JOINT ECDC–EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Listeria monocytogenes* clonal complex 8 infections linked to consumption of cold-smoked fish products

4 June 2019

Summary

A prolonged multi-country outbreak of 22 listeriosis cases caused by *Listeria monocytogenes* sequence type (ST) 1247, clonal complex (CC) 8 has been identified through whole genome sequencing (WGS) in five EU countries: Denmark (9 cases), Estonia (6), Finland (2), France (1) and Sweden (4). Five patients have died due to, or with, the disease. The first case had symptom onset in July 2014 in Estonia, and the most recent case occurred in Denmark in February 2019. Eight patients, out of twelve for whom a food consumption history was available, confirmed the consumption of cold-smoked fish products.

L. monocytogenes food isolates, matching the human outbreak strain by WGS, were detected at wholesale and retail level in four countries (i.e. France, Denmark, Italy and Sweden) from 13 batches of cold smoked or gravad salmon and from six batches of cold smoked trout products. Traceability information of the contaminated batches pointed to the Estonian processing Company A as the single common manufacturer of these fish products. The raw fish was received from suppliers in Norway and Finland. Environmental investigations and food testing at the Estonian processing plant showed the presence of *L. monocytogenes* that matched the outbreak strain in two samples on the processing line and in four batches of the final product.

The presence of *L. monocytogenes* matching the outbreak strain over several years in the fish products suggests the persistence of the microorganism at the Estonian company's premises. Further investigation is needed to identify points of (cross-)contamination in the food processing plant. Control measures were implemented in Estonia, Denmark, France and Italy following the RASFF (Rapid Alert System for Food and Feed) notifications, but until the source of infection has been identified and controlled, new invasive listeriosis cases associated with this event may still occur.

In general, pregnant women, the elderly and immunocompromised individuals are at increased risk of invasive listeriosis, which is associated with severe clinical course and potentially death.

Options for response

Competent authorities are encouraged to report new human cases associated with this event (as well as the findings of public health investigations) to the Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses (EPIS-FWD). They should also consider interviewing new and recent listeriosis cases that are possibly related to this event. In order to assess exposure history, interviews should include questions about the consumption of ready-to-eat fish products, including brand information.

Suggested citation: European Centre for Disease Prevention and Control and European Food Safety Authority. Multi-country outbreak of *Listeria monocytogenes* clonal complex 8 infections linked to consumption of cold-smoked fish products – 4 June 2019. Stockholm and Parma: ECDC/EFSA; 2019.

ECDC supports the WGS analysis of human *L. monocytogenes* isolates from cases possibly related to this outbreak and reported in countries that are not routinely performing WGS. Countries that routinely perform WGS are kindly requested to share sequences (reads) from possibly related isolates with ECDC for inclusion in the multi-country analysis. Sequences from a representative outbreak strain are available in the European Nucleotide Archive (ENA), ERR2223569.

The European Union Reference Laboratory for *L. monocytogenes* (EURL-*Lm*) provides support to Member States that do not have WGS capacity to perform WGS of non-human isolates for strains possibly related to this outbreak. In order to identify the source of contamination, the involved Member States are advised to carry out environmental and food sampling and testing at critical sites along the production lines in processing plants and primary production facilities.

Competent public health and food safety authorities in affected EU countries should share information at the European level on epidemiological, microbiological and environmental investigations (including tracing information) and issue relevant notifications through the Early Warning and Response System (EWRS)[1] and the RASFF[2].

Source and date of request

The Directorate General for Health and Food Safety requested the production of an EFSA–ECDC rapid outbreak assessment on 25 March 2019; the proposal was accepted by EFSA and ECDC on 26 March 2019. Production took several weeks due to the collection of sequences of all relevant non-human *L. monocytogenes* isolates across the food chain.

Public health issue

This document provides an assessment of the cross-border public health risk related to *L. monocytogenes* infections linked to the consumption of cold-smoked fish products, possibly originating from a single food processing company.

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- France: Marie-Pierre Donguy (Ministère de l'Agriculture et de l'Alimentation)

[1] 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. EWRS enables the European Commission and competent authorities of the Member States to be in permanent communication for the purposes of alerting, assessing public health risks and determining measures that may be required to protect public health. National competent authorities should notify an alert in EWRS when the development or emergence of a serious cross-border threat to health fulfils the criteria listed in Article 9 of Decision 1082/2013/EU.

[2] RASFF is the official EU system for sharing information on hazards found in food and feed, trade of potentially contaminated batches between Member States and 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.

- Norway: Asne Sangolt (Norwegian Food Safety Authority)
- Sweden: Mats Lindblad (National Food Agency).

RASFF contact points in Belgium, Denmark, Estonia, Finland, France, Germany, Italy, Norway and Sweden were consulted by EFSA to clarify and validate their national data on food and environmental investigations.

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. 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 ECDC's and EFSA's knowledge as of 23 May 2019. 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

From 2013 to 2017, between 1 905 and 2 527 listeriosis cases were reported to The European Surveillance System (TESSy) annually by 30 EU/EEA countries [1]. Germany, France and Spain accounted for 26%, 17% and 10%, respectively, of the reported cases in this period. Severe *L. monocytogenes* infections were more common in males (54%) and in people over 65 years of age (65% of cases) in both genders. The majority of the cases (98%) were of domestic origin [1].

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

Food-borne outbreaks caused by *Listeria monocytogenes*

This section presents information on food-borne outbreaks caused by *L. monocytogenes* reported to EFSA under the framework of Directive 2003/99/EC. Although mandatory, not all Member States report the complete information on food-borne outbreaks to EFSA. Consequently, the information in this section may not be exhaustive for certain countries.

From 2010 to 2017, four *L. monocytogenes* outbreaks strongly associated with the consumption of fish and fishery products were reported to EFSA by Germany (one outbreak in 2010) and Denmark (three outbreaks: 2010, 2014 and 2017), involving a total of 44 cases. In 2015, the Netherlands reported one outbreak with weak evidence linking the consumption of smoked salmon to three human cases.

The 2017 Danish outbreak was caused by *L. monocytogenes* ST8 (CC8) and part of a larger multi-country outbreak involving 12 human cases in Denmark, France and Germany, all related to the consumption of ready-to-eat cold-smoked salmon produced in Poland [5,6].

