



RAPID RISK ASSESSMENT

Multi-country outbreak of *Salmonella* Enteritidis phage types 56 and 62, MLVA profile 2-11-3-3-2 and 2-12-3-3-2 infections

20 July 2017

Main conclusions and options for response

A multi-country outbreak of *S. Enteritidis* delineated through whole genome sequencing (WGS) analysis is currently ongoing, with 314 confirmed cases in Austria, France, Ireland, Luxembourg and the United Kingdom. Additionally, Austria, Belgium, Denmark, the Netherlands, Norway and the United Kingdom reported 21 probable and 50 historical probable cases. Confirmed cases belong to three closely related genetic clusters; probable cases belong to the multi-locus variable-number tandem repeat (MLVA) profiles 2-11-3-3-2, 2-12-3-3-2; however, an additional confirmed isolate has a MLVA profile of 2-9-3-3-2.

Isolates sharing the three t5-level single nucleotide polymorphism (SNP) addresses (t5.151; t5.718; t5.783) associated with this outbreak have been identified since 2014. At least two countries participating in the investigation (Austria and the UK) report human isolates within the same WGS cluster from cases without a travel history, indicating that the vehicle of infection is distributed to different EU countries. On the other hand, a large part of the confirmed travel-associated cases reported travelling to Spain or Portugal, which makes these two countries likely to be affected by the outbreak and having the vehicle of infection distributed there as well.

Public Health England reports that investigations into these genetic clusters have shown that the outbreaks could be associated with the consumption of poultry products, i.e. meat or eggs.

Since MLVA and WGS are not performed in all EU/EEA countries, additional EU countries may unknowingly be affected by this outbreak if the vehicle of infection was distributed there. This stresses the need to perform MLVA typing for *S. Enteritidis* in order to identify cases associated with this outbreak and perform WGS on isolates with the outbreak MLVA profiles in order to confirm their participation in the ongoing outbreak. ECDC will offer WGS services to those countries that do not yet have WGS capacity in order to ensure that they can analyse human isolates linked to the outbreak.

All countries identifying new confirmed or probable cases should also consider interviewing the new cases in a timely fashion.

Public health authorities in the affected EU/EEA countries are encouraged to consult the food safety authorities to support a joint investigation.

New cases and critical developments should be reported to EPIS-FWD, the Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses.

The competent authorities in affected Member States are encouraged to continue sharing information on this outbreak investigation, including issuing relevant notifications using the Early Warning and Response System (EWRS), the official channel to notify cross-border threats.

Source and date of request

ECDC Internal Decision, 12 July 2017.

Public health issue

Multi-country outbreak of *Salmonella* Enteritidis strains due to a persistent source of infection, with current or recent human cases in at least nine EU/EEA Member States.

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Disease background information

Salmonella Enteritidis (*S. Enteritidis*) is the most commonly detected serovar in cases of reported human non-typhoidal salmonellosis in the 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 [1]. 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 from <http://ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx> [2].

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, following a standardised 5-loci *S. Enteritidis* MLVA protocol [3].

As of 17 July 2017, eight countries have submitted MLVA data for *S. Enteritidis* to TESSy. Five of these countries reported 35 *S. Enteritidis* isolates with the MLVA profile 2-12-3-3-2 and six isolates with the MLVA profile 2-11-3-3-2.

Table 1. Number of isolates of *S. Enteritidis* reported to TESSy with an MLVA profile identical to the outbreak case definition or to additional MLVA profiles described in this investigation, July 1997 – June 2017 (dates used for statistics for all *S. Enteritidis* isolates with MLVA data)

MLVA profile	Isolates in TESSy	
	Number	Proportion of all <i>S. Enteritidis</i> isolates with MLVA data (%)
2-11-3-3-2	6	0.2
2-12-3-3-2	35	0.9
Other MLVA profiles	3780	98.9
Total	3821	100.0

Event background information

On 9 June 2017, the United Kingdom (Public Health England) reported in EPIS FWD a re-emergence of cases of genetically closely related *S. Enteritidis* infections. The cases fell into three 5-SNP single linkage clusters that were genetically related at 10-SNP level. Most cases that were phage-typed were characterised as PT56 or PT62, and two corresponding MLVA profiles are 2-11-3-3-2 and 2-12-3-3-2.

As of 17 July 2017, ten EU/EEA countries replied to the urgent inquiry; eight countries reported cases that could be possibly associated.

In collaboration with the affected countries, ECDC formed an international outbreak investigation team. ECDC also prepared a case definition and a line list to gather information on cases associated with this event.

