

## RAPID RISK ASSESSMENT

# Re-emerging multi-country WGS-defined outbreak of *Salmonella* Enteritidis, MLVA type 2-12-7-3-2 and 2-14-7-3-2

3 February 2017\*

### Main conclusions and options for response

The detection of a WGS-defined cluster of *S. Enteritidis* in the United Kingdom (UK) in the autumn of 2016 highlights the re-emergence of a strain that was first identified in 2014 and caused an outbreak that was investigated from May to October 2015 in Spain and the UK.

In 2016, France, Ireland, Spain and the UK reported cases belonging to the same single nucleotide polymorphism (SNP) cluster as the 2015 outbreak cases, suggesting that the cases in both years are part of a common source outbreak.

This 2016 WGS cluster highlights a multi-country outbreak involving at least four countries that has been ongoing since 2014. The 2016 investigations in the UK pointed to an exposure outside the home. Since non-travel related cases outside the UK have been linked through whole genome sequencing (WGS), it is expected that the contaminated vehicle(s) of infection has been or is being distributed in at least four EU/EEA Member States.

Since Multi Locus Variable-Number Tandem Repeat (MLVA) and WGS are not routinely performed in all EU/EEA countries, those countries experiencing increases in *S. Enteritidis* or in *S. Enteritidis* PT59 could consider enhancing their surveillance and performing MLVA or WGS.

Isolates from shell and the interior of eggs sampled in Spain between July and September 2016 from the homes of a sporadic case and a family cluster were found to belong to the WGS cluster associated with this outbreak.

To support the investigation of this outbreak, ECDC is offering support to sequence isolates from probable cases as per the EU/EEA case definition. Contact ECDC at [FWD@ecdc.europa.eu](mailto:FWD@ecdc.europa.eu) for detailed information. ECDC would also encourage EU/EEA Member States to collect food exposure information from confirmed cases.

### Source and date of request

ECDC decision on 19 January 2017 following the EWRS message from the United Kingdom on 18 January 2017.

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Suggested citation: European Centre for Disease Prevention and Control. Re-emerging multi-country WGS-defined outbreak of *Salmonella* Enteritidis, MLVA type 2-12-7-3-2 and 2-14-7-3-2, 3 February 2017, Stockholm. ECDC: 2017.

\*Erratum: On 17 February 2017 this risk assessment was shortened to remove information pertaining to the food production cycle.

## Public health issue

Recurrent multi-country outbreak of *Salmonella* Enteritidis due to a persistent source of infection, with current or recent exposure in at least four EU/EEA Member States.

## Consulted experts

### ECDC experts

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- Ireland: Patricia Garvey (Health Service Executive), Martin Cormican (University Hospital Galway)
- The Netherlands: Lapo Mughini Gras (Rijksinstituut voor Volksgezondheid en Milieu)
- Norway: Lin Thorstensen Brandal (Norwegian Institute of Public Health)
- Slovenia: Eva Grilc (Nacionalni inštitut za javno zdravje), Marija Trkov (National Laboratory for Health, Environment and Food)
- Spain: Carmen Varela Martinez and Silvia Herrera Leon (Instituto de Salud Carlos III)
- Sweden: Cecilia Jernberg and Moa Rehn (Folkhälsomyndigheten)
- United Kingdom: Lesley Larkin, Jacquelyn McCormick and Tim Dallman (Public Health England), Gillian Hawkins and Alison Smith-Palmer (Health Protection Scotland), John Coia (NHS Greater Glasgow and Clyde), Derek Brown and Henry Mather (NHS Greater Glasgow and Clyde), Deb Morgan (Food Standards Agency).