Listeria monocytogenes isolation in food

L. monocytogenes is an environmental bacterium that can enter the food-processing environment via incoming raw materials and the movement of personnel and equipment, colonising food-processing equipment and food-contact surfaces. By forming biofilms, *L. monocytogenes* can persist in food-processing environments for prolonged periods, contaminating a wide range of foodstuffs, especially during the processing stage. Food business operators are requested to perform their own checks to monitor and control possible contamination by *L. monocytogenes* of the food-processing environment and the final product [7].

L. monocytogenes can multiply at refrigerator temperatures and listeriosis is usually associated with the ingestion of contaminated fish, dairy, meat or vegetable products that have been kept at refrigeration temperatures and eaten as such or without being cooked properly [8].

In accordance with Zoonoses Directive 2003/99/EC, Member States are required to report specific data on the occurrence of *L. monocytogenes* in food that are published annually in the EU summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks [9].

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 RTE foods and which has been in force since 1 January 2006 [7]. According to EU Regulation (EC) No 2073/2005 ready-to-eat (RTE) foods are defined as food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organism of concern.

This section focuses on data from 2010 to 2017 on *L. monocytogenes* in fish and fishery products, which represent the suspected food category in this investigation.

In 2017, *L. monocytogenes* isolations were reported in 365 RTE fish and 30 RTE fishery products sample units, from 5 255 and 1 423 total sample units, respectively. *L. monocytogenes*-positive units were mostly sampled at the retail level (203; 11% of the total units sampled) and at processing plants (175; 4% of the total units sampled) while only a few isolations were reported from samples collected at catering facilities (1; 2% of total units sampled) or at wholesalers (4; 13% of the total units sampled). Most *L. monocytogenes* isolations reported in 2017 were from smoked fish (363; 8% of the total units sampled), including hot- and cold-smoked fish. In 2017, the following 11 Member States reported *L. monocytogenes*-positive units from RTE fish or fishery products based on various detection methods: Belgium (2), Cyprus (1), Denmark (16), Estonia (3), Germany (198), Hungary (6), Italy (21), Netherlands (9), Poland (121), Spain (17) and Sweden (1).

Table 4 (see Annex 1) summarises the information reported on *L. monocytogenes* in fish and fishery products from 2010 to 2017 related to RTE regardless to the sampling stage. Results obtained by the detection method (presence or absence of *L. monocytogenes*) are presented in Table 4 (see Annex 1).

Information on multilocus sequence typing (MLST) is usually not reported in the context of monitoring zoonoses and zoonotic agents in food as required by Directive 2003/99/EC. Specifically, no data on *L. monocytogenes* ST1247 or CC8 have been reported to EFSA (this information is not mandatory and is only reported on a voluntary basis).

Event background information

On 19 December 2017, Denmark (through EPIS-FWD) reported a national outbreak of listeriosis with six cases identified through WGS analysis over the previous 12 months. At the time, no vehicle of infection was identified. EPIS FWD was updated in February 2019 after the identification of additional cases and food isolates that matched the human strains.

Following the new Danish alert, additional human *L. monocytogenes* isolates, matching the cluster identified through WGS-based cgMLST analysis within seven allelic differences using Institut Pasteur scheme (including 1748 loci) were identified in Denmark and four other EU Member States between July 2014 and February 2019.

This rapid outbreak assessment is linked to the EPIS FWD urgent inquiry UI-452 and RASFF notifications 2018.0394, 2018.1833, 2018.2003, 2018.2870, 2018.3687, 2018.3808, 2019.0806, 2019.0999.

Multi-country investigations

EU outbreak case definition

Outbreak-confirmed case:

- An EU/EEA resident with laboratory-confirmed invasive listeriosis, with symptom onset on or after 1 January 2016 (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 a *L. monocytogenes* isolate within 7 cgMLST allelic differences using Institut Pasteur scheme [10] from any other isolate assessed to be part of the multi-country outbreak (representative sequence ERR2223569, assembly uploaded in EPIS-FWD UI-452) based on the ECDC WGS pipeline.

Outbreak-historical confirmed case:

- An EU/EEA resident with laboratory-confirmed invasive listeriosis, with symptom onset before 1 January 2016 (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 a *L. monocytogenes* isolate within 7 cgMLST allelic differences using Institut Pasteur scheme [10] from any other isolate assessed to be part of the multi-country outbreak (representative sequence ERR2223569, assembly uploaded in EPIS-FWD UI-452) based on the ECDC WGS pipeline.

Epidemiological and microbiological investigations of humans

As of 23 May 2019, five EU countries have identified 19 outbreak-confirmed cases reported on or after 1 January 2016 and 3 historical outbreak-confirmed cases reported in 2014 and 2015 (Table 1).

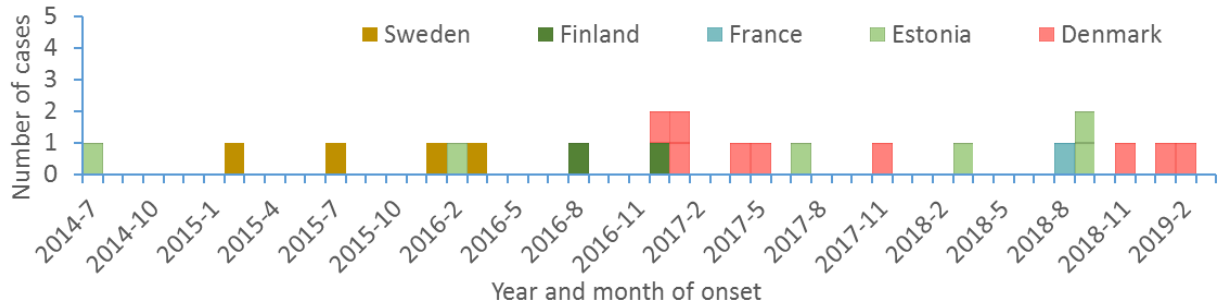
Table 1. *L. monocytogenes* CC8 outbreak cases by country and case classification, 2014–2019, as of 23 May 2019

Reporting country	Outbreak-confirmed cases (reported on or after 1 January 2016)	Historical outbreak-confirmed cases (reported before 1 January 2016)	Total number of cases
Denmark	9	0	9
Estonia	5	1	6
Finland	2	0	2
France	1	0	1
Sweden	2	2	4
Total	**19**	**3**	**22**

Information on age and gender is available to ECDC for 20 patients: median age is 76 years (interquartile range): 64–83 years). All patients are older than 50 years of age, except for one neonatal case reported in 2014. Eleven patients are male and nine are female. At least five patients died due to or with the disease.

