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Multi-country outbreak of new *Salmonella enterica* 11:z41:e,n,z15 infections associated with sesame seeds

European Food Safety Authority European Centre for Disease Prevention and Control

Abstract

An outbreak of a new serovar of Salmonella enterica subspecies enterica with antigenic formula 11:z41:e,n,z15, initially detected in Greece, affected 47 individuals in five European Union countries (the Czech Republic, Germany, Greece, Luxembourg and the United Kingdom) between March 2016 and May 2017. Epidemiological and microbiological evidence linked some of the outbreak cases to a sesame paste produced by a Greek manufacturer. The sesame seeds used for the production of the sesame paste were traced back to an African country. The same Greek manufacturer also processed a batch of sesame seeds imported from another African country that tested Salmonella positive after processing. The most recent cases associated with this outbreak were reported in Germany and Luxembourg in March 2017. Since the withdrawal of the implicated sesame paste in March and April 2017, no new cases were reported. If new cases with the outbreak serovar are reported, competent authorities in the European Union/European Economic Area (EU/EEA) should consider rapidly interviewing the cases to assess the exposures and attempt to identify potential links to the outbreak. In line with established procedures - and as carried out so far - new cases and important developments should be reported to EPIS-FWD (Epidemic Intelligence Information System for Foodand Waterborne Diseases and Zoonoses) and to EWRS (Early Warning and Response System). The latter represents the official channel to notify cross-border threats. Information from food investigations should be shared by issuing relevant notifications in the Rapid Alert System for Food and Feed (RASFF). National public health and food safety authorities should exchange relevant information with each other.

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Key words: *Salmonella enterica*, sesame seeds, sesame paste, multi-country outbreak, Whole Genome Sequencing (WGS)

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RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of new *Salmonella enterica* 11:z41:e,n,z15 infections associated with sesame seeds

14 June 2017

Conclusions and options for response

An outbreak of a new serovar of *Salmonella enterica* subspecies *enterica* with antigenic formula 11:z41:e,n,z15, initially detected in Greece, affected 47 individuals in five European Union countries (the Czech Republic, Germany, Greece, Luxembourg and the United Kingdom) between March 2016 and May 2017.

Epidemiological and microbiological evidence linked some of the outbreak cases to a sesame paste produced by a Greek manufacturer. The sesame seeds used for the production of the sesame paste were traced back to an African country. The same Greek manufacturer also processed a batch of sesame seeds imported from another African country that tested *Salmonella* positive after processing.

The most recent cases associated with this outbreak were reported in Germany and Luxembourg in March 2017. Since the withdrawal of the implicated sesame paste in March and April 2017, no new cases were reported.

If new cases with the outbreak serovar are reported, competent authorities in the European Union/European Economic Area (EU/EEA) should consider rapidly interviewing the cases to assess the exposures and attempt to identify potential links to the outbreak.

In line with established procedures – and as carried out so far – new cases and important developments should be reported to EPIS-FWD (Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses) and to EWRS (Early Warning and Response System). The latter represents the official channel to notify cross-border threats.

Information from food investigations should be shared by issuing relevant notifications in the Rapid Alert System for Food and Feed (RASFF). National public health and food safety authorities should exchange relevant information with each other.

Source and date of request

Decision by ECDC and EFSA, 15 March 2017.

Public health issue

A multi-country outbreak of a new serovar of *Salmonella* linked to consumption of sesame-based food products.

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- Luxembourg: Joël Mossong (Laboratoire National de Santé)
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ECDC issued this risk 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, and with the contribution of the European Food Safety Authority (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 and laying down procedures in matters of food safety.

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

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

All data published in this rapid outbreak assessment are correct to the best of our knowledge on 7 June 2017. 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.

Event background information

On 10 May 2016, Greece reported a cluster of cases of *Salmonella enterica* subspecies *enterica* with antigenic formula 11:z41:e,n,z15, not previously described in the White-Kauffmann-Le Minor-scheme [1]. The isolates from these cases were susceptible to 16 antimicrobial agents, and their pulsed field gel electrophoresis (PFGE) profiles were indistinguishable (The European Surveillance System (TESSy) reference XbaI.2460). https://tessy.ecdc.europa.eu/TessyWeb/OnlineQueryTool/Query.aspx?type=ClusterAnalysis&subject=SALMISO&me thod=Cluster&clustercode=2016-07.SALM.03.XBAI.2460

On 26 May 2016, the Pasteur Institute in Paris confirmed the results of the Greek laboratory verifying a putative new serovar of *Salmonella enterica* subspecies *enterica*. If this finding is confirmed by the ongoing additional testing, a name will be given to the new serovar.