## Disease background information

*Salmonella* Enteritidis (*S. Enteritidis*) is the most commonly detected serovar in humans of non-typhoidal salmonellosis in the European Union/European Economic Area (EU/EEA). The number of human cases of *S. Enteritidis* has declined substantially in recent years, most likely as a result of successful control measures implemented under Regulation (EC) No. 2160/2003\* in poultry populations, and measures in food businesses to improve hygiene implemented under EU Food law. The number of confirmed cases of *S. Enteritidis* by year and by EU/EEA Member State is presented in the ECDC Surveillance Atlas of Infectious Diseases, available at: <http://ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx>

ECDC has been collecting EU/EEA-wide Multi Locus Variable-Number Tandem Repeat (MLVA) data for *S. Enteritidis* through The European Surveillance System (TESSy) since June 2016. ECDC is using a standardised protocol of a 5-loci *S. Enteritidis* MLVA scheme [1] developed by Public Health England (PHE) in the United Kingdom (UK) and validated by PHE and ECDC.

As of 25 January 2017, eight countries had submitted MLVA data for *S. Enteritidis* to TESSy. Six of these countries have reported 130 *S. Enteritidis* isolates with the MLVA type 2-12-7-3-2 and six isolates with the MLVA type 2-14-7-3-2.

## Event background information

Between May and October 2015, Public Health England (PHE) in the UK investigated an outbreak of *S. Enteritidis* Phage Type 59 (PT59) detected through WGS [2]. The UK identified 136 cases, 12 of which had travelled to Spain. PHE contacted the Spanish public health authorities who reported a five-fold increase in the number of *S. Enteritidis* PT59 cases compared to the previous three years, although the total number of *Salmonella* cases reported had not significantly increased overall during the period. A set of 19 PT59 isolates sampled in Spain were sent to PHE for analysis. Eleven isolates were from sporadic cases and eight from two unrelated outbreaks linked to eggs in April and October 2015. Isolates from these outbreaks included: four isolates originating from humans during one outbreak, and three human and one food isolate (from a leftover of an omelette) originating from the other outbreak. WGS analysis showed that all 19 Spanish PT59 strains, including the food isolate, shared the outbreak 15-level SNP address with isolates from the UK. In summary, the UK investigations indicated hen eggs as the vehicle of infection and an exposure which had occurred outside of the home for the case in question. Detailed information about the investigation was published in 'Epidemiology & Infection' and is available at: <https://doi.org/10.1017/S0950268816001941> [2].

On 8 November 2016, the UK reported a cluster of 17 cases of *S. Enteritidis* PT59, with MLVA profile 2-12-7-3-2 through the Epidemic Intelligence Information System for Food- and Water-borne Diseases and Zoonoses (EPIS-FWD). This cluster was detected in Scotland between early September and late October 2016. Seven cases were

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\* Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents.

linked to a point source outbreak in a restaurant and three cases reported travel to Spain (2) and Portugal (1). WGS analysis performed by PHE showed that the strain causing this outbreak belonged to the same WGS cluster as the strain of the outbreak investigated in the UK and Spain in 2015 [2]. Isolates within 5-SNPs of another cluster isolate were considered as part of the 1.2.3.151.362.363.% (t5.363) cluster. Based on the WGS analysis, additional isolates with different phage types – i.e. PT59, PT56, PT8, PT14b, PT62, RDNC (reacts but does not conform) and untypeable isolates exhibiting the MLVA profiles 2-12-7-3-2 or 2-14-7-3-2 were determined to be part of the cluster.

Seven EU/EEA Member States replied through EPIS-FWD, four of these reporting isolates with the implicated MLVA profiles. No country reported a significant increase in the number of cases over the preceding months. At that stage, no additional investigations were conducted at EU/EEA level, in the absence of clear evidence of an excess of cases outside the UK.

## Epidemiological and microbiological investigation on humans

On 18 January 2017, the UK gave notification through the Early Warning and Response System (EWRS) of the re-emergence of the strain causing the 2015 and 2016 outbreaks. WGS analysis performed on human isolates from France, Ireland and Spain and the UK revealed a multi-country cluster sharing the same t5-level SNP address (1.2.3.151.362.363.%).