Epidemiological and microbiological investigation

European outbreak case definition

Confirmed outbreak case

A laboratory-confirmed *S. Enteritidis* case fulfilling the following laboratory criterion: with an isolate from a clinical specimen that by WGS analysis belong to one of the 5-SNP single linkage clusters (t5 cluster), as defined by the Public Health England analysis pipeline [4-6]:

1.2.3.151.151.151.% (t5:151)
OR 1.2.3.151.151.718.% (t5:718)
OR 1.2.3.151.151.783.% (t5:783)

Probable outbreak case

A laboratory-confirmed *S. Enteritidis* case with symptoms onset on or after 1 January 2017 (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 an isolate from a clinical specimen of MLVA profile 2-11-3-3-2 or 2-12-3-3-2 [3]

OR

A laboratory-confirmed *S. Enteritidis* case with symptoms onset on or after 1 January 2017 (date of sampling or date of receipt by the reference laboratory if date of onset is not available)

AND

with an isolate from a clinical specimen that by WGS analysis belong to the 10-SNP single linkage cluster 1.2.3.151.151.% (t10:151), as defined by Public Health England WGS analysis pipeline, but not belonging to the t5 clusters defined above in the confirmed outbreak case definition.

Historical probable case

A laboratory-confirmed *S. Enteritidis* case with an isolate from a clinical specimen of MLVA profile 2-11-3-3-2 or 2-12-3-3-2 and date of disease onset from 1 January 2012 to 31 December 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 outbreak cases that have had person-to-person contact with an outbreak case and no known exposure to a potential common source.
- Cases from a clinical specimen of MLVA profile 2-11-3-3-2 or 2-12-3-3-2, but not sharing the t10-level SNP address as the defined Public Health England probable outbreak cluster based on WGS analysis (t10:151).

Note: The definition of a confirmed case based on WGS does not include any criteria based on date of onset because WGS offers very strong evidence to associate cases with the same outbreak. By contrast, the definition of a probable case includes only cases detected on or after 1 January 2017.

Epidemiological investigation

As of 17 July 2017, nine EU/EEA countries reported 314 confirmed cases, 21 probable cases and 50 historical probable cases (Table 2). The country reporting the most cases is the UK, with 81% of all outbreak cases and 94% of the confirmed cases.

Table 2. Number of *S. Enteritidis* isolates by case classification and reporting country, EU/EEA, May 2014 – 13 June 2017 (n=385)

Country	Confirmed cases	Probable cases	Historical probable cases	Total
Austria	4	2	0	6
Belgium	0	2	14	16
Denmark	0	2	5	7
France	10	0	0	10
Ireland	3	0	0	3
Luxembourg	1	0	0	1
Netherlands	0	13	9	22
Norway	0	0	8	8
United Kingdom	296	2	14	312
Total	314	21	50	385

In 2014, 2015 and 2016, outbreak cases peaked in September and October, whereas in 2017 an early peak occurred in March. This out-of-season peak was the second largest identified in the four-year period. While in the previous three years the vast majority (>93%) of cases in peak months was reported by the UK, in 2017 the proportion of UK cases decreased to 71% (Figures 1 and 2).

Figure 1. Distribution of *S. Enteritidis* isolates by month of reporting and case classification, EU/EEA, May 2014 – June 2017 (n=385)

