JOINT RAPID OUTBREAK ASSESSMENT

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

27 October 2016

## 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 at the EU level to facilitate the coordination of investigation and response measures.

From 1 May 2016 to 12 October 2016, seven EU/EEA countries have reported 112 confirmed cases belonging to two distinct WGS clusters, and 148 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, Denmark, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom. Nine of the confirmed cases are associated with a travel history to Hungary or Poland, both of which countries are also considered to be affected by this outbreak. In addition, Croatia reported a cluster of *S.* Enteritidis cases, including a fatal case, with an epidemiological link to the outbreak. The characterisation of the Croatian isolates is currently ongoing.

The food safety authorities in Belgium, Croatia, the Netherlands and Scotland have carried out extensive environmental and food investigations to identify the source of this outbreak. Since *S.* Enteritidis is mostly associated with chickens, the investigations focused, among other food items, on the tracing of eggs and chicken meat supply chains into food establishments where outbreak cases had reported eating. As of 25 October 2016, these investigations showed that a number of food establishments and at least a retail chain in Croatia received eggs supplies from packing centre B in Poland. Additionally, the cases in the Croatian cluster had consumed eggs originating from packing centre B in Poland. Additional sampling of eggs intended for direct human consumption originating from the implicated Polish packing centre B was performed in the Netherlands in October 2016 yielding positive results for *S.* Enteritidis contamination. Eight non-human *S.* Enteritidis isolates from the abovementioned eggs were found to have MLVA type 2-9-7-3-2 and to belong to the two WGS clusters associated with this outbreak. *S.* Enteritidis isolates from food testing in Norway, sampled in May and November 2015 and identified through a company's own check controls, were also demonstrated to be part of one of the WGS clusters defining the outbreak. The isolates originated from unpasteurised (before processing) liquid eggs, which were made from fresh eggs imported to Norway from the Polish packing centre B.

The available evidence from WGS, food and environmental investigations, as well as from tracing-back investigation of eggs, establishes a link between this multi-country foodborne outbreak and the packing centre B in Poland, pointing at eggs as the most likely vehicle of infection for at least part of the outbreak cases. Additional information from epidemiological, food and WGS investigations might bring further evidence on the possible vehicles and sources of infection associated with this outbreak. The molecular typing of isolates deriving

Suggested citation: European Centre for Disease Prevention and Control and the European Food Safety Authority. Multi-country outbreak of *Salmonella* Enteritidis phage type 8 MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections, 27 October 2016. ECDC: Stockholm; 2016.

from other countries, not yet confirmed to be affected by this outbreak, could help in understanding the actual geographical distribution of the outbreak.

Competent authorities in Poland and in the Member States where eggs from the packing centre B were distributed, have already introduced restrictive measures to withdraw and stop placing implicated eggs on the market and investigations are ongoing to eliminate the source. Due to the large outbreak that is currently ongoing, national authorities, particularly in affected countries, could also consider reminding food establishments and food providers of good practices for safely preparing and handling food at risk of *Salmonella* contamination.

In order to monitor the circulation of the outbreak strains and keep track of the number of affected countries, all EU/EEA countries should consider enhancing their surveillance for *S.* Enteritidis infections acquired within the EU/EEA. It would be of particular value to perform MLVA typing and/or WGS.

To support the investigations in countries, ECDC and the European reference laboratory for *Salmonella* continue offering WGS services for isolates suspected to be linked to the outbreak, particularly if the isolates originate from countries not yet confirmed to be affected by this outbreak. 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, 11 October 2016.

# Public health issue

This document assesses the risk associated with a multi-country outbreak of *Salmonella* Enteritidis PT 8 associated with two MLVA types, 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. That assessment was previously updated on 5 September 2016.

