

JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of monophasic *Salmonella* Typhimurium sequence type 34 linked to chocolate products – first update

18 May 2022

Abstract

On 17 February 2022, the United Kingdom (UK) reported a cluster of cases with monophasic *Salmonella* Typhimurium sequence type 34 infection. As of 18 May 2022, 324 cases had been reported in 12 EU/EEA countries and the UK, including two distinct strains. Most cases are below ten years of age and 41% of all cases have been hospitalised. The two strains are multidrug-resistant and some tested isolates also carry resistance to disinfectants that are based on quaternary ammonium compounds and hydrogen peroxide, but remain susceptible to azithromycin, ciprofloxacin, meropenem, and third generation cephalosporins. Epidemiological investigations suggested specific chocolate products of Brand A, produced by Company A in Processing Plant B in Belgium, as likely vehicles of infection.

Two strains of monophasic *Salmonella* Typhimurium matching the outbreak strains were identified in the anhydrous milk fat (AMF) line at Plant B between December 2021 and January 2022. The AMF was provided by an Italian supplier where *Salmonella* was not detected. This supplier delivered the AMF to other plants of Company A where, based on the available evidence, *Salmonella* was not detected.

On 8 April 2022, based on official controls, the food safety authority in Belgium decided to withdraw the authorisation for production of the Plant B due to lack of transparency and insufficient guarantees for safe production. Company A globally recalled all products of Brand A produced at Plant B. Public warnings were issued by the competent national authorities in different countries.

This outbreak has evolved rapidly, with children most at risk for severe infection. The closure of Plant B and the global recall of all their products have reduced the risk of exposure. However, eight cases cannot be explained by consumption of chocolate products such as those manufactured at Plant B, suggesting that there may also be other sources of infection.

Amendment: this report was amended on 8 June 2022 to replace 'buttermilk' with 'anhydrous milk fat' (AMF). In the section 'Public health experts consulted for data and facts validation' the national experts for Italy were added. A reference was added to the reference list. The names of the genes were corrected from the first version.

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Supporting publications by EFSA: Technical report approved by EFSA on 18 May 2022; doi:10.2903/sp.efsa.2022.EN-7352; Key words: *Salmonella*, chocolate products, multi-country outbreak, Whole Genome Sequencing (WGS). Requestor: European Commission; Question number: EFSA-Q-2022-00288; correspondence: roa-efsa@efsa.europa.eu, ISSN: 2397-8325.

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Event background

On 17 February 2022, the United Kingdom (UK) reported in ECDC's EpiPulse system (event ID 2022-FWD-00014) a 5-single nucleotide polymorphism (SNP) cluster of 18 cases with monophasic *Salmonella* Typhimurium infection of eBURST Group 1 (eBG1).

Comparisons of national sequences in other countries with the outbreak strains in the UK revealed cases mostly in young children, with isolates genetically close to the UK strains. Interviews with parents and guardians of the cases raised suspicions that chocolate products of Brand A might be the vehicle of infection. This was corroborated by the identification of a genetically close monophasic *S. Typhimurium* ST34 strain (cluster 1) from the production environment of specific chocolate products of Brand A. The increasing number of case reports from several EU/EEA countries and the UK, as well as a finding of a genetically close monophasic *S. Typhimurium* ST34 strain (cluster 1) in chocolate products, triggered the investigations and initiation of a joint ECDC-EFSA Rapid Outbreak Assessment (ROA), which was published on 12 April 2022.

The investigations performed by food safety authorities in Belgium had identified another strain of monophasic *S. Typhimurium* ST34 in five non-human isolates, forming a second but distinct cluster (cluster 2). Subsequently, comparisons of sequences of the second strain with isolates from humans identified additional human cases in several countries, thus triggering an update of the ROA.

Resistance profile of the human isolates in cluster 1

According to the analyses in the United Kingdom Health Security Agency (UKHSA), the outbreak strains in cluster 1 are **susceptible** to amikacin (MIC = 4 mg/L), azithromycin (MIC = 4-8 mg/L), ciprofloxacin (MIC = <0.06 mg/L), meropenem (MIC = 0.03-0.06 mg/L), and third generation cephalosporins cefotaxime (MIC = 0.25 mg/L) and ceftazidime (MIC = 0.25 mg/L).

The outbreak strain in cluster 1 is **resistant** to six classes of antimicrobials listed as follows with associated resistant determinants:

- penicillins (*bla_{TEM-1}*);
- aminoglycosides (streptomycin, spectinomycin, kanamycin, and gentamicin (variable gene combinations); *aph(3'')-Ib/strA*, *aac(6')-Iaa*, *aac(3)-IId*, *aph(6)-Id/strB*, *aadA-2*, *aadA-8b*, *aadA-12*, *aadA-15* and *aadA-17*);
- phenicols (*cmIA1*, *flrR*);
- sulfonamides (*sul2* with some strains having an additional *sul3* gene);
- trimethoprim (*dfrA12*);
- tetracyclines (*tetB* and *tetM*).

In addition, some strains in cluster 1 contained the *Inu(F)* gene encoding resistance to lincosamides.

The resistance to kanamycin/gentamicin, phenicols, and trimethoprim is rare in monophasic *S. Typhimurium* and could therefore be used for screening of probable cases. However, France and Belgium have noted that a minor proportion of their isolates matching the outbreak sequence have lost a DNA fragment containing several genes, including those encoding for this rare resistance profile. Also, Spain reports that one of their confirmed cases belonging to cluster 1 has the following resistance profile: ampicillin (*bla_{TEM-1}*), sulfamethoxazole (*sul2* and *sul3*), tetracycline (*tetB*), kanamycin (*aph(3')-Ia*) and streptomycin (*aadA17*, *aph(3'')-Ib*, *aph(6)-Id*) as well as *InuF*, and *aac(6')-Ia*.

In addition, some of the isolates in cluster 1 possess *qac* genes (*qacL*) encoding resistance to quaternary ammonium compounds (QAC) and *sitABCD* genes encoding resistance to hydrogen peroxide.

Resistance profile of the human isolates in cluster 2

The outbreak strain in cluster 2 is **resistant** to four classes of antimicrobials listed as follows, with associated resistant determinants (not tested phenotypically):

- penicillins/beta lactams (*bla_{TEM-1}*)
- tetracyclines (*tetA* or *tetB*)
- sulphonamides (*sul-2*)
- aminoglycosides (*aph(6)-Id/strB* (streptomycin); *aph(3'')-Ib/strA* (streptomycin); *aph(3')-Ia* (kanamycin); *aac(6')-Iaa*).

European outbreak case definitions

Cluster 1

Two representative UK isolates are publicly available in the European Bioinformatics Institute/European Nucleotide Archive (EBI/ENA) with accession numbers SRR17830210 and SRR18021617. These represent isolates in cluster 1 (C1), which are part of Enterobase cgMLST hierarchical cluster designation HC5_296366 ([1,2]).

A confirmed outbreak case in cluster 1

A laboratory-confirmed monophasic *Salmonella* Typhimurium case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

Fulfilling at least one of the following laboratory criteria for cluster 1: a monophasic *S. Typhimurium* ST34 isolate:

- clustering with any of the representative outbreak isolates by the national cgMLST pipeline within five allelic differences (AD); OR
- clustering in a centralised whole genome sequencing (WGS) analysis within five ADs in a single linkage analysis; OR
- belonging to the cgMLST HC5_296366 according to the Enterobase scheme; OR
- falling into the 5-SNP (single nucleotide polymorphism) single linkage cluster 1.1.1.124.6096.7575.% (t5.7575) (eburst group 1), according to the UKHSA pipeline [3]; OR
- clustering according to a national SNP pipeline within five SNPs.

A probable outbreak case in cluster 1, when WGS had not yet been performed to confirm or rule out the case

A laboratory-confirmed monophasic *S. Typhimurium* case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

By phenotypical testing expressing resistance to ampicillin/amoxicillin, kanamycin/gentamicin, trimethoprim/co-trimoxazole (trimethoprim-sulmethoxazole) and chloramphenicol.

OR

MLVA (multilocus variable number tandem repeat analysis) profile 3-11-14-NA-0211 [4].

Cluster 2

Two representative UK isolates are publicly available in the EBI/ENA with accession numbers SRR18488397 and SRR18590198. These represent isolates in cluster 2 (C2), which is part of Enterobase cgMLST hierarchical cluster designation HC5_298160.

A confirmed outbreak case in cluster 2

A laboratory-confirmed monophasic *Salmonella* Typhimurium case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

Fulfilling at least one of the following laboratory criteria for 'cluster 2': a monophasic *S. Typhimurium* ST34:

- clustering with any of the representative outbreak isolates by the national cgMLST pipeline within five allelic differences (AD); OR
- clustering in a centralised whole genome sequencing (WGS) analysis within five ADs in a single linkage analysis; OR
- belonging to the cgMLST HC5_298160 according to the Enterobase scheme; OR
- falling into the 5-SNP (single nucleotide polymorphism) single linkage cluster 1.1.1.2765.6144.7643.% (t5.7643) (eburst group 1), according to the UK Health Security Agency (UKHSA) pipeline [3]; OR
- clustering according to a national SNP pipeline within five SNPs.

A probable outbreak case in cluster 2, when WGS had not yet been performed to confirm or rule out the case

A laboratory-confirmed monophasic *S. Typhimurium* case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

By phenotypical testing expressing resistance to ampicillin/amoxicillin, kanamycin, and tetracyclines.

OR

MLVA (multilocus variable number tandem repeat analysis) profile 3-8-10-NA-0211 for cluster 2 [4].

Epidemiological and microbiological investigations of human cases

As of 18 May 2022, 324 cases of monophasic *S. Typhimurium* ST34, of which 266 are confirmed and 58 probable, have been reported in 12 EU/EEA countries and the UK (Table 1). In addition, cases in clusters 1 and 2 have been identified in Canada (C1 n=1), Switzerland (C1 n=41, C2 n=2), and the United States (C1 n=1). Globally, cluster 1 comprises 274 confirmed cases, of which 231 cases were reported in 11 EU/EEA countries and the UK. Cluster 2 includes 37 cases from eight EU/EEA countries, the UK, and Switzerland. Altogether, 369 cases linked to the outbreak have been identified globally (Table 1).

The first case (cluster 1) was reported in the UK on 7 January 2022 with a sampling date on 21 December 2021, week 51/2021 (Figure 1). The most recent sampling date was reported for a case (cluster 1) in the UK on 27 April 2022. In cluster 2, the first case was sampled on 24 January 2022 in the UK and the most recent case on 25 April 2022 in Belgium. Since January 2022, case reports increased steadily until week 15, when the case number dropped from 41 in week 14 (4 April) to 12 in week 15 (11 April) (Figures 1-2). The median delay between disease onset and notification to the national public health authority was 21 days (range 4 to 40 days) among 156 cases with both dates reported.