Epidemiological and microbiological investigations of humans

For the purpose of this Rapid Outbreak Assessment the following case definition is used:

A **confirmed outbreak case** is defined as an EU/EEA resident, with or without gastrointestinal symptoms, that either had:

• a laboratory-confirmation of *Salmonella* isolates with an antigenic formula 11:z41:e,n,z15 according to the White-Kauffmann-Le Minor-scheme.

• a laboratory-confirmation of *Salmonella* with a whole-genome sequence matching any of the reference sequences provided below.

Sequence data from representative *Salmonella* isolates with the sequence identification code 239-2016 were uploaded to Enterobase (<u>https://enterobase.warwick.ac.uk/</u>) and the NCBI short read archive (accession numbers SRR5413117, SRR5413119 and SRR5413121).

Epidemiological situation

From May 2016 to 1 June 2017, five EU Member States reported 47 confirmed cases infected by this new *Salmonella* serovar.

Greece has reported 22 confirmed cases since March 2016. Two cases were detected in 2017, with the latest case at the end of February 2017. The Greek National Reference Centre received about 200 *Salmonella* isolates in the last quarter of 2016, which now await serotyping, which could lead to the detection of additional cases that match the outbreak case definition.

Germany has reported 13 confirmed cases with the new *Salmonella* serovar since May 2016. The latest case was notified in March 2017.

The **Czech Republic** reported five confirmed cases, with the most recent case notified at the end of June 2016.

Luxembourg reported four confirmed cases, with the latest notification in March 2017.

The **United Kingdom** reported three confirmed cases, with sampling dates between May and September 2016.

The first three cases associated with this outbreak were reported in March 2016 by Greece, and the last two cases were reported in March 2017 by Germany and Luxembourg. The outbreak peaked in April 2016, and since August 2016, one to four cases were reported per month (Figure 1).

Information on age was available for 46 of the 47 cases: median age is eight years (interquartile range 3-46 years). Twenty-six cases were female. Four German cases had an epidemiological link to Greece, either through travelling to Greece or through a Greek relative visiting Germany. There were no other cases who had a travel history or any other epidemiological link to another country.

Five of the 34 cases with available clinical information were asymptomatic: three in Luxembourg, one in Germany and one in Greece. Of the 26 cases with available information on hospitalisation, 12 patients were hospitalised. No deaths were reported associated with this outbreak.



Figure 1. Distribution of cases by reporting country and month of report*, EU, as of 24 May 2017 (N=43)

For four cases, no information was available on month of report. * Month of sampling or month of receipt by the reference laboratory when month of onset not available.

In Greece, a case-case study comprising 11 cases with the new serotype and 22 cases of *Salmonella* Enteritidis indicated an association between the occurrence of disease and the consumption of tahini (a sesame-based product). Interviews of five German and two Luxembourgish cases identified exposure to a common food item, a specific sesame paste sold in a screw-lid glass jar.

Microbiological information

At least 17 isolates from the Czech Republic, Germany, Greece and Luxembourg share an indistinguishable PFGE profile (TESSy reference XbaI.2460, Figure 2). In addition to WGS performed in public health laboratories in Germany (human isolates from eight cases and one non-human isolate from a food sample), Luxembourg (human isolates from three cases) and England (human isolates from three cases), the ECDC contractor has performed WGS for nine isolates (human isolates from three Czech cases, three German cases, and three Greek cases). Multi-country WGS data analysis, performed in Germany, concluded that 22 human isolates from Germany (exposed either in Greece or in Germany), Greece, Luxembourg, the Czech Republic and the United Kingdom clustered tightly together by cgMLST (Figure 3).

Figure 2. PFGE-*Xba*I profile for TESSy reference type XbaI.2460 (2) alongside a *Salmonella* Braenderup H9812 size standard [1]

1			11		
2			1	11	

In August 2016, Greece reported an extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* 11:z41:e,n,z15 which carried a blaSHV-5-like gene isolated from a previously reported relapse case.