# Consulted experts

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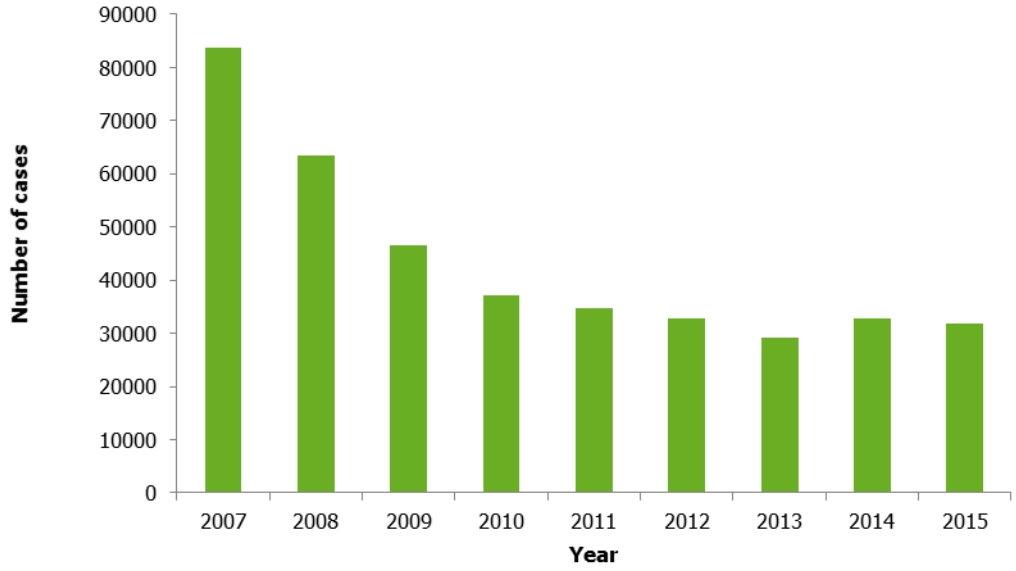
# Disease background information

## *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 at food business operators to improve hygiene implemented under the EU Food law, including the introduction of microbiological criteria for *Salmonella*.

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




*Source: ECDC [1]*

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 3 709 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 with available information were domestically acquired. Cases occurred among all age groups, and the median age was 24 years (IQR=6–51). No gender difference could be observed. Eight countries reported 1 034 cases of *S.* Enteritidis PT8 to TESSY in 2015. Five countries reported 157 cases in the first two quarters of 2016.

## *Salmonella* Enteritidis MLVA types 2-9-7-3-2 and 2-9-6-3-2 in TESSy

Public Health England (PHE) and ECDC validated and published a standardised protocol for a 5-loci *S.* Enteritidis MLVA scheme [3]. EU/EEA-wide data collection for *S.* Enteritidis MLVA data was started in TESSy in June 2016. As of 12 October 2016, five countries have submitted MLVA data for *S.* Enteritidis, including 126 *S.* Enteritidis isolates with the MLVA type 2-9-7-3-2.