Only three cases (1.8%) among those with available information on travelling (n=165) reported travel prior to symptom onset. Two cases had travelled to Spain (one case from the Netherlands and one case from the UK) and one UK case reported travel to India.

The majority of infections (86.3%) are among children at or younger than 10 years, and for all cases in the EU/EEA and the UK with information available, 41.3% of them have been hospitalised (Table 1, Figure 3). No deaths were reported. Females, with 63.3% of cases, are more frequently reported than males and the female-to-male ratio is 1.7.

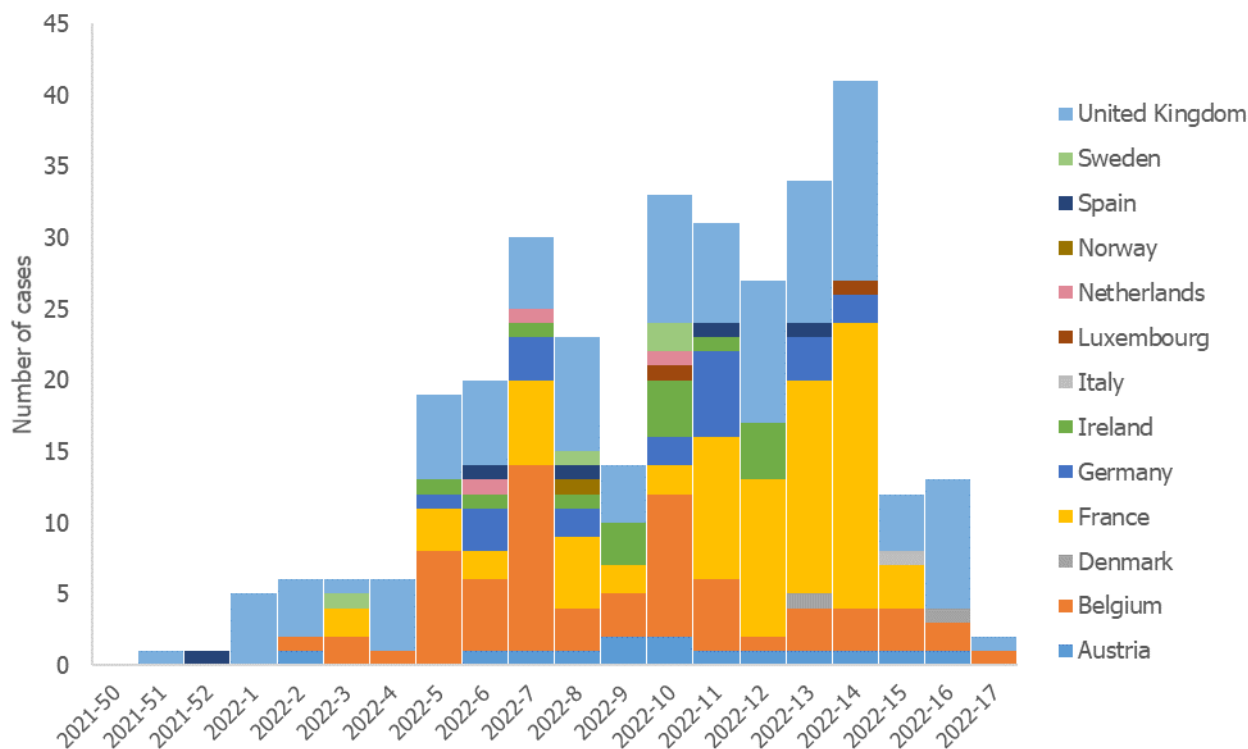
The Canadian case is a child under the age of ten with a symptom onset date of 19 February 2022 and with no reported travel outside of Canada prior to becoming ill. In Switzerland, 40/41 cases in cluster 1 were children below ten years with a mean age of four years. Of 41 cases, 24 were females and 17 males. The two Swiss cases in cluster 2 were four-year-old females. The laboratory received the first sample of a Swiss case in cluster 1 on 16 February and in cluster 2 on 29 March 2022. The case in the US was reported from the state of Illinois but no further information about the case is available.

Table 1. Number of confirmed and probable cases of monophasic *S. Typhimurium* and hospitalisations by country, as of 18 May 2022

Country	Confirmed cases		Probable cases	Total number of cases	Hospitalised (among cases with available data)
	Cluster 1	Cluster 2			
Austria	7	7	0	14	35.7%
Belgium	7	5	52	64	39.2%
Denmark	1	1	0	2	NA
France	73	8	0	81	42.9%
Germany	15	4	3	22	66.7%
Ireland	15	1	0	16	30.8%
Italy	0	1	0	1	NA
Luxembourg	2	0	0	2	NA
Netherlands	2	1	0	3	NA
Norway	1	0	0	1	NA
Spain	2	0	3	5	NA
Sweden	4	0	0	4	NA
Total EU/EEA	129	28	58	215	41.9%
United Kingdom	102	7	0	109	39.7%
Total EU/EEA and UK	231	35	58	324	41.3%
Canada	1	0	0	1	NA
Switzerland	41	2	0	43	NA
United States	1	0	0	1	NA
Total	274	37	58	369	NA

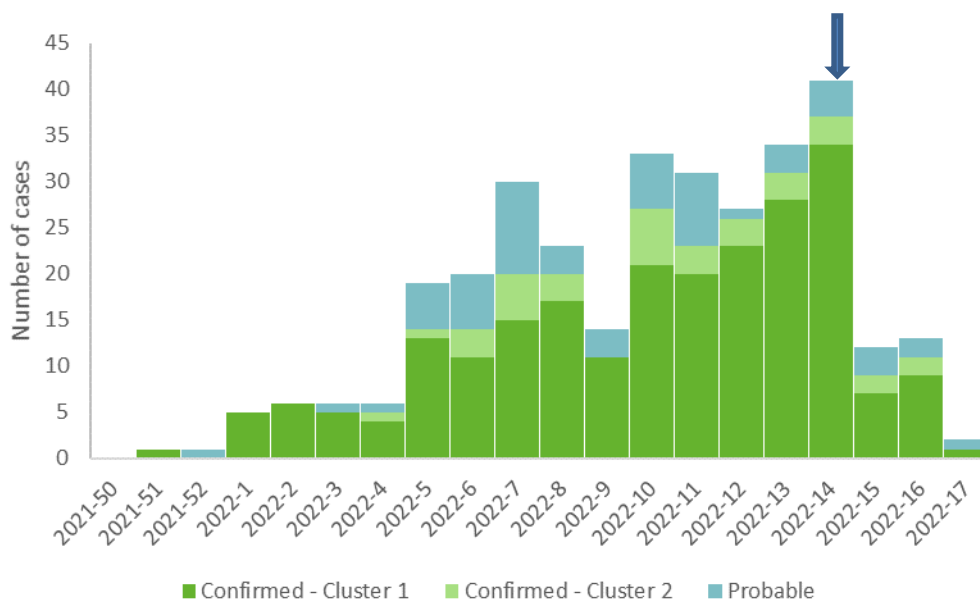
NA= Data not available or not applicable due to ≤ 5 cases

Figure 1. Distribution of 324 confirmed and probable monophasic *S. Typhimurium* cases by week-year* and country in 12 EU/EEA countries and the United Kingdom, as of 18 May 2022



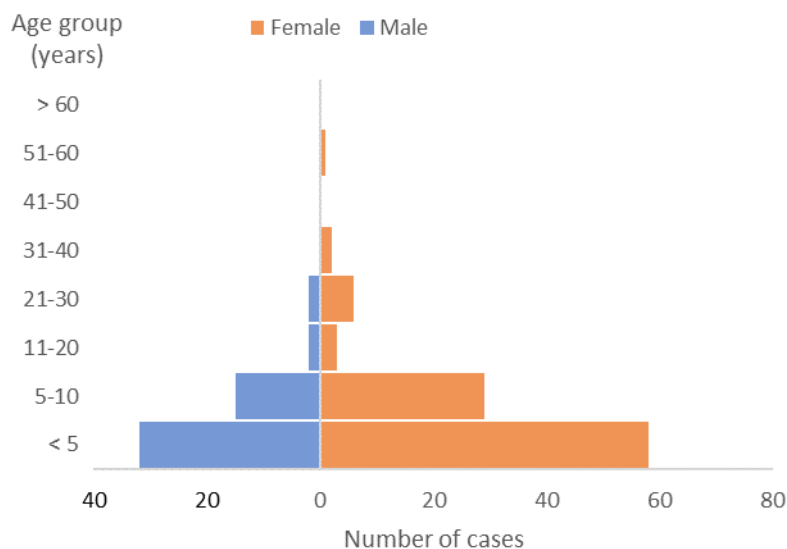
*Date of sampling (n=295), date of receipt to the laboratory (n=26), or date of notification (n=3)

Figure 2. Distribution of 324 confirmed (cluster 1 + cluster 2) and probable monophasic *S. Typhimurium* cases by week-year* in 12 EU/EEA countries and the United Kingdom, as of 18 May 2022 (arrow shows the closure of the Belgian Processing Plant B)



*Date of sampling (n=295), date of receipt to the laboratory (n=26), or date of notification (n=3)

Figure 3. Distribution of 322* confirmed (cluster 1 + cluster 2) or probable monophasic *S. Typhimurium* cases in 12 EU/EEA countries and the United Kingdom by age group and gender, as of 18 May 2022



*Gender unknown for two cases in Belgium

Information from patient interviews

Among the 324 cases under study, 191 (59.0%) were interviewed. Among 179/191 interviewed cases with information available, 170 (95.0%) reported consumption of any chocolate product of Company A and nine reported no consumption of products of Company A. Two cases with no reported consumption of products of Company A were suspected to be secondary cases.

Among the 170 cases reporting consumption of chocolate products of Company A, the most commonly reported products that were also listed as products produced in the Belgian Processing Plant B were Product A (n=136) and Product B (n=48 cases), and 34 cases reported consumption of both Product A and Product B. Fourteen cases reported consumption of Product E and two cases reported consumption of Product C.

Consumption of other types of chocolate products, like mini bars of Brand A (n=48 cases), Product I (n=29 cases) and Product N of Company A were also reported.

There are eight cases who did not report consumption of types of chocolate products produced at the Belgian Processing Plant B of Company A, but consumption of other products produced by Company A. Five of these eight cases (two cases in France and one each in Germany, Ireland, and the UK) reported that the only Company A product they had consumed was Product N. Two of these eight cases, in Belgium reported the only Company A products they had consumed were Product I and Product N. One of these eight cases, a one-year-old child in Ireland, had consumed Company A's Product J and Product K before symptom onset. Among these eight cases, five belonged to cluster 1 and three to cluster 2. The production plants of products consumed by these eight cases have not been traced.

There were six cases below one year of age and the parents of three of them were interviewed. Two babies had no exposure to chocolate products whereas one had consumed Product A.

For 19 cases, the frequency of chocolate consumption was reported and among those, 47.4% reported consumption every day whereas 26.3% of cases reported consumption of the implicated chocolate products about once a week.

