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Multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA profile 2-9-7-3-2 and 2-9-6-3-2 infections

European Food Safety Authority
European Centre for Disease Prevention and Control

Abstract

A multi-country outbreak of *Salmonella* Enteritidis phage type (PT) 8 with multiple locus variable-number tandem repeat analysis (MLVA) profiles 2-9-7-3-2 and 2-9-6-3-2, linked to eggs, is ongoing in the EU/EEA. Based on whole genome sequencing (WGS), isolates are part of two distinct but related genetic clusters. ECDC and EFSA are liaising with relevant authorities in the Member States and the European Commission to facilitate the coordination of investigation and response measures. From 1 May 2016 to 24 February 2017, 14 EU/EEA countries have reported 218 confirmed cases belonging to two distinct WGS clusters, and 252 probable cases sharing the *S. Enteritidis* MLVA profiles 2-9-7-3-2 or 2-9-6-3-2. Outbreak cases, both confirmed and probable, have been reported by Belgium, Croatia, Denmark, Finland, France, Greece, Hungary, Italy, Luxembourg, the Netherlands, Norway, Slovenia, Sweden and the United Kingdom. Eleven confirmed cases are reported to have travelled to Poland during the incubation period. Poland is therefore likely to be affected by this outbreak as well. Croatia and Hungary reported a fatal case each. The outbreak peaked at the end of September 2016, shortly before the vehicle of infection was identified and control measures were implemented at the farm and distribution level. Since then, the number of cases caused by the outbreak types of *S. Enteritidis* has steadily decreased: two or fewer new cases per week were reported in January and in the first week of February 2017. As of 24 February 2017, 21 confirmed and probable cases have been reported since 1 December 2016 by the United Kingdom (n=9), Norway (n=4), Belgium (n=3), the Netherlands (n=3), Hungary (n=1) and Sweden (n=1). The last case was reported by Norway, with the date of sampling being 2 February 2017. It is likely that contaminated food items were circulating in these countries until recently. The European outbreak case definition relies on a limited number of *S. Enteritidis* types (WGS for the definition of confirmed cases and MLVA for the definition of probable cases), and is therefore extremely specific. In countries where MLVA or WGS is not undertaken routinely, additional cases are expected to be associated with this outbreak. Available evidence from epidemiological, microbiological, environmental and tracing investigations identified eggs originating from three Polish packing centres as the vehicle of infection in this outbreak. Investigations in the farms identified 18 *S. Enteritidis*-positive laying hen farms in Poland. Most of these farms, as well as the three packing centres, belong to the same Polish consortium and are considered to be interlinked. According to information provided by Polish authorities, no *S. Enteritidis*-positive breeding flocks were detected in 2016. Based on the current evidence, the source of infection of this outbreak is likely to be at the level of the laying hen farms. However, due to the nature of egg production there is the possibility that *S. Enteritidis* might have entered at a higher level in the food chain. It is therefore recommended that Poland conducts further checks on the *S. Enteritidis* status of the hatcheries and breeding flocks, especially those linked to *S. Enteritidis*-positive farms. In addition to the control measures already in place, it is recommended that Poland applies all measures that can reduce the risk of *Salmonella* contamination in laying hen farms. In order to monitor the public health impact of control measures, an enhanced surveillance period has been established by the international outbreak investigation team. During the coming months, all countries should consider performing WGS on any human isolate characterised by MLVA profiles 2-9-7-3-2 or 2-9-6-3-2. ECDC will be offering 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. EU/EEA countries should also consider performing WGS on non-human *S. Enteritidis* isolates that have a link to the *S. Enteritidis*-positive farms in Poland. New cases and critical developments should be reported to EPIS-FWD (Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses). The competent authorities in the food safety and the public health sectors in affected Member States and at the European level are encouraged to continue sharing information on the epidemiological, microbiological and environmental investigations, including issuing relevant notifications using the Rapid Alert System for Food and Feed (RASFF) and the Early Warning and Response System (EWRS), the latter representing the official channel to notify cross-border threats.

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Key words: *Salmonella* Enteritidis, eggs, multi-country outbreak, multiple locus variable-number tandem repeat analysis (MLVA), Whole Genome Sequencing (WGS)

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JOINT RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA profile 2-9-7-3-2 and 2-9-6-3-2 infections

First update, 7 March 2017

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) profiles 2-9-7-3-2 and 2-9-6-3-2, linked to eggs, is ongoing in the EU/EEA. Based on whole genome sequencing (WGS), isolates are part of two distinct but related genetic clusters. ECDC and EFSA are liaising with relevant authorities in the Member States and the European Commission to facilitate the coordination of investigation and response measures.

From 1 May 2016 to 24 February 2017, 14 EU/EEA countries have reported 218 confirmed cases belonging to two distinct WGS clusters, and 252 probable cases sharing the *S. Enteritidis* MLVA profiles 2-9-7-3-2 or 2-9-6-3-2. Outbreak cases, both confirmed and probable, have been reported by Belgium, Croatia, Denmark, Finland, France, Greece, Hungary, Italy, Luxembourg, the Netherlands, Norway, Slovenia, Sweden and the United Kingdom. Eleven confirmed cases are reported to have travelled to Poland during the incubation period. Poland is therefore likely to be affected by this outbreak as well. Croatia and Hungary reported a fatal case each.

The outbreak peaked at the end of September 2016, shortly before the vehicle of infection was identified and control measures were implemented at the farm and distribution level. Since then, the number of cases caused by the outbreak types of *S. Enteritidis* has steadily decreased: two or fewer new cases per week were reported in January and in the first week of February 2017.

As of 24 February 2017, 21 confirmed and probable cases have been reported since 1 December 2016 by the United Kingdom (n=9), Norway (n=4), Belgium (n=3), the Netherlands (n=3), Hungary (n=1) and Sweden (n=1). The last case was reported by Norway, with the date of sampling being 2 February 2017. It is likely that contaminated food items were circulating in these countries until recently.

The European outbreak case definition relies on a limited number of *S. Enteritidis* types (WGS for the definition of confirmed cases and MLVA for the definition of probable cases), and is therefore extremely specific. In countries where MLVA or WGS is not undertaken routinely, additional cases are expected to be associated with this outbreak.

Available evidence from epidemiological, microbiological, environmental and tracing investigations identified eggs originating from three Polish packing centres as the vehicle of infection in this outbreak. Investigations in the farms identified 18 *S. Enteritidis*-positive laying hen farms in Poland. Most of these farms, as well as the three packing centres, belong to the same Polish consortium and are considered to be interlinked. According to information provided by Polish authorities, no *S. Enteritidis*-positive breeding flocks were detected in 2016. Based on the current evidence, the source of infection of this outbreak is likely to be at the level of the laying hen farms. However, due to the nature of egg production there is the possibility that *S. Enteritidis* might have

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entered at a higher level in the food chain. It is therefore recommended that Poland conducts further checks on the *S. Enteritidis* status of the hatcheries and breeding flocks, especially those linked to *S. Enteritidis*-positive farms.

In addition to the control measures already in place, it is recommended that Poland applies all measures that can reduce the risk of *Salmonella* contamination in laying hen farms.

In order to monitor the public health impact of control measures, an enhanced surveillance period has been established by the international outbreak investigation team. During the coming months, all countries should consider performing WGS on any human isolate characterised by MLVA profiles 2-9-7-3-2 or 2-9-6-3-2. ECDC will be offering 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.

EU/EEA countries should also consider performing WGS on non-human *S. Enteritidis* isolates that have a link to the *S. Enteritidis*-positive farms in Poland.

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

The competent authorities in the food safety and the public health sectors in affected Member States and at the European level are encouraged to continue sharing information on the epidemiological, microbiological and environmental investigations, including issuing relevant notifications using the Rapid Alert System for Food and Feed (RASFF) and the Early Warning and Response System (EWRS), the latter representing the official channel to notify cross-border threats.

Source and date of request

European Commission Directorate General for Health and Food Safety request, 4 February 2017.

Public health issue

This document updates the assessment of the public health risk associated with a multi-country outbreak of *Salmonella* Enteritidis PT 8 which is epidemiologically and microbiologically linked to contaminated eggs from Poland and can be characterised by at least two MLVA profiles and two clusters defined by whole genome sequencing. Previous assessments of this event were published in a first ECDC rapid risk assessment (published by ECDC on 21 March 2016 in EPIS-FWD), an updated rapid risk assessment published by ECDC on 5 September 2016, and a joint rapid outbreak assessment published by ECDC and EFSA on 27 October 2016 [1].

