RAPID RISK ASSESSMENT

Multi-country outbreak of *Salmonella* Enteritidis phagetype 8, MLVA type 2-9-7-3-2 infections

First update, 5 September 2016

Conclusions and options for response

A multi-country outbreak of *Salmonella* Enteritidis phage type (PT) 8 with multiple locus variable-number tandem repeat analysis (MLVA) profile 2-9-7-3-2 has been ongoing in the EU/EEA since at least July 2015. The outbreak has been further defined on the basis of whole genome sequencing (WGS) analysis. Isolates were initially part of two distinct but relatively close clusters, but recent isolates from the United Kingdom for which WGS was performed fall only into one cluster.

Since May 2016, 16 confirmed and 132 probable cases have been reported to ECDC by six EU/EEA countries. Between February 2012 and April 2016, 101 historical isolates with outbreak strain characteristics (either based on WGS or MLVA) were reported. Outbreak cases, both confirmed and probable, were reported by Belgium, Denmark, the Netherlands, Norway, Sweden and the United Kingdom. At least 24 of these cases are associated with a travel history to EU countries that do not routinely perform MLVA or WGS; it is therefore likely that these EU countries are also affected by this outbreak.

In order to determine the extent of this outbreak, ECDC will continue supporting WGS for a selected number of isolates with the MLVA profile 2-9-7-3-2, regardless of travel history.

To improve the capability to identify the source of the outbreak, EU/EEA countries should consider interviewing recent and new cases that have the same MLVA profile by using a common questionnaire. ECDC will provide WGS support for a number of isolates from these cases. Interviews of probable and confirmed cases should be held as soon as possible, even if WGS results from the isolates are not yet available. Only questionnaires from confirmed cases will be included in the analysis.

In order to monitor the circulation of this strain and keep track of the number of affected countries, all affected EU/EEA countries should consider enhancing surveillance for *S.* Enteritidis. It would be particular helpful to perform MLVA typing and/or WGS.

To monitor the magnitude and severity of this cross-border health threat, new cases and critical developments should be reported to EPIS-FWD (Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses). Outbreak investigations should be harmonised across EU/EAA countries and utilise a multidisciplinary approach for detecting the source of the outbreak.

Suggested citation: European Centre for Disease Prevention and Control. Multi-country outbreak of *Salmonella* Enteritidis phagetype 8 MLVA type 2-9-7-3-2 infections – first update, 5 September 2016. ECDC: Stockholm; 2016.

Source and date of request

ECDC internal decision, 29 August 2016

Public health issue

This document assesses the risk associated with a multi-country outbreak of *Salmonella* Enteritidis PT 8 with identical MLVA type, initially confirmed and delineated by whole genome sequencing methods. A first rapid risk assessment on this event was published by ECDC on 21 March 2016 in EPIS-FWD.

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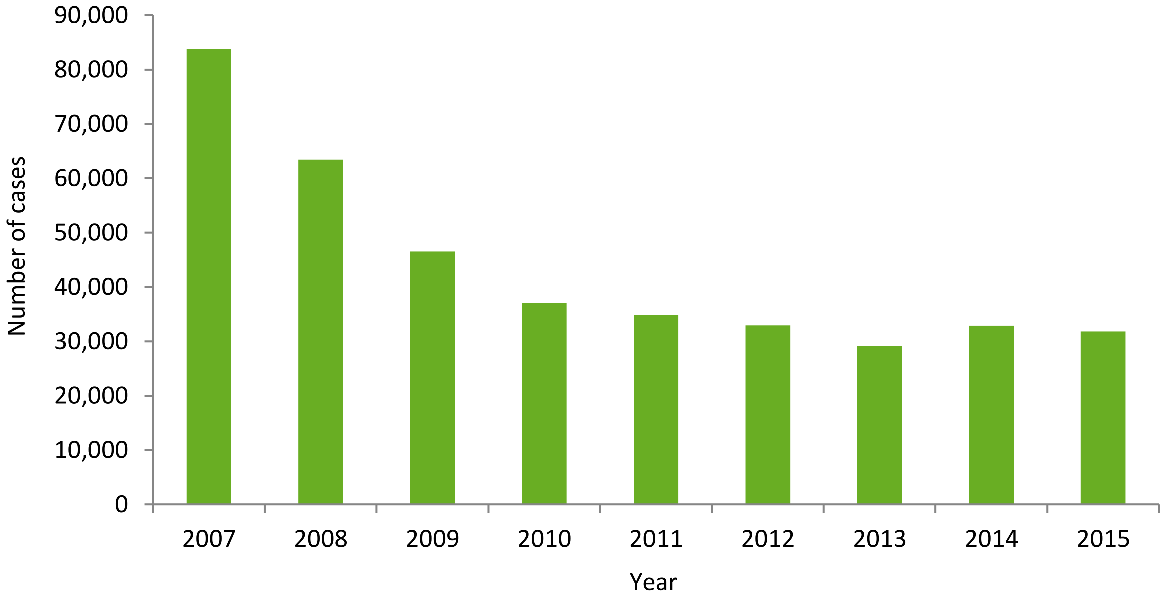
Disease background information

