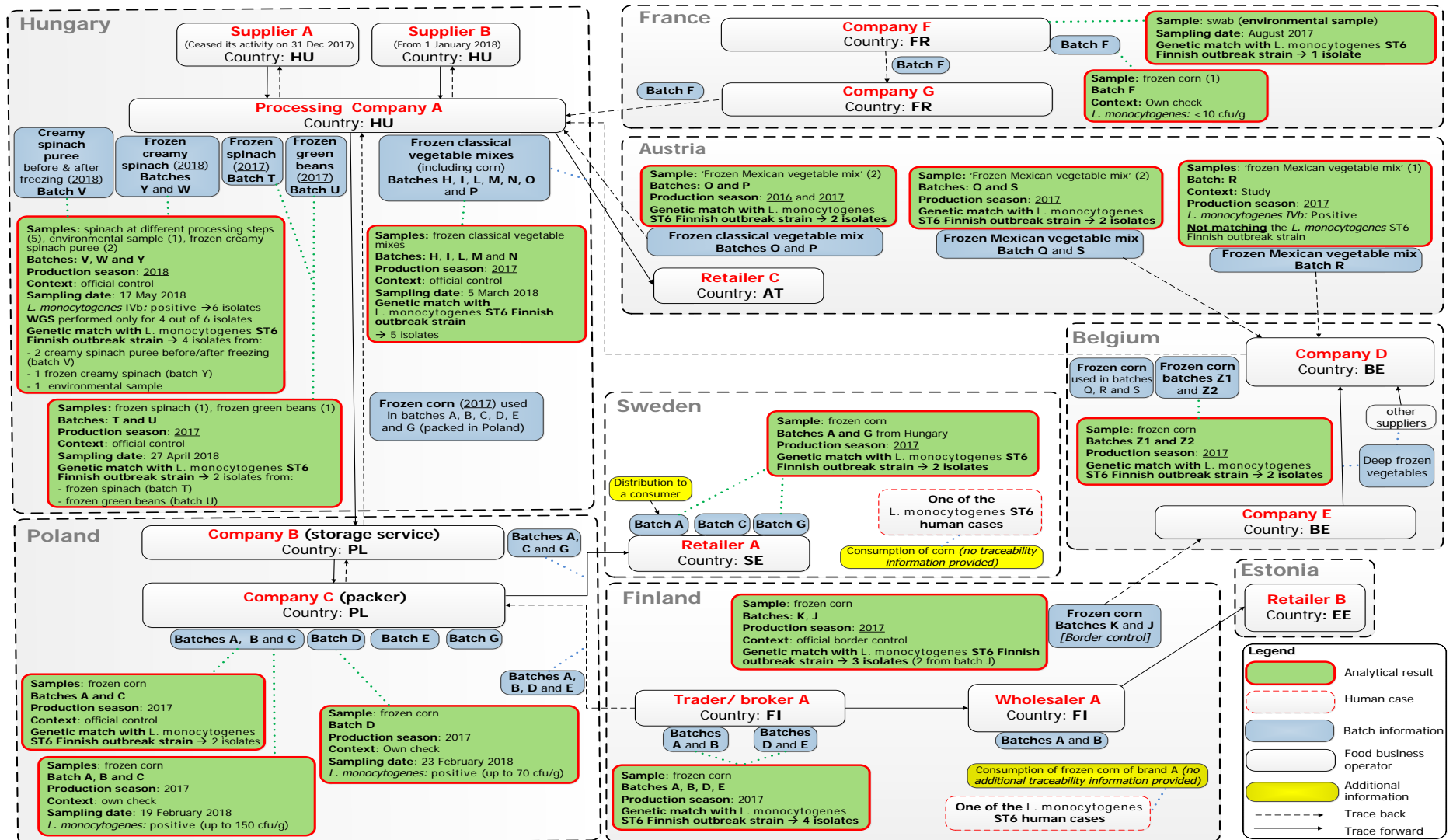
New information

L. monocytogenes was also isolated from a batch of frozen corn (batch F) sampled at French company F and supplied by French company G, which received this batch from the Hungarian company A (RASFF news 17-849, follow-up 26). The isolate obtained from batch F was not kept and therefore it was not possible to compare it with the outbreak strain.

United Kingdom

Food and environmental investigations are ongoing in the plants belonging to Company group A, which source corn from the Hungarian company A. No food or environmental isolates have yet been made available for further testing or comparison with the outbreak strain.

Figure 3. Graphical representation of traceability and testing information available in RASFF or provided to EFSA by Member States, as of 29 June 2018



Note: cfu/g: colony-forming unit per gram. AT: Austria, BE: Belgium, EE: Estonia, FI: Finland, FR: France; HU: Hungary, PL: Poland, SE: Sweden

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 WGS results from the EURL *Lm* are presented below, and only major differences in the results obtained with other pipelines are indicated (see Table 3 footnote).