Consulted experts

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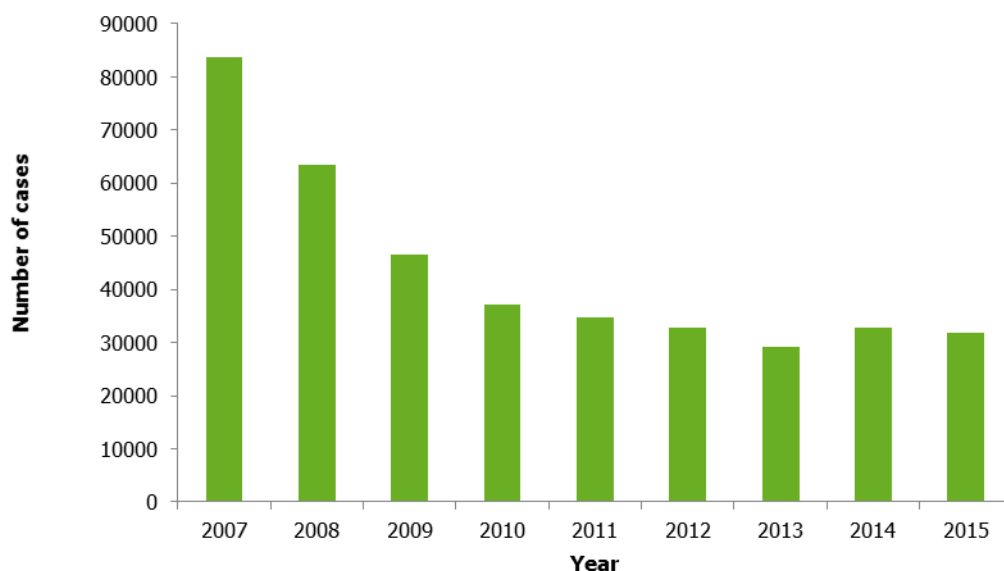
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 United Kingdom: Lesley Larkin, Hassan Hartman (Public Health England – PHE), Gill Hawkins (Health Protection Scotland – HPS), Drazenka Tubin-Delic (Food Standards Agency), Deb Morgan, Joanne Edge (Food Standard Agency – FSA).

Disease background information

Salmonella Enteritidis phage type 8 isolations in humans

S. Enteritidis is the most commonly detected serovar in cases of 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, measures aimed at food business operators to improve hygiene implemented under the EU Food Law, including the introduction of microbiological criteria for *Salmonella* [2]. Phage type 8 is the most commonly reported *S. Enteritidis* phage type in human cases in EU/EEA.

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



Source: ECDC [3]

From 2007 to 2015, 392 302 cases of *S. Enteritidis* were reported to The European Surveillance System (TESSy) (mean number of cases per year 43 586, range 29 090 to 83 760) by 27 countries, with Germany and the Czech Republic reporting 51% of all cases. The median age for all cases with information available was 28 years (interquartile range IQR=5–49); 52% (n=201 437) were female, and 89% (n=294 057) were acquired in the reporting country. Symptom onset was distributed over the year, with a peak from July to September. To date, 14 124 *S. Enteritidis* cases have been reported by 23 countries to TESSy for 2016 (data not yet published) [3].

During the period 2011–2015, 13 EU/EEA countries (Austria, Belgium, Denmark, Estonia, Hungary, Ireland, Italy, 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 was reported by the United Kingdom (35%) which, together with Austria and Hungary, accounted for 84% of reported cases. Seventy-nine percent of the cases with available information were acquired domestically. Cases occurred among all age groups, and the median age was 30 years (IQR=6–50). No gender difference could be observed. A total of five countries reported 509 cases of *S. Enteritidis* PT8 to TESSy in 2016.

Salmonella Enteritidis MLVA profiles in TESSy

Public Health England (PHE) developed a protocol for a *S. Enteritidis* MLVA, which was validated by an inter-laboratory study in collaboration with ECDC and published as a standard protocol for a 5-loci *S. Enteritidis* MLVA scheme [4]. EU/EEA-wide collection of *S. Enteritidis* MLVA data started in TESSy in June 2016. As of 28 February 2017, eight countries have submitted MLVA data for *S. Enteritidis*, including 152 *S. Enteritidis* isolates with the

MLVA profile 2-9-7-3-2. Of these, 145 are part of a multi-country cluster involving Denmark, Greece, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom, with dates used for statistical purposes to denote time of occurrence ranging from 28 May 2015 to 2 February 2017. The remaining seven are isolates from 2012–2014 which were reported by the Netherlands and Scotland. Additional cases with isolates of this MLVA profile were reported to ECDC through EPIS-FWD. In addition, eleven *S. Enteritidis* isolates with MLVA profile 2-9-6-3-2, a single-locus variant of the original outbreak MLVA profile, were reported. Isolates belonging to MLVA profile 2-9-6-3-2 were reported to TESSy by Norway and Scotland. The dates used for statistical purposes to denote time of occurrence ranged from 12 March 2015 to 29 September 2016.

Additional isolates from TESSy with MLVA profiles are presented in Table 1 and described below under 'Microbiological and environmental investigations of food'.

Table 1. Number of isolates reported to TESSy with an MLVA profile identical to the outbreak case definition and the MLVA profiles identified in the *S. Enteritidis*-positive farms in Poland (see Table 3)

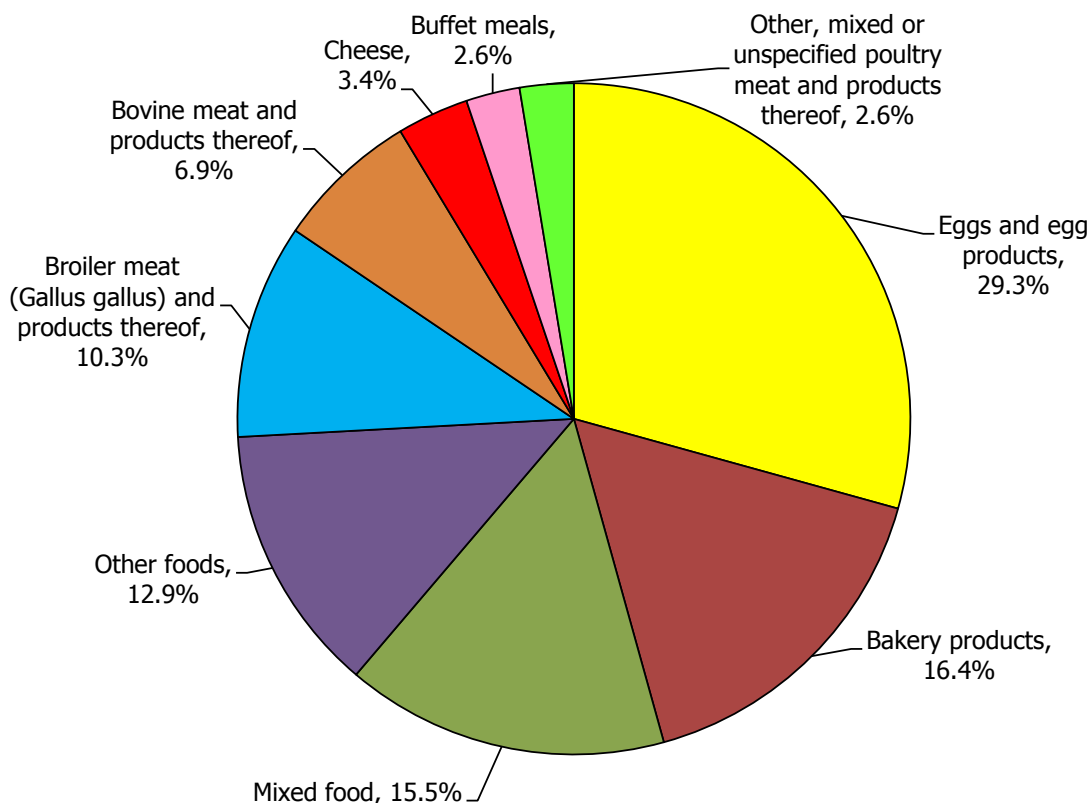
MLVA profile	Isolates in TESSy	
	Number	Proportion
2-9-6-3-2	11	0.3%
2-9-7-3-2	152	3.8%
2-10-6-3-2	28	0.7%
2-10-8-3-2	67	1.7%
2-11-8-3-2	4	0.1%
Total isolates with MLVA characterisation in TESSy	3962	100%

Food-borne outbreaks caused by *Salmonella* Enteritidis

S. Enteritidis is the predominant serovar associated with *Salmonella* outbreaks [5]. In 2015, *S. Enteritidis* accounted for 575 outbreaks (60.3% of all *Salmonella* outbreaks) and 4 042 human cases (61.1% of all cases in *Salmonella* outbreaks) in the EU. For 116 of these outbreaks, the evidence supporting the association with the suspected food vehicle was reported to be strong. As in previous years, 'eggs and egg products' were the food vehicles most frequently associated with *S. Enteritidis* outbreaks with strong evidence. The proportion of outbreaks with strong evidence decreased from 46.1% in 2014 to 29.3% in 2015 (Figure 2). In 2015, no food-borne *S. Enteritidis* outbreaks were reported by non-EU Member States.

In 2015, information on phage type was only provided for 39 food-borne outbreaks caused by *S. Enteritidis*. Twenty of these outbreaks were due to *S. Enteritidis* PT8 and involved a total of 96 human cases. For one of the 20 *S. Enteritidis* PT8 outbreaks, the evidence supporting the association with the suspected food vehicle was reported to be strong: it was associated with the consumption of pig meat in a household setting.

Figure 2. Distribution of food vehicles in strong-evidence food-borne outbreaks caused by *Salmonella* Enteritidis, EU, 2015 (n=116)



Source: The European Union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2015, EFSA [5]

Note: Data from 116 outbreaks included Austria (1), Belgium (1), Croatia (12), France (17), Germany (2), Greece (1), Hungary (1), Latvia (1), Lithuania (20), Poland (48), Portugal (1), Romania (2), Slovakia (7), Sweden (1) and the United Kingdom (1). Other food items (N = 15) included other foods (5), vegetables and juices and other products thereof (2), pig meat and products thereof (2), meat and meat products (2), fish and fish products (2), herbs and spices (1), and sweets and chocolate (1). In 2015, no food-borne *S. Enteritidis* outbreaks were reported by non-EU Member States.