Outbreak cases have been reported every year since 2014, with a small clustering of cases between the end of 2015, 2016 and 2018, and the start of the following year. Estonia, Finland and Sweden reported cases in the first two years of this outbreak; since January 2017, all cases (with the exception of one case in France in 2018) have been reported in Estonia and Denmark (Figure 1).

Figure 1. Distribution of outbreak-confirmed listeriosis cases by country and month of onset*, EU, 2014–2019, as of 23 May 2019 (n=22)



** Month of sampling or month of receipt by the reference laboratory if month of onset unavailable*

Austria, the Czech Republic, Germany, Luxembourg, the Netherlands, Norway, Spain and the United Kingdom reported no cases suspected to be related to this outbreak.

Information from patient interviews

Information from patient interviews was available for 12 patients in Denmark (9), France (1) and Sweden (2). Eight patients (seven in Denmark and one in France) reported consumption of either cold-smoked salmon or cold-smoked trout. Two of the remaining 10 patients died before being interviewed, and the information obtained from their family members was not complete. Exposure information from three cases that occurred before 2016 was not available and could not be retrieved due to the long delay between onset and current investigation.

Microbiological and environmental investigations of food

This section summarises country-specific information on food and environmental investigations and traceability of the products suspected to be associated with this outbreak that were reported through RASFF (under notification numbers 2018.0394, 2018.1833, 2018.2003, 2018.2870, 2018.3808, 2018.3687, 2019.0806, 2019.0999) between 25 March 2019 and 23 May 2019 (see Table 3 and Figure 2 for details on testing and traceability).

Denmark

Overall, *L. monocytogenes* isolates matching the outbreak strain were reported from 13 batches of cold-smoked fish products:

- Batch C of cold-smoked trout sampled at Wholesaler G in 2018 (Batch C; originated from Batch C1).
- Batches E and F of cold-smoked trout sampled at Wholesaler C in 2019.
- Batches G, H, I, L, M, N, O, P, Q, R of cold-smoked salmon products sampled at Wholesaler C in 2019.

On 10 October 2018, Denmark reported on RASFF (2018.2870) that frozen cold-smoked trout products belonging to Batch C of Brand B were sampled (6 September 2018) at storage in the context of official controls at Wholesaler G; a microbiological analysis was conducted on 4 October 2018 on five samples. The analysis revealed the presence of *L. monocytogenes* (between less than 10 cfu/g and 570 cfu/g). The Danish Veterinary and Food Administration (RASFF 2019.0999) reported that an isolate of *L. monocytogenes* detected from Batch C was genetically closely related to the outbreak strain; hqSNP (CSI Phylogeny version 1.4.) and cgMLST was used for the analysis [10,11].

Batch C of Brand B (derived from Batch C1) was produced by Estonian processing Company A; the entire batch was delivered frozen to the Danish Wholesaler C on 10 August 2018. Products belonging to the Batch C were thawed and relabelled by Wholesaler G, contractor of Wholesaler C (until January 2019); the relabelled batch received a new expiry date which relied on the shelf life documentation provided by Estonian processing Company A. Neither Wholesaler C nor its contractor Wholesaler G manipulated the fish products during thawing and labelling. No control actions took place for Batch C as the analyses were done at the end of the products' shelf life.

On 15 March 2019, Denmark reported (RASFF 2019.0999) that, as part of an official control, 65 samples from 13 batches of smoked fish products (including salmon and trout products) were sampled (8 March 2019) at Wholesaler C. *L. monocytogenes* was detected in 49 samples, corresponding to 12 batches (batches E, F, G, H, I, L, M, N, O, P, Q, and R) with an enumeration of less than 10 cfu/g. All *L. monocytogenes* isolates matched the outbreak strain [10] and clustered within 1 to 8 SNP differences to the outbreak strain (CSI Phylogeny version 1.4.). In five of the 12 positive batches (E, F, G, P and R), pH and a_w were measured and ranges were 6.1–6.5 and 0.96–0.98, respectively.

The 13 tested batches were produced by Estonian processing Company A, imported by Wholesaler C, thawed, and relabelled by its contractor Wholesaler G; new expiry dates that relied on the shelf life documentation provided by Estonian processing company A were added. The 13 tested batches were delivered to Retailer G and Retailer H, both based in Denmark, and to Retailer I, based in Germany, but the exact distribution for each batch shipped to these retailers was not available. On 15 March 2019, the Danish Veterinary and Food Administration withdrew all products from the Danish market that originated from Estonian processing Company A and recalled them from consumers. Moreover, a recall at Retailer I in Germany was initiated.

As of February 2019, Wholesaler C started to perform thawing and relabelling under its own capacity.

France

L. monocytogenes isolates matching the outbreak strain were reported from two batches of cold-smoked fish products:

- Batches A and B of chilled cold-smoked trout sampled at retailer A in 2018.

In addition, *L. monocytogenes* isolates were reported from Batch D: chilled cold-smoked salmon of Brand C sampled at retailer B; sequencing was not available.

Two food isolates of *L. monocytogenes* of the same cgMLST cluster of the outbreak strain (L2-SL8-CC8-CT4158, Inst. Pasteur scheme [10], analysis performed by Institut Pasteur) were detected in France in 2018 during official and the company's own checks at the retail level. Isolates were obtained from two batches of chilled cold-smoked trout products, namely Batch A of Brand A (sampled during an official control) and Batch B of Brand A (sampled during a company check, as notified in RASFF (2018.2870)). They were sampled at two different stores of the same French retailer A on 2 July 2018 and 19 July 2018, respectively. Following microbiological analyses, a total of 400 cfu/g of *L. monocytogenes* were enumerated in Batch A, and less than 10 cfu/g of *L. monocytogenes* were enumerated in Batch B.

On 29 June 2018, the French authorities released (RASFF 2018.1833) the analytical results of Batch D (chilled smoked salmon labelled as 'Norwegian smoked salmon' by Brand C) and sampled at French Retailer B during an official control (26 June 2018). *L. monocytogenes* was present in 25 g and measured at a level of less than 10 cfu/g.

A withdrawal from the market and a recall from consumers was implemented as of 18 July 2018 for Batch A and as of 25 July 2018 for Batch B.