The Canadian case reported consuming a 'large' chocolate egg with a surprise toy inside prior to symptom onset.

Microbiological and environmental investigations of food and control measures

This section summarises country-specific information on microbiological and traceability investigations as reported through the Rapid Alert System for Food and Feed (RASFF) alert notification 2022.1799 as of 16 May 2022 (275 EC validated follow-ups - *fup*), RASFF news 2022.2201 (16 *fups* as of 16 May 2022) and RASFF information notification for attention 2022.2452 (45 *fups* as of 16 May 2022). A visual representation describing the traceability of raw material, chocolate products, and testing information linked to Company A is provided in Figure A1 (Annex 1). Table A1 (Annex 1) describes the number of own-check samples tested *Salmonella*-positive and taken at the Belgian Processing Plant B of Company A. The number of official samples tested *Salmonella*-negative and collected in the involved Member States is provided in Table A2 (Annex 1). A summary of the countries that reported the implementation of control measures in RASFF 2022.1799 is outlined in Annex 1.

On 25 March 2022, the European Commission sent an alert through RASFF (RASFF alert notification 2022.1799) of an ongoing foodborne outbreak of infections caused by monophasic *Salmonella* Typhimurium. The alert notification was triggered by epidemiological investigation performed by the public health authority in the UK that pointed to chocolate products of Brand A from Company A as a possible vehicle of the infection. The UK's food safety authority and public health authorities had discussed the issue with Company A and provided additional information that was uploaded in RASFF (*fup42*, 2022.1799). Consequently, according to the food exposure information reported by the public health authorities in Germany, France, Ireland and Sweden, different types of chocolate products from Brand A produced by Company A were suspected to be the vehicle of the infection. Company A has plants in Belgium (Belgian Processing Plant B), France, Germany, Ireland, Italy and Poland that produce different products, including chocolate products of Brand A (*fup23*, 2022.1799).

On 8 April 2022, the food safety authority in Belgium informed through RASFF that the investigation in the Belgian Processing Plant B had shown that the plant had not been able to provide the necessary guarantees concerning its management of the contamination and was therefore not able to guarantee the safety of its products. The food safety authority decided to withdraw the authorisation for production of the Belgian Processing Plant B due to lack of transparency and insufficient guarantees for safe production. Company A decided to extend the recall to all batches of Brand A products manufactured in this facility and linked to the contaminated production line, regardless of lot number or expiration date. Company A informed all its business units in the different countries. They, in turn, informed the local authorities. The food safety authority in Belgium has taken all the necessary measures concerning the Belgian Processing Plant B (*fup71*, 2022.1799; *fup13*, 2022.2201).

Belgium

In Belgium, Company A produced products of Brand A (Product A, Product B, Product D, Product E) and other chocolate products of a different brand (Product F) in a single plant (Belgian Processing Plant B) (*fup23*, 2022.1799) up to 8 April 2022.

Belgian Processing Plant B

At the Belgian Processing Plant B, the leading time from manufacture to shelf was between 55 and 60 days (*fup42*, 2022.1799). Company A communicated that some of the raw materials used were similar across various products. The suppliers of the raw materials for products of Brand A are batch-dependent (two or three suppliers for raw materials), but there have been no changes in suppliers, recipes or manufacturing process in the past year (*fup42*, 2022.1799). In addition, Company A communicated that the shelf-life of Product A and of other products of Brand A was 270 days for the UK, while in Europe this was 225 days (*fup42*, 2022.1799).

Outcome of the official controls performed by the food safety authority in Belgium

Following the official control performed on 8 April 2022, the food safety authority in Belgium said that a total of 81 samples from finished products, semi-finished products, intermediate products, raw materials, environmental swabs and rinse oil samples taken in own-check at the Belgian Processing Plant B over a production period of two months between 3 December 2021 and 25 January 2022 tested positive for *Salmonella* (2022.2201). The food safety authority in Belgium was informed about part of these *Salmonella* findings at the beginning of April 2022 (*fup20*). A summary of the positive findings are displayed in Table A1 (Annex 1). In particular, the monophasic *Salmonella* Typhimurium isolate from 3 December 2021 was identified in a sample of Product A (finished product reanalysed by a French laboratory on 3 April 2022 and received by the national reference laboratory in Belgium on 19 April 2022). The food safety authority informed that WGS analysis of the detected strain indicates that the strain belongs to cluster 2 (RASFF 2022.2201) (*fup13*, 2022.2201).

Out of the 81 *Salmonella* positive samples, eight samples with nine isolates (*fup10*, *fup13*, 2022.1799) were submitted between 5 January 2022 and 8 February 2022 by Company A to an Italian laboratory for serological and molecular typing. Four isolates were WGS-typed as monophasic *Salmonella* Typhimurium ST34 belonging to cluster 1 and five as monophasic *Salmonella* Typhimurium ST34 belonging to cluster 2. The four isolates from cluster 1 had been detected from three samples, namely an environmental swab collected from the preparation area on 28

December 2021 of Product B (two isolates); a finished product (Product B) related to the production process of 6 January 2022 (one isolate); and an intermediate product intended for Product A related to the production process of the 7 January 2022 (one isolate).

The five isolates from cluster 2 had been detected in five samples collected from: an intermediate product related to the production process of the 13 December 2021 and intended for Product B (one isolate); anhydrous milk fat (AMF) collected from the AMF filter of both Tank 1 and Tank 2 of the AMF circuit (one isolate) that was sampled on 9 January 2022; some residual material from the AMF filter common to Tank 1 and Tank 2 (one isolate) collected on 8 January 2022; AMF collected from the spout of the Tank 2 (one isolate) on 9 January 2022; and residual material (one isolate) collected on 8 January 2022 from Tank 2 (*fup152*, 2022.1799).

The food safety authority also said that a total of 229 samples of raw materials and finished food products had been collected and analysed for *Salmonella* using real-time PCR and/or ISO methods during the official control in April. Seven of the analysed food products (finished products) tested positive for *Salmonella*. Whole genome sequencing analysis of the isolated strains indicated matches with both clusters.

Timeline of positive findings and identification of the point of contamination at Belgian Processing Plant B

The Belgian Processing Plant B had received the results from the first positive finding (four positive samples) (from the production of 13 December 2021) on 15 December 2021. The positive findings belonged to the two semi-finished products and two intermediate products, and they were related to the production line of Product E, Product A, Product B and another chocolate product. The analysis was performed by PCR at the end of the batch production. After confirmation of the positive findings, the affected products were destroyed (*fup23*, 2022.1799) and a cleaning and an internal investigation (sampling of intermediate, semi-finished, finished products and work shift used for these productions) was started. Samples were collected on the days following the 13 December 2021. Between 14 December 2021 and 16 December 2021, 26 positive samples were identified at the plant. Following the positive findings, production was stopped on 16 December 2021 (except for the production line of Product F which was physically separated). From 18 December 2021, a deep cleaning of the lines was started, excluding the part of the production line where the positivity was detected on 16 December 2021, which was bypassed until the end of February 2022. To evaluate the efficiency of the cleaning performed on 18 December and of further cleanings, environmental swab and/or rinsing oil samples were taken and analysed, leading to six non-compliant results (six samples collected on 18, 20, 21, 23 and 28 December 2021 tested *Salmonella* positive). The production was restarted on 3 January 2022, excluding part of the production line for which the cleaning was finalised on 8 January 2022. All final products were released after negative *Salmonella* results of the tested batch.

On 5 January 2022, a *Salmonella* positive result related to the production of 3 January 2022 was received (confirmed on 7 January by official method). Therefore, as of 5 January 2022, the internal investigation ordered an hourly sampling. The samples collected hourly between 3 and 4 January 2022 yielded negative results except for one positive result (also confirmed on 7 January 2022 by official method). On 7 January 2022, identification of a further 23 positive results (semi-finished products related to the production line of Product E, Product B, and finished products - Product A) led to stopping the production. This investigation was performed by testing the products across the two production days of 6 and 7 January 2022 hourly. On 8 and 9 January 2022, environmental samples and raw material samples (residues of AMF from the tank filter) were collected, and they yielded positive results. These results indicated that the AMF circuit, namely Tank 2, was the point of origin of the contamination. Therefore, the AMF contained in both Tank 1 and Tank 2 was discarded and the AMF circuit was bypassed. The production was restarted after the implementation of cleaning of the lines and bypass of the AMF circuit. Between 11 and 14 January 2022, seven additional samples (environmental swabs, intermediate products related to the production line of Product E, Product B, and semi-finished products related to the production line of Product E) were found positive. A further cleaning was performed. One more positive result was obtained from a semi-finished product related to the production line of Product E on 25 January 2022, after which a further cleaning was performed. The increased sampling plan was kept in place until 13 March 2022.

In response to the contamination event, the sampling frequency was increased at all phases of the production process. The raw material sampling was increased from testing 250 g of samples collected at a single point per month to testing 750 g of samples collected at three sampling points (during the offloading) per delivery. Therefore, the testing frequency was increased to each delivery and the sampling amount was increased by three times. The semi-finished products and the final product sampling frequency was increased nine times and the sampling amount was increased by three times (from 250 g to 750 g of samples collected throughout the production).

Sampling and monitoring plan for anhydrous milk fat

Following the positive findings at the anhydrous milk fat (AMF) circuit, the Belgian Processing Plant B upgraded the sampling and testing monitoring plan on AMF from one sampling per month to one sampling per delivery. Therefore, the sampling was performed on top of the truck (analysis performed on a sample amount of 250g) and on the unloading system on the truck (sampling done by swabbing). The Belgian Processing Plant B said there was no unloading of the truck before a confirmation of a negative result was received. The flexible pipe was sampled every week and it was changed every six months. During the unloading of AMF, the Belgian Processing Plant B

performed the sampling at three sampling points (point after the pump). Moreover, samples (analysis of 750 g) were taken twice from the bottom of Tank 1 and Tank 2 per work shift.

On 25 January 2022, the two tanks which were part of the AMF circuit were sampled to verify the efficiency of the post-cleaning intervention (cleaning and sanitisation performed by an external company between 21 January and 22 January 2022). Tank 1 and Tank 2 were sampled by swabbing the bottom, middle, and top on the internal surfaces and they tested negative. On 26 January 2022, two samples of oil after flushing were taken from the two outflow pipes from the two silos as verification of the cleaning and they tested negative.

Cleaning protocol, verification activities and release of the production line

The Belgian Processing Plant B stopped the production line when a *Salmonella* positive sample was found and implemented cleaning of the production line. The cleaning protocol consisted of three steps. Step A Wet Cleaning is a combination of dis-assembly, wet cleaning with detergent and sanitiser. Step B Dry Cleaning consisted in the emptying of machinery, followed by mechanical cleaning (in place) with detergent and sanitiser. Step C Hot Oil Flushing consisted of the flushing of piping and equipment with oil over 75°C.