Salmonella Enteritidis isolations in food, animals and feed

EU/EEA countries have frequently reported *S. Enteritidis* from food and animals in the framework of the monitoring on zoonoses and zoonotic agents based on Directive 2003/99/EC [6].

From 2004 to 2015, the EU/EEA Member States reported a total of 6 330 units positive for *S. Enteritidis* from several food categories (Annex 1, Table 1), mainly from chicken meat (*Gallus gallus*) (n=3 042), followed by eggs and egg products (n=1 075), meat from other or unspecified poultry species (n=1 069), meat from pigs (n=277) and other types of meat, including bovine meat, mixed and unspecified meat (n=353). *S. Enteritidis* was reported less frequently from additional food categories, such as 'fish and fishery products' (n=44), 'dairy products (including cheese) and milk' (n=33), fruits and vegetables (including 'spices and herbs' and 'sprouted seeds') (n=37) and several other foods (n=400).

In the same period, the EU/EEA Member States reported a total of 29 068 units positive for *S. Enteritidis* from several animal species, primarily from chickens (*Gallus gallus*) (n=24 735), followed by cattle (n=1 255), geese (n=496), ducks (n=420), pigs and wild boars (n=391), turkeys (n=372), and 'other or unspecified poultry or game birds' (n=639). *S. Enteritidis* isolations were less frequently reported from other animal species.

From 2004 to 2015, *S. Enteritidis* was also detected in 254 units of different feed categories: compound feedingstuffs for poultry (n=69), other or unspecified types of feedingstuffs/feed material (n=46), pet food (n=34), feed material of land animal origin (n=32), feed material of oil seed or fruit origin (n=21), feed material of marine animal origin (n=18), feed material of cereal grain origin (n=17), compound feedingstuffs for pigs (n=9), and compound feedingstuffs for cattle (n=8).

In 2015, 259 units positive for *S. Enteritidis* were reported from food of which the majority (n=136, 52.5%) was from broiler meat (*Gallus gallus*). *S. Enteritidis* isolations from broiler meat were provided by 12 Member States, mainly by Poland and the Czech Republic that reported 36.8% and 34.6% of the total 136 *S. Enteritidis* isolations from broiler meat, respectively. In 2015, isolations of *S. Enteritidis* from eggs (n=22) and egg products (n=1) were

reported by six Member States, mainly Germany, which reported 12 isolations from table eggs. Additional *S. Enteritidis* isolations from table eggs were reported by Slovakia (n=4), Spain (n=4), the Czech Republic (n=1) and Bulgaria (one isolate from unspecified eggs). The only isolation of *S. Enteritidis* from liquid egg products was reported by Austria.

The majority of the 2015 *S. Enteritidis* isolations in animals (n=1 594) were obtained from fowl (*Gallus gallus*) (n=1 237 isolations, representing 77.6% of the total), followed by cattle (n=170), other poultry species or game birds (including ducks, geese and turkeys) (n=109), and pigs (n=19). *S. Enteritidis* isolations were less frequently reported from several other animal species, including pigs and wild boar, domestic solipeds, reptiles, cats, dogs, and other animals (Annex 1, Table 1). Out of the 1 237 *S. Enteritidis* isolations reported from fowls (*Gallus gallus*), 765 isolations were obtained from broiler flocks, 420 isolations were reported from laying hens flocks, and 52 isolations came from breeding flocks (including parent and grandparent breeding flocks). In 2015, *S. Enteritidis* was widely isolated in chickens in the EU, with the following Member States reporting the highest number of isolates: the Czech Republic (n=216), France (n=216), Germany (n=213), Poland (n=169), the United Kingdom (n=73), Slovakia (n=63), Romania (n=57), and Spain (n=44).

According to Regulation 2160/2003 [2], EU Member States are subject to national control programmes for *Salmonella* in poultry populations. Data on *S. Enteritidis* isolations from broiler flocks were reported by 16 EU Member States, with the majority of isolates reported by the Czech Republic (n=210), France (n=158), Poland (n=86), the United Kingdom (n=69), Germany (n=61), Slovakia (n=48) and Romania (n=47). Eighteen EU Member States and one non-EU Member State reported *S. Enteritidis* isolates from laying hen flocks; among these countries, the highest numbers of isolates from laying hen flocks were reported by Germany (n=132), Poland (n=65), the Netherlands (55), France (n=50), and Spain (n=28). *S. Enteritidis* isolates from breeding flocks were reported by seven EU Member States, mostly from Germany (n=20), Poland (n=18) and France (n=8).

In 2015, 12 *S. Enteritidis* isolations in feed were reported by four Member States and two non-Member States: three isolations from pet food, one isolation from feed material of oil seed or fruit origin, one isolation from feed material of cereal grain origin, one isolation from compound feedingstuffs for cattle, and six isolations from other or unspecified feedingstuffs/feed materials. *S. Enteritidis* isolations from feed were reported by the United Kingdom (n=4), Bulgaria (n=3), Iceland (n=2), Austria (n=1), Croatia (n=1) and Norway (n=1).

Information on the *S. Enteritidis* isolations reported in food, animals and feed from 2004 to 2015 are summarised in Annex 1, Table 1.

Information on phage type is usually not reported in the context of the monitoring zoonoses and zoonotic agents in food, animals and feed based on Directive 2003/99/EC. Isolations of *S. Enteritidis* PT8 in food and animals were only provided by two Member States (Austria and the United Kingdom) in 2013, three Member States (Austria, Slovakia and the United Kingdom) in 2014, and two Member States (Ireland and the United Kingdom) in 2015. In 2014, 22 isolations of *S. Enteritidis* PT8 were reported, 18 of which were from fowl (*Gallus gallus*), two from domestic solipeds, and two from pig meat. In 2015, only four *S. Enteritidis* PT8 isolations were reported: two isolations from broiler meat reported by Ireland and two isolations from feed reported by the United Kingdom.

Until 2012, Member States provided EFSA with isolate level information on *Salmonella* serovars and phage types from the laboratories*, in addition to monitoring data on *Salmonella* reported in the framework of Directive 2003/99/EC. During the period 2004–2012, the laboratories provided information on 239 isolations of *S. Enteritidis* PT8 from food – mainly from broiler meat (n=119), followed by eggs (n=37) – whereas only few *S. Enteritidis* PT8 isolations from other types of foods were reported. During the same period, the laboratories provided information on 1 369 *S. Enteritidis* PT8 isolations from animals, primarily fowl (*Gallus gallus*) from which 1 160 isolations were reported.

Event background information

In January and August 2016, two urgent inquiries describing increases of human isolates characterised by an uncommon MLVA profile (2-9-7-3-2) were launched in EPIS-FWD, first by Scotland and then by the Netherlands. Following the urgent inquiries, a number of EU countries reported previous detections of this MLVA profile.

* Any comparison between the reporting of *S. Enteritidis* PT8 isolations in food and animals from the laboratories and the isolations of *S. Enteritidis* collected in the framework of the Directive 2003/99/EC (as summarised in Table 1 of Annex 1) should be avoided because data are derived from two different sources and are not comparable.

After a WGS analysis of Scottish isolates, England and Wales reported genetically linked isolates identified in and after 2012, the majority of which were reported after July 2015. In order to confirm and delineate the multi-country outbreak, ECDC offered WGS services to possibly affected countries. WGS analysis was performed simultaneously in the Netherlands, Scotland and England on isolates sequenced by reference laboratories in the affected countries or through ECDC support. The results confirmed a multi-country outbreak involving two genetically distinct clusters.

Using the PHE in-house pipeline and nomenclature for WGS analysis, the threshold for inclusion in any of the WGS clusters was set to ≤ 5 single nucleotide polymorphisms (SNP) difference (t5 level) to at least one other case in the outbreak [7-9]. The SNP addresses at t5 level for the two WGS clusters were 1.2.3.175.175.175.% (WGS cluster 175, accession number SRR3285443) and 1.2.3.18.359.360.% (WGS cluster 360; accession number: SRR4063700). The %-sign in the SNP address represents a wildcard character, i.e. it represents any number denoting the 0-SNP level, all variations of which are included for isolates clustering at the 5-SNP threshold level.

Following the second EPIS FWD urgent inquiry in August 2016, ECDC convened an international outbreak investigation team involving all countries that reported cases with isolates that had the same MLVA profile or matched one of the WGS clusters, with the goal to agree on an outbreak case definition, assess the magnitude of the outbreak, and identify response options. The case definition was updated in October 2016 following the identification of isolates with MLVA profile 2-9-6-3-2 belonging to WGS cluster 360, one of the clusters associated with this outbreak.

The European outbreak case definition is as follows:

A confirmed outbreak case

- A laboratory-confirmed *Salmonella* Enteritidis 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 United Kingdom outbreak clusters based on WGS analysis: 1.2.3.175.175.175.% (WGS cluster 175) OR 1.2.3.18.359.360.% (WGS cluster 360).

A probable outbreak case

- A laboratory-confirmed *Salmonella* Enteritidis 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 OR 2-9-6-3-2 corresponding to the MLVA protocol with 5 loci [4].