The reads 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. The cgMLST analysis was performed using assembly-based and assembly-free allele calling with the Moura scheme [11] 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. Extracted fasta format allele sequences generated through mapping to reference alleles were used (replacing the assembly) to analyse data generated with IonTorrent platform not passing the QC. This was to mitigate the issues of indels that are inherent in the Ion Torrent assemblies generated from raw reads. Results from this analysis are described in Table 3 below and visualised in Figure 2.

Two strains from Sweden provided poor alignment results (i.e. Swedish non-human isolate only has 80% cg-allele coverage) and were analysed following an allele mapping extraction protocol provided by the SE NPHL. These results provided 99% cg allele coverage.

Four additional food isolates matching the outbreak strain from Finland (two isolates, 0-1 allele differences) and Belgium (two isolates, 1-2 allele differences) were not provided to EURL for *Lm* and were not included in this joint analysis (details on the origin of the isolates are described in the respective country sections on food and environmental investigations).

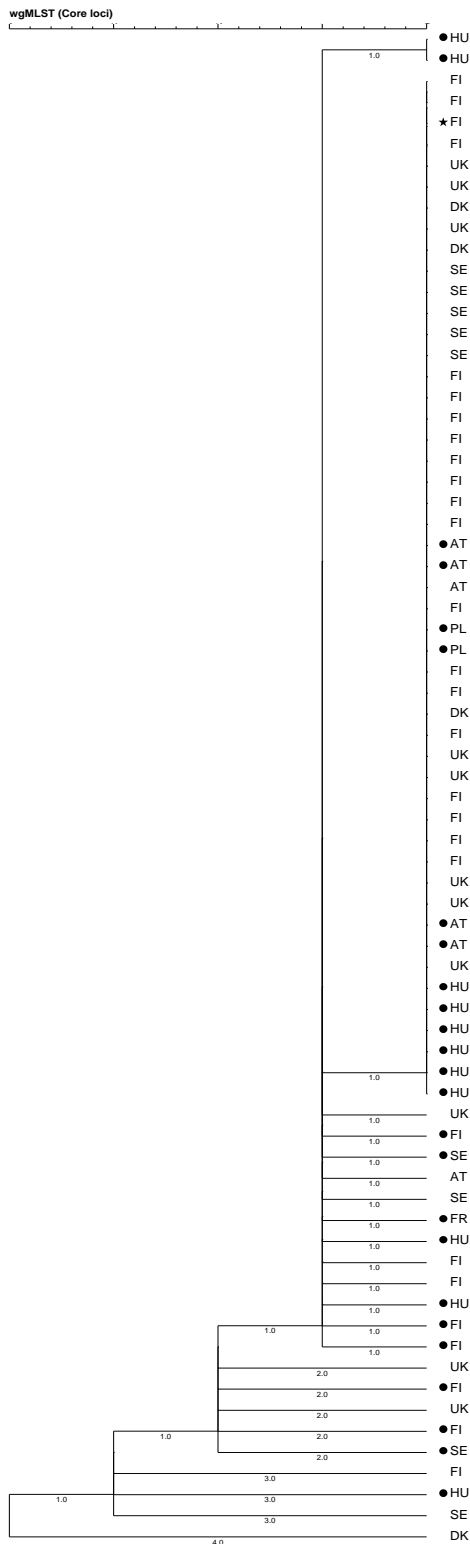
Table 3. *Listeria monocytogenes* isolates within seven allelic differences from the *L. monocytogenes* ST6 Finnish representative outbreak strain by cgMLST, 2015–2018*

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 (3–4)	4 (3–4)
Denmark	4 (3–7)	–
Finland	23 (0–5)	5 (4–5)
France	–	1 (4)
Hungary	–	11 (3–6)
Poland	–	2 (3)
Sweden	7 (3–6)	2 (4–5)**
United Kingdom	11 (1–6)	–
Total	47 (0–7)	25 (3–6)

* The non-human isolates were obtained from samples during the 2016, 2017 and 2018 production seasons.

** One of the Swedish non-human isolates differed by nine allelic differences from the *L. monocytogenes* ST6 Finnish representative outbreak strain in the ECDC pipeline.