* 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

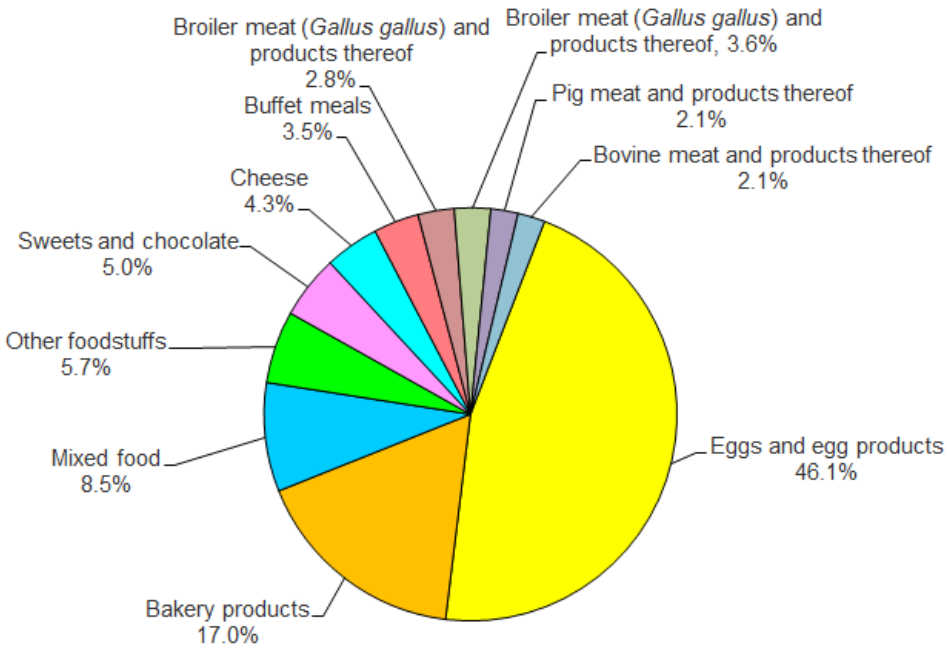
Of these, 119 are part of a multi-country cluster involving Denmark, Luxembourg, the Netherlands, Norway and the United Kingdom, with dates used for statistical purposes to denote time of occurrence ranging from 28 May 2015 to 20 September 2016. The remaining seven are historical isolates from 2012-2014 reported by the Netherlands and Scotland. Additional identified cases with isolates of this MLVA type have also been reported to ECDC through EPIS-FWD. In addition, nine *S.* Enteritidis isolates with MLVA type 2-9-6-3-2, a single locus variant of the original outbreak MLVA profile, have been reported. By WGS, isolates with this single locus variant profile belong to one of the WGS-based clusters included in the case definition. Isolates belonging to MLVA type 2-9-6-3-2 have been reported to TESSy by Norway and Scotland. The dates used for statistical purposes to denote time of occurrence range from 12 March 2015 to 26 August 2016.

# Foodborne outbreaks caused by *Salmonella* Enteritidis

*S.* Enteritidis is the predominant serovar associated with the *Salmonella* outbreaks [4]. Overall in the EU, in 2014, *S.* Enteritidis accounted for 658 outbreaks (62.5% of all *Salmonella* outbreaks) and 6 059 human cases (64.8% of all cases in *Salmonella* outbreaks). For 141 of them, the evidence supporting the implication of the food vehicle was reported to be strong. As in the previous years, most of these strong-evidence *S.* Enteritidis outbreaks reported in 2014 were attributed to eggs and egg products (46.1% of 141 strong-evidence *S.* Enteritidis outbreaks, see Figure 2). In addition, one water-borne strong-evidence outbreak was reported in 2014. In the same year, egg and egg products were implicated in 108 outbreaks (18.2%) out of 594 strong-evidence outbreaks reported at EU level, of which 91.7 % were caused by *Salmonella* spp. [4]. The majority of these outbreaks were associated with *S.* Enteritidis (65.7%), as in previous years. No *S.* Enteritidis food-borne outbreaks were reported by non-EU Member States in 2014.

In 2014, phage type level information was only provided for 124 foodborne outbreaks caused by *S.* Enteritidis, of which 18 outbreaks were due to *S.* Enteritidis PT8 and involved 403 human cases, 81 hospitalisations and 2 deaths. For 8 of the 18 *S.* Enteritidis PT8 outbreaks, the evidence supporting the association with the suspected food vehicle was reported to be strong. The most frequently reported food vehicle in the eight strong-evidence *S.* Enteritidis PT8 foodborne outbreaks was 'mixed food' (four outbreaks), followed by 'eggs and egg product' (two outbreaks), 'broiler meat and product thereof' and 'bakery products' (responsible of one outbreak each).