A historical confirmed case

- A laboratory-confirmed *Salmonella* Enteritidis case with a strain sharing the same t5-level SNP address as one of the defined United Kingdom 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).

A historical probable case

- A laboratory-confirmed *Salmonella* Enteritidis case with a strain with MLVA profile 2-9-7-3-2 OR 2-9-6-3-2 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 profile 2-9-7-3-2 or 2-9-6-3-2, but not sharing the t5-level SNP address as one of the defined United Kingdom outbreak clusters based on WGS analysis (e.g. 1.2.3.175.175.175.% or 1.2.3.18.359.360.%).

Epidemiological and microbiological investigation of humans

As of 24 February 2017, 14 EU/EEA countries have reported 218 confirmed and 252 probable cases associated with this outbreak (Table 2). The number of reported outbreak cases peaked in week 39 of 2016 (last week of September 2016) and then decreased steadily (Figure 3).

The 218 confirmed cases belong mostly to WGS cluster 175 (168 cases) and cluster _360 (50 cases) and were reported between May 2016 and February 2017 (Figure 3). Based on the PHE WGS analysis pipeline, the maximum SNP difference between the two clusters is approximately 80 SNPs. Within the clusters, there is a variation of 27 SNPs for cluster 175 and of 12 SNPs for cluster 360. Eighteen isolates with MLVA profile 2-9-7-3-2 and one isolate with MLVA profile 2-9-6-3-2, identified in the same time period, were sequenced and found not to belong to either of the two WGS clusters so far associated with this outbreak.

Table 2. Number of isolates by case classification and country, EU/EEA, February 2012 to February 2017 (n=601)

Country	Number of isolates					
	Confirmed cases		Probable cases	Historical confirmed cases		Historical probable cases
	Cluster_175	Cluster_360		Cluster_175	Cluster_360	
Belgium	12	2	119	0	0	0
Croatia	4	0	0	0	0	0
Denmark	5	1	0	0	0	0
Finland	0	0	1	1	0	0
France	3	2	0	0	0	0
Greece	0	0	2	0	0	0
Hungary	0	0	12	0	0	0
Italy	1	0	0	0	0	0
Luxembourg	1	2	0	0	0	0
Netherlands	55	18	98	2	13	5
Norway	6	2	16	0	0	19
Slovenia	2	1	0	0	0	0
Sweden	6	3	0	2	0	0
UK (England, Wales and Isle of Man)	57	4	0	26	33	0
UK (Northern Ireland)	1	0	0	0	0	0
UK (Scotland)	15	15	4	14	16	0
Total	168	50	252	45	62	24

Since 1 December 2016, a more accentuated decrease in the number of newly reported cases per week has been observed. As of 24 February 2017, from 1 December 2016 to 2 February 2017 (date of sampling of the last reported case), 21 confirmed and probable cases were reported by the United Kingdom (n=9), Norway (n=4), Belgium (n=3), the Netherlands (n=3), Hungary (n=1) and Sweden (n=1). Of these, one confirmed case from England and one confirmed case from Sweden had travelled to Poland during the incubation period.

Most confirmed and probable cases with available information on MLVA profile (n=398) have MLVA profile 2-9-7-3-2 (n=368). The 30 isolates with MLVA profile 2-9-6-3-2 were identified in Belgium (n=1), the Netherlands (n=21), Norway (n=2) and Scotland (n=6). The 16 isolates (11 from the Netherlands, two from Norway and three from Scotland) with MLVA profile 2-9-6-3-2 that were sequenced and confirmed by WGS were found to belong to WGS cluster 360.

Figure 3. Distribution of outbreak cases by week and case classification (n=579), EU/EEA, January 2015 to February 2017

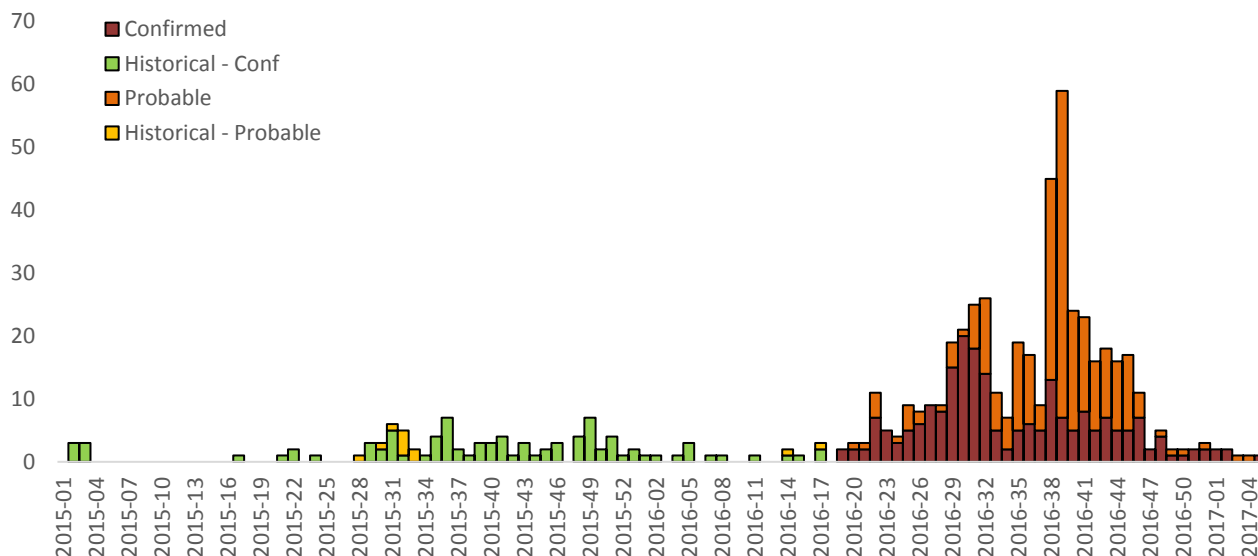
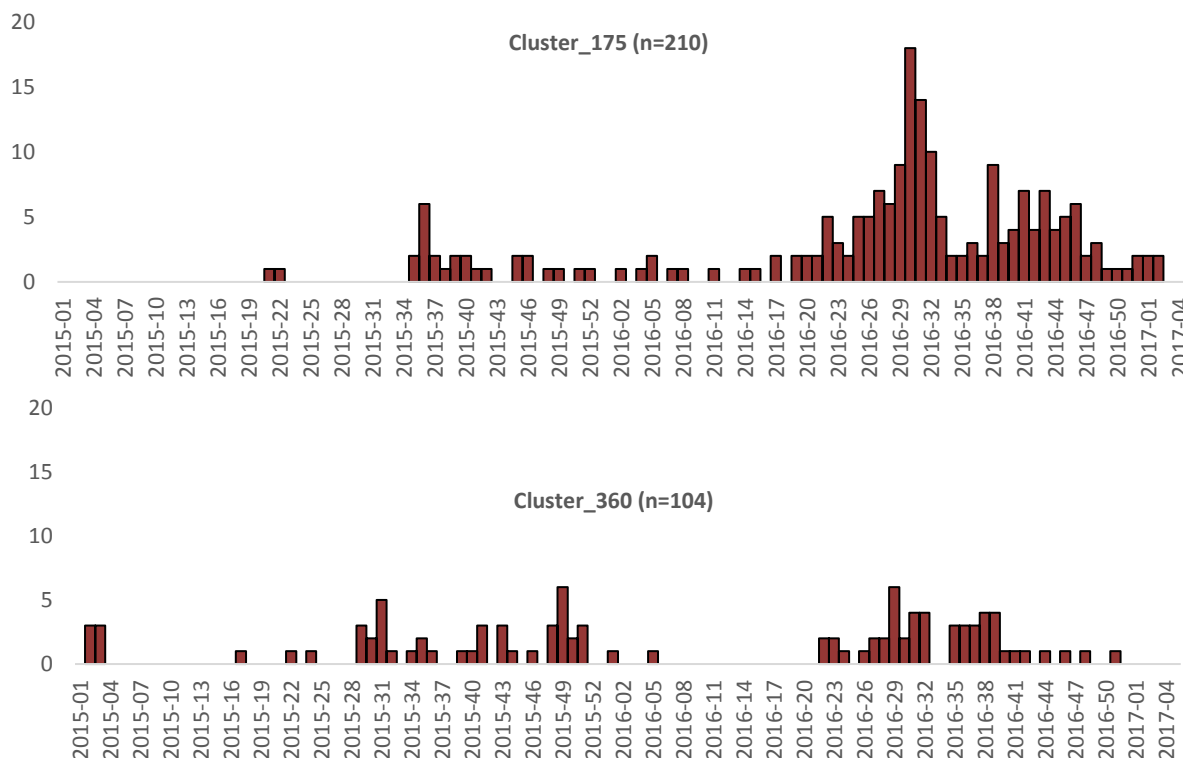


Figure 4. Distribution of outbreak confirmed and historical confirmed cases by week and genetic cluster (n=314), EU/EEA, January 2015 to February 2017



Information on hospitalisation was available for 90 confirmed and probable cases, 26 of which were reported as hospitalised. One confirmed case in Croatia and one probable case in Hungary were in patients who died due to or with a *S. Enteritidis* infection.