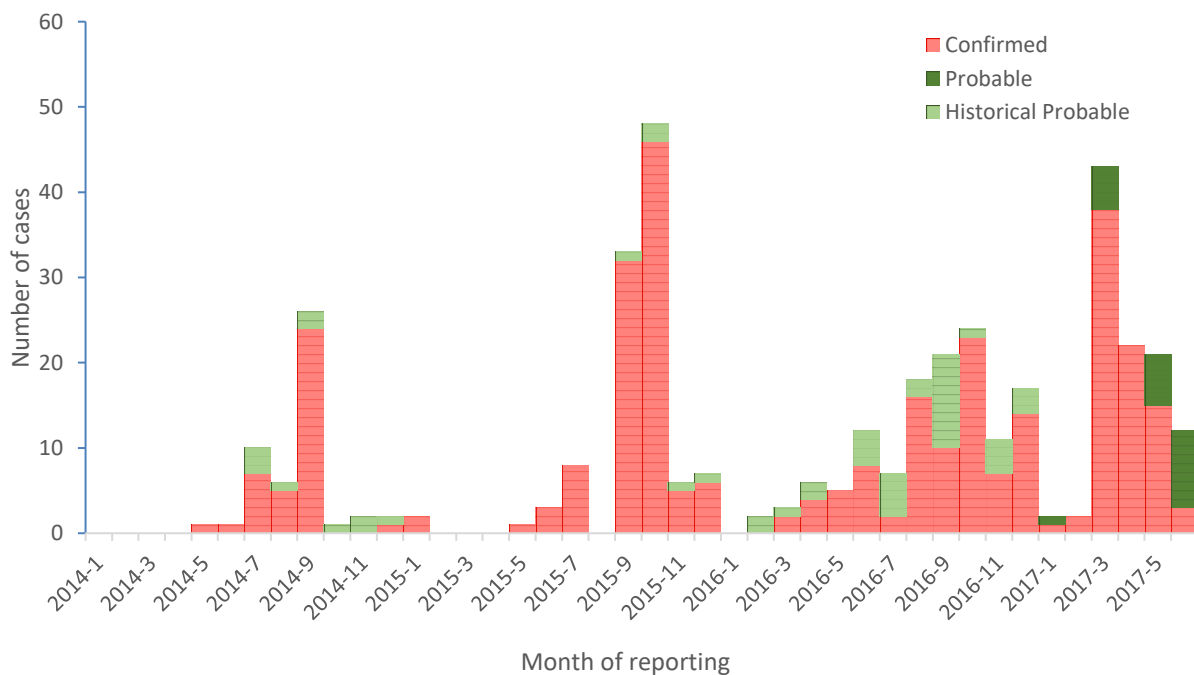
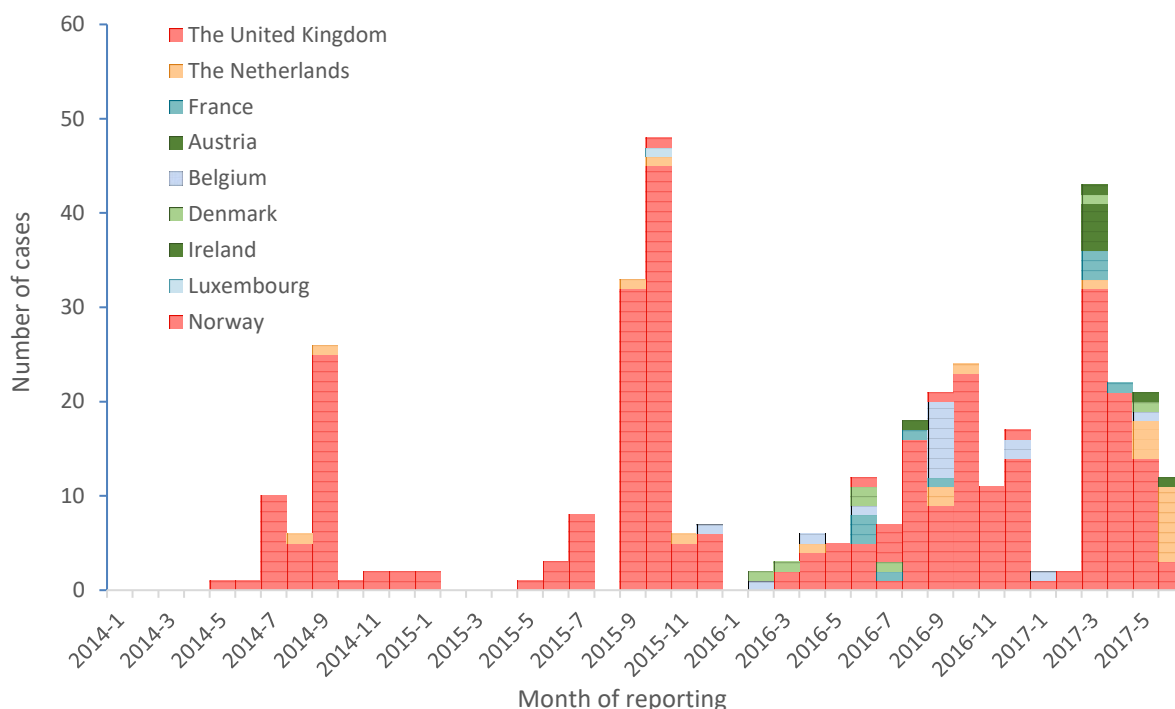


Figure 2. Distribution of *S. Enteritidis* isolates by month of reporting and reporting country, EU/EEA, May 2014 – June 2017 (n=385)



Of the 374 cases with information available on gender, 189 (51%) are male. The median age for male cases is 31 years (interquartile range (IQR) 11–53); for female cases the median age is 30 years (IQR 16–51).