**Figure 2. Distribution of food vehicles in strong-evidence food-borne outbreaks caused by *Salmonella* Enteritidis in the EU, 2014 (n=141)**




*Source: EFSA and ECDC, 2015.*

*Note: Data from 141 food-borne outbreaks included: Austria (n=5), Belgium (n=3), Croatia (n=16), France (n=13), Germany (n=3), Hungary (n=4), Latvia (n=2), Lithuania (n=5), Poland (n=43), Romania (n=3), Slovakia (n=7), Slovenia (n=4), Spain (n=30) and United Kingdom (n=3). This graph does not include one water-borne outbreak caused by S. Enteritidis.*
*Other foodstuffs (n=8) include: other foods (n=5), vegetables and juices and other products thereof (n=1), crustaceans, shellfish, molluscs and products thereof (n=1), and cereal products including rice and seeds/pulses (nuts, almonds) (n=1).*
*Other meat and meat products (n=6) include: meat and meat products (n=2), Pig meat and products thereof (n=2), and other, mixed or unspecified poultry meat and products thereof (n=2). In 2014, no S. Enteritidis food-borne 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.

From 2004 to 2014, the EU/EEA Member States reported a total of 6 071 units positive for *S.* Enteritidis from several food categories (Table 1 in Annex 1), mainly from chicken meat (*Gallus gallus*) (n=2 906), followed by eggs and egg products (n=1 052), meat from other or unspecified poultry species (n=1 009), meat from pigs (n=267) and other types of meat, including bovine meat, mixed and unspecified meat (n=344). *S.* Enteritidis was reported less frequently from additional food categories, such as 'fish and fishery products' (n=43), 'dairy products (including cheese) and milk' (n=30), fruits and vegetables (including 'spices and herbs', 'sprouted seeds') (n=29) and several other foods (n=391).

In the same period, the EU/EEA Member States reported a total of 27 474 units positive for *S.* Enteritidis from several animal species, primarily from chickens (*Gallus gallus*) (n=23 498), followed by cattle (n=1 085), geese (n=469), ducks (n=387), pigs and wild boars (n=372), turkeys (n=329), 'other or unspecified poultry or gamebirds' (n=633). *S.* Enteritidis isolations were less frequently reported from other animals species.

From 2004 to 2014, *S.* Enteritidis was also detected in 242 units of different feed categories: compound feedingstuffs for poultry (n=69), other or unspecified types of feedingstuffs/feed material (n=40), feed material of land animal origin (n=32), pet food (n=31), feed material of oil seed or fruit origin (n=20), marine animal origin (n=18) and cereal grain origin (n=16), compound feedingstuffs for pigs (n=9) and cattle (n=7).

In 2014, 693 units positive for *S.* Enteritidis were reported from food, of which the majority (n=551, 79.5%) was from broiler meat (*Gallus gallus*). *S.* Enteritidis isolations from broiler meat were provided by 26 Member States and one non-Member State, mainly by Poland that reported 74.8% of the total 551 *S.* Enteritidis isolations from broiler meat, followed by the Czech Republic (12.7%), Spain (3.3%) and the other countries (0.0–1.6%). In 2014, isolations of *S.* Enteritidis from table eggs (n=23) and egg products (n=3) were reported by six Member States, mainly Spain, who reported 14 isolations from table eggs and all the three isolations from egg products (of which two isolations were from ready-to-eat egg products). Additional *S.* Enteritidis isolations from table eggs were reported by Germany (n=4), Romania (n=2), the Czech Republic (n=1), Hungary (n=1) and Slovakia (n=1).

The majority of the 2014 *S.* Enteritidis isolations in animals (n=1 388) were obtained from fowl (*Gallus gallus*) (n=954 isolations, representing 68.7% of the total), followed by other poultry species or game birds (including ducks, geese and turkeys) (n=207), cattle (n=148) and pigs (n=20). *S.* Enteritidis isolations were less frequently reported from several additional animal species, including pigs and wild boar, domestic solipeds, reptiles, cats, dogs, and other animals (Table 2 in Annex 1). Out of the 954 *S.* Enteritidis isolations reported from fowls (*Gallus gallus*), 557 isolations were obtained from broiler flocks, 332 isolations from laying hens flocks, 59 isolations from breeding flocks and the remaining 6 *S.* Enteritidis isolations from unspecified fowl (*Gallus gallus*). In 2014, *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=282), Poland (n=118), the Netherlands (n=82), Germany (n=68), Romania (n=51), Hungary (n=49), France (n=47) and Spain (n=46).