Twelve countries (Austria, Cyprus, Denmark, Finland, France, Ireland, Italy, the Netherlands, Norway, Scotland, Sweden and Switzerland) reported not having observed human *Salmonella* isolates with this antigenic formula. Before April 2016, no *Salmonella* isolates/cases with antigenic formula 11:z41:e,n,z15 or with PFGE profile XbaI.2460 have been reported to TESSy.

Figure 3. Minimum spanning tree of sequencing analysis showing 22 human and one food *Salmonella* isolates with antigenic formula 11:z41:e,n,z15 from the Czech Republic, Germany, Greece, Luxembourg and the United Kingdom, March 2016 – March 2017, as of 31 May 2017



Ridom SeqSphere+ MST for 25 Samples based on 2150 columns, pairwise ignoring missing values Distance based on columns from S. enterica cgMLST 2143 targets LT2 (2143), S. enterica MLST (7) Comparison Table definition: Ssp.I_11_z41_e,n,z15

Comparison Table created: 18.04.2017 14:54

Task Templates: S. enterica cgMLST 2143 targets LT2, S. enterica MLST, S. enterica Accessory 2201 targets LT2

Grouped by column "Country of Isolation"

- Czech Republic
- Germany
- Germany_food
- Greece
- Luxembourg



Note: Figure provided by Robert Koch Institute (RKI, Germany). The tree was created with Ridom SeqSphere+ (Ridom GmbH, Münster, Germany) based on a preliminary (ad hoc) cgMLST scheme for Salmonella enterica developed at RKI. All isolates were isolated from human samples, except for one isolated from food (sesame seeds, sample collected in Germany). One human isolate from Germany belonging to the main cluster is missing in the figure.

Microbiological and environmental investigations of food

This section summarises, by country, all information on food and environmental investigations related to this outbreak that the Member States reported to RASFF. Also included is information reported to EFSA by the national competent authorities of the affected Member States in the context of this outbreak (as of 7 June 2017). Figure 4 provides an overview of traceability and testing information as provided by Member States or available in RASFF.

Greece

Sesame seeds

On 20 January 2016, a shipment (five containers, net weight 90 038 kg) of sesame seeds (expiry date 9 Jan 2018) was shipped from a Nigerian producer (Producer A) to a Greek harbour where it was unloaded in February 2016. The seeds were sampled in Nigeria and tested negative for *Salmonella*. On 15 September 2016, the containers were forwarded to a Greek company (Company A).

The shipment was delivered to Greek Company A on 16 September 2016, where the product was tested before processing and the negative result for *Salmonella* was confirmed. The sesame seeds were used to make two different products: a) hulled and pasteurised sesame seeds (Lots 1, 2, 3 and 4), and b) natural cleaned and washed sesame seeds (Lots 5, 6 and 7). Each lot was processed on a different day (between 4 October 2016 and 19 January 2017). Processing was identical, except for Lots 1, 2, 3, 4, which were also hulled. The overall process included the following steps:

- Foreign material removal
- Washing
- Hulling (only for the hulled and pasteurised sesame seeds)
- Drying (temperature >90 °C for 10 minutes)
- 'Sterilisation' (115 °C for 8–9 minutes, proven to deliver a 7-log bacterial count reduction)
- Roaster chamber (90 °C, validated for a 6-log bacterial count reduction).

On 6 October 2016, Lot 1 of the hulled and pasteurised sesame seeds (production date 5 October 2016) was sampled at Greek Company A and tested negative for *Salmonella*. The remaining lots of hulled sesame seeds and the three lots of natural sesame seeds were sampled close to the date of shipping for further distribution; testing for *Salmonella* was negative.

In the period October 2016–January 2017, Company A delivered these lots to Germany through a German trader (Trade A) which distributed the lots to another German company (Company B).

Between October 2016 and January 2017, all seven lots were tested for *Salmonella* at German company B during own checks. Lots 2, 3, 4, 5, 6, and 7 tested negative for *Salmonella*. But on 2 November 2016, Lot 1 (hulled sesame seeds) tested positive for *Salmonella* 11:z41:e,n,z15, and on 10 November 2016 the entire positive lot was shipped back through German trader A to the Greek supplier (Company A).