Belgium

Batches A, B and D, produced by Estonian Processing Company A on 14 June, 1 July and 29 May 2018, respectively, were traded chilled (-5 °C) to Belgian Wholesaler B. Belgian Wholesaler B relabelled the products with a new storage temperature of 0–4 °C and new expire dates (Batch D), after shelf-life tests. The products were then distributed to French Retailers A (Batch A and B (both under Brand A labels)) and to French Retailer B (Batch D, labelled as Brand C). Moreover, Belgian Wholesaler B delivered products belonging to Batch D to several other French retailers in June 2018. The use-by-dates for Batch D included in the delivery notes provided by the Belgian competent authority varied between June and July 2018.

Italy

L. monocytogenes isolates matching the outbreak strain were reported from two batches of cold-smoked fish products:

- Batch T of cold-smoked salmon sampled at Wholesaler F in 2019.
- Batch S of chilled cold-smoked trout sampled at Retailer D.

In addition, *L. monocytogenes* isolates (without information on sequencing) were reported from:

- Batch Z of chilled smoked salmon sampled at Retailer E.
- Batch W of chilled Norwegian smoked salmon products sampled at Retailer F.

On 27 February 2019, the Italian competent authority reported (RASFF 2019.0806) that during an official control at the wholesale level (Wholesaler F), a frozen smoked salmon product from Batch T was collected on 6 February 2019. Microbiological analysis showed the presence of *L. monocytogenes* in four aliquots (25 g each) and an enumeration of less than 10 cfu/g. All 13 *L. monocytogenes* isolates obtained from the four aliquots matched the outbreak strain (based on cgMLST using chewBBACA allele calling [12] with the *Listeria* scheme described by Moura et al. 2016 [10]); they also clustered at maximum 3 allele differences with the reference genome of the outbreak strain. Measurements of pH and a_w ranged between 5.99–6.09 and 0.974– 0.976, respectively. The Batch T was produced by Estonian Processing Company A and was shipped in its entirety, and in frozen form, to Wholesaler F on 29 January 2019. As of 6 February 2019, Batch T was officially impounded by the Italian competent authority, therefore Batch T never reached the consumers.

On 15 December 2018, Italy reported (RASFF 2018.3687) that during an official control at Retailer E samples of chilled smoked salmon of Batch Z were collected on 29 November 2018. Microbiological analyses showed *L. monocytogenes* enumeration of less than 10 cfu/g and the presence in 25 g. Measurements of pH and a_w ranged between 5.96–5.99 and 0.966–0972, respectively. Batch Z was produced by Estonian Processing Company A on 25 July 2018 and was shipped in its entirety (frozen, -18 °C) to Italian Wholesaler F on 1 August 2018. Italian Wholesaler F sold the whole batch to Italian Wholesaler A, which thawed, repacked and relabelled the fish products with a different use-by date after shelf-life testing. Italian Wholesaler A finally consigned them to Retailer E and other Italian retailers.

On 28 December 2018, Italy reported (RASFF 2018.3808) that in the context of an official control at Retailer F, samples of Norwegian thawed smoked salmon products from Batch W were collected on 14 December 2018. Microbiological analyses revealed *L. monocytogenes* enumeration of less than 10 cfu/g and presence in 25 g. The isolate was serotyped as 1/2a. Physical-chemical measurements of pH and a_w ranged between 6.10–6.11 and 0.979–0.984, respectively. Smoked salmon products belonging to the Batch W were produced by Estonian processing Company A and delivered frozen to Wholesaler G, a contractor of Wholesaler C (until January 2019). Wholesaler G thawed the product and relabelled it with a new expiry date, relying on the shelf life documentation provided by Estonian Processing Company A. Batch W was then distributed to Italian Retailer F. Batch W was impounded by the Italian competent authority on 28 December 2018 and never reached the consumer's table.

On 7 February 2018, the Italian competent authority reported (RASFF 2018.0394) that a sample of chilled smoked rainbow trout product from Batch S was collected as part of an official control plan at retail level (Retailer D). Analyses showed *L. monocytogenes* enumeration of less than 10 cfu/g and the presence in 25 g. Physical-chemical measurements of pH and a_w were 6.25 and 0.96, respectively. The smoked trout product of Batch S was produced on 19 September 2017 by Estonian processing Company A as frozen products and was delivered, also frozen, to Italian Wholesaler E where it was thawed it in its original package and further distributed to Wholesaler D who in turn distributed it to the Italian Retailer D.

Sweden

L. monocytogenes isolates matching the outbreak strain were reported from two batches of cold-smoked or gravad fish products:

- Batch U of sliced cold gravad salmon sampled at Retailer C,
- Batch V of sliced cold-smoked salmon sampled at Retailer C.

On 28 March 2019, the Swedish competent authority informed its counterparts (RASFF 2018.2870) that one sample from a batch of sliced gravad (Batch U) of Brand E and one sample from a batch of cold-smoked salmon (Batch V) of

Brand E collected at the retail level were positive for *L. monocytogenes*. The samples were collected on 22 March 2016 (Batch U) and 14 April 2016 (Batch V) during an official control at Retailer C in the context of a nationwide survey performed in 2016. In both samples, the presence of *L. monocytogenes* was detected using qualitative analyses at the end of the shelf life, but at a level of less than 10 cfu/g. The two food isolates of *L. monocytogenes* are MLST ST-1247 and genetically linked to the outbreak strain as shown by SNP analysis performed at the national level. The products were produced by Estonian processing Company A and shipped to Sweden under Brand E. The food products were not withdrawn from the market in 2016.

Estonia

Information on Estonian processing Company A

Estonian processing Company A produces chilled and frozen products from salmon and trout: fresh, cold and hot smoked, salted, and gravad.

Depending on the type of products, different processing steps are applied (not necessarily in the order given below): 1) removal of fish from boxes and ice (room 1), 2) removal of head and tail (room 1), 3) washing of fish (room 1), 4) filleting line for fish (room 2), 5) manual trimming, removal of bones and washing (room 2), 6) skinning of fillet (for products with skin) (room 2), 7) washing the fish fillet (room 2), 8) salting/marinating (dry salt or injection) (room 2), 9) packaging of the fresh products (room 2), 10) fish curing (room 3), 11) salt flashing (room 4), 12) smoking in three different smoking chambers (room 5), 13) chilling (room 6), 14) holding (room 7), 15) fish slicing (room 8), 16) weighing, packaging, labelling of smoked, salted and marinated products (room 8), 17) chilling at 0/+4 °C (room 9) or freezing (room 10 or 11) at -35 °C (product temperature is -18°C by the end of freezing). All products are processed in the same line up to step 7 (included).