In 2014, twenty-one *S.* Enteritidis isolations in feed were reported by six Member States: 10 isolations from compound feedingstuffs for poultry (9 from feed for laying hens and 1 from feed for breeders), 9 isolations from pet food and 2 isolations from feed material of land animal origin. *S.* Enteritidis isolations from feed were mainly reported by Germany (n=8) and Romania (n=6).

Information on the *S.* Enteritidis isolations reported in food, animals and feed from 2004 to 2014 have been summarised in Table 1 in Annex 1.

Phage type level information are usually not reported in the context of the monitoring of 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 indeed only provided by two Member States (Austria and the United Kingdom) in 2013 and by three Member States (Austria, Slovakia and the United Kingdom) in 2014. In 2014, 22 isolations of *S.* Enteritidis PT8 were reported, of which 18 isolations were from fowl (*Gallus gallus*), 2 from domestic solipeds and 2 from pig meat.

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 [5].*

# Event background information

## Past episode

On 18 January 2016, Scotland launched an urgent inquiry in EPIS-FWD, reporting 21 cases of *S.* Enteritidis PT8 that shared an uncommon MLVA profile (2-9-7-3-2). Scotland indicated that the first case dated from late August 2015 and that there was (at the time of the urgent inquiry) a substantial increase compared with previous years. Prior to this outbreak, Scotland had last observed that MLVA profile in August 2014. Three EU countries responded to the urgent inquiry by reporting human cases with the same MLVA profile. 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 identified prior to 2015 in Denmark and Slovenia.

After a WGS analysis of Scottish isolates, England and Wales reported 52 genetically linked isolates identified between June 2014 and 15 January 2016, the majority of which 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, the analysis of 106 isolates detected between February 2012 and April 2015 was as follows:

- Forty-seven isolates shared the t5-level SNP address 1.2.3.175.175.175.% (WGS cluster 1). The 47 matches included 41 isolates from the United Kingdom, three from Sweden, two from the Netherlands and one from Finland.
- Fifty-four isolates shared the t5-level SNP address 1.2.3.18.359.360.% (WGS cluster 2). The 54 matches included 47 isolates from the United Kingdom and seven from the Netherlands.

The countries involved did not report additional cases after the WGS analysis was finalised and the outbreak described. No vehicle or source of the outbreak could be identified, and national and international investigations were discontinued.

## Current episode

On 25 August 2016, the Netherlands launched another urgent inquiry 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 United Kingdom reported recent cases with the same MLVA pattern or associated WGS profiles. ECDC convened an outbreak investigation team, 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 case definition was updated in October 2016 following the identification of three isolates with MLVA type 2-9-6-3-2 belonging to the WGS cluster 2 associated with this outbreak.

* 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 2) should be avoided, as these data derive from two different sources and are not comparable.

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 1) OR 1.2.3.18.359.360.% (WGS cluster 2).

**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 [3].

**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-type 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-type 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 on humans

Between 1 May and 12 October 2016, 112 confirmed and 148 probable cases have been reported by Belgium, Denmark, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom (Table 1). The distribution of reported outbreak cases steadily increased since the first week of May 2016 and peaked in the last week of September (Figure 3). The 112 confirmed cases were part of two genetic clusters associated with this outbreak (Figure 4). Ninety-one isolates belonging to the WGS cluster 1 and 21 isolates belonging to the WGS cluster 2 have been identified between 1 May and 12 October 2016 (Figure 3, Annex 2a and 2b). Based on the PHE WGS analysis pipeline, a 26-50 SNP difference has been identified between the two WGS clusters. Six isolates with MLVA profile 2-9-7-3-2, identified in the same time period, were sequenced and found not to belong to either of the two WGS clusters associated with this outbreak.