The *Salmonella*-positive Lot 1 of hulled sesame seeds was returned back to Greek Company A, where it was tested again for *Salmonella* in November 2016 and in December 2016, with negative results (five samples were tested in November, and 14 samples in December). The entire lot was seized by the Greek competent authority and stored in the Greek supplier's warehouse (Company A).

Sesame paste

On 22 June 2015, a Sudanese company (Producer B), on behalf of a company from the United Arab Emirates, shipped whitish sesame seeds to Company A in Greece (five containers, gross weight 95 190 kg). After receiving the seeds on 2 November 2015, Company A sampled the seeds, which were negative for *Salmonella* (samples were taken on 10 November 2015, results obtained on 16 November 2015). On 18 March 2016, some of these seeds were used to make tahini. On the same day, but on a separated production line, the remaining seeds were used to produce hulled sesame seeds and natural roasted seeds; all products were further distributed.

The production process for tahini is roughly identical to the one described above (hulled sesame seeds). Two additional steps at the end of the production process are carried out:

- Grinding (>95 °C)
- Additional 'sterilisation' process (>120 °C for 12–14 minutes, validated for a 6-log bacterial count reduction).

All processing steps applied during production on 18 March 2016 were validated and, according to the audit findings, there was no evidence of underperformance during the decontamination process. However, the usually entirely closed production line for the sesame paste was brought to a halt, and the tahini was temporarily stored in plastic pallet tanks. There is no information available on the sanitation conditions of these tanks before they were used.

Some of the tahini was packaged in plastic containers, some was used to make sesame oil; both products were further distributed. The remaining amount was used as an ingredient for sesame paste, branded exclusively for a German company (Company C). The sesame paste was produced on 21 of March 2016 by adding sugar, cottonseed oil, and soya lecithin to the tahini that earlier had been stored in plastic pallet tanks. The finished product was pumped through a closed pipeline system into a stainless steel holding tank and kept at 45 °C for up to two days. The sesame paste was packaged in glass jars on 21, 22 and 23 March 2016. The glass jars used for packaging were UV-treated, but the lids were not.

Due to the novelty of this serovar and the geographical distance of Nigeria and Sudan, it seems unlikely that the contamination occurred independently in the seeds from the two countries. It is possible that the mentioned serovar originated in one area, and cross-contamination took place at Greek Company A. Investigations showed that the seeds from Nigeria had not even arrived at Company A (delivery was in September 2016) when the *Salmonella*-positive sesame paste – made with Sudanese sesame seeds – was produced and packaged (March 2016). This means that Nigerian seeds could not have been the source of the contamination.

During the processing of the Sudanese seeds (delivered on 2 November 2015) for the production of sesame paste, the usually entirely closed production line was brought to an unscheduled halt, and the tahini was temporarily stored in plastic pallet tanks; for this step, no information on sanitation conditions was available. This could be a contributory factor to the contamination of the sesame paste. Moreover, considering that the Nigerian sesame seeds were not mixed with the Sudanese seeds, it is conceivable that the environment might have played a role in the cross-contamination.

Germany

Sesame seeds

German Company B received four individual shipments of Nigerian seeds via Greece, delivered through German Trader A, for a total of seven lots: Lots 1, 2, 3, 4 hulled and pasteurised sesame seeds) and Lots 5, 6, 7 (natural cleaned and washed sesame seeds).

During own checks on 19 October 2016, Company B sampled Lot 1 (hulled and pasteurised sesame seeds), which tested positive for *Salmonella* 11:z41:e,n,z15. The isolate was later sequenced and matched the isolates from human cases (from Germany, Greece and the Czech Republic): maximum two loci pairwise difference with cgMLST. Due to the detection of *Salmonella*, on 10 November 2016, the German Company B sent the entire lot back to Company A in Greece, again enlisting the services of Trader A. Lots 2, 3, 4, 5, 6 and 7 were *Salmonella*-negative during own checks conducted between October 2016 and January 2017 and were further distributed.