After labelling, the final product is stored and then shipped to customers (chilled, product temperature: +4 °C; or frozen: -18 °C).

Traceability of suspected products

The fish used to produce the implicated batches originated from Norwegian Suppliers A, B, C, D, E, G and from Finnish Supplier F. The implicated products were distributed to wholesalers in Belgium, Denmark and Italy. During 2016–2019, the Estonian processing Company A sold fish products to Swedish Wholesaler H but an exact distribution list of batches was not available.

Information on food and environmental sampling and testing

Overall, *L. monocytogenes* isolates matching the outbreak strain were reported from four batches of processed fish products sampled at the premises of Estonian Processing Company A in 2019:

- Batch J of salted salmon sampled in the context of own check on 15 January 2019
- Batch K of cold-smoked salmon sampled in the context of own check on 15 January 2019
- Batch X of cold-smoked trout sampled in the context of own check on 6 March 2019
- Batch Y of cold-smoked trout sampled in the context of official control on 18 March 2019.

In addition, *L. monocytogenes* isolates matching the outbreak strain were reported from two environmental samples collected at the premises of Estonian Processing Company A in 2019:

- A surface sample from a washed blue box in room 12 (storage room), collected in the context of company check on 12 March 2019
- A surface sample from the conveyor belt of a slicing machine in room 8, collected in the context of an official control on 18 March 2019.

As part of quality control checks carried out between January 2016 and April 2019, the Estonian processing Company A performed a total of 1 143 microbiological analyses for *L. monocytogenes* on cold-smoked and salted final products. Overall, *L. monocytogenes* was detected in three batches of the final products (batches J, K and X) sampled between 15 January and 6 March 2019. The company also performed own checks on batches A, C1, D, H, T, and Z, with a total count of *L. monocytogenes* being less than 10 cfu/g in all samples (excluding in one from Batch D where the count was reported to be less than 40 cfu/g).

During the same period (January 2016 to April 2019), Estonian processing Company A performed a total of 589 shelf-life tests on cold-smoked and salted products, 55 of which (9%) exceeded the limit of 100 cfu/g.

Between 30 November 2017 and 4 April 2019, a total of 284 company-performed checks on environmental samples were collected at the premises of the Estonian Processing Company A (i.e. 268 samples from surfaces, 15 from the hands of operators at different steps of the production line, and one from clothes). Among these 284 samples, 11 and 5 samples were taken during the production of batches G and L, respectively. *L. monocytogenes* was detected in five samples: four samples collected in September and October 2018 from the salting machine during production in room 2, the line after skinning in room 2, skinning machine buttons and a conveyor belt; and one sample collected in March 2019 from a washed blue box in room 12.

On 18 March 2019, the Estonian competent authority carried out an official control at Estonian processing Company A, identifying Batch Y as positive for the presence of *L. monocytogenes*. Batch Y was not released by the company. On the

same day, the competent authority collected 22 samples from surfaces throughout the production lines. *L. monocytogenes* was detected in two surface samples from the slicing machine conveyor belt with needles in room 8 (Table 2).

Table 2. Positive environmental samples for *Listeria monocytogenes* collected in Estonian processing Company A between September 2018 and April 2019

Sample date	Place	Room	Process (Step number)	Matching the outbreak strain[&]	No sequenced isolates*
18 Sep 2018	Salting machine screen	2	Salting (8)	n.a.	n.a.
27 Sep 2018	Transporting line after skinning machine	2	Skinning (6)	n.a.	n.a.
16 Oct 2018	Skinning machine button	2	Skinning (6)	n.a.	n.a.
24 Oct 2018	Blue transporting line	2	Not for cold-smoked products	n.a.	n.a.
12 Mar 2019	Washed blue plastic box	12 (storage room)	Not for cold-smoked products	yes	1
18 Mar 2019	Slicing machine conveyor belt with needles	8	Slicing (15)	yes	1
18 Mar 2019	Slicing machine conveyor belt with needles	8	Slicing (15)	n.a.	n.a.

n.a. = not available;
[&]Seven or fewer core gene alleles, cgMLST Institut Pasteur scheme, BioNumerics version 7.6.3 (Applied-Maths, Sint-Martens-Latem, Belgium) performed by EURL Lm.
**Number of sequenced isolates provided to EURL Lm for the joint analysis.*

Control measures implemented at Estonian processing Company A

As of 20 March 2019, the Estonian competent authority requested Estonian processing Company A to comply with the food safety criterion that specifies the absence of *L. monocytogenes* in 25 g in cold-smoked and salted products. The authorities insisted that before products can leave the food processing plant, the company needs to present the results of an analysis for *L. monocytogenes* that verifies the following: absence in 25 g, n=5, c=0. No batch can be released to the market if the results of the analysis are positive.

Table 3. Batches of fish products where *Listeria monocytogenes (Lm)* was detected

Processing company					Sampling			*Lm* (cfu/g)	Outbreak strain match[&]	No. of sequenced isolates*
Batch	Product type	Brand	Supplier (country)	Storage condition	Context (year)	Stage	Storage condition			
A	Cold-smoked trout	A	A (NO)	chilled	company's own check (2018)	Processing company A (EE)	chilled	< 10	n.a.	n.a.
					official control (2018)	Retailer A (FR)	chilled	400	yes(a)	1
B	Cold-smoked trout	A	A (NO)	chilled	company's own check (2018)	Retailer A (FR)	chilled	< 10	yes(a)	1
C1	Cold-smoked trout	B	F (FI)	frozen	company's own check (2018)	Processing company A (EE)	frozen	< 10	n.a.	n.a.
C	Cold-smoked trout	B	F (FI)	frozen	official control (2018)	Wholesaler G (DK)	frozen	≤ 570	yes(a)	0
D	Smoked salmon	C	D (NO)	chilled	company's own check (2018)	Processing company A (EE)	chilled	<10; < 40	n.a.	n.a.
					official control (2018)	Retailer B (FR)	chilled	< 10	n.a.	n.a.
E	Cold-smoked trout	D	A (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	0
F	Cold-smoked trout	D	F (FI)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1
G	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	0
H	Cold-smoked salmon	n.a.	B (NO)	frozen	company's own check (2018)	Processing company A (EE)	chilled	< 10	n.a.	n.a.
					Official control (2019)	Wholesaler C (DK)	chilled	≤ 10	yes(a)	1
I	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	3
J	salted salmon	n.a.	E (NO)	chilled	company's own check	Processing company A (EE)	chilled	< 10; < 40	yes(b)	1
K	cold-smoked salmon	n.a.	H (NO)	chilled	company's own check	Processing company A (EE)	chilled	< 10; < 40	yes(b)	1
L	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1
M	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	2
N	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	2
O	Cold-smoked salmon	n.a.	B (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1
P	Cold-smoked salmon	n.a.	C (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1
Q	Cold-smoked salmon	n.a.	C (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1
R	Cold-smoked salmon	n.a.	C (NO)	frozen	official control (2019)	Wholesaler C (DK)	chilled	< 10	yes(a)	1