**Table 1. Number of isolates by case classification and country, EU/EEA, February 2012 to October 2016 (n=372)**

| Country | Number of isolates | | | | | |
|---|---|---|---|---|---|---|
| | Confirmed cases | | Probable cases | Historical confirmed cases | | Historical probable cases |
| | Cluster 1 | Cluster 2 | | Cluster 1 | Cluster 2 | |
| Belgium | 0 | 0 | 61 | 0 | 0 | 0 |
| Denmark | 0 | 0 | 5 | 0 | 0 | 0 |
| Finland | 0 | 0 | 0 | 1 | 0 | 0 |
| Luxembourg | 1 | 0 | 0 | 0 | 0 | 0 |
| Netherlands | 51 | 6 | 50 | 2 | 13 | 5 |
| Norway | 2 | 0 | 17 | 0 | 0 | 0 |
| Sweden | 0 | 0 | 11 | 2 | 0 | 0 |
| UK (England, Wales and Isle of Man) | 22 | 0 | 0 | 26 | 33 | 0 |
| UK (Scotland) | 15 | 15 | 4 | 14 | 16 | 0 |
| Total | **91** | **21** | **148** | **45** | **62** | **5** |

Most confirmed and probable cases with available information on MLVA type (n=238) have MLVA profile 2-9-7-3-2 (n=219). The 19 isolates with MLVA profile 2-9-6-3-2 had been identified in the Netherlands (n=11), Norway (n=1), Sweden (n=1) and Scotland (n=6). Three of the six Scottish isolates were the only ones confirmed by WGS and were found to belong to WGS cluster 2.

**Figure 3. Distribution of outbreak cases by week and case classification (n=372), EU/EEA, January 2015 to October 2016**