To investigate this outbreak, ECDC suggested the following case definition, which is based on the case definition used in the UK:

### A confirmed outbreak case

A laboratory-confirmed *Salmonella* Enteritidis case with a strain sharing the same t5-level SNP address as the defined United Kingdom outbreak cluster based on WGS analysis: 1.2.3.151.362.363.% (t5.363)

### A probable outbreak case

A laboratory-confirmed *Salmonella* Enteritidis case with symptom onset on or after 1 August 2016 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

### AND

With a strain matching the MLVA profile 2-12-7-3-2 OR 2-14-7-3-2 corresponding to the MLVA protocol with five loci [1].

### Exclusion criteria

- Cases with travel history outside of the EU/EEA.
- Secondary cases defined as confirmed cases having had person-to-person contact with a confirmed case and no exposure to a common source.
- Cases infected with MLVA-profile 2-12-7-3-2 or 2-14-7-3-2, but not sharing the same t5-level SNP address as the defined United Kingdom outbreak cluster based on WGS analysis (e.g. 1.2.3. 151.362.363.%).

*Note: the definition of a confirmed case based on WGS does not include an inclusion criteria based on date of onset because the WGS offers very strong evidence to associate cases with the same outbreak. EU-wide collection of MLVA data for *S. Enteritidis* was initiated in June 2016 and the probable case definition therefore only covers the re-emergence of the outbreak strain in late 2016.*

Information is currently still being gathered on probable and confirmed cases in the EU/EEA. As of 26 January 2017, 329 confirmed cases and 37 probable cases had been reported by:

- France: eight confirmed cases between November 2015 and October 2016, including two sporadic cases and six cases from two foodborne outbreaks:
  - Outbreak 1, November 2015: Eight individuals developed symptoms of salmonellosis after sharing a 'gratin' made with fresh eggs. Six of them had stool cultures identifying *S. Enteritidis* strains with MLVA profile 2-12-7-3-2. One human strain was sequenced and shared the outbreak t5-level SNP address (t5.363). Non-human isolates from the gratin were also positive for *S. Enteritidis* MLVA 2-12-7-3-2.

- Outbreak 2, September 2016: 23 individuals developed symptoms of salmonellosis after eating 'tiramisu' made with fresh eggs in a restaurant. Five of them had stool cultures identifying *S. Enteritidis* strains. The strains were sequenced and shared the outbreak t5-level SNP address (t5.363).
- Ireland: two confirmed cases with date of onset in August 2016
- Spain: 34 confirmed cases reported from April 2015 to September 2016
- United Kingdom:
  - 285 confirmed cases since April 2014: England (255), Scotland (24), Wales (5) and Northern Ireland (1). Median age is 30 years (range 0–99 years), 52% are female.
  - 22 probable cases in Scotland.
- Denmark: four probable cases reported in August (1), September (2) and November (1) 2016
- The Netherlands: four probable cases reported in August (1), September (1) and October (2) 2016
- Norway: two probable cases with date of sampling in August 2016
- Slovenia: one probable case reported in October 2016
- Sweden: four probable cases with date of onset in August–September (3) and December (1) 2016.

In the UK, cases are being interviewed using questionnaires looking at food habits and food consumption before the date of onset. Analysis of completed questionnaires points to consumption outside of the home.

*S. Enteritidis* isolates sharing the outbreak t5-level SNP address were detected in the interior and shell of two samples from eggs taken from the fridge of one Spanish sporadic case and a Spanish family cluster of three cases between July and September 2016. Sequences were closely related to sequences of the UK cluster, with one isolate indistinguishable and the remainder within 1–2 SNPs from UK human isolates. All isolates fell into the same 5-SNP cluster.

## ECDC risk assessment for the EU

A multi-country outbreak of *S. Enteritidis* delineated through WGS analysis with 329 confirmed cases from four EU countries is currently ongoing. Additionally, six EU/EEA countries reported 38 probable cases belonging to the MLVA type 2-12-7-3-2 and 2-14-7-3-2.