Salmonella Enteritidis phagetype 8 isolations in humans

S. Enteritidis is the most commonly detected serovar in human non-typhoidal salmonellosis in Europe. It has declined substantially in recent years (Figure 1), most likely as a result of successful control measures implemented under Regulation (EC) No. 2160/2003* in poultry populations.

Figure 1. **Distribution of confirmed cases of *Salmonella* Enteritidis by year, EU/EEA, 2007–2015**



Source: ECDC [1]

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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

From 2007 to 2014, 364 450 cases of *S.* Enteritidis were reported to The European Surveillance System (TESSy) (mean cases per year 45 056, range 29 090 to 83 760) by 27 countries, with Germany and the Czech Republic reporting 51% of all cases. In 2014, the Czech Republic observed an increase in the number of foodborne outbreaks of *Salmonella,* contributing to a slight increase in the number of cases reported for the year at EU level. The median age for all cases with information available was 19 years (interquartile range IQR=5-49); 52% (n=184 906) were female and 89% (n=268 865) were acquired within the reporting country. Symptom onset was distributed over the year, with a peak from July to September. To date, 31 829 *S.* Enteritidis cases have been reported to TESSy for 2015 (data not yet published), and 18 countries reported 3 536 cases in the first two quarters of 2016 [2].

During the period 2011–2014, 12 EU/EEA countries (Austria, Belgium, Denmark, Estonia, Hungary, Ireland, the Netherlands, Romania, Slovakia, Spain, Sweden and the United Kingdom) reported between 1 025 and 1 705 confirmed cases of *S.* Enteritidis PT8 to TESSy annually. The majority of PT8 cases were reported by the United Kingdom (40%) which, together with Austria and Hungary, accounted for 84% of reported cases. Seventy-seven percent of the cases for which this type of information was known were domestic. Cases occurred among all age groups, and the median age was 24 years (IQR=6-51). No gender difference could be observed. A total of 1 034 *S.* Enteritidis PT8 cases was reported to TESSY by eight countries in 2015; five countries reported 98 cases in the first two quarters of 2016.

S. Enteritidis is the predominant serovar associated with *Salmonella* outbreaks in Europe. *S.* Enteritidis accounted for 142 outbreaks in 2014, accounting for 46.1% of all *Salmonella* outbreaks. Phage type PT8 was the most frequently reported phage type among documented *S.* Enteritidis outbreaks, with 18 outbreaks identified [3].

Microbiological background

Public Health England (PHE) and ECDC validated and published a standardised protocol for a 5-loci *S.* Enteritidis MLVA scheme [4]. EU/EEA-wide data collection for *S.* Enteritidis MLVA data has been started in TESSy in June 2016. As of 30 August 2016, four countries have submitted data. Ninety-nine *S.* Enteritidis isolates with the MLVA type 2-9-7-3-2 have been submitted to TESSy. Of these, 92 are part of a multi-country cluster involving the Netherlands, Norway and the United Kingdom, with dates used for statistics ranging from 28 May 2015 to 19 August 2016.

ECDC is offering WGS services to support outbreak confirmation and delineation. In connection with outbreak investigations between January and April 2016, the Dutch public health agency (RIVM) analysed WGS data with identical MLVA types from the multi-country outbreak of *S.* Enteritidis PT8. A cross comparison of the analysis and its interpretation was performed by PHE and Health Protection Scotland, with similar results.