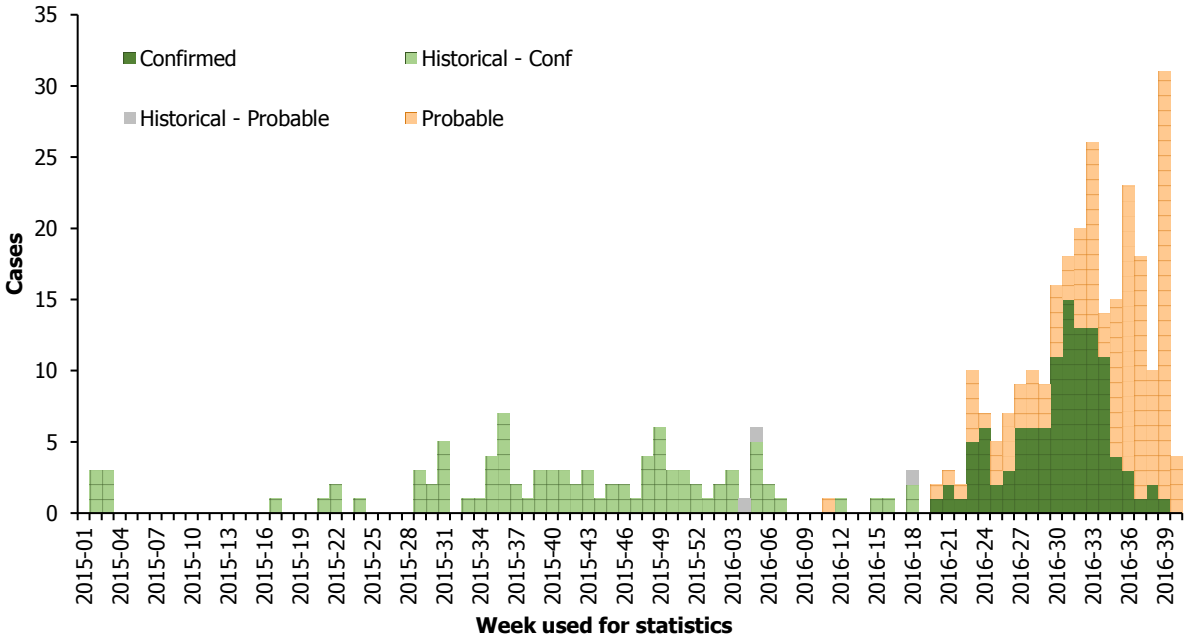
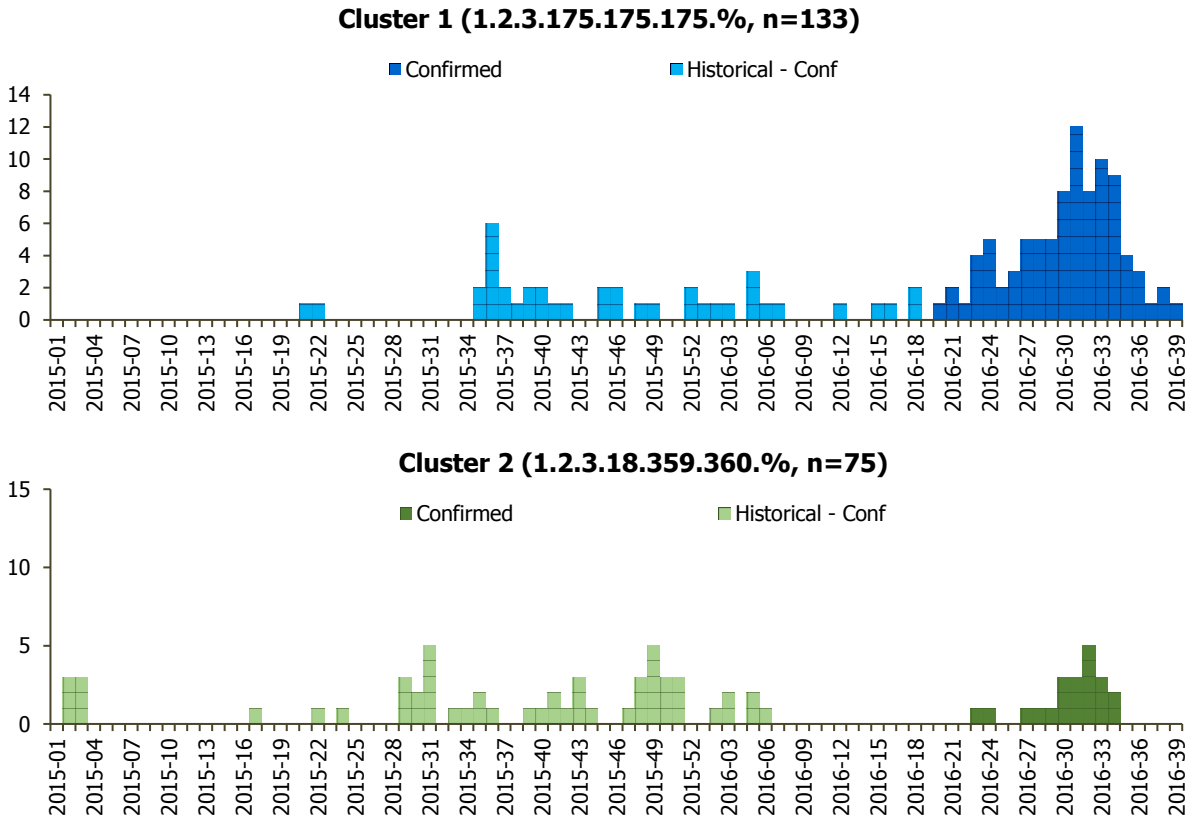


**Figure 4. Distribution of outbreak confirmed cases by week, case classification and genetic cluster (n=208), EU/EEA, January 2015 to October 2016**




All or part of the cases reported by each affected country were interviewed. A case-control study is currently being performed in the Netherlands. So far, 44 cases and 43 controls have been interviewed. A preliminary analysis of the study identified cases more likely than controls to have eaten in a food establishment, but no specific food exposures have been identified yet. A number of food establishments were associated with cases belonging to the WGS cluster 1. Descriptive epidemiological data from the Incident Management Team in the United Kingdom indicated a link between cases and specific food establishments in Scotland. Additional case interviews linked clusters of cases to food establishments in Denmark and to two butchers in Belgium.