Isolates sharing the t5-level SNP address (t5.363) associated with this outbreak have been identified since 2014. Non-human isolates sampled from an omelette in 2015, and from the interior and shell of eggs found at two different case-related homes in Spain in 2016 also shared the outbreak t5-level SNP address (t5.363). Isolates sharing the same t5 WGS SNP address provided a very strong indication of a common source outbreak. The presence of isolates from food and raw eggs, particularly from the interior part of an egg, support the hypothesis that eggs could be the vehicle of this outbreak. Case interviews have so far not confirmed the hypothesis in any of the currently affected countries. However identifying strong evidence of case exposure to eggs in cases associated with large multi-country outbreaks is always challenging. A recent EU-wide investigation highlighted that cases associated with large multi-country egg outbreaks were associated with eating outside of the home [5]. The current investigation in the UK identified the same risk factor.

Human isolates within the same WGS cluster from different countries participating in the investigation may indicate that the vehicle of infection is or has until recently been distributed to different EU countries. Since MLVA and WGS are not performed in all EU countries, additional EU countries may unknowingly be affected by this outbreak, if the vehicle of infection has been distributed there.

Until the source and vehicle(s) of infection has been identified and the outbreak controlled, new cases associated with this outbreak may be reported. Elderly people are at risk of a more severe outcome if infected.

In 2014, an EU multi-country outbreak was found to be associated with consumption of German eggs characterised by isolates with the same MLVA profile (2-12-7-3-2) as for this outbreak [3,4]. However, there were more than 100 SNPs difference between the two outbreaks, indicating no association between the current and the previous outbreak. This stresses the need to sequence probable cases in order to confirm their association with the ongoing outbreak.

## References

1. European Centre for Disease Prevention and Control. Laboratory standard operating procedure for multiple-locus variable-number tandem repeat analysis of *Salmonella enterica* serotype Enteritidis. Stockholm: ECDC; 2016. Available from: <http://ecdc.europa.eu/en/publications/Publications/Salmonella-Enteritidis-Laboratory-standard-operating-procedure.pdf>.
2. Inns T, Ashton PM, Herrera-Leon S, Lighthill J, Foulkes S, Jombart T, et al. Prospective use of whole genome sequencing (WGS) detected a multi-country outbreak of *Salmonella* Enteritidis. *Epidemiology and Infection*. 2016 Oct 26:1-10.

3. European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA). Multi-country outbreak of *Salmonella* Enteritidis infections associated with consumption of eggs from Germany – 25 August 2014. Stockholm: ECDC, 2014. Available at: <http://ecdc.europa.eu/en/publications/Publications/salmonella-enteritidis-rapid-outbreak-assessment-270814.pdf>
4. Inns T, Lane C, Peters T, Dallman T, Chatt C, McFarland N, et al. A multi-country *Salmonella* Enteritidis phage type 14b outbreak associated with eggs from a German producer: 'near real-time' application of whole genome sequencing and food chain investigations, United Kingdom, May to September 2014. Euro Surveill. 2015 Apr 23;20(16). Available at: <http://eurosurveillance.org/ViewArticle.aspx?ArticleId=21098>
5. European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA). Multi-country outbreak of *Salmonella* Enteritidis phage type 8 MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections, 27 October 2016. Stockholm: ECDC; 2016. Available at: <http://ecdc.europa.eu/en/publications/Publications/ROA-ECDC-EFSA-Salmonella%20Enteritidis.pdf>

## Annex 1

Annex 1 can be found as a separate PDF accompanying the rapid outbreak assessment at the ECDC website [http://ecdc.europa.eu/en/publications/risk\\_assessment/Pages/default.aspx](http://ecdc.europa.eu/en/publications/risk_assessment/Pages/default.aspx)

Single Nucleotide Polymorphism (SNP)-based phylogenetic trees of *S. Enteritidis* isolates from France, Ireland, Spain and the United Kingdom. SNP address 1.2.3.151.362.363%.

*Isolates from France are highlighted in blue font.*

*Isolates from Ireland are highlighted in green font.*

*Isolates from Spain are highlighted in red font.*

*Isolates from the United Kingdom are highlighted in black font.*

*Non-human isolates presented in purple font.*

*IQA = Internal quality assessment*

*Taxa label is COUNTRY -> YEAR -> SNP ADDRESS -> Foreign Travel if known*

*Source: Public Health England, United Kingdom*