The cleaning step was followed by the verification of the cleaning activities. The effectiveness of cleaning was verified by surface swabbing for cleaning protocols described in Step A and Step B, while for cleaning protocol described in Step C, a sample of oil was analysed as well as samples from surface swabbing (when access was possible). Company A used RT-PCR to analyse the samples. The confirmation of positive or suspected positive samples for *Salmonella* was performed according to the ISO 6579-1 method.

Upon verification of the efficiency of the cleaning protocol i.e. yielding a *Salmonella* negative result of the tested samples, the production line was restarted. When *Salmonella* was detected, the cleaning protocol was repeated. The efficiency of this activity was verified by swabbing and/or oil flush analysis.

Italy

In Italy, Company A produces products of Brand A in four processing plants: Italian Processing Plant C, Italian Processing Plant D, Italian Processing Plant E and Italian Processing Plant F. Company A said that none of the semi-finished products produced at the Belgian Processing Plant B is used at the Italian Processing Plants (*fup23*, 2022.1799).

The Italian Supplier A

The traceback analysis performed at Belgian Processing Plant B on the raw material (AMF) used at the plant which tested positive for monophasic *S. Typhimurium* cluster 2 in January 2022, pointed towards the Italian Supplier A (2022.2201). The Italian Supplier A supplies AMF to five plants of Company A i.e., the German Processing Plant A (AMF in tank), the Belgian Processing Plant B (AMF in tank), the Italian Processing Plant C (AMF in tank), the Italian Processing Plant E (packed in 25 kg cartons), and the Polish Processing Plant H (packed in 25 kg cartons).

On 13 April 2022, the food safety authority in Italy performed an official control at the Italian Supplier A and informed in RASFF that *Salmonella* was not identified at the Italian Supplier A (own-check between October 2021 and April 2022) (*fup2*, 2022.2201). On 19 and 20 April 2022, the food safety authority carried out a second official control and verified the hygiene-sanitary status of the whole production line starting from the tank for milk reception to the AMF production and storage, including control of the supplier's own-check plan. During the official control, samples were taken from the AMF production area (four environmental swabs collected on 20 April 2022) and from available batches of AMF (three samples collected on 20 April 2022). The microbiological analyses (by PCR) did not detect *Salmonella* (*fup5*, *fup10*, 2022.2201).

Anhydrous milk fat production and distribution

Anhydrous milk fat is a cream with 99.8% fat with a maximum moisture residue of 0.1% and 0.1% protein, lactose, and salts residues (*fup4*, 2022.2201). Anhydrous milk fat is produced starting from raw cow milk that is collected from farms located in an Italian region. For the AMF production, the use of butter and fat originating from milk whey is not permitted. The AMF is produced by the Italian Supplier A following Company A's specifications (*fup2*, 2022.2201).

According to the flow diagram for AMF production, after the raw milk skimming step, the cream undergoes a pasteurisation step at 80°C per 12 seconds. Subsequently, the cream with 40% fat undergoes a concentration step by centrifugation (75% fat) and an inversion phase by homogenisation. When the liquid component is removed, the cream reaches the fat concentration of 99.5%. With the subsequent washing step (polishing), the cream reaches the fat concentration of 99.8%. The AMF is stored in bags of 900 kg at 5°C. Before being delivered, the AMF is re-processed and melted at 98°C. It is then kept for one minute at this temperature (degaser step) and it is cooled down to 45°C and stored in silos (tanks) intended for deliveries (*fup2*, 2022.2201).

At Italian Supplier A, the daily batch production of AMF is 24 tons. The Italian Supplier A delivered part of the daily batch production to Belgian Processing Plant B (storing capacity of 15.3 tons) and part of the same daily batch production to other plants. The last delivery to Belgian Processing Plant B was on 28 March 2022. Between 1 October 2021 and 28 March 2022, the Italian Supplier A supplied Belgian Processing Plant B 66 times, the Italian Processing Plant C 75 times and the German Processing Plant A 18 times. The food safety authority in Italy said

that AMF was also produced and delivered in the same period to companies in Germany and France other than Company A (*fup2*, 2022.2201).

Anhydrous milk fat storage conditions and shelf life

Upon its production, the AMF is transferred directly from the silos in which it is contained to tanks used for storage within a closed circuit. The AMF is stored at temperature between 40°C and 46°C in its liquid status for ten days. The AMF is also stored in drums at room temperature for 12 months (*fup4*, 2022.2201).

The AMF has a shelf life of 12 months when kept at +4°C and transported at +4°C (packed in 25 kg cartons), ten days when kept at +45°C and transported at +45°C (in bulk), and 24 months when kept at -18°C and transported at -18°C (packed in 25 kg cartons) (*fup2*, 2022.2201).

Anhydrous milk fat chemical and microbiological testing foreseen by the own-check plan

During the AMF production, chemical and microbiological analyses are performed. The chemical analysis refers to arsenic, lead, and benzopyrene (*fup2*, 2022.2201). The routine microbiological analysis of AMF consists of testing one sample per batch for the total colony count at 30°C, *Enterobacteriaceae*, *E. coli*, coliforms at 37°C, yeasts, and moulds. In addition, as requested by Company A, the Italian Supplier A tests the AMF twice a year for the following pathogens: coagulase positive *Staphylococci* and *Listeria* (analysis on 1 g), lipolytic bacteria, *Salmonella* (five aliquots of AMF). Regarding *Salmonella*, the food safety authority said that the Italian Supplier A performs additional analyses on each batch intended to be delivered to Company A (*fup2*, 2022.2201). As of 23 February 2022, five aliquots of AMF are tested for *Salmonella* per delivery intended for export to China.

Moreover, the Italian Supplier A performs microbiological monitoring of the surfaces of tanks intended for the AMF by testing for total colony count at 30°C, *Enterobacteriaceae*, coliforms (by water filtration), moulds and yeasts (*fup2*, 2022.2201).

During official controls, the food safety authority checked the results from the analytical reports (from 19 March 2021, 15 September 2021, 28 February 2022, 10 March 2022, 14 March 2022, 23 March 2022) of the own-check samplings related to the AMF deliveries, and said that *Salmonella* was not detected (*fup2*, 2022.2201). Furthermore, the food safety authority said that all the microbiological analysis (coliforms, yeasts, moulds, *E. coli*, *Enterobacteriaceae*, total colony count at 30°C), including those for *Salmonella* that had been performed on the AMF batches produced between 1 June 2021 to 28 February 2022, did not reveal non-compliant results (*fup2*, 2022.2201).

Regarding the results from environmental testing (sponge sampling of the area where the AMF is produced), from the washing and sterilisation procedures, and from the wastewater used at the plant, the food safety authority reported that *Salmonella* was not detected (*fup2*, 2022.2201).

Anhydrous milk fat equipment cleaning procedure

All the equipment used to produce AMF is cleaned every day for each tank. The cleaning is followed by a disinfection step with peracetic acid or hydrogen peroxide. Before starting the production and before the storage of AMF in silos, the Italian Supplier A flushes the line with AMF at 80°C to remove moisture residues. Then, the AMF used for flushing is discarded.

The Italian Processing Plant C

The Italian Processing Plant C produces different products of Brand A (including Product A, Product I, other chocolate products from Brand A) and other brands, and products other than chocolate products (*fup23*, 2022.1799).

On 19 April 2022, the food safety authority in Italy performed an official control at the Italian Processing Plant C and reported the outcome of the investigation on the AMF (*fup4*, 2022.2201) in RASFF.

At the Italian Processing Plant C, AMF is used for the production of chocolate products from Brand A, namely Product A, Product I, Product E and other chocolate products from Brand A and from another brand of the same Company A (*fup4*, 2022.2201).

The Italian Processing Plant C receives the AMF from the Italian Supplier A via Transport A of Company A. Company A carries out the transport of AMF from Italian Supplier A to its plants in tanks that are cleaned by authorised operators, according to Company A's requirements (*fup4*, 2022.2201).

The food safety authority in Italy said the Italian Processing Plant C has received the AMF from the Italian Supplier A since August 2009, and that from September 2021, it only received the AMF from the Italian Supplier A.

At the Italian Processing Plant C, the AMF tanks are quarantined for 48 hours waiting for results of the microbiological analysis. The tanks in which the AMF is stored are cleaned every time the tanks are emptied for a total washing time of 122 minutes divided into six steps. There is a maximum of ten days between two consecutive washings (*fup4*, 2022.2201). The efficiency of the cleaning of the tanks is verified annually by means of surface samples (sponge) tested for *Enterobacteriaceae* and total colony counts (*fup2*, 2022.2201). Before and after unloading the AMF from the tanks in which AMF is contained, microbiological analyses on samples (sponge samples) automatically collected from some components of the tanks are performed to test for *Enterobacteriaceae* (analysis carried out at each tank arrival) (*fup4*, 2022.2201).

In addition, the food safety authority said that the Italian Processing Plant C had received the AMF from other suppliers i.e. the German Supplier B (manufacturer) that delivered it in November 2016 and is authorised to deliver it in case there is a need; the Dutch Supplier C (only trader), the German Supplier D (only trader), and the German Supplier E (manufacturer).

Sampling plan for anhydrous milk fat monitoring

The sampling plan on AMF foresees the testing for *Salmonella* in 250g with a frequency of once per month and testing for *Enterobacteriaceae* with frequency of one per tank (until February 2022). Starting from February 2022, testing for *Salmonella* in 250g and for *Enterobacteriaceae* is done with the frequency of one per tank (one per delivery). The food safety authority said that since September 2021, 42 analyses were performed at the Italian Processing Plant C and that *Salmonella* was not detected. In addition, the food safety authority said that in its ten-year history of deliveries to Company A, all own-check analyses done for *Salmonella* in 250g were negative (*fup4*, 2022.2201).

The Italian Processing Plant E

The Italian Processing Plant E produces other chocolate products (mainly snacks) from Brand A and other brands (*fup7*, 2022.2201) and receives the AMF from Italian Supplier A.

On 21 April 2022, the food safety authority in Italy carried out an official control at the Italian Processing Plant E and said that six samples had been collected. These samples were four finished products (snacks) and two raw materials (chocolate) used to produce the snacks (*fup7*, 2022.2201). Results from the microbiological analysis revealed no *Salmonella* detection (*fup12*, 2022.2201).

The food authority also performed an inspection of the analytical reports from the own-check plan of the Italian Processing Plant E. The reports showed no *Salmonella* detection. The sampling plan for AMF foresees that each received batch of AMF is tested against *Salmonella* (*fup7*, 2022.2201).

Moreover, no *Salmonella* was detected in previous years in both finished products and raw materials (*fup7*, 2022.2201).

Poland

The Polish Processing Plant H

In Poland, Company A produces different products of Brand A (including Product A, Product I, and other chocolate products (*fup23*, *fup85*, 2022.1799) in the Polish Processing Plant H.

With regard to Product I that had been mentioned as consumed by one interviewed case in Ireland (*fup85*, 2022.1799), the food safety authority in Poland said in RASFF 2022.1799, that Product I is manufactured at the Polish Processing Plant H as well as in Italy, at the Italian Processing Plant C, and in France, at the French Processing Plant G (*fup23*, *fup85*, 2022.1799).