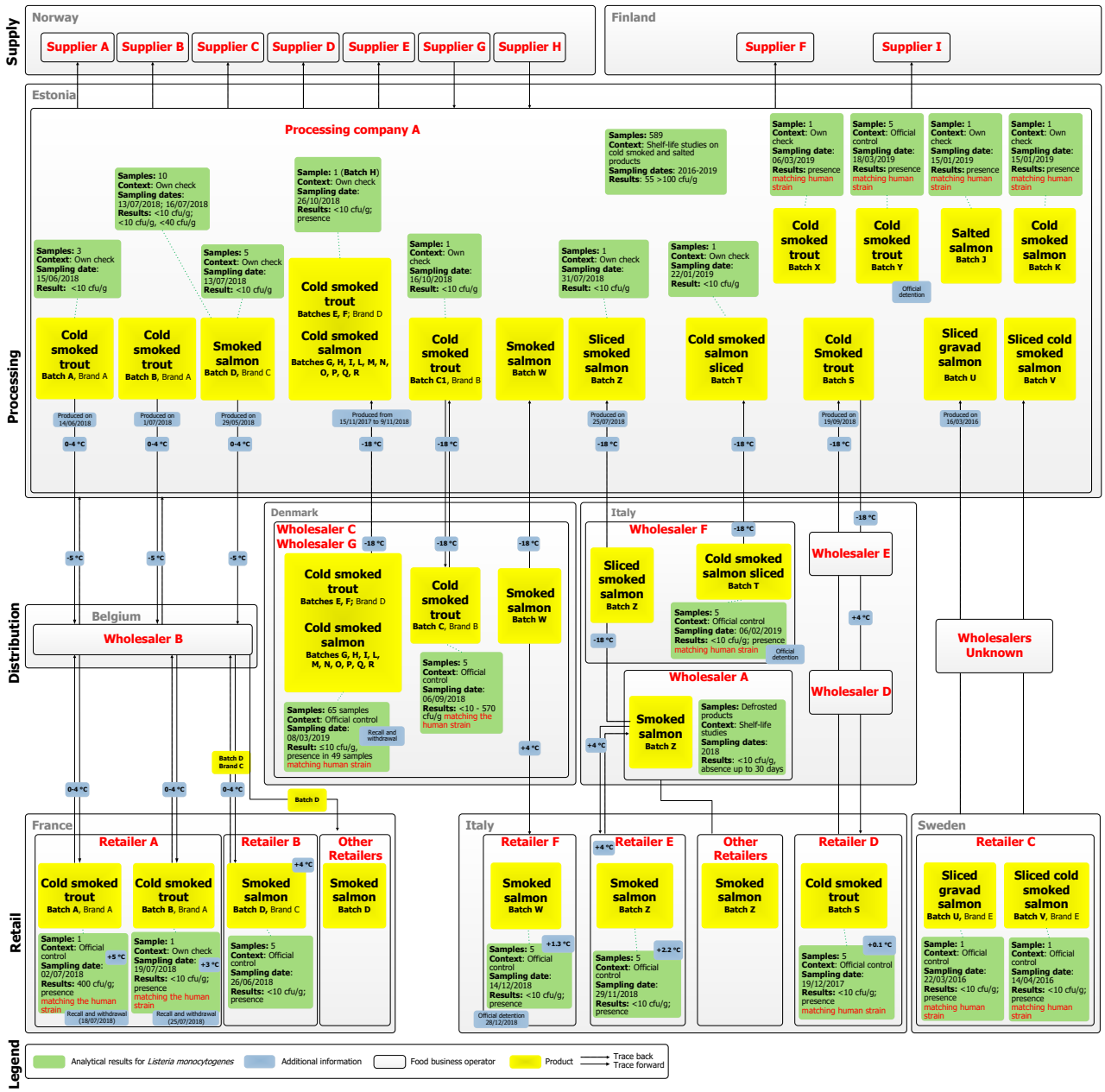
Processing company					Sampling			*Lm* (cfu/g)	Outbreak strain match[&]	No. of sequenced isolates*
Batch	Product type	Brand	Supplier (country)	Storage condition	Context (year)	Stage	Storage condition			
S	Cold-smoked trout	n.a.	A (NO)	frozen	official control (2017)	Retailer D (IT)	chilled	< 10	yes(a)	2
T	Cold-smoked salmon sliced	n.a.	E (NO)	frozen	company's own check (2019)	Processing company A (EE)	frozen	< 10	n.a.	n.a.
					official control (2019)	Wholesaler F (IT)	frozen	< 10	yes(a)	13
U	Sliced gravad salmon	E	n.a.	n.a	official control (2016)	Retailer C (SE)	n.a.	< 10	yes(a)	1
V	Sliced cold-smoked salmon	E	n.a.	n.a	official control (2016)	Retailer C (SE)	n.a.	< 10	yes(a)	1
W	Smoked salmon	n.a.	n.a.	n.a.	official control	Retailer F	chilled	< 10	n.a.	n.a.
X	cold-smoked trout	n.a.	I (FI)	chilled	company's own check	Processing company A (EE)	chilled	< 10; < 40	yes(b)	1
Y	cold-smoked trout	n.a.	I (FI)	chilled	official control	Processing company A (EE)	chilled	< 10	yes(b)	5
Z]	Smoked salmon	n.a.	n.a.	frozen	official control	Retailer E (IT)	chilled	< 10	n.a.	n.a.

n.a. = not available

[&]Criteria for the assessment of the association as reported by the a) National Competent Authorities using either SNP or cgMLST methodologies as described in the chapters above and b) EURL Lm [Seven or fewer core gene alleles, cgMLST Institut Pasteur scheme, BioNumerics version 7.6.3 (Applied-Maths, Sint-Martens-Latem, Belgium)]

** Number of sequenced isolates provided to EURL Lm for the joint analysis*

Figure 2. Graphical representation of traceability and testing information reported by Member States under the following RASFF notifications 2018.3808, 2018.3687, 2018.2870, 2018.2003, 2018.1833, 2018.0394, 2019.0999, 2019.0806



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

Using the Danish representative sequence ERR2223569, initial WGS-based searches for identifying possible outbreak-related isolates were performed in the Member States' public health institutes and national reference centres using national WGS pipelines. In addition, ECDC searched for possibly outbreak-related isolates in TESSy. The Finnish (2), French (1) and Swedish (3) public health institutes and national reference centres identified altogether five closely related isolates. In addition, ECDC identified altogether three isolates from Estonia (2) and Sweden (1) among the TESSy data. Estonia sent four additional human isolates for centralised analysis. Raw sequence data (Denmark, Estonia, Finland) or assemblies (France, Sweden) from all human *L. monocytogenes* isolates (22) closely related to the Danish representative sequence ERR2223569 were collected by ECDC from national reference laboratories (NRLs)/centres of the FWD network.