Figure 2. CgMLST-based (Moura scheme) single-linkage tree including sequences from 47 human and 25 non-human *Listeria monocytogenes* isolates from six countries, 2015–2018 (software: BioNumerics version 7.6.2); data as of 20 June 2018



Note: 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) are 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=47) and non-human (n=25) isolates (Figure 2) shows that all of them are within six cg-allelic differences (and within 4-5 cg-allelic differences from the *L. monocytogenes* ST6 Finnish representative outbreak strain (122265)), indicating close genetic relatedness. The Finnish representative outbreak strain (122265) has 1 730 of the 1 748 core loci (98.97%), and 1583 of the 1 748 core loci (90.56%) were shared by all isolates – i.e. the unique loci were detected in each of the 72 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 origin of the non-human isolates included in the joint WGS analysis is provided in the country specific sections on food and environmental investigations.

Information on patient interviews

Information on patients' food exposure is captured at national level through interviews using national questionnaires. However, information on exposure to corn, the only common food item identified at the time which matched with non-human isolates, was not routinely requested in national questionnaires used in the affected countries.

Questions on consumption of corn and mixed vegetables were introduced into the national questionnaires after the identification of these as possible food vehicles. Recent and new cases were re-interviewed/interviewed with the updated questionnaires.

There were 26 patients interviewed from Denmark (2), Finland (11), Sweden (7) and the United Kingdom (6). Of the 26, 11 reported consumption of corn, nine patients reported no consumption (5)/possibly no consumption of corn (4) and six patients were unable to remember whether they had consumed corn.

Of the 11 patients reporting corn consumption, three reported consumption of frozen corn products, four reported having consumed both frozen and non-frozen corn and four only non-frozen corn. Of the four patients who had only consumed non-frozen corn, three had consumed vegetable mix and one reported only having consumed canned corn.

One of the Finnish patients confirmed having consumed 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 case. Two cases from the United Kingdom consumed frozen corn from the UK supermarket(s) known to be supplied by Hungary; both had frozen corn in their home freezers and the results of the microbiological tests performed are still pending.

Of the 15 cases that did not report corn consumption, two replied that they had consumed non-frozen mixed vegetables, three cases reported no consumption of corn or mixed vegetables, six cases did not know if they consumed corn or mixed vegetables, four cases had possibly not consumed corn and one of these four had possibly consumed frozen mixed vegetables.

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 47 human cases reported since 2015. Nine patients have died. Eighteen outbreak cases have been reported in 2018 with the latest cases having disease onset in May 2018. Thus, the outbreak is continuing, or has been ongoing until very recently. 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/EEA countries routinely use this advanced technique to characterise *L. monocytogenes* isolates.

WGS analysis confirmed that all 47 human *L. monocytogenes* isolates have 0–7 allelic differences from the Finnish representative outbreak isolate FI 122265 based on cgMLST. In addition, 29 non-human isolates from Austria, Belgium, Finland, France, Poland, Hungary and Sweden were found to be closely related to the 47 human outbreak strains using cgMLST (≤ 6 allelic differences). The majority of the non-human isolates were obtained in products from the 2017 production season: mainly frozen corn (13 samples), frozen vegetable mixes including corn (8 samples), frozen spinach (1) and frozen green beans (1). Only one isolate was reported from a frozen vegetable mix produced in 2016, while three isolates were obtained in spinach products produced in 2018. In addition, two isolates were also obtained from two environmental samples collected in two different plants freezing and handling frozen vegetables in France and Hungary during the 2017 and the 2018 production seasons, respectively.

The WGS analysis provides a strong microbiological link between the human and the non-human isolates and this is indicative of a common source related to frozen corn and other frozen vegetable mixes including corn persisting in the food chain. Traceability information of the contaminated products pointed to the source of contamination at a freezing plant in Hungary (company A). As *L. monocytogenes* IVb ST6 matching the outbreak strain has been isolated from frozen spinach and frozen green beans sampled at the Hungarian plant, it is possible that frozen

vegetables other than corn, which have been processed at this plant, could also be implicated as a vehicle of human infection.

The finding of *L. monocytogenes* IVb, ST6 matching the outbreak strain in frozen corn and other frozen vegetables produced in the 2016, 2017 and 2018 production seasons at the plant of the Hungarian company A suggests that the strain could be persisting in the processing environment after standard cleaning and disinfection procedures carried out in conjunction with periods of inactivity in the plant, as well as the rotation of the processed products. In particular, the finding of *L. monocytogenes* in three out of five spinach samples from one batch collected during the different phases of the 2018 spinach production further supports the hypothesis that contamination has occurred within the plant, during one and/or multiple processing stages. It is important to note that the grinding stage (where the first positive sample was taken) occurs after blanching (96°C for 110 seconds) and cooling of the spinach. WGS was only performed for two of the three isolates and both matched with the outbreak strain. The isolation of the *L. monocytogenes* IVb ST6 matching the outbreak strain in a sample from a floor drain at the packaging area confirms the environmental contamination of the Hungarian processing plant. It is also important to note that even though some environmental samples from different bands (i.e. sorter and blanching bands, band leading to the grinding machine) tested negative for *L. monocytogenes*, other sites in the processing line, less accessible and more difficult to clean (e.g. slicers, grinder, refrigerator systems, etc.) could be contaminated and could maintain *L. monocytogenes* contamination in the plant. Further investigations, including thorough sampling and testing [1a], are needed to identify the source of contamination at the Hungarian processing plant.