Event background information

On 18 January 2016, Scotland launched an urgent inquiry (UI) in EPIS-FWD, reporting 21 cases of *S.* Enteritidis PT8 that shared an uncommon MLVA profile (2-9-7-3-2). The first cases had appeared in late August 2015 and represented a substantial increase compared with previous years. Prior to this outbreak, this MLVA profile had last been seen in August 2014, also in Scotland. Following the launch of the UI in EPIS-FWD, three EU countries reported human cases with the same MLVA profile during the same period: between May 2015 and January 2016, 17 isolates sharing the same MLVA profile were recorded in the Netherlands; Denmark and Finland recorded one case each (August 2015 and September 2015, respectively). In addition, a number of human cases with the same MLVA profile were discovered prior to 2015 in Denmark and Slovenia.

After a WGS analysis of several Scottish isolates, England and Wales reported 52 genetically linked isolates (reported between June 2014 and 15 January 2016), with the majority reported after July 2015.

In February 2016, in order to confirm and describe the outbreak, ECDC offered WGS support for isolates of human origin with the MLVA profile 2-9-7-3-2 that were detected in EU/EEA countries. WGS data analysis for these isolates was performed simultaneously in the Netherlands, Scotland, England and Wales until February 2016. The results confirmed a multi-country outbreak with two genetically distinct clusters.

A total of 47 isolates shared the t5-level SNP address 1.2.3.175.175.175.%, based on the PHE pipeline (PHE in-house WGS data analysis scheme) and a threshold set to a difference of ≤5 single nucleotide polymorphisms (SNP) to at least one other case in the outbreak. The 47 matches included (Table 1):

- Thirty-seven isolates from the UK, three from Sweden, two from the Netherlands and one from Finland, all detected between July 2015 and April 2016.
- Four isolates were detected in the UK between February 2012 and June 2015. A total of 54 isolates shared the t5-level SNP address 1.2.3.18.359.360.% (Table 1):
- Thirty-two isolates from the UK and seven from the Netherlands detected between July 2015 and April 2016.
- Fifteen isolates were detected in the UK between February 2012 and June 2015.

By the time the WGS analysis was finalised and the outbreak described, all involved countries reported that no new cases had been identified over the last few weeks. No vehicle or source of the outbreak could be identified, and national and international investigations were discontinued.

On 25 August 2016, the Netherlands launched a new UI in EPIS-FWD reporting a new increase in cases of *S.* Enteritidis characterised by the same MLVA profile 2-9-7-3-2. In the following days, Belgium, Denmark, Norway, Sweden and the UK reported recent cases with the same MLVA pattern or associated WGS profile. ECDC convened an outbreak investigation meeting, involving all countries that reported cases with the goal to confirm the re-emergence of the outbreak, agree on an updated case definition, and discuss response options.

The European outbreak case definition is as follows:

A confirmed outbreak case

- With symptoms onset on or after 1 May 2016 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

  AND

- Fulfilling the following laboratory criterion: with a strain sharing the same t5-level SNP address as one of the defined UK outbreak clusters based on WGS analysis (e.g. 1.2.3.175.175.175.% or 1.2.3.18.359.360.%).

A probable outbreak case

- With symptoms onset on or after 1 May 2016 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

  AND

- Fulfilling the following laboratory criterion: with a strain matching the MLVA profile 2-9-7-3-2 corresponding to the MLVA protocol with 5 loci [4].

A historical case

- A laboratory-confirmed *Salmonella* Enteritidis case with MLVA-type 2-9-7-3-2 or a strain sharing the same t5-level SNP address as one of the defined UK outbreak clusters based on WGS analysis and date of disease onset from February 2012 to April 2016 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

Exclusion criteria

- Cases with travel history outside of the EU/EEA.
- Secondary cases defined as those confirmed cases that have had person-to-person contact with a confirmed case and no exposure to a common source.
- Cases infected with MLVA-type 2-9-7-3-2, but not sharing the t5-level SNP address as one of the defined UK outbreak clusters based on WGS analysis (e.g. 1.2.3.175.175.175.% or 1.2.3.18.359.360.%).

Epidemiological investigation

Between 1 May and 30 August 2016, 16 confirmed and 132probable cases were reported by Belgium, Denmark, the Netherlands, Norway, Sweden and the United Kingdom (Table 1). The 16 confirmed cases were reported by England and Wales (n=15) and Scotland (n=1), and were all part of the t5-level SNP address 1.2.3.175.175.175.% previously described.