Regarding the AMF, the food authority also informed that at the Polish Processing Plant H, AMF is used for the production of Product I and that *Salmonella* was not detected from Product I or from raw materials used at the plant (laboratory reports not available from RASFF) (*fup85*, 2022.1799).

On 21, 22, and 25 April 2022, the food safety authority performed an official control at the Polish Processing Plant H and on 2 May 2022 said in RASFF that the Plant received the AMF from the Italian Supplier A (in 25 kg boxes) via Transporter A of Company A (*fup6*, *fup11*, 2022.2201). Between October 2021 and December 2021, the Polish Processing Plant H received nine batches of AMF from the Italian Supplier A (20.7 tons per delivery) (*fup15*, 2022.2201) accompanied by laboratory reports (own-check) showing bacterial counts < 1 CFU/g for coliforms, yeasts, moulds, *E. coli*, and *Enterobacteriaceae*, and <10 CFU/g for total colony count at 30°C (*fup6*, 2022.2201). There were no deliveries between January and April 2022 (*fup15*, 2022.2201). The food authority also said on 2 May 2022 that no *Salmonella* positive findings were reported at the plant (laboratory reports not available in RASFF). During the inspection, samples from all batches (95 samples from 19 batches) of AMF from the Italian Supplier A and 12 samples from three batches of finished products from another brand from Company A and containing exclusively AMF from the Italian Supplier A were collected and tested *Salmonella* negative (*fup16*, 2022.2201). In addition, the food safety authority said that other finished products contained AMF from the German Supplier B or a mix of AMF from both suppliers (*fup11*, 2022.2201).

The food authority also said that the Polish Processing Plant H only receives the AMF from German Supplier B and Italian Supplier A (*fup173*, 2022.1799).

Germany

German Processing Plant A

In Germany, Company A produces different chocolate products of Brand A (including Product A, Product L, Product J, Product K) and other chocolate products of different brands in a single plant (German Processing Plant A) (*fup23*, 2022.1799).

The German Processing Plant A performs 150 microbiological own-checks daily and 35 000 per year (*fup1*, 2022.1799). The German headquarters of Company A is exclusively responsible for the traceability within Germany (*fup29*, 2022.1799).

On 25 March 2022, the food safety authority in Germany reported in RASFF that the German Processing Plant A was inspected. Over 30 official food samples were collected from different batches of chocolate products of Brand A (*fup1*, 2022.1799).

Regarding Product J and Product K that had been mentioned as consumed by an interviewed case in Ireland (*fup219*, 2022.1799), the food safety authority in Germany informed in RASFF 2022.1799 that five samples from Product J and five samples of Product K (production dates between January and February 2022) had been tested in an official control on 25 March 2022 and no *Salmonella* was detected (*fup1*, *fup226*, 2022.1799) by PCR or by culture (*fup167*, 2022.1799).

Regarding Product L which had been mentioned in RASFF 2022.1799 by the authorities in France (*fup73*, 2022.1799), the food safety authority in Germany said that the products had been manufactured on 11 June 2021 with expiration dates in January 2022 (*fup96*, 2022.1799). At the German Processing Plant A, microbiological analysis of own check samples collected from Product L, from its raw materials, and from its semi-finished products did not detect *Salmonella* since June 2021 (*fup96*, 2022.1799). The food safety authority also said that all semi-finished products intended for Product L are manufactured exclusively at the German Processing Plant A and there are no raw materials or intermediate products shared with the Belgian Processing Plant B (*fup96*, 2022.1799).

On 11 April 2022, the food safety authority performed an official control at the German Processing Plant A and said in RASFF that samples had been collected from Product L and from the environment (*fup96*, 2022.1799) (sponge and swab samples) (28 environmental samples), especially from Product L's production line (*fup167*, 2022.1799). Samples were also collected from raw materials and semi-finished products (nine samples). No *Salmonella* was detected by PCR (*fup167*, 2022.1799) (laboratory reports not available in RASFF). In addition, the food authority said that only raw materials supplied directly to the German Processing Plant A by the supplier are used for the production of the Product L (*fup167*, 2022.1799).

Concerning the AMF, the German Processing Plant A receives the AMF from the Italian Supplier A and from other suppliers i.e. the German Supplier B, the Dutch Supplier C (only trader), the German Supplier D (only trader), and the German Supplier E. The last deliveries from the German Supplier E to the German Processing Plant A were on 1 April 2022 and to the Italian Processing Plant C on 1 March 2021.

The official samples collected in Germany from chocolate products (Table A2, Annex 1) from Brand A of Company A (Product A, Product B, Product C, Product I, Product J, Product K, Product L, and other chocolate products) tested *Salmonella* negative.

The German Supplier B

On 14 April 2022, the food safety authority in Germany said that the German Supplier B supplied AMF exclusively to the German Processing Plant A and the Polish Processing Plant H. The two last deliveries to the German Processing Plant A were on 25 March 2021 and on 7 October 2021 and had been accompanied by laboratory reports showing *Salmonella* negative results (*fup173*, 2022.1799).

France

In France, Company A produces a product of Brand A namely Product I and another chocolate product (Product N) of a different brand in the French Processing Plant G (*fup23*, 2022.1799).

On 26 April 2022, the food safety authority reported the outcome of an official control performed at a French company that had received a batch of AMF from the Italian Supplier A on 30 November 2021. The batch was entirely used between 3 and 9 December 2021 to make 11 products. Ten product samples had been taken from the company's stock (one recipe was not available). The food authority reported that laboratory results were *Salmonella* negative (*fup9*, 2022.2201).

The French Processing Plant G

On 6 April 2022, the food safety authority in France reported that official controls were ongoing at the French Processing Plant G of Company A and that the cases' purchases were being investigated too (*fup6*, *fup44*, 2022.1799).

Global distribution

On 27 April 2022, the food safety authority in Belgium provided in RASFF 2022.2452 the global distribution of the chocolate products of Brand A manufactured at the Belgian Processing Plant B and intended to be distributed to 99 EU and non-EU countries: Albania, Andorra, Argentina, Armenia, Australia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Canada, Chile, Costa Rica, Croatia, Cyprus, Czechia, Denmark, Dominican Republic, Egypt, El Salvador, Estonia, Ethiopia, Faeroe Islands, Finland, France, French Guiana, French Polynesia, Georgia, Germany, Greece, Guadeloupe, Guatemala, Honduras, Hong Kong, Hungary, Iceland, India, Iraq, Ireland, Israel, Italy, Jordan, Kazakhstan, Kosovo, Kuwait, Kyrgyzstan, Latvia, Lebanon, Lithuania, Luxembourg, Madagascar, Malaysia, Malta, Mauritius, Mayotte, Mexico, Moldova, Mongolia, Montenegro, Morocco, the Netherlands, New Caledonia, New Zealand, Nicaragua, Norway, Oman, Panama, Paraguay, Poland, Portugal, Qatar, Republic of North Macedonia, Romania, Russia, Réunion, San Marino, Saint Martin, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Taiwan, Tajikistan, Tunisia, Turkey, Turkmenistan, Ukraine, United Arab Emirates, the United Kingdom, United States, Uruguay, Uzbekistan and the Vatican City (2022.2452).

The global distribution of the concerned products included the deliveries between 1 June 2021 and April 2022 and the list of operators performing and receiving the deliveries. The shipment of the products was done via Company A platforms. Therefore, the food safety authority said that the concerned products may have transited through other countries before arriving at Company A's platform responsible for the final distribution, and that the concerned products may have been redistributed from the recipient country to other countries not included in the list above (Figure A1, Annex 1) (2022.2452).

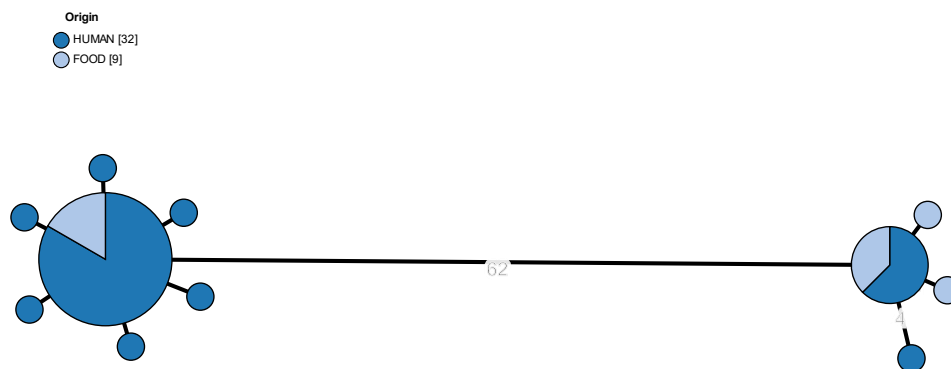
European whole genome sequencing analysis of human and non-human isolates

The EURL-*Salmonella* launched a consultation for submitting sequences of monophasic *S. Typhimurium* isolated from food between January 2021 and June 2022. As of 8 April 2022, National Reference Laboratories (NRL) in 11 countries had replied: Czechia, Cyprus, Germany, Ireland, Latvia, Malta, Portugal, Slovenia, Spain, Switzerland and the Netherlands. None of the identified sequenced isolates either matched the outbreak strains or were epidemiologically linked to the outbreak.

The Italian laboratory that typed the isolates from the Belgian Processing Plant B submitted the genome sequences of nine monophasic *S. Typhimurium* isolates from samples of semi-finished products to EFSA. The nine non-human isolates were analysed together with 32 human isolates of HC5_296366 and HC5_298160, including the representative isolates of the outbreak strains, shared publicly by UKHSA and available in European Nucleotide Archive (ENA) on 16 May 2022. CgMLST analysis was performed in EFSA using chewBBACA version 2.8.5 (<https://github.com/B-UMMI/chewBBACA>) using the schema as described by Rossi et al. 2018 [5] for *Salmonella enterica* made available by chewie Nomenclature Server [6] at <https://chewbbaca.online/species/4>. Isolates with more than 1% of missing loci (32 over a total of 3 225 loci) were excluded from the analysis. The minimum spanning tree was constructed using MSTree V2 algorithm as implemented in GrapeTree v1.5.0 [7] and shown in Figure 2.

The analysis confirms the presence of two distinct clusters at 62 allelic differences from each other. One cluster (left cluster in Figure 2) includes the representative isolates of the cluster 1 outbreak strain (SRR17830210 and SRR18021617), and additionally 24 human isolates from HC5_296366 and four non-human isolates from the Belgian Processing Plant B. The second cluster (right cluster in Figure 2) includes the representative isolates of the cluster 2 outbreak strain (SRR18488397 and SRR18590198) and additionally, four human isolates from HC5_298160 and five non-human isolates from the Belgian Processing Plant B.