Information on travel history was available for 112 confirmed cases, of which 27 reported having travelled to one of the following EU countries during the relevant exposure period: Poland (n=11), Hungary (n=4), Greece (n=4), Belgium (n=3), Spain (n=2), Austria (n=1), Cyprus (n=1) and Italy (n=1).

In September and October 2016, a case-control study was performed in the Netherlands with 67 cases and 89 controls interviewed. The study concluded that cases were more likely than controls to have eaten out, but no exposure to specific food items could be established with strong evidence.

A number of food establishments were associated with cases belonging to the WGS cluster 175 and WGS cluster 360. Descriptive epidemiological data collected earlier by the Incident Management Team in the United

Kingdom indicated a link between cases and specific food establishments in Scotland. Case interviews in Belgium and France linked sporadic cases and clusters of cases to two butchers in Belgium. Case interviews in Denmark linked four cases to the same restaurant. Hungary investigated four outbreaks that were later found associated with *S. Enteritidis* MLVA profile 2-9-7-3-2 through MLVA analysis of a selection of isolates. The first outbreak took place in August 2016 in Nógrád County, with 44 associated cases; the second outbreak occurred in September 2016 in Budapest (57 associated cases); the third outbreak was in Békés County (41 associated cases), also in September; the last outbreak was in December 2016 in Csongrád County, with 12 associated cases. As of 2 February 2017, information on interviews performed on cases is available for 127 confirmed or probable cases reported by five countries (Belgium, the Netherlands, Sweden, Slovenia and United Kingdom), with 98 cases (77%) reporting exposure to eggs or to products containing eggs.

Microbiological and environmental investigations of food

This section summarises country-specific information on food and environmental investigations associated with this outbreak which has been reported to RASFF (Rapid Alert System for Food and Feed) and/or directly to EFSA by national authorities (up to 1 March 2017). See Figure 5 below for details on traceability and testing.

Netherlands

The Netherlands Food and Product Safety Authority (NVWA) and the National Institute for Public Health and the Environment (RIVM) investigated a cluster of 19 cases infected with *S. Enteritidis* MLVA profile 2-9-7-3-2 associated with 16 Dutch restaurants. Since *S. Enteritidis* is mostly associated with a chicken reservoir, the main focus of the investigation was on eggs. A trace-back investigation revealed that the 16 restaurants received contaminated eggs from 11 Dutch wholesalers (Figure 5) who had obtained the eggs from a Dutch packing centre (packing centre A). Packing centre A distributed eggs originating from three Dutch farms that tested negative for *S. Enteritidis* and one Polish packing centre (packing centre B). The *Salmonella* status of the Polish packing centre B is reported below (section on Poland).

At packing centre A, the NVWA sampled 5 000 eggs that originated from packing centre B and were intended for direct human consumption (class A). The eggs, divided in eggshells and egg contents, were analysed in pools of 10, resulting in a total of 1 000 pooled samples (500 samples of eggshell and 500 samples from egg content). Of the 70 pools of eggshells that tested positive for *Salmonella* spp., 66 were positive for *S. Enteritidis*. Two pools of egg content were positive for *S. Enteritidis*; in both cases, the pools of the corresponding shells were positive too, indicating possible cross-contamination from the shells.

MLVA typing was performed on the 66 *S. Enteritidis* isolates from egg samples collected at packing centre A: 62 isolates with MLVA profile 2-9-7-3-2, two isolates with MLVA profile 2-10-8-3-2, one isolate with MLVA profile 2-9-6-3-2, and one with MLVA profile 2-10-7-3-2. WGS of 10 isolates showed that three isolates (all MLVA 2-9-7-3-2) belonged to WGS cluster 175 and six isolates (five isolates of MLVA 2-9-7-3-2, one isolate 2-9-6-3-2) to WGS cluster 360, linking them to the human outbreak cases. One isolate of MLVA 2-10-8-3-2 did not belong to either WGS cluster 175 or WGS cluster 360. Consequently, at that time, the NVWA deemed all eggs with a common origin unsafe for human consumption in accordance with Article 14 of Regulation (EC) 178/2002.

Based on information from tracing-forward investigations provided by the Dutch national authorities, suspected eggs were distributed to approximately 2 200 food business operators in the Netherlands. Suspected eggs and (unpasteurised) egg products were also shipped to the following EU Member States: Belgium, Denmark, France, Germany, Italy, Poland, Sweden, and the United Kingdom. Suspected batches of eggs were also distributed to Angola, Bahrein, Congo, The Gambia, Oman, Sierra Leone, Qatar and the United Arab Emirates.

Belgium

At the end of September 2016, the Federal Agency for the Safety of the Food Chain in Belgium investigated two butchers associated with a cluster of cases. In total, 51 food samples from both implicated butchers were analysed, 20 of which were positive for *S. Enteritidis* MLVA 2-9-7-3-2 profile; food items included minced beef/pork (containing eggs), spaghetti sauce (with minced meat), vole au vent (contains raw eggs), pork chops, and *filet américain* (with minced meat). A WGS analysis was successfully performed on six food isolates with the MLVA profile 2-9-7-3-2 and, among these, four isolates from minced beef/pork meat, spaghetti sauce, vol-au-vent, and pork chop belonged to WGS cluster 175; two isolates from steak tartare and an unspecified meat product belonged to cluster 360.

A trace-back investigation identified two wholesalers (wholesalers F and G) who had sold the eggs to the two butchers. A third wholesaler (wholesaler H) delivered eggs to wholesalers F and G. Tracing showed that the eggs from wholesaler H came indirectly (via another Belgian wholesaler – wholesaler I) from the Polish packing centre B (Figure 5).

Croatia

On 11 October 2016, the central competent authority in Croatia was informed by the public health service about a cluster of *S. Enteritidis* cases. Five members of a family were infected, and a five-year-old child died. The epidemiological investigation of the outbreak showed that the event was household-related and that all members of the family had eaten eggs bought at a chain retailer. During the course of the investigation, the remaining eggs from the household were sampled and the content was analysed: of 22 eggs, two were positive for *S. Enteritidis*. Typing of the *S. Enteritidis* isolates was not performed. A number of samples were taken in the family household, e.g. from food leftovers (cooked rice and peas, eggshells, leftover meat), kitchen surfaces, and kitchen utensils. Some of these samples tested positive for *Salmonella* (i.e. cooked rice and peas, kitchen utensils, and eggshells). One isolate from eggshells was positive for *S. Enteritidis*. No further information on MLVA/WGS was provided.

The investigations identified a specific batch of eggs that came from packing centre B in Poland as the one possibly associated with the cases. The Croatian competent authority sampled eggs from the same batch and the same company at the retail chain where the family had bought the eggs; the samples (including both the eggshell and the egg content) tested negative. Through a trace-back investigation a Croatian wholesaler could be identified (wholesaler J) that had sold the suspected batch of eggs to the retail chain on 3 October; the suspected eggs originated from packing centre B in Poland (Figure 5). At the premises of wholesaler J, 1 070 samples of eggshell and egg content were taken and analysed in pools of 10. Nine pooled samples were positive for *S. Enteritidis*. Isolates were sent to the European Union Reference Laboratory (EURL) for *Salmonella* at RIVM for further typing. Out of nine MLVA 2-9-7-3-2 isolates, five isolates belong to cluster 175 and three to cluster 360. One isolate of MLVA 2-9-7-3-2 did not belong to either WGS cluster 175 or WGS cluster 360.

Norway

In the beginning of October 2016, Norway reported two non-human isolates of *S. Enteritidis* MLVA profile 2-9-7-3-2, which were identified during a routine check at an egg-processing company that produces homogenised liquid egg products. The samples were taken in May and November 2015 from unpasteurised liquid eggs made from class B whole eggs imported to Norway from the Polish packing centre B. A sequence analysis performed in October 2016 showed that the isolates belonged to WGS cluster 360.

In October 2016, Norway received eggs from Polish packing centre C. All eggs from this packing centre were heat-treated.

Poland

The Polish competent authority established that in 2016, eggs from 52 farms from seven Polish regions were supplied to three packing centres (packing centres B, C, and D). Official sampling was carried out in 48 farms (four farms turned out not to keep animals). Eighty-two flocks of laying hens in 18 farms located in three different regions were *S. Enteritidis* positive. According to the available information, most of the *S. Enteritidis*-positive farms, as well as the three packing centres, belonged to the same Polish consortium.

The authorities took a number of samples at packing centre B; the collected swabs taken from surfaces were negative for *S. Enteritidis*.

In addition, the Polish State Sanitary Inspection provided information on five samples that were positive for *S. Enteritidis* (four eggshells and one egg content) from eggs collected at retail locations and originating from three *S. Enteritidis*-positive farms (included in the list of 18 *S. Enteritidis*-positive farms mentioned above) that had delivered eggs to the three packing centres. Information on positive results was immediately provided to the Veterinary Inspection, which is the competent authority responsible for the official control of farms.

Contaminated eggs from farm B – one of the 18 *S. Enteritidis*-positive farms in Poland – were distributed in Hungary through a Polish trader (trader A), where they were associated with a cluster of human cases in August 2016 (for details see section on Hungary). According to the information provided by the Polish authorities, farm B also delivered eggs to packing centre D in 2016. It was established that the eggs associated with the Hungarian human cases were shipped to Hungary without the involvement of any Polish packing centres.