In all countries, cases were reported as sporadic or in clusters of less than 10 cases. At least 24 cases were reported as travel-associated: one to Austria, one to the Czech Republic, one to Germany, 13 to Greece, seven to Poland and one each to France and the Netherlands. All Norwegian cases were travel-related (to Greece and Poland).

Three of the historical cases had been previously reported with a travel history to Bulgaria, Poland and Portugal; the fourth case was connected to a journey to the Dutch Antilles.

In August 2016, an outbreak investigation was launched in the Netherlands. In the United Kingdom, case interviews that use a previously prepared trawling questionnaire are planned. Currently, national investigations have not been able to implicate a common source or vehicle of infection for this outbreak.

Table 1. Number of isolates by case classification and country, EU/EEA, February 2012 to August 2016

Country	Number of isolates				
	Confirmed cases (after 30 April 2016)		Probable cases (after 30 April 2016)	Historical cases (from February 2012 to April 2016)	
	SNP address 1.2.3.175.175.175.%	SNP address 1.2.3.18.359.360.%		SNP address 1.2.3.175.175.175.%	SNP address 1.2.3.18.359.360.%
Belgium	0	0	15	0	0
Denmark	0	0	1	0	0
Finland	0	0	0	1	0
Netherlands	0	0	64	2	7
Norway	0	0	14	0	0
Sweden	0	0	10	3	0
UK (England, Wales and Isle of Man)	15	0	0	25	33
UK (Scotland)	1	0	28	16	14
Total	**16**	**0**	**132**	**47**	**54**

ECDC threat assessment for the EU

Since 1 May 2016, six EU/EEA countries have reported 148 *S.* Enteritidis cases either with an identical MLVA profile (n=132) or belonging to a t5-level SNP address cluster (n=16) previously shown to be associated with the same MLVA profile. An international outbreak has been confirmed by high resolution WGS analysis in March 2016 on isolates of the same MLVA type. Although the outbreak had been considered ended in the first months of 2016, the new cases reported since May with identical MLVA type or falling in one of the two previously described WGS clusters confirm that the outbreak is still ongoing and that additional cases have to be expected. Although WGS analysis was performed only on recent isolates from England and Wales, it has to be considered very unlikely that the majority of the other probable cases would not be associated with this outbreak, particularly in the Netherlands, Scotland and Sweden which already identified outbreak cases in the past. WGS analysis on all or a sample of isolates from probable cases will clarify the extent of this international outbreak. In addition, WGS of isolates from probable cases with a travel history to a different EU country than the reporting one, may allow identifying additional countries affected by this outbreak among those not routinely performing neither MLVA nor WGS typing. Currently, all EU/EEA countries to which confirmed or probable cases reported travel should be considered as affected by this outbreak.

Based on the available information, it is likely that all cases are part of an outbreak caused by a common source which has been active since at least July 2015. In order to better identify the possible source of the outbreak, the updated European outbreak case definition now focuses only on those cases that were reported on or after 1 May 2016.

The outbreak initially consisted of two distinct SNP address clusters, but over the last few months only cases from one address cluster could be identified. Limiting the case definition to cases reported in May 2016 and later, and splitting the outbreak into two separately investigated clusters (based on WGS results), helps investigators to develop a strong hypothesis on the source (or sources) of the outbreak.