Information on travel during the incubation period was available for 181 cases, 57 (31%) of whom travelled abroad. Among those, the most commonly reported countries of travel were Spain (60%) and Portugal (14%) (Table 3). All cases from Ireland and Luxembourg were travel-associated. A confirmed case from Luxembourg who travelled to Spain in 2015 had a fatal outcome. This is the only patient reported to have died in this outbreak.

Table 3. Number of *S. Enteritidis* isolates by case classification and probable country of infection, EU/EEA, May 2014 to June 2017 (n=57)

Country of travel	Confirmed Cases	Probable cases	Historical probable	Total
Cyprus	1	0	0	1
Greece	0	0	2	2
France	1	0	0	1
Italy	2	1	0	3
Norway	0	0	2	2
Portugal	6	0	2	8
Spain	25	6	3	34
Several EU countries	2	0	1	3
Unknown	2	1	0	3
Total	39	8	10	57

Public Health England reports that non-travel related UK cases are distributed throughout the country. In the current and past years, investigations into this outbreak pointed toward an association with eating in food outlets. Poultry products (i.e. poultry meat or eggs) were suggested as potential vehicles of infections; however, there was not enough evidence to link a specific product to the infections.

In Spain, no MLVA or WGS is performed, and it is therefore not possible to determine whether there is also an outbreak in Spain. However, phage typing is being performed on isolates from outbreaks under investigation, and in January and March 2017, two outbreaks of *S. Enteritidis* PT56 were identified in Spain.

Microbiological investigation

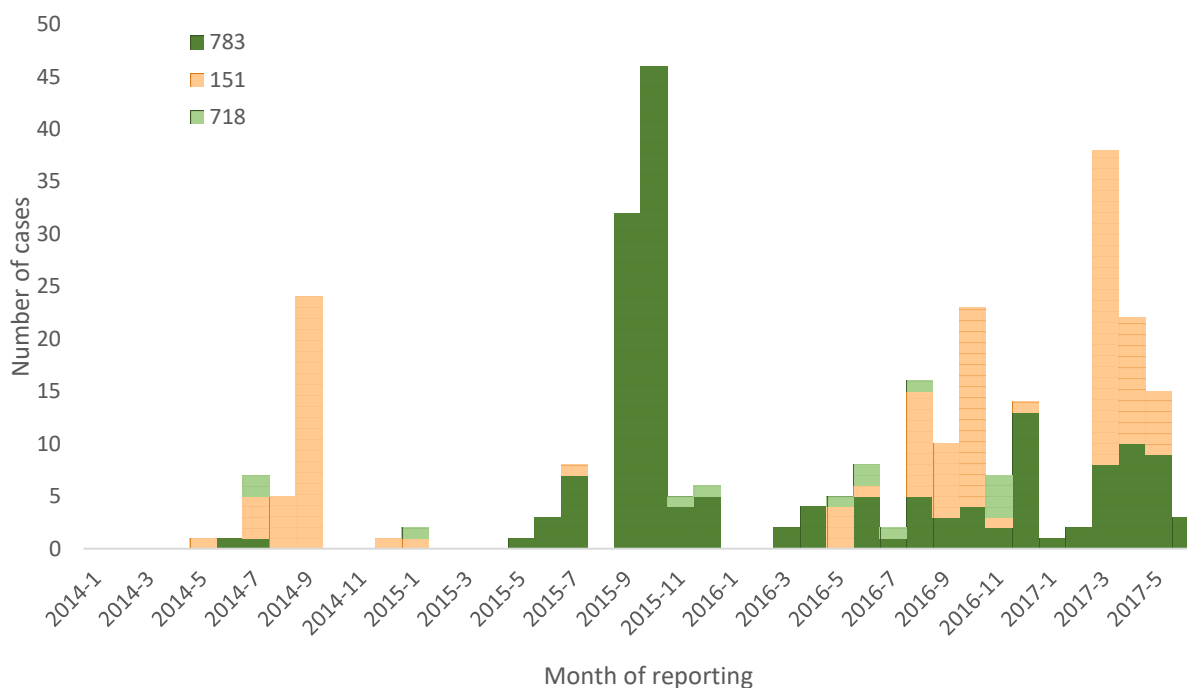
As of 17 July 2017, 314 isolates are part of the three t5 clusters (t5.151; t5.718; t5.783) following Public Health England nomenclature (Figure 3).