A trace-forward investigation by Polish authorities, based on information provided by the involved Member States, was initiated to identify the distribution of the suspected batches of eggs. Poland could establish that the contaminated eggs were distributed in Poland and exported to the following EU/EEA countries: Belgium, Bulgaria, Croatia, the Czech Republic, Denmark, Germany, Greece, France, Hungary, Ireland, Italy, the Netherlands, Norway, Romania, Slovakia, Sweden and the United Kingdom. Batches of eggs from *S. Enteritidis*-positive farms were also exported to eight non-EU countries: Angola, Djibouti, Gambia, China (Hong Kong), Iraq, Liberia, Oman and the United Arab Emirates.

According to information provided by the Hungarian and Austrian authorities, *S. Enteritidis*-positive eggs sampled at two packing centres in Hungary (packing centre E) and Austria (packing centre G) originated from the same *S. Enteritidis*-positive farm in Poland (farm C). Based on information available in RASFF, farm C was neither added

to the list of *S. Enteritidis*-positive farms nor to the list of 48 farms delivering eggs to the three Polish packing centres provided by Poland. Polish authorities later reported that company checks and official samples taken at farm C were negative for *Salmonella*.

According to information provided by Poland, the breeding farms which provided pullets to the *S. Enteritidis*-positive laying farms were negative for *S. Enteritidis*, based on the results from routine tests performed between January and November 2016 by food business operators and official competent authorities in the framework of the *Salmonella* control programme [2].

Poland provided information on WGS analyses that were performed on 10 non-human isolates from three *S. Enteritidis*-positive laying hen farms that provided eggs to packing centre B. Of seven isolates with MLVA profile 2-9-7-3-2, four isolates belonged to WGS cluster 175, and three isolates belonged to WGS cluster 360. One isolate with MLVA profile 2-10-6-3-2 belonged to cluster 175. The remaining two isolates had MLVA profiles 2-10-8-3-2 and 2-11-8-3-2, which do not belong to either WGS cluster 175 or WGS cluster 360.

According to the WGS results, the above-mentioned new MLVA profiles 2-10-8-3-2 and 2-11-8-3-2 have the following new WGS SNP addresses: 1.2.3.18.455.2440.6301 (MLVA 2-10-8-3-2) and 1.2.3.18.455.469.6307 (MLVA 2-11-8-3-2), which cluster at t25-level with WGS cluster 360. Information on MLVA and WGS analysis performed on the 10 isolates originated from three *S. Enteritidis*-positive Polish farms of laying hens is summarised in Table 3. WGS analysis on isolates from all the remaining positive farms is planned.

Table 3. MLVA/WGS information on 10 isolates originating from three *S. Enteritidis*-positive laying hen farms in Poland

Origin of the isolate(s)	Number of isolates	MLVA profiles	WGS SNP addresses
Laying hen farm (faeces or dust)	4	2-9-7-3-2	1.2.3.175.175.175.%
Laying hen farm (faeces)	3	2-9-7-3-2	1.2.3.18.359.360.%
Laying hen farm (faeces)	1	2-10-6-3-2	1.2.3.175.175.175.%
Laying hen farm (faeces)	1	2-10-8-3-2	1.2.3.18.455.2440.6301
Laying hen farm (faeces)	1	2-11-8-3-2	1.2.3.18.455.469.6307

Note: Three isolates MLVA 2-9-7-3-2 belonging to WGS cluster 175 were collected from one single S. Enteritidis-positive laying hen farm, where the isolates 2-10-6-3-2 and 2-11-8-3-2 also originated from. Four isolates MLVA 2-9-7-3-2 (three belonging to WGS cluster 360 and one belonging to WGS cluster 175) were collected from a second S. Enteritidis-positive farm. The isolate MLVA 2-10-8-3-2 originated from a third farm.

Restrictive measures have been applied by the Polish Competent Authority since 20 October 2016. The restrictive measures taken consisted of banning the placing on the market of table eggs originating from the positive farms and from the concerned Polish packing centres as a precautionary measure until comprehensive monitoring could demonstrate the absence of *Salmonella*. All the specific requirements concerning flocks of laying hens as laid down in Regulation 2160/2003 were applied as soon as a positive flock was identified by the Polish authorities. According to the aforementioned Regulation the ban did not refer to eggs used in the production of heat-treated products.

New pullets were introduced after the culling of *Salmonella*-positive flocks and the cleaning and disinfection of the poultry house, all under the supervision of the Polish authorities.

United Kingdom

An incident management team led by Health Protection Scotland (with members from Public Health England, Food Standards Scotland and the Food Standards Agency) was convened in the United Kingdom to investigate a national outbreak of *S. Enteritidis* PT 8 MLVA 2-9-7-3-2 (WGS clusters 175 and 360) between July 2015 and February 2016. As part of that investigation, interviews of cases in Scotland identified that the majority of cases (18/24, 75%) reported eating out in the week before onset of symptoms. This included two small restaurant clusters (seven cases in total). An investigation of these two restaurant clusters by local environmental health officers identified the potential for cross contamination within the restaurants, particularly in relation to the practice of batching eggs for use in various dishes. Interviews of cases in England and Wales did not identify any common exposures or exposure settings, so no targeted egg supply investigations were carried out in England and Wales.

Egg supply chain investigations carried out at 12 of the restaurants in Scotland where cases had reported eating showed that these restaurants bought their eggs from multiple food business operators and, as such, it was difficult to identify a specific batch that may have caused the infections. However, for nine of the 12 restaurants, the investigations did identify a common wholesaler (wholesaler K) based in Scotland which was sourcing eggs from multiple suppliers, including eggs from packing centre B in Poland. Approximately 1 000 eggs from wholesaler K in Scotland were sampled. These related to six different suppliers, and all tested negative for *Salmonella*. An investigation of poultry supply chains for restaurants did not identify a common supply chain.

Since July 2016, there have been four small restaurant clusters (10 cases in total) of *S. Enteritidis* PT8 MLVA 2-9-7-3-2 or 2-9-6-3-2 reported in Scotland. Food Standards Scotland investigated the egg supply chains of these restaurants.

The United Kingdom has acted on traceability information provided by competent authorities in other Member States to ensure that any implicated table eggs were traced in the distribution chain, withdrawn from the UK market and disposed of as necessary, or had undergone further heat treatment.

Hungary

Official investigations were carried out in Hungary to investigate the potential source of infection for two clusters of *S. Enteritidis* human cases in August and September 2016; both clusters were traced back to two different Polish laying hen farms (farms A and B).

The cluster of human cases that occurred in August 2016 was associated with the consumption of a meal prepared in a summer holiday resort. The eggs for the meal came from packing centre B in Poland, which delivered eggs via a Polish trader (trader A) to a Hungarian packing centre (packing centre E). According to the information provided by Poland, packing centre B received the eggs from another packing centre (packing centre F) via an intermediary; packing centre F received the eggs directly from laying hen farm A (Figure 5).

The Hungarian authorities sampled three different types of food containing eggs at the summer holiday resort that subsequently tested positive for *S. Enteritidis*. These strains and the human isolates were identified as MLVA profile 2-9-7-3-2. WGS is ongoing. Based on information provided by Poland, farm A was negative for *S. Enteritidis* (official testing performed by food business operators and competent authorities in the framework of the *Salmonella* control programme).

The cluster of human cases that occurred in September 2016 was associated with the consumption of eggs in a restaurant. The consumed eggs originated from farm B in Poland, which delivered eggs to trader A, who directly supplied the Hungarian packing centre E. Farm B was confirmed as *Salmonella*-positive after official testing and added to the list of *S. Enteritidis*-positive farms in Poland (see also Figure 5). A laboratory analysis performed by the Hungarian authorities on different samples collected in the kitchen of the restaurant identified MLVA profile 2-9-7-3-2 in *S. Enteritidis* isolates, i.e. from an egg packaging tray with broken eggs from Poland and feathers, milk rice (rice pudding), and the surface of a plastic bowl. Based on WGS analysis performed by EURL-*Salmonella*, the isolates do not belong to either WGS cluster 175 or 360.

The Hungarian competent authority reported that an official sample was taken in the Hungarian packing centre E from an egg consignment originating from Polish farm C. *S. Enteritidis* was detected in a pooled sample of eggshells (15 pieces) with MLVA profile 3-10-5-4-1. WGS analyses are ongoing. According to information provided by Poland, both own checks and official samples taken at farm C were negative. Farm C was neither added to the list of *S. Enteritidis*-positive farms nor to the list of 48 farms delivering eggs to the three Polish packing centres provided by Poland. Farm C delivered the eggs to the Hungarian packing centre E via Polish trader A.

The Hungarian authorities ordered the recall and withdrawal of the concerned eggs from the market and ordered an immediate rigorous cleansing and disinfection of the Hungarian packing centre E. The withdrawn quantities were destroyed. The Hungarian authorities reported that packing centre E did not ship contaminated eggs from the implicated Polish farms to other countries.

Austria

As a result of an official control carried out in an Austrian packing centre (packing centre G), *S. Enteritidis* was detected from eggs originating from the Polish farm C. Information on MLVA typing was only available for two isolates from eggshells sampled at packing centre G: both were both MLVA profile 3-10-5-4-1. WGS analysis was successfully carried out to only one strain, which had a different WGS genotype than the ones associated with this outbreak. According to information provided by Poland, both own checks and official samples taken at farm C were negative.