Figure 2. Minimum spanning tree of 32 human (dark blue) and nine non-human (light blue) monophasic *Salmonella* Typhimurium isolates



ECDC and EFSA risk assessment for the EU/EEA and the United Kingdom

As of 18 May 2022, 324 cases (266 confirmed and 58 probable) of monophasic *S. Typhimurium* ST34 have been identified in 12 EU/EEA countries and the UK since 21 December 2021 (sampling date for a first identified case in cluster 1 in the UK), and 369 cases globally. This is more than double the number compared with the previous risk assessment on 12 April 2022, partly due to the addition of the second strain to the case definition. There are two distinct clusters, of which cluster 1 comprises 231 cases reported in 11 EU/EEA countries and the UK, and cluster 2 includes 35 cases from eight EU/EEA countries and the UK. The most recent case in cluster 1 has a sampling date of 27 April 2022 in the UK and the most recent case in cluster 2, of 25 April 2022 in Belgium. Most cases (86.3%) are children younger than ten years, and a high proportion of cases (about 40%) have been hospitalised. No deaths have been reported. Female cases are more frequent than males with a female-to-male ratio of 1.7.

Out of 170 (95.0%) of the 179 interviewed cases with available information, consumption of chocolate products of Company A, as listed by the Belgian food safety authority, were reported as follows; Product A (n=136) and Product B (n=48 cases), and 34 cases reported consumption of both Product A and Product B. Fourteen cases reported consumption of Product E and two cases reported consumption of Product C. There are eight cases, who did not report consumption of the above-mentioned products (Products A, B, C and/or E). Five cases reported consumption of only Product N of Company A: two cases in France and one each in Germany, Ireland, and the UK. Two cases in Belgium reported only consumption of Product I and Product N of Company A. One case, a one-year-old child in Ireland, had consumed Product J and Product K of Company A before symptom onset. Among these eight cases, five belonged to cluster 1 and three to cluster 2.

At the Belgian Processing Plant B of Company A, during the production period between 3 December 2021 and 25 January 2022, monophasic *S. Typhimurium* and *Salmonella* spp. were detected in 81 samples collected from the production lines of Brand A. The *Salmonella* positive products were destroyed, cleaning of the production line was performed and an internal investigation was started. Production was restored after negative test results for *Salmonella*, and all final products were released after a negative *Salmonella* result of the tested batch. Among the 81 collected samples, 10 monophasic *S. Typhimurium* isolates matching the representative outbreak strains from cluster 1 (four isolates) and cluster 2 (six isolates) were identified in samples collected from residual raw materials from the AMF line, and from finished and intermediate products of Brand A. On 8 April, additional official samples were collected from finished products and seven tested positive for *Salmonella*, matching either cluster 1 or cluster 2.

The AMF line was identified by the Belgian Processing Plant B as the point of the contamination involving the production of both Products A and B. It is worth noting that Product A was identified in several countries, including the UK, as the most frequently reported product consumed by cases and that for the UK market it is only produced at the Belgian Processing Plant B. Moreover, Product B was one among those products mentioned by the cases and also originated from the Belgian Processing Plant B. In the absence of WGS typing of the other *Salmonella* isolates detected at the plant, there is a need for further investigation to exclude the presence of additional strains.

Considering that the average time from production to retail is approximately 60 days, the first sampled case in the UK on 21 December 2021 is very unlikely to be explained by the contamination with the outbreak strains detected in the Belgian Processing Plant B in December 2021. This suggests that the contamination in the Belgium plant occurred earlier than December 2021.

The traceback analysis of the AMF used at the Belgian Processing Plant B during the contamination event identified the Italian Supplier A as the single AMF provider. At the Italian Supplier A, *Salmonella* was not detected in own-checks (October 2021-April 2022) and from official samples taken by the food safety authority in Italy on 20 April 2022.

The Italian Supplier A, among other companies, delivered the AMF to other plants of Company A located in Germany, Italy, and Poland, where, based on the available evidence, *Salmonella* was not detected. The Italian Supplier A also delivered AMF to other companies located in France and Germany.

On 8 April 2022, the food safety authority in Belgium decided to withdraw the authorisation for production of the Belgian Processing Plant B due to lack of transparency and insufficient guarantees for safe production. At the same time, Company A decided to extend the recall to all batches of all products of Brand A produced at the Belgian Processing Plant B that had been distributed globally, including Canada, USA, and Switzerland where cases linked to this outbreak were also reported. Following Company A's recall, countries reported the implementation of withdrawal and recall of all products of Brand A produced at the Belgian Processing Plant B.

This is a very extensive outbreak of monophasic *S. Typhimurium* linked to chocolate products, which has evolved rapidly with children at most risk for severe infection. The high hospitalisation rate warrants specific attention. Despite multidrug resistance to several antibiotic classes, the outbreak strains seem to be susceptible to azithromycin, ciprofloxacin, meropenem and third generation cephalosporins, including cefotaxime and ceftazidime. The closure of the Belgian Processing Plant B on 8 April 2022 and the global recall and withdrawal of all their products have reduced the risk of exposure, but new cases may occur due to the long shelf life and possible storage of products at home. The public health impact of the rigorous control measures implemented can be reflected in the significant drop of reported cases between weeks 14 and 15 in April 2022. However, among 156 cases reported in this outbreak with available dates, the median delay between disease onset and notification to the national surveillance system was three weeks with a maximum of five weeks. It is also likely that cases in countries that have not reported cases may have remained undetected if cases were not sequenced routinely.

Finally, there are eight cases, which cannot be explained by consumption of chocolate products from the Belgian Processing Plant B suggesting that there may also be other sources of infection, albeit secondary infections, which cannot be excluded.

Options for response

ECDC continues the prospective monitoring of reported cases on its website and encourages Member States to be alert for, and investigate, human infections with monophasic *S. Typhimurium* strains that have multi-drug resistance profiles, particularly showing resistance or having resistance markers to kanamycin/gentamicin, trimethoprim or co-trimoxazole (trimethoprim-sulfamethoxazole), and chloramphenicol. Further sequencing of such isolates is recommended. ECDC encourages countries to interview those cases for possible exposure to chocolate products described in this ROA. Any new information on human cases linked to this event should be reported in EpiPulse under the event 2022-FWD-00014. ECDC offers sequencing support for countries with limited or no WGS capacity. Notifications in the Early Warning and Response System should be issued if the appropriate criteria are fulfilled. ECDC further encourages public health authorities to cooperate closely with food safety authorities.

EFSA encourages Member States to perform sequencing of monophasic *S. Typhimurium* food isolates related to the RASFF notifications 2022.1799, 2022.2201, and 2022.2452, and/or linked to the present clusters either microbiologically (serotype or ST) or epidemiologically (e.g. consumption of chocolate products by human cases or isolates linked to the company involved as in RASFF 2022.1799, 2022.2201, and 2022.2452), and to share these sequences with EFSA and the EURL for *Salmonella*. Further information about food traceability investigation can be requested from the RASFF contact point for the relevant countries. EFSA can offer sequencing support to those countries that have no capacity (on request via roa-efsa@efsa.europa.eu).

Source and date of request

On 21 April 2022, EFSA and ECDC agreed to produce an update of the Rapid Outbreak Assessment that was published on 12 April 2022. ECDC sent an official request to EFSA on 25 April 2022 and EFSA accepted it on 26 April 2022.

Consulted experts and national contact points

ECDC experts (in alphabetical order): Áine Collins, Johanna Takkinen, Therese Westrell

EFSA staff (in alphabetical order): Lorena Corredor Barrera, Ernesto Liebana, Denise Pezzutto, Valentina Rizzi, Mirko Rossi, Eleonora Sarno.

Public health experts consulted for data and facts validation:

Austria: Christian Kornschober (Austrian Agency for Health and Food Safety)

Belgium: Dieter Van Cauteren, Valeska Laisnez, Wesley Mattheus, Inne Nauwelaers (Sciensano);

Canada: Russell Forrest, Cynthia Misfeldt (Public Health Agency of Canada)

Czechia: Ondřej Daniel (National Reference Laboratory for Salmonella, National Institute of Public Health), Michaela Špačková (Department of Infectious Diseases Epidemiology, National Institute of Public Health);

Denmark: Luise Müller, Emily Dibba White, Pernille Gymoese (Statens Serum Institute)

France: Maria Pardos de la Gandara and François-Xavier Weill (Institut Pasteur, Centre National de Référence des *E. coli*, *Shigella* et *Salmonella*); Nathalie Jourdan-da Silva, Henriette de Valk, and Hugues Delamare (Santé publique France)

Germany: Sandra Simon (National Reference Centre for Salmonella and other bacterial enteric pathogens, Robert Koch Institute); Gerhard Falkenhorst and Raskit Lachmann (Department of Infectious Disease Epidemiology, Robert Koch Institute)

Ireland: Patricia Garvey, Sarah Gee, and Paul McKeown (Health Protection Surveillance Centre); Martin Cormican and Niall De Lappe (National *Salmonella*, *Shigella* and *Listeria* Reference Laboratory, University Hospital Galway); Charlotte Salgaard Nielsen, ECDC Fellowship Programme, Field Epidemiology path (EPIET) (European Centre for Disease Prevention and Control and Health Protection Surveillance Centre)

Italy: Lena Raffaello, Stefania D'amato, Claudia Lucarelli, Giovanni Mattalia, and Laura Villa (Istituto Superiore di Sanità)

Luxembourg: Joël Mossong and Anne Vergison (Health Directorate); Catherine Ragimbeau (National Health Laboratory)

The Netherlands: Maaike van den Beld and Roan Pijnacker (National Institute for Public Health and the Environment)

Norway: Lin T. Brandal and Heidi Lange (Institute of Public Health)

Spain: Silvia Herrera León (National Centre of Microbiology, Instituto de Salud Carlos III), Carmen Varela Martinez (National Centre of Epidemiology, Instituto de Salud Carlos III)

Sweden: Rikard Dryselius and Nadja Karamehmedovic (Public Health Agency)

Switzerland: Roger Stephan (Institute for Food Safety and Hygiene, University of Zürich), Mirjam Maeusezahl (Federal Office of Public Health)

The United Kingdom: Marie Chattaway, Gauri Godbole, Suzanne Gokool, Ann Hoban, Lesley Larkin, Jacquelyn McCormick, Anaïs Painset, and Caisey Pulford (United Kingdom Health Security Agency), Derek Brown, Lynda Browning, and John Cowden (Public Health Scotland)

The United States: Morgan Schroeder (Centers for Disease Control and Prevention)

RASFF contact points: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland.