From the 87 cases with MLVA information, 18 had MLVA profile 2-11-3-3-2, and 68 cases had MLVA profile 2-12-3-3-2. One isolate from a confirmed case from Ireland had MLVA profile 2-9-3-3-2. No isolates with MLVA profile 2-9-3-3-2 have been reported to TESSy.

Most confirmed cases in the UK with information available on phage type were PT56 or PT62, which are not commonly isolated in the UK. In addition, Austria, Ireland and the UK reported a small number of confirmed cases as PT8, PT22, PT23 and untypable.

The three t5 clusters associated with this outbreak are genetically related at the 25-SNP level (t25), with a cluster of cases investigated in the UK between 2015 and 2017: t5 cluster SNP address is 1.2.3.151.362.363.% (t5.363). The t5.363 was investigated between May and October 2015 by Public Health England (PHE) and found to be associated with 154 human isolates from the UK and Spain and one non-human isolate from an egg omelette in Spain [6]. The same genetic cluster was later investigated in 2016 and January 2017 [7].

Figure 3. Distribution of *S. Enteritidis* outbreak cases by month of reporting and t5 cluster, EU/EEA, May 2014 – June 2017 (n=325)



ECDC threat assessment for the EU

A multi-country outbreak of *S. Enteritidis* associated with three WGS defined 5-SNP clusters is ongoing. Since at least 2014, 314 confirmed cases have been identified from five reporting countries.

The outbreak was detected through WGS and is characterised by its long duration with relatively low numbers of cases reported intermittently and peaks of re-emergence in late summer/early autumn between 2014 and 2016. In 2017 this pattern changed, with a peak observed in March.

The 5-SNP clusters are genetically closely related (belonging to the same t10 cluster) and are potentially epidemiologically linked to a common source of infection. This could indicate that there are one or more vehicles of infection associated with a single source contaminated with multiple strains of *S. Enteritidis*. Alternatively, there could be more than one vehicle of infection from different sources distributed through the same supply networks.

Austria, France and the United Kingdom report isolates belonging to the same genetic clusters from cases without history of travel abroad in 2017. This is suggestive of a circulation of vehicles of contamination in all three countries.

A large proportion (60%) of cases with known travel history during the incubation period travelled to Spain. The second most commonly visited country (by cases with a travel history) was Portugal. The two countries in the Iberian Peninsula are likely to be affected by this outbreak. In Spain and Portugal, WGS and/or MLVA are not routinely performed, thus the two countries did not report cases associated with this outbreak. However, the

presence of such a high proportion of travel-related cases and the reporting of outbreaks of *S. Enteritidis* PT56 early in 2017 in Spain strongly suggest that Spain is also affected by this outbreak.

Based on patient interviews, investigators in the United Kingdom hypothesise that poultry products are the vehicle of infection of this outbreak. Further investigations are ongoing.

Until the source of infection has been identified and the outbreak is controlled, new cases associated with this outbreak are likely to occur. Elderly people and immunocompromised patients are at risk of a more severe outcome if infected. Infants are at increased risk for invasive infection [8].

The European outbreak case definition relies on WGS, which is performed on a routine basis by only a small number of EU/EEA countries. This case definition is extremely specific, but can leave cases undetected in countries that do not use WGS. MLVA is a standardised typing method and used in several EU/EEA countries, thus enabling the detection of probable cases that can be subjected to WGS. However, cases may remain undetected, particularly in countries where serotyping and MLVA or WGS are not routinely performed.

References

1. 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. 2003; OJL, L325:[15 p.]. Available from: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R2160&rid=2>.
2. 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>.
3. Peters T, Bertrand S, Bjorkman JT, Brandal LT, Brown DJ, Erdosi T, et al. Multi-laboratory validation study of multilocus variable-number tandem repeat analysis (MLVA) for *Salmonella enterica* serovar Enteritidis, 2015. Euro Surveill. 2017 Mar 02;22(9).
4. Ashton P, Nair S, Peters T, Tewolde R, Day M, Doumith M, et al. Revolutionising Public health reference microbiology using whole genome sequencing: *Salmonella* as an exemplar. bioRxiv. 2015.
5. Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A, et al. Identification of *Salmonella* for public health surveillance using whole genome sequencing. PeerJ. 2016;4:e1752.
6. 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.
7. European Centre for Disease Prevention and Control (ECDC). 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.
8. Heymann D, editor. Salmonellosis. In: Control of communicable diseases manual – Salmonellosis. Washington, D.C.: American Public Health Association; 2015. p. 523-39.