The use of the contaminated production lines for several frozen vegetables may represent an additional risk for potential cross-contamination of the final products processed at Hungarian company A's plant (e.g. frozen corn and frozen vegetable mixes). Cross-contamination of the frozen fruit appears to be less probable, as no freezing of fruit is carried out at the plant and fruit frozen by other factories are packed in a completely separate area of the plant. The plant of the Polish company C, which was initially [1] considered one of the possible points of contamination together with Hungarian company A's plant, was then excluded as a result of the intense environmental sampling and testing that made it possible to identify the points of contamination by *L. monocytogenes* IIa (not related to this outbreak) and carry out thorough cleaning and disinfection at the plant of Polish company C.

Food business operators in Estonia, Finland, Poland and Sweden have withdrawn and recalled the implicated frozen corn products from the market. Since March 2018, the implicated Hungarian plant has been under increased official control and no frozen vegetable products from the 2018 production season have been distributed to the market yet. Following the positive findings from food and environmental samples collected during the 2018 production, freezing activities were recently halted at the plant concerned. On 29 June 2018, the Hungarian Food Chain Safety Office banned the marketing of all frozen vegetable and frozen mixed vegetable products produced by the Hungarian plant between August 2016 and June 2018, and ordered their immediate withdrawal and recall. This restrictive measure is likely to significantly reduce the risk of human infections and contain this outbreak.

As the outbreak is still continuing or at least has been ongoing until very recently, there are indications that contaminated products may still be on the market or that contaminated products purchased before the recalls are still being consumed. Any potentially contaminated frozen vegetables (e.g. frozen corn, frozen vegetable mixes including corn, frozen spinach and frozen green beans) from the 2017 and 2016 production seasons could still represent a possible risk to consumers until completely withdrawn and recalled. This risk may exist, even at a low level of contamination, if the products are not properly cooked before consumption. It is worth noting that thawed and fresh corn and green peas have been shown to support the growth of *L. monocytogenes* at refrigeration temperature (4°C) [12,13]. In addition, new invasive listeriosis cases may be identified due to the long incubation period (1–70 days), the long shelf-lives of frozen corn products, and potential consumption of frozen vegetable products bought by consumers before the recalls and eaten without being cooked properly.

Information on corn consumption was not routinely requested during patient interviews in the affected countries. Questions on consumption of corn and vegetable mixes were introduced in the questionnaires once the match between human and non-human isolates was found. Consumption of frozen or non-frozen corn has been confirmed by 11 out of 26 patients interviewed from Denmark, Finland, Sweden and the United Kingdom. Of the 15 cases that did not report corn consumption, two replied that they had consumed non-frozen mixed vegetables, three cases reported no consumption of corn or mixed vegetables, six cases did not know if they consumed corn or mixed vegetables, four cases had possibly not consumed corn and one of these four had possibly consumed frozen mixed vegetables. The matching *L. monocytogenes* isolations in other types of frozen vegetables (spinach and green beans) give grounds to expand the collection of exposure data from patients with *L. monocytogenes* isolation matching the outbreak strain.

It is worth noticing that the frozen corn, frozen vegetable mixes and frozen creamy spinach were considered by the producer to be 'non ready-to-eat' food. However, consumers may have eaten these thawed products without having cooked them properly or at all. For example, foods cooked in the microwave may still have cold spots where the bacteria could survive. Moreover, the consumption of thawed corn and thawed vegetables without cooking them is not an unusual practice (e.g. in salads, smoothies, etc.)

Positive findings of other strains of *L. monocytogenes* which are different from the outbreak strain, have been reported in food (from frozen corn, frozen vegetable mixes, etc.) and environmental samples in the Hungarian company A (serogroup

IIa), the Polish company C (serogroup IIa), and the Belgian company D (serotype 1/2b). Although these strains are serologically different from the one related to the present outbreak, further sequencing of these *L. monocytogenes* isolates may provide information on additional potential links to human cases of relevance to public health.

Further studies on the risk of *L. monocytogenes* associated with the consumption of frozen vegetables could help clarify some important aspects, such as the growth kinetics of *L. monocytogenes* in frozen and thawed vegetables.

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