National experts consulted by the RASFF contact points:

Belgium: Laurence Delbrassinne, Bavo Verhaegen (Sciensano, NRL for Foodborne Outbreaks)

Czechia: Lenka Bartošová, Pavla Kundrliková – Burešová (Czech Agriculture and Food Inspection Authority)

Finland: Mika Varjonen, Paula Hietanen (Finnish Food Authority)

Hungary: György PLEVA, Dr. (Directorate for Food and Feed Safety, National Food Chain Safety Office), Zsuzsanna LANCZ SRÉTERNÉ, Dr. (National Reference Laboratory for Microbiology, National Food Chain Safety Office)

Italy: Raffaello Lena, Giovanni Mattalia (office 8 DG SAN Ministry of Health)

Luxembourg: François Zimer, Josiane Dahm, Fabienne Clabots, (Division de la Sécurité alimentaire, Ministère de la Santé).

Other experts exceptionally consulted for the provision of technical data:

The United Kingdom: Tina Potter, Christine Gathumbi (The Food Standards Agency).

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 18 May 2022. 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.

Annex 1. Food traceability and analyses

Control measures

On 2 April 2022, Company A performed a recall in the United Kingdom and Ireland on a precautionary basis based on the strong epidemiological links between the Brand A's products and cases in the UK. A further recall was implemented on 7 May 2022 for additional distributed products.

As of 7 April 2022, the food safety authority in Belgium said in a RASFF that the Belgian Processing Plant B of Company A had been conducting a recall in the countries concerned by the product distribution. Company A performed a voluntary and precautionary recall based on the *Salmonella* detection in December 2021 at the Belgian Processing Plant B, and on the epidemiological studies performed by the public health authorities in different countries, including the first case detection in the UK on 21 December 2021 (*fup20*, 2022.1799).

From 8 April 2022, based on the outcome of investigations performed by the food safety authority in Belgium showing that the plant had not been able to provide the necessary guarantees concerning its management of the contamination and was therefore not able to guarantee the safety of its products, the food safety authority decided to withdraw the authorisation for production of the Belgian Processing Plant B. At the same time, Company A decided to extend the recall to all batches regardless the production date and batch number of all products of Brand A produced at the Belgian Processing Plant B (*fup71*). Company A said that Product F produced at Belgian Processing Plant B was not involved in the recall since the production line of Product F is separated from Brand A's production lines and Brand A semi-finished products are not used (*fup101*, 2022.1799).

Control measures consisting of the withdrawal and recall of the chocolate products from Brand A manufactured at the Belgian Processing Plant B were implemented by the food safety authorities of the countries involved in their distribution (Belgium, Croatia, Czechia, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Romania, Slovakia, Spain, Sweden and the United Kingdom) as reported in RASFF 2022.1799 up to 8 April 2022 (up to *fup72* EC validated) and as published in the Rapid Outbreak Assessment on 12 April 2022. In addition, other countries (Albania, Austria, Bulgaria, Canada, Cyprus, Estonia, Kosovo, Israel, Latvia, Lithuania, Malta, Poland, Republic of San Marino, Slovenia and Switzerland) reported implementation of the control measures (2022.1799 after *fup72*). Public warnings to inform consumers were issued in the involved countries.

Table A1. Overview of *Salmonella*-positive own-check samples spanning a production period of two months between 3 December 2021 to 25 January 2022, collected at the Belgian Processing Plant B

Sampling date	Sampled product	Aim of sampling*	Intended for final product	WGS analysis (cluster 1 or cluster 2)
3 Dec 2021	Finished product	N/A	Product A	Cluster 2
13 Dec 2021	Semi-finished	Normal sampling	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Intermediate		Product A	
	Intermediate		Product B, Product E	Cluster 2
14 Dec 2021	Semi-finished	Normal sampling	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Finished product		Product A	N/A
	Intermediate		Product E, Product B	N/A
15 Dec 2021	Semi-finished	Normal sampling	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E and other product	N/A
	Finished product		Product A	N/A
	Finished product		Product D	N/A
	Finished product		Product B	N/A
	Intermediate	Investigation	Product E, Product B	N/A
	Intermediate		Product E, Product B	N/A
	Intermediate		Product E and other product	N/A
	Intermediate		Product E, Product B	N/A
	Intermediate		Product A	N/A
	Intermediate		Product A	N/A
16 Dec 2021	Intermediate	Investigation	Product A	N/A
	Intermediate		Product A	N/A
	Intermediate		Product B, Product E	N/A

Sampling date	Sampled product	Aim of sampling*	Intended for final product	WGS analysis (cluster 1 or cluster 2)
	Intermediate		Product B, Product E	N/A
	Intermediate		Product A	N/A
	Intermediate		Product A	N/A
	Intermediate		Product B, Product E	N/A
	Intermediate		Product B, Product E	N/A
		Intermediate	Normal sampling	Product B
18 Dec 2021	Environmental Swab	Post-cleaning	N/A	N/A
	Rinse Oil		Product D	N/A
20 Dec 2021	Intermediate	Investigation	Product B, Product E	N/A
	Intermediate		Product B, Product E	N/A
	Rinse oil	Post-cleaning	N/A	N/A
21 Dec 2021	Environmental swab	Post-cleaning	Product B, Product E	N/A
23 Dec 2021	Semi-finished	Investigation	Product E and other product	N/A
	Semi-finished		Product E and other product	N/A
	Semi-finished		Product E and other product	N/A
	Semi-finished		Product E and other product	N/A
	Semi-finished		Product E and other product	N/A
	Rinse oil	Post-cleaning	N/A	N/A
28 Dec 2021	Environmental Swab	Investigation	Product B, Product E	Cluster 1 (x2)
03 Jan 2022	Semi-finished	Reinforced sampling	Product E, Product B	N/A
04 Jan 2022	Semi-finished	Investigation	Product B	N/A
06 Jan 2022	Finished	Reinforced sampling	Product B	Cluster 1
	Semi-finished	Investigation	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
07 Jan 2022	Semi-finished	Reinforced sampling	Product E, Product B	N/A
	Semi-finished		Product E and other product	N/A
	Finished product		Product A	N/A
	Semi-finished	Investigation	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Finished product		Product A	N/A
	Intermediate		Reinforced sampling	Product A
Semi-finished	Investigation	Product E and other product	N/A	
08 Jan 2022	Raw material	Investigation	AMF	Cluster 2
	Environmental swab		AMF	Cluster 2
09 Jan 2022	Raw material	Investigation	AMF	Cluster 2
10 Jan 2022	Raw material	Investigation	AMF	Cluster 2
11 Jan 2022	Environmental swab	Investigation	AMF	N/A
	Environmental swab		AMF	N/A
	Environmental swab		AMF	N/A
13 Jan 2022	Intermediate	Reinforced sampling	Product E, Product B	N/A
14 Jan 2022	Semi-finished	Reinforced sampling	Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A
	Semi-finished		Product E, Product B	N/A

Sampling date	Sampled product	Aim of sampling*	Intended for final product	WGS analysis (cluster 1 or cluster 2)
25 Jan 2022	Semi-finished	Reinforced sampling	Product E, Product B	N/A

***Normal sampling:** routine, sampling for finished and semi-finished products every three hours, analysis in batch, pool of 250 g; for intermediate products pool of daily batch 250 g. **Post-cleaning:** oil, swabs or material after cleaning, sampling of minimum 1 kg, single analysis on 750 g (not for swabs). **Investigation:** extra analysis due to deviations, to investigate on the root cause analysis and beginning of contamination; analysis on 750 g. **Reinforced sampling:** every hour, 1 kg sampling, analysis in 750 g.

Table A2. Overview of official samples testing *Salmonella* negative and collected from products within their durability dates by food safety authorities in countries involved in the distribution of chocolate products from Company A

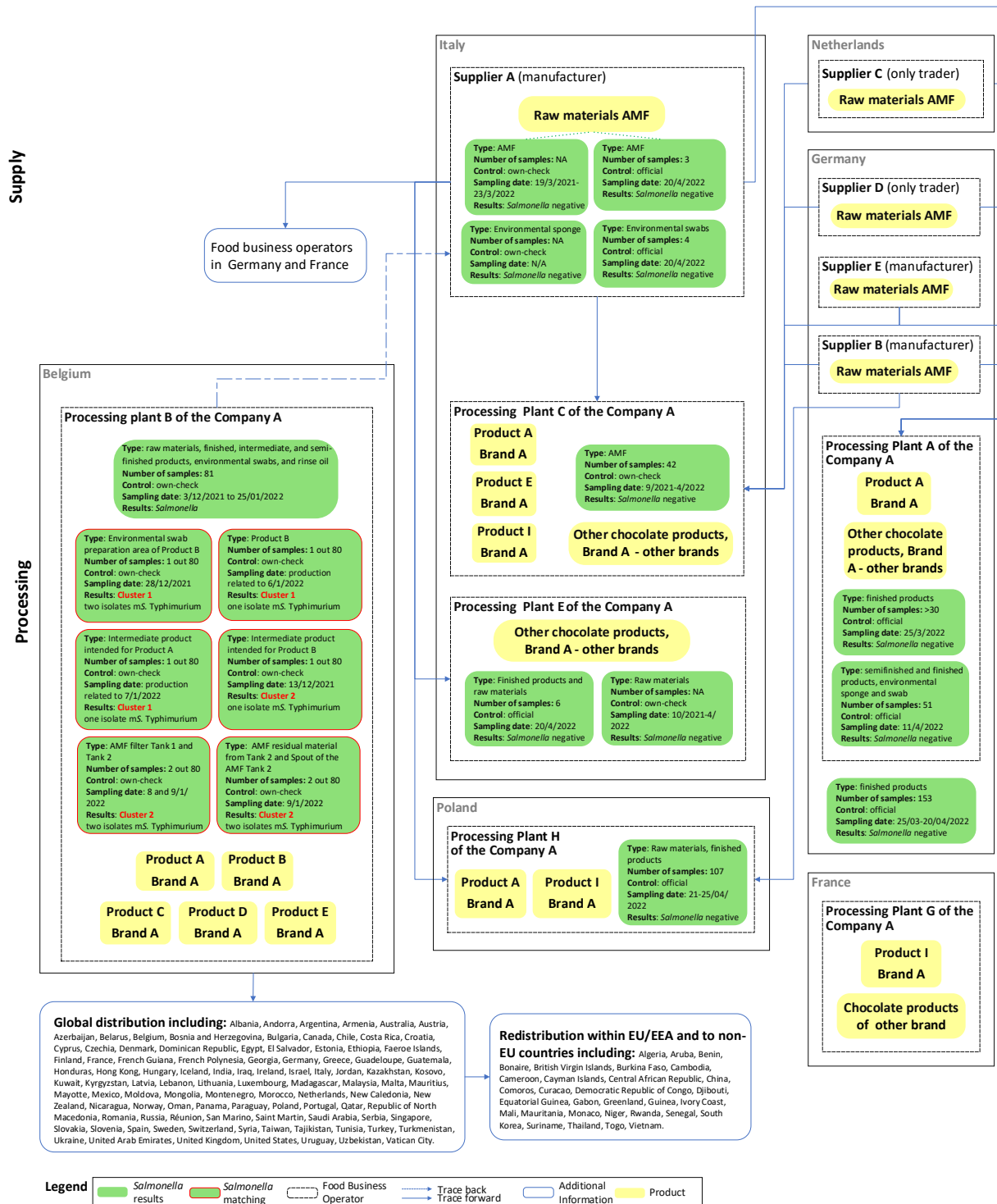
Country	Sampling place	Sampling date*	No. of samples	Sampled product
Germany	German Processing Plant A	25 March 2022	5	Product A
			5	Product J
			5	Product K
			3	Product L
			8	Other chocolate products from Brand A
	Consumer level and retail level	94	One opened bag of Product C that contained: <ul style="list-style-type: none"> Product B (5 samples) Other chocolate products from Brand A (7 samples) (9 samples) (12 samples) (9 samples) Three bags of other chocolate products from Brand A that contained: <ul style="list-style-type: none"> Product I (21 samples) Product L (3 samples) Other chocolate products from Brand A (9 samples) (4 samples) (15 samples) 	
	Retail and consumer level (Hesse)	30 and 31 March 2022	14	Product B
	Retail and consumer level (Hesse)	5 and 8 April 2022	23	Product A Product B Product L
N/A (Hamburg)	28 March 2022, 29 March 2022	17	Product A Product B Product I Product L Product N Other chocolate products	
German processing plant A	11 April 2022	14	Product B (1 sample) Product L (10 samples) Other chocolate products	
N/A (Berlin)	20 April 2022	5	Product I Product B Product K Other chocolate products	
Czechia	Wholesale level	07 April 2022	10	Product A Product B Product I Other chocolate products Other chocolate products from another brand
	Retail level	8 – 12 April 2022	15	Product A Product D Product I Other chocolate products Other chocolate products from another brand Product N
Cyprus	N/A	05 April 2022	4	Product A
		27 April 2022	7	Product C Product I
			7	Product A Other chocolate products Other chocolate products from another brand
The United Kingdom	Consumer Level	N/A	N/A	Product A Other chocolate products Product L
Italy	Italian processing plant E	21 April 2022	4	Other chocolate products Other chocolate products from another brand
	Consumer level	10 May 2022	1	One opened pack of Product M