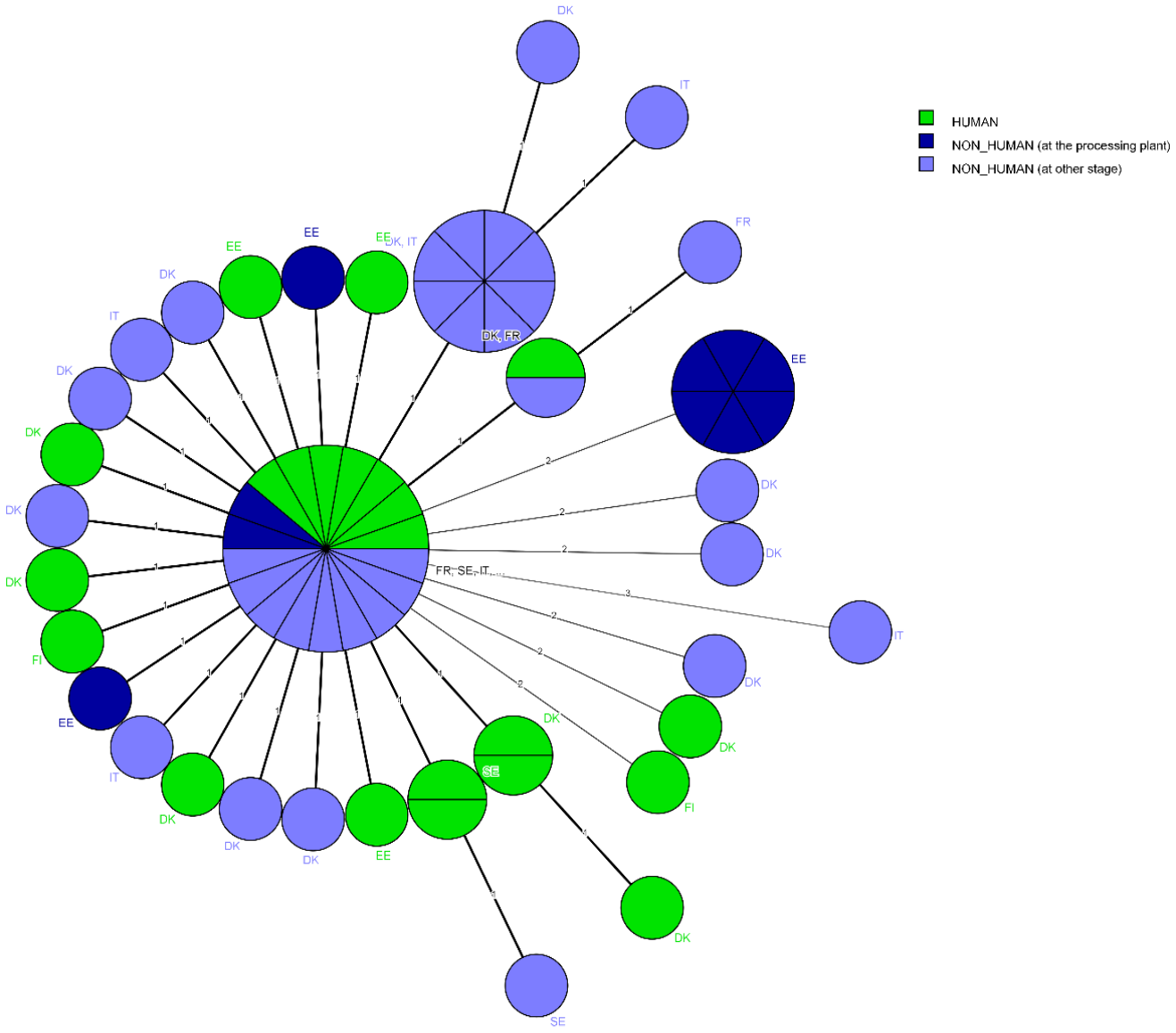
A total of 43 non-human isolates were included in the analysis. The EURL-Lm collected sequence data on 33 non-human isolates from the Danish (14), Italian (15), Swedish (2) NRLs and the French National Reference Centre (NRC, Institut Pasteur) for France (2). In addition, the EURL-Lm performed the sequencing of 10 isolates provided by the Estonian NRL (Table 3).

Human and non-human isolates from Denmark, France, Italy, Estonia and Finland were sequenced using Illumina technology while the three Swedish isolates were sequenced using Ion Torrent technology.

WGS data analysis of human and non-human isolates was performed jointly by ECDC, EFSA and the EURL-*Lm* using the same software and harmonised parameters. Sequences were analysed with BioNumerics version 7.6.3 (Applied-Maths, Sint-Martens-Latem, Belgium). The analysis of raw reads included trimming using the default Bionumerics 7.6.3 settings; *de novo* assembly using SPAdes v.3.7.1; post-assembly optimisation by mapping reads back onto the assembly and keeping the consensus (using MismatchCorrector implemented in SPAdes v3.7.1). The default settings of BLAST parameters for allele calling were used. cgMLST analysis was performed using assembly-based allele calling using Institut Pasteur scheme [10] in BioNumerics. Isolates were retained in the analysis if at least 1 661 (95%) of the 1 748 core loci were detected. Isolates differing by seven or fewer core gene alleles from any other outbreak isolate were considered as part of the outbreak cluster.

The single-linkage tree including all human (n=22) and non-human (n=43) isolates (Annex 2) shows that all of them are within 3 core gene allelic differences (based on cgMLST pairwise similarity), indicating close genetic relatedness.