Sesame paste

Thirteen salmonellosis cases were identified in Germany. Five of those cases said in an interview that they ate sesame paste that was manufactured by Greek Company A. The sesame paste was delivered to German Company C which sold it to Wholesaler A who distributed it to three Member States (Austria, Belgium, Estonia). The sesame paste was also distributed to a German food retailer, where some of the human cases purchased it. Company C also delivered the sesame paste to another five countries (Austria, France, Luxembourg, Portugal, Switzerland), either directly or through other wholesalers (Wholesalers B, C and D) (see Figure 4).

On 22 March 2017, samples were taken from sealed jars of sesame paste from the household of one human case and from the German food retailer where the human case purchased the product. A test performed at the German national reference laboratory for *Salmonella* at the Federal Institute for Risk Assessment (BfR) was positive for *Salmonella* serotype 11:z41:e,n,z15 (5 April 2017). These results strongly suggest a connection between the contaminations detected in the paste produced with Sudanese seeds and the one concerning the sesame seeds imported from the Nigerian producer A.

After the detection of *Salmonella*, German Company C issued a food safety alert and initiated a recall of the sesame paste. After that, Germany and the other affected countries (Austria, Belgium, Estonia, France, Luxembourg, Portugal, and Switzerland) issued a consumer warning and started a product withdrawal.

Luxembourg

Sesame paste

Two human cases in Luxemburg reported exposure to the sesame paste traded by the German Company C. A sample of the incriminated lot of sesame paste was taken in a retail shop and tested positive for *Salmonella* 11:z41:e,n,z15. After a food safety alert issued by Company C, the incriminated lot of sesame paste was recalled in Luxembourg on 31 March 2017.

Figure 4. Traceability and testing information either available in RASFF or provided by Member States to EFSA, as of 7 June 2017



ECDC and EFSA threat assessment for the EU/EEA

Five EU Member States reported a total of 47 cases infected with a new *Salmonella* serovar. Infections were reported between March 2016 and May 2017; the antigenic formula was 11:z41:e,n,z15 and was never described before. The distribution of cases points at a continuous common-source outbreak. PFGE and WGS analyses confirmed that the isolates from these cases are genetically very close, and thus they are likely associated with a common source of infection.

An epidemiological analytical study performed in Greece found an association between the infection with the new *Salmonella* serovar and sesame paste. This hypothesis was later confirmed in Germany, where the same *Salmonella* serovar was found in sesame seeds imported to Germany from West Africa via Greece. Epidemiological investigations in Germany and Luxembourg also found cases exposed to the same lot of sesame paste, which was distributed in both countries.

Possible *Salmonella* contamination of sesame seeds is a known risk. In this particular case, tracing focused on two products which were positive for *Salmonella* serotype 11:z41:e,n,z15: hulled and pasteurised sesame seeds (produced with seeds from Nigeria) and a sesame paste (produced with seeds from Sudan). The tracing activities were restricted to back-tracing in order to identify a common source of the contamination. The food chain analysis showed that a Greek company ('Company A') was the unique link between the two contaminated food items (Figure 4).

Due to the novelty of this serovar and the geographical distance of Nigeria and Sudan, it seems unlikely that the contamination occurred independently in the seeds from the two countries. It is possible that the mentioned serovar originated in one area, and cross-contamination took place at Greek Company A. Investigations showed that the seeds from Nigeria had not even arrived at Company A (delivery was in September 2016) when the *Salmonella*-positive sesame paste – made with Sudanese sesame seeds – was produced and packaged (March 2016). This means that Nigerian seeds could not have been the source of the contamination.

During the processing of the Sudanese seeds (delivered on 2 November 2015) for the production of sesame paste, the usually entirely closed production line was brought to an unscheduled halt, and the tahini was temporarily stored in plastic pallet tanks; for this step, no information on sanitation conditions was available. This could be a contributory factor to the contamination of the sesame paste. Moreover, considering that the Nigerian sesame seeds were not mixed with the Sudanese seeds, it is conceivable that the environment might have played a role in the cross-contamination. Further investigations are needed to clarify how the environmental contamination occurred.

Although sesame seeds have a long shelf life and cases associated with this outbreak were reported over a period of one year, the non-distribution of the *Salmonella*-positive lot of hulled sesame seeds, the withdrawal of the implicated sesame paste lot in March and April 2017, and the national warnings to consumers appear to have successfully stopped the occurrence of new cases.

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