Country	Sampling place	Sampling date*	No. of samples	Sampled product
	Retail level	10 May 2022	12	Product M (from the same batch of the sample collected at consumer level)
Hungary	Consumer level	05 May 2022	11	Other chocolate products
	Retail level	05 May 2022	50	Other chocolate products
Luxembourg	Consumer level	15 and 25 April 2022	6	Product A (1 sample) Product E (1 sample) Product I (1 sample) Other chocolate products (3 samples)
	Wholesale and retail level	15 April 2022	25	Product A (5 samples) Product B (8 samples) Product D (6 samples) Product E (6 samples)
Poland	Processing plant H	21-25 April 2022	12	Other chocolate products from another brand

N/A not available in RASFF 2022.1799.

**Date of sampling OR date of reporting in RASFF 2022.1799, 2022.2201, 2022.2452.*

Figure A1. Graphical representation of the traceability, testing information and distribution of raw materials and chocolate products from the Belgium Processing Plant B, as reported by the involved countries under RASFF notification 2022.1799, 2022.2201, and 2022.2452



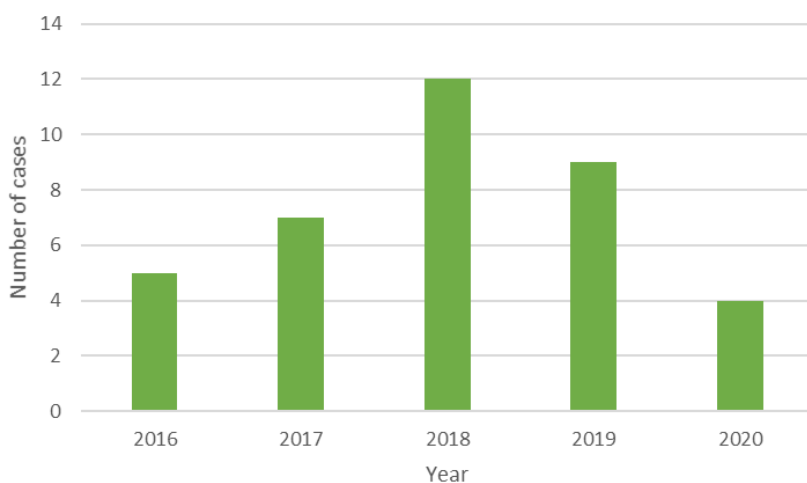
Annex 2. Disease background

Epidemiology of MDR monophasic *S. Typhimurium* in the EU

Monophasic *Salmonella* Typhimurium 1,4,[5],12:i:- is the third most common serovar (or rather variant of Typhimurium) among human *Salmonella* infections in the EU/EEA [8]. Between 2016 and 2020, it accounted for 8.9% of reported salmonellosis cases. The serovar is known for expressing resistance to ampicillin, streptomycin, sulfonamides and tetracycline (resistance pattern ASSuT). However, the strain in the current outbreak is also expressing resistance to additional aminoglycosides (such as gentamicin and kanamycin), phenicols (such as chloramphenicol), and trimethoprim. Genes associated with resistance to lincosamides have also been identified but are not expressed in most tested isolates. The isolates are susceptible to azithromycin, ciprofloxacin, and third generation cephalosporins.

Between 2016 and 2020, 14 EU countries (Austria, Belgium, Denmark, Estonia, France, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Slovenia, Spain and Sweden) reported antimicrobial susceptibility results for monophasic *S. Typhimurium* 1,4,[5],12:i:- on the antimicrobial classes mentioned above to TESSy. Of 7 157 tested isolates, only 37 (0.5%) were resistant to ampicillin, sulfonamides, tetracycline, gentamicin (streptomycin and kanamycin data not collected), chloramphenicol and trimethoprim while being susceptible to ciprofloxacin and cefotaxime/ceftazidime, with the highest number of cases in 2018 (Figure A2). (Resistance here is considered as either non-wild type, according to EUCAST epidemiological cut-off values, or as decreased susceptibility, according to EUCAST clinical breakpoints). The 37 isolates were reported by 10 of the 14 countries and the cases were either domestically acquired (20 cases) or without information on travel status. Spain accounted for one third of the cases (n=12) but Luxembourg and Ireland reported the highest proportion of monophasic *S. Typhimurium* with this resistance pattern (3.6% and 2.9% of tested isolates, respectively). Children aged 0-4 years accounted for 30% of the cases, and the male-female ratio was 1:1.8. It is worth noting that the COVID-19 pandemic has had an impact on salmonellosis in 2020, with significantly fewer cases being reported in the EU/EEA.

Figure A2. Number of cases with resistance to the seven antimicrobial classes reported to TESSy, 2016-2020 (N=37)



Foodborne outbreaks caused by monophasic *S. Typhimurium*

This section summarises country-specific data on foodborne outbreaks associated with monophasic *S. Typhimurium* from 2015 to 2020, as reported to EFSA by EU Member States in accordance with the Zoonoses Directive 2003/99/EC. During the years 2015–2019, the United Kingdom was an EU Member State.

For the years 2015–2020, no monophasic *S. Typhimurium* food-borne outbreaks were reported linked to the food categories 'Sweets and chocolate', 'Chocolate', and 'Confectionery products and pastes - chocolate-based product'.

In 2020, a total of 13 foodborne outbreaks (three strong-evidence outbreaks and 10 weak-evidence outbreaks) due to monophasic *S. Typhimurium* were submitted to EFSA by four EU countries: Austria, Denmark, France, and Italy. Among the three strong-evidence outbreaks, eight cases and four hospitalisations were reported. No deaths were reported. Among the 10 weak-evidence outbreaks, 55 cases, 12 hospitalisations and no deaths were reported.

During the period 2015–2019, a total of 73 foodborne outbreaks (25 strong-evidence outbreaks and 48 weak-evidence outbreaks) caused by monophasic *S. Typhimurium* were submitted to EFSA by 10 EU Member States: Austria, Czechia, Denmark, France, Germany, Greece, Italy, Slovenia, Spain, and Sweden. Among the 26 strong-evidence outbreaks, 746 cases and 179 hospitalisations were reported. No patients died due to or with the disease. Among the 50 weak-evidence outbreaks, 331 cases, 44 hospitalisations and no deaths were reported. During the period 2015–2019, five foodborne outbreaks (one strong-evidence and four weak-evidence outbreaks) caused by monophasic *S. Typhimurium* were submitted to EFSA by three non-EU countries: Iceland, Norway, and the United Kingdom. In the one strong-evidence outbreak, 58 cases, two hospitalisations, and no deaths were reported. Among the four weak-evidence outbreaks, 80 cases and one hospitalisation were reported. No deaths were reported.

Occurrence of monophasic *S. Typhimurium* in food

This section summarises country-specific data on the occurrence of monophasic *S. Typhimurium* from 2015 to 2020, as reported to EFSA by EU Member States in accordance with the Zoonoses Directive 2003/99/EC. During the years 2015–2020, the United Kingdom was an EU Member State.

For the years 2015–2020, no units belonging to the food categories 'Chocolate' and 'Confectionery products and pastes - chocolate-based product' were reported to be positive for monophasic *S. Typhimurium*.

In 2020, 228 units positive for monophasic *S. Typhimurium* out of 80 549 total units tested (0.28%) were reported to EFSA by 16 EU Member States (Austria, Belgium, Bulgaria, Croatia, Czechia, Denmark, Estonia, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Netherlands, Slovenia, and Spain) and by two non-EU Member States (Norway and the United Kingdom). The units positive for monophasic *S. Typhimurium* belonged to 'Fish', 'Meat from bovine animals', 'Meat from broilers (*Gallus gallus*)', 'Meat from other animal species or not specified', 'Meat from pig', 'Meat from sheep', 'Meat from spent hens (*Gallus gallus*)', 'Meat from turkey', 'Meat, mixed meat', 'Meat, red meat (meat from bovines, pigs, goats, sheep, horses, donkeys, bison and water buffalos)', and 'Molluscan shellfish'.

In the years 2015–2019, 1 625 units positive for monophasic *S. Typhimurium* out of 286 612 total units tested (0.57%) were reported to EFSA by 24 EU Member States (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and Sweden) and by three non-EU Member States (Iceland, Switzerland, and the United Kingdom). The units positive for monophasic *S. Typhimurium* belonged to 'Eggs', 'Fish (food)', 'Live bivalve molluscs', 'Meat from bovine animals', 'Meat from bovine animals and pig', 'Meat from broilers (*Gallus gallus*)', 'Meat from duck', 'Meat from goat', 'Meat from horse', 'Meat from other animal species or not specified', 'Meat from other animal species or not specified', 'Meat from other poultry species', 'Meat from pig', 'Meat from poultry, unspecified', 'Meat from sheep', 'Meat from turkey', 'Meat, mixed meat', 'Milk from other animal species or unspecified', 'Molluscan shellfish', 'Other food', 'Other processed food products and prepared dishes', 'Seeds, dried', and 'Spices and herbs'.

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