References

1. European Centre for Disease Prevention and Control. Surveillance atlas of infectious diseases. Stockholm: ECDC; 2016 [cited 2016 March 10]. Available from: http://atlas.ecdc.europa.eu/public/index.aspx?Dataset=27&HealthTopic=46&Indicator=109848&GeoResolution=2&TimeResolution=Month&StartTime=2011-01&EndTime=2014-12&CurrentTime=2014-12&Distribution=109852&DistributionRepresentation=B&TimeSeries=109848&TimeSeriesRepresentation=T&FixDataset=1.
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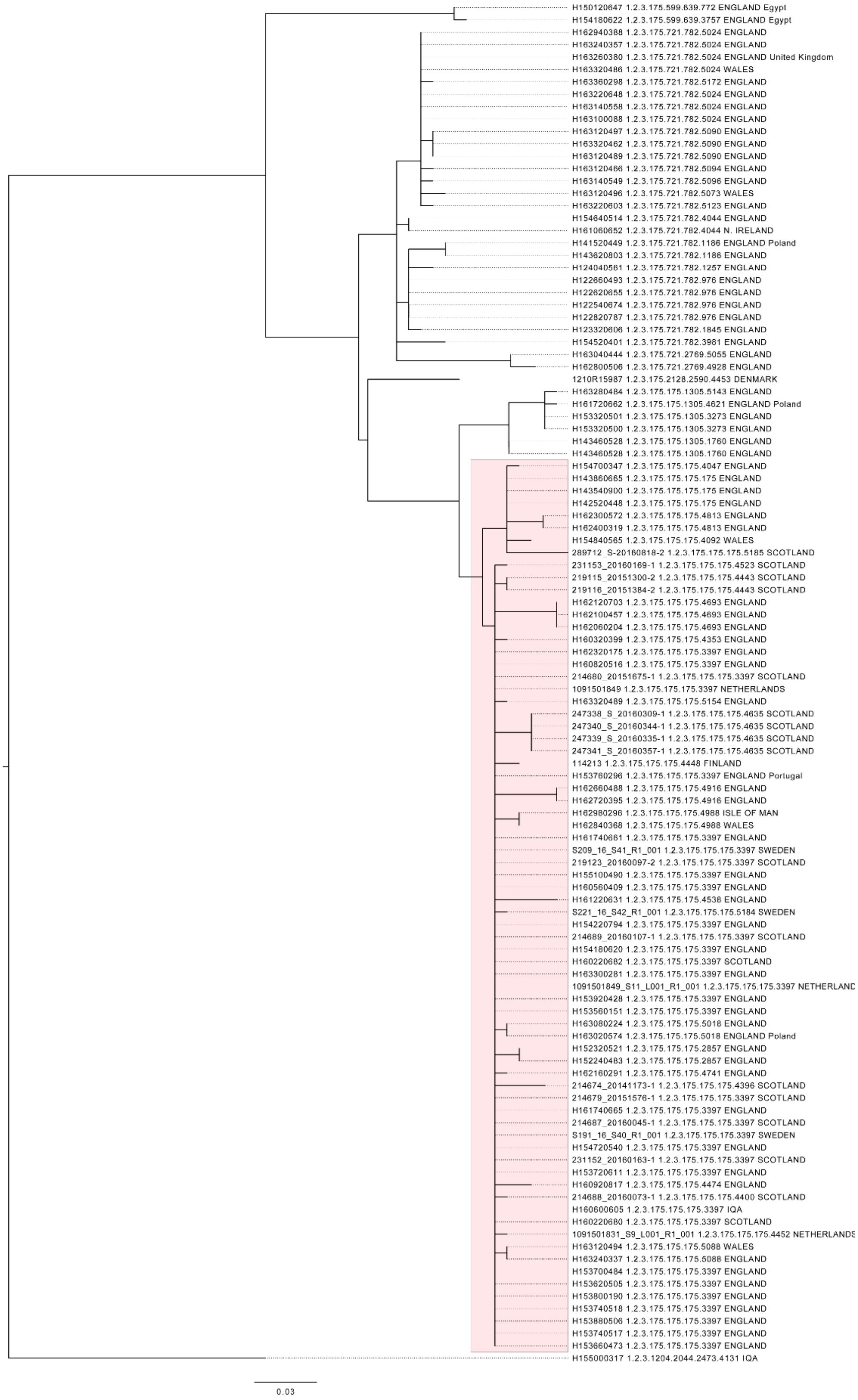
4. European Centre for Disease Prevention and Control. Laboratory standard operating procedure for multiple-locus variable-number tandem repeat analysis of *Salmonella enterica* serotype Enteritidis. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/en/publications/Publications/Salmonella-Enteritidis-Laboratory-standard-operating-procedure.pdf.

Annex 1

SNP-based phylogenetic trees of S. Enteritidis 2-9-7-3-2 isolates from Finland, the Netherlands, Sweden and the United Kingdom. SNP address 1.2.3.175.%.



Outbreak type is highlighted red.
IQA: internal quality assessment
Country name in uppercase: country where sample was taken
Country name in lower case: country of potential exposure

Source: Public Health England, United Kingdom