France

The French authorities provided information about a cluster of human cases in the north of France that had an epidemiological link with a butcher in Belgium. The *S. Enteritidis* MLVA profile 2-9-7-3-2 was identified from food samples that originated from a Belgian butcher and were collected in the homes of patients. WGS analysis is ongoing.

Analyses performed by the European Union Reference Laboratory for *Salmonella*

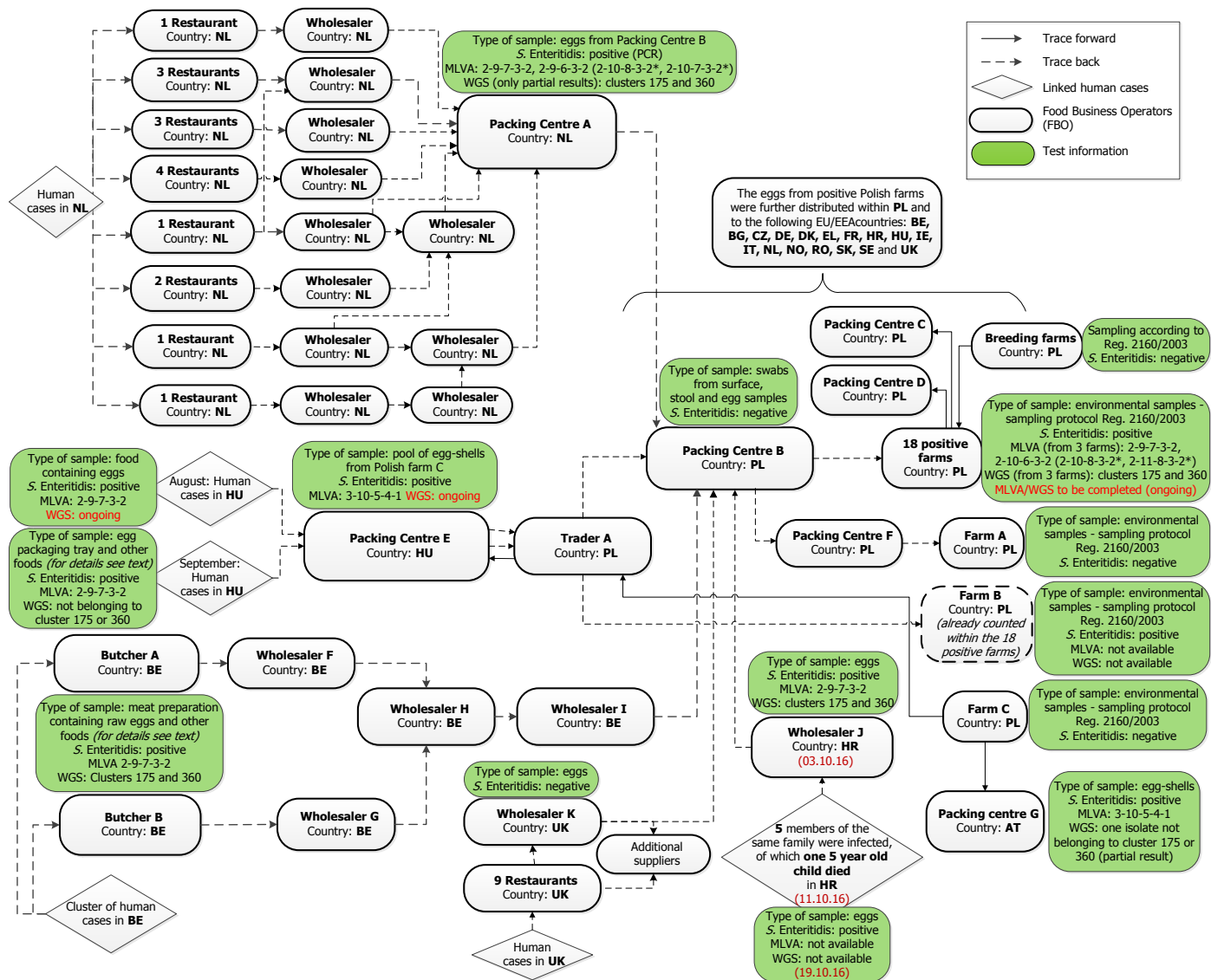
After a request from national RASFF focal points, backed up by information on the findings of national investigations, the European Union Reference Laboratory (EURL) for *Salmonella* received 63 isolates of *S. Enteritidis* for MLVA and/or WGS analysis results from 10 EU Member States: Austria (2 isolates), Belgium (7 isolates), Croatia (9 isolates), the Czech Republic (6 isolates), Denmark (9 isolates), France (2 isolates), Greece (5 isolates), Hungary (3 isolates), the Netherlands (10 isolates) and Poland (10 isolates). Results on the isolates from Austria, Belgium, Croatia, Hungary, the Netherlands and Poland are presented above.

An MLVA analysis of the six isolates provided by the Czech Republic, the two isolates from France and the five isolates from Greece showed no match with the MLVA profiles of the outbreak so that no further WGS analysis was carried out. Of the six Czech *S. Enteritidis* isolates from poultry meat products originating from Poland, the following MLVA profiles were identified: 2-12-7-3-2 (1 isolate), 3-9-5-4-1 (2 isolates), 3-10-5-4-1 (1 isolate), 3-11-1-4-1 (1 isolate) and 3-11-5-4-1 (1 isolate). Out of the two French *S. Enteritidis* isolates originating from poultry meat, one isolate was MLVA profile 3-9-5-4-1 and one isolate was MLVA profile 3-11-5-4-1. Out of the five Greek *S. Enteritidis* isolates originating from a *S. Enteritidis*-positive laying hen flock in Greece, one isolate was MLVA profile 2-10-7-3-2, and four isolates were MLVA profile 3-9-5-4-1.

Out of the nine Danish *S. Enteritidis* isolates from chicken meat originating from Poland, one isolate was MLVA profile 2-9-7-3-2, three isolates were MLVA profile 2-10-7-3-2, and two isolates were MLVA profile 2-10-6-3-2; three isolates were without an identified MLVA profile. The nine isolates from Denmark were sequenced, and the WGS genotypes were different from the ones associated with this outbreak.

To ensure compatibility with the WGS analysis of the isolates from humans, most of the data from anonymised non-human isolates were also analysed at Public Health England.

Figure 5. Graphical representation of traceability and testing information available in RASFF or provided by Member States to EFSA, as of 1 March 2017



Source: RASFF or Member States (as reported to EFSA), as of 1 March 2017

(*) MLVA profiles indicated between brackets and marked with * are different from WGS cluster 175 and WGS cluster 360.

Almost all information from the previously published version of this graph (see Figure 5 in the previous version of this rapid outbreak assessment) was kept in the updated graph above. Due to the large amount of information, some information presented in the previously published version of Figure 5 could not be included in the updated version above (e.g. information related to Norway, *S. Enteritidis*-negative farms in the Netherlands). This information is, however, included in the text above.

This risk assessment uses the same letters used to distinguish farms, packing centres, wholesalers and restaurants as the one published on 27 October 2016. The only exception is the Netherlands, where, due to the increase in the number of the of restaurants and wholesalers involved in the trace-back investigation, it was considered appropriate to remove the letters previously used to identify the Dutch restaurants and wholesalers.

According to information provided by the French authorities, a cluster of human cases in France was also associated with a butcher in Belgium.

ECDC and EFSA threat assessment for the EU

A multi-country outbreak of *S. Enteritidis* associated with contaminated eggs from Poland was confirmed by epidemiological, microbiological and WGS analysis in 14 EU/EEA countries. The outbreak, first detected in January 2016, is still ongoing, with the most recent case reported by Norway on 2 February 2017 (date of sampling).

Based on the existing EU outbreak case definition, the human isolates belong to two distinct genetic clusters. Following PHE nomenclature, the clusters were given the designations 1.2.3.175.175.175.% (WGS cluster 175) and 1.2.3.18.359.360.% (WGS cluster 360).

The first isolates belonging to one of the WGS clusters associated with this outbreak were identified in 2012 in the United Kingdom. The MLVA profiles associated with these two genetic clusters and denoting probable cases are 2-9-7-3-2 and 2-9-6-3-2. The verification of this outbreak was published on 27 October 2016, along with some early investigation results [1].

The number of confirmed and probable cases increased steadily between May 2016 and the last week of September 2016, when the outbreak reached its peak with 59 new reported cases. During the subsequent months the number of newly reported cases steadily decreased to between zero and two reported cases per week in January and February 2017. The decline in the number of reported cases in recent weeks is encouraging; however, due to reporting delay and the limited information currently available from the Polish investigations (MLVA/WGS analyses from affected farms not complete), it is expected that additional cases associated with this outbreak will continue to be reported.

New cases with a date of onset or sampling between December 2016 and February 2017 and no or unknown travel history were reported by Belgium, Hungary, the Netherlands, Norway and the United Kingdom (England and Northern Ireland). Two additional confirmed cases were reported during the same time, both with a travel history to Poland.

The food safety authorities in Belgium, Croatia, Hungary, the Netherlands, Poland and the United Kingdom carried out extensive environmental and food investigations to identify the source of this outbreak. These investigations showed that a number of food establishments (the Netherlands, Hungary and the United Kingdom), food shops (Belgium), a summer resort (Hungary) and two retail chains (one in Croatia and one in Poland) received eggs from contaminated laying hen farms in Poland. According to the available information, most of the *S. Enteritidis*-positive farms, as well as the three implicated Polish packing and distribution centres, belong to the same consortium and are therefore considered to be interlinked.