Figure 2. cgMLST-based (Institut Pasteur scheme) minimum spanning tree including sequences from 22 human *L. monocytogenes* isolates and 43 non-human isolates from six countries, EU/EEA, 2015 to 2019



ECDC and EFSA threat assessment for the EU/EEA

In February 2019, Denmark reported a cluster of eight *L. monocytogenes* CC8 (ST 1247) cases between 2016 and 2019. WGS-based cgMLST analyses performed at the national level and at ECDC identified a total of 22 cases from five EU countries in the same cluster, with onset of symptoms between July 2014 and February 2019. The *L. monocytogenes* CC8 isolates were detected from Denmark (9), Estonia (6), Finland (2), France (1) and Sweden (4). Most cases were over 65 years of age, and at least five died due to, or with, the disease.

The human isolates belonged to the same cgMLST cluster, with a maximum of 2 allelic differences in single linkage clustering. The close genetic relatedness suggests a common source of infection. The temporal distribution of cases indicates a prolonged intermittent common-source outbreak taking place in different EU Member States. Since the last patient had disease onset in February and was reported in March 2019, the source of contamination of this outbreak may still be active or has been active until very recently.

In Denmark, where half of the outbreak cases (and the most recent ones) were reported and a thorough investigation was performed, most (7/9) patients were found to have consumed cold-smoked fish products during the incubation period. However, there was no specific information on the brands and batches of these products at the time of publication of this report to verify direct epidemiological link to exposure.

Furthermore, the competent authority in Denmark found matching *L. monocytogenes* strains in cold-smoked fish products imported from Estonian processing Company A. Non-human isolates matching the outbreak strain were also identified in France, Italy and Sweden – all in fish products imported from the same Estonian producer. In total,

L. monocytogenes isolates matching the human strain were detected at wholesale and retail levels from 13 batches of cold-smoked or gravad salmon products and from six batches of cold-smoked trout products. Among them, 13 batches were sampled in Denmark at the wholesale level (10 batches of smoked salmon and three batches of smoked trout), two in France at the retail level (from smoked trout products), two in Italy at the wholesale and retail levels (from cold-smoked and gravad salmon products), and two in Sweden at the retail level (smoked and gravad salmon). In 16 batches, *L. monocytogenes* was detected at <10 cfu/g, in one batch at <40 cfu/g, and in two cold-smoked trout batches, *L. monocytogenes* was enumerated at 400 cfu/g and 570 cfu/g.

Traceability information of the contaminated batches pointed to an Estonian processing plant run by Company A as the single common manufacturer of all fish products that were produced with raw materials originating from suppliers in Norway and Finland. Environmental investigations and food testing at the Estonian processing plant run by Company A showed the presence of *L. monocytogenes* that matched the outbreak strain in two samples found at the processing line and in four batches of the final product (two batches of cold-smoked trout, one batch of cold-smoked salmon, one batch of salted salmon products).

The environmental contamination with *L. monocytogenes* matching the outbreak strain has been verified in two rooms along the processing lines: in room 12, which is dedicated to the storage of plastic boxes not used for cold-smoked products, and in room 8 on a conveyor belt used for the processing of cold-smoked and salted products. Moreover, *L. monocytogenes* (with no sequencing information) was identified on the button of the skinning machine and on the transporting line after skinning in room 2 (step 6, Table 2). These findings suggest that the contamination with *L. monocytogenes* matching the outbreak strain might have happened through food contact surfaces shared by fresh and smoked/salted products (steps 1 through 7), upstream of the crossing point of the different lines of production.

L. monocytogenes is ubiquitous in the environment (e.g. moist environments, soil, and water) and it may persist in food processing facilities for years, representing a risk for food safety, especially in RTE food products, and is a serious threat to public health [8,13]. Environmental factors (e.g. hard-to-clean facilities and equipment) and intrinsic strain characteristics (e.g. biofilm formation and better adaptation to environmental stress) can contribute to the *Listeria* persistence in food processing plants [14]. Furthermore, *L. monocytogenes* CC8 strains may possess virulence genes and therefore have a high pathogenic potential [14].

The presence of *L. monocytogenes* matching the outbreak strain over a few years' time in fish products suggests persistence of the microorganism in the Estonian company's premises. Further investigation is needed to assess the role of raw materials and to identify and control points of (cross-)contamination in the food processing plant. Control measures were implemented at wholesale and retail levels in Denmark, France and Italy. In addition, as of 20 March 2019, the Estonian processing company (Company A) now only releases cold-smoked and salted fish products after the verification of 'absence of *L. monocytogenes* in 25 g', following a request from the Estonian competent authority. However, until the source of infection has been identified and controlled, new invasive listeriosis cases may still occur. Pregnant women, the elderly and immunocompromised people are at higher risk of invasive listeriosis, which is associated with severe clinical course and potentially death.

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Annex 1

Table A-1. Number of positive units for *L. monocytogenes* from RTE fish and fishery products reported to EFSA under the framework of Directive 2003/99/EC, EU/EFTA countries, 2010–2017

Food category		Number of positive units							
		2010	2011	2012	2013	2014	2015	2016	2017
Fish, RTE	smoked	185	944	1182	1111	1197	64	37	346
	marinated	0	0	11	23	0	0	55	1
	gravad /slightly salted	0	2	0	4	6	26	9	1
	smoked – hot-smoked	0	0	8	40	0	10	2	3
	smoked – cold-smoked	3	0	12	10	0	5	2	14
	Total positive units of RTE fish	**188**	**946**	**1213**	**1188**	**1203**	**105**	**105**	**365**
Fishery products RTE	unspecified – cooked	6	0	0	0	1	2	0	21
	unspecified – ready-to-eat - chilled	7	5	6	0	0	1	7	1
	unspecified – seafood pâté	0	9	0	0	0	0	0	
	unspecified – smoked	29	2	6	0	6	4	12	5
	unspecified – ready-to-eat	86	19	3	9	3	57	27	3
	unspecified – cooked - chilled	0	0	0	0	0	0	0	0
	unspecified – ready-to-eat - frozen	0	0	0	0	0	0	0	0
	Total positive units of RTE fishery products	**128**	**35**	**15**	**9**	**10**	**64**	**46**	**30**

Annex 2

Figure A-1. cgMLST-based single-linkage cluster analysis including sequences from 22 human (the dot represents the reference human isolate) and 43 non-human *L. monocytogenes* isolates, EU/EEA, 2014–2019