Ten *S. Enteritidis* isolates from three of 18 *Salmonella*-positive farms in Poland were characterised further. Of the seven isolates with MLVA profile 2-9-7-3-2, four isolates belong to WGS cluster 175, and three isolates belong to WGS cluster 360. Three new MLVA profiles were identified: 2-10-6-3-2, 2-10-8-3-2, and 2-11-8-3-2. WGS analysis confirmed that the MLVA 2-10-6-3-2 isolate belongs to WGS cluster 175, while the other two isolates had different SNP addresses (MLVA 2-10-8-3-2: SNP 1.2.3.18.455.2440.6301; MLVA 2-11-8-3-2: SNP 1.2.3.18.455.469.6307). The two new SNP addresses cluster at t25 level with the WGS cluster 360. The two new SNP addresses suggest that there are potentially more strains linked to this outbreak than those considered in the current case definition.

Based on the current evidence, the source of infection in this outbreak is likely to be at the level of the laying hen farms. However, the possibility that *S. Enteritidis* might have been introduced at a higher level in the food chain cannot be excluded, and further investigation is needed.

The new MLVA profiles 2-10-6-3-2, 2-10-8-3-2 and 2-11-8-3-2 cluster with a number of human isolates reported in TESSy from 2010 to 2016. Furthermore, four human isolates reported in 2015 in the United Kingdom cluster at t5 level with the following new WGS SNP addresses: one with 1.2.3.18.455.2440.% and three with 1.2.3.18.455.469.%. The match between human and non-human isolates from the new strains should be explored further, as it is likely that more human cases infected with the new strains are part of this outbreak.

To date, only cases from countries performing WGS or MLVA typing of *S. Enteritidis* isolates have been identified. Since typing is only done in a small number of countries, it is likely that more countries and substantially more cases are part of this outbreak. Based on information from the WGS investigation, it is also likely that this multi-country outbreak has existed in the EU/EEA since at least 2012.

The detection of a completely different MLVA profile 3-10-5-4-1 in *S. Enteritidis* isolates originating from Polish eggs sampled in two packing centres in Hungary and Austria is likely not to be associated with this outbreak. Indeed, these eggs originated from a Polish farm that is not included in the list of *Salmonella*-positive farms provided by Poland; the farm is also not on the list of farms that delivered eggs to the three implicated Polish packing centres. According to the preliminary WGS result reported by Austria, one MLVA 3-10-5-4-1 isolate had a different WGS genotype than the ones associated with this outbreak.

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Annex 1

Table 1. Reported isolations of *Salmonella* Enteritidis from food, animals and feed, EU Member States and other reporting countries, 2004–2015

Source	No. of positive units, 2004–2010	No. of positive units in 2011	No. of positive units in 2012	No. of positive units in 2013	No. of positive units in 2014	No. of positive units in 2015	Total no. of positive units, 2004–2015
Food							
Eggs ^(a)	765	25	102	22	23	22	959
Egg products ^(b)	94	7	7	4	3	1	116
Meat from broilers	1 451	107	298	499	551	136	3 042
Meat from spent hens	0			28	40	37	105
Meat from turkey	41		14	7	8	5	75
Meat from other poultry ^(c)	739	84	37	7	4	18	889
Meat from pig	196	21	22	17	11	10	277
Meat from bovine animals	125	2	4	18	13	2	164
Meat from other animal species ^(d)	109	16	22	17	18	7	189
Fish and fishery products ^(e)	31	9	1	2		1	44
Dairy products (including cheeses) and milk	20	1	1	5	3	3	33
Fruits and vegetables ^(f)	22	2	1		4	8	37
Other food ^(g)	324	26	15	11	15	9	400
Total isolations in food	3 917	300	524	637	693	259	6 330
Animals							
<i>Gallus gallus</i> (fowl)	1 8728	1 381	1 533	902	954	1 237	24 735
Ducks	284	8	39	32	24	33	420
Geese	296	23	60	63	27	27	496
Turkeys	243	34	16	21	15	43	372
Other poultry or game birds ^(h)	375	5	96	16	141	6	639
Other birds ⁽ⁱ⁾	59	7	4	3	2	4	79
Cattle	792	23	62	60	148	170	1255
Other ruminants ^(l)	14	3	4	8		3	32
Pigs and wild boars	283	18	17	34	20	19	391
Domestic solipeds	24	8	4	5	6	19	66
Cats and dogs ^(m)	63	8	20	3	2	10	106
Reptiles	14		8	13	4	1	40
Other animals ⁽ⁿ⁾	180	50	20	120	45	22	437
Total isolations in animals	21 355	1 568	1 883	1 280	1 388	1 594	29 068
Feed							
Compound feedingstuffs for poultry	57	1	1	0	10		69
Feed material of land animal origin	26		3	1	2		32
Pet food	18	2	2		9	3	34
Feed material of oil seed or fruit origin	12	4	1	3		1	21
Feed material of marine animal origin	13			5			18
Feed material of cereal grain origin	12	2	2			1	17
Compound feedingstuffs for pigs	6	1		2			9
Compound feedingstuffs for cattle	7					1	8
Other feedingstuffs/feed material or unspecified ^(o)	33	1	6	0	0	6	46
Total isolations in feed	184	11	15	11	21	12	254
Total isolations (food, animals, feed)	25 456	1 879	2 422	1 928	2 102	1 865	35 652

Note: Information summarised in this table is based on Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC.

Eggs (2010–2015): 959 *S. Enteritidis* isolates distributed as follows: 867 from table eggs, 84 from liquid eggs for egg products, 8 from unspecified eggs. (b) Egg products (2010–2015): 116 *S. Enteritidis* isolates distributed as follows: 89 from unspecified egg products, 17 from liquid egg products, 7 from ready-to-eat egg products, 3 dried egg products. (c) 'Meat from other poultry' includes meat from ducks, geese, other poultry species and meat from unspecified poultry. (d) 'Meat from other animal species' includes 'meat from bovine animals and pig', 'meat from deer (venison)', 'meat from rabbits', 'meat from sheep', 'meat from wild

boars', 'meat from wild game', 'meat from wild game – birds', 'meat from wild game – land mammals', 'meat, mixed meat', 'meat, red meat (meat from bovines, pigs, goats, sheep, horses, donkeys, bison and water buffalos)' and 'meat from other animal species or not specified'. (e) 'Fish and fishery products' includes crustaceans, fish, unspecified fishery products, live bivalve molluscs, molluscan shellfish. (f) 'Fruits and vegetables' includes vegetables, 'fruits and vegetables', 'ready-to-eat salads', 'sprouted seeds, and 'spices and herbs'.

(g) Other food includes bakery products, 'cereals and meals', 'confectionery products and pastes', 'frog legs', 'infant formula', 'sauce and dressings', sweets, 'other processed food products and prepared dishes', 'other food of non-animal origin' and 'other food'. (h) 'Other poultry or gamebirds' includes ostriches, other poultry, partridges, pheasants, pigeons, unspecified poultry, quails. (i) 'Other birds' includes canary, parrots, psittacidae and unspecified birds. (l) 'Other ruminants' includes deer, goats, sheep, 'sheep and goats'. (m) 'Cats and dogs' includes cats, dogs and the generic animal category 'pet animals, all'. (n) 'Other animals' includes badgers, bears, chinchillas, falcons, finches, fish, foxes, fur animals, guinea pigs, hedgehogs, land game mammals, leopards, lions, lynx, mice, minks, moose, rabbits, raccoon dogs, rodents, squirrels, water buffalos, wild animals, zoo animals and other animals. (o) 'Other feedingstuffs/feed material or unspecified' includes 'compound feedingstuffs for fish', 'compound feedingstuffs for fur animals', unspecified compound feedingstuffs, 'other feed material' and 'other feedingstuffs'.

Annex 2a

Annex 2a can be found as a separate PDF on the ECDC website at:

http://ecdc.europa.eu/en/publications/Report%20Assets/Annex_2_a_1.2.3.175_060317.nexus.pdf

Single nucleotide polymorphism (SNP)-based phylogenetic trees of *S. Enteritidis* isolates from Belgium, Croatia, Denmark, Finland, France, Hungary, Italy, Luxembourg, the Netherlands, Norway, Poland, Slovenia, Sweden, and the United Kingdom. SNP address 1.2.3.175.% (N=249)

Outbreak type is highlighted blue.

Non-human isolates highlighted in red.

IQA: internal quality assessment

Country name in uppercase: country where sample was taken

Country name in lowercase: country of potential exposure

Source: Public Health England, United Kingdom

Annex 2b

Annex 2b can be found as a separate PDF on the ECDC website at:

http://ecdc.europa.eu/en/publications/Report%20Assets/Annex_2_b_1.2.3.18.359_030317.nexus.pdf

SNP-based phylogenetic trees of *S. Enteritidis* isolates from Belgium, Croatia, Denmark, France, Luxembourg, the Netherlands, Norway, Poland, Slovenia, Sweden and the United Kingdom. SNP address 1.2.3.18.359.% (N=134)

Non-human isolates highlighted in red.

IQA: internal quality assessment

Country name in uppercase: country where sample was taken

Country name in lowercase: country of potential exposure

Source: Public Health England, United Kingdom