Nine confirmed cases were reported as travel-associated: six cases had travel history to Poland, two to Hungary and one is unknown. All isolates from confirmed cases with travel association belonged to WGS cluster 1. Twenty additional probable cases with a history of travel were identified: thirteen travelled to Greece, three to Poland, two to Croatia, one to Austria and one to the Netherlands. All 19 Norwegian cases were travel-related (mostly to Greece or Poland) apart from one possibly domestically acquired case, but this case has not been available for an interview.

In addition to the confirmed and probable cases described above, on 11 October, the Central Competent Authority (CCA) in Croatia was informed by the public health service about a cluster of *S.* Enteritidis cases. Five members of the same family were infected, including a five-year old child who died. The epidemiological investigation of the outbreak showed that the event was related to home-consumed food and that all members of the family had eaten eggs bought in a retail chain.

# Microbiological and environmental investigations on food

Following an analysis of the Rapid Alert System for Food and Feed (RASFF) in September 2016, 16 notifications of non-human isolates of *S.* Enteritidis since 1 May 2016 were identified as reported by the Czech Republic (n=6), Denmark (n=2), Finland (n=1), France (n=2), Italy (n=2), the Netherlands (n=1), and Slovakia (n=2). Fifteen notifications were related to poultry meat products of which 13 were linked to products originating from Poland, one from the Netherlands, and one from the Czech Republic. One notification was related to fruits and vegetables originating from China.

This section summarises by country all the information on food and environmental investigations related to this outbreak that have been reported by Member States to RASFF (up to 25 October 2016), as well as additional information reported by the competent authorities of the interested Member States to EFSA and ECDC in the context of this outbreak. The graphical representation of the traceability information available in RASFF is illustrated in Figure 5.

## Netherlands

The Netherlands Food and Product Safety Authority (NVWA) investigated seven restaurants associated with a cluster of cases which were infected with *S.* Enteritidis MLVA 2-9-7-3-2. Since *S.* Enteritidis is mostly associated with chickens, the investigation focussed, among other food items, on the tracing of chicken meat and eggs. The tracing-back investigation of the eggs carried out by NVWA revealed the link to a possible source of contamination (Figure 5). The eggs were delivered directly or indirectly to the seven restaurants from five Dutch wholesalers (wholesalers A, B, C, D and E). These wholesalers obtained the eggs from a Dutch packing centre (packing centre A). Based on the delivery documents from the Dutch packing centre A to the wholesalers it was determined that the eggs originated from three Dutch farms, one Polish packing centre (packing centre B) and one Polish farm. The *Salmonella* status of the Dutch farms was verified through faecal samples (using socks) as part of the national control program and all farms were negative. The *Salmonella* status of the Polish packing centre B is reported in the Polish section. The *Salmonella* status of the Polish farm is unknown.

The National Institute for Public Health and the Environment (RIVM) started to execute a case-control study. Preliminary results showed that the outbreak was restaurant-related. Various Dutch restaurants were identified at which cases had eaten within one week before the onset of disease. These restaurants were subsequently inspected by the NVWA to obtain information related to the origin of various ingredients of the meals the cases had eaten. Salt, onions and eggs were the ingredients commonly used in meals eaten by cases. Since *S.* Enteritidis is mostly associated with chicken reservoir, the main attention was on eggs. Via this approach, two restaurants (of the seven restaurants mentioned above) were identified that obtained eggs from wholesaler A.

The NVWA had sampled 5 000 eggs intended for direct human consumption (class A), originating from the Polish packing station B, at the Dutch packing centre A. In total, 5 000 eggs were analysed in pools of 10. The egg-shell has been analysed separately from the inside of the eggs, resulting in a total of 1 000 pooled samples (500 samples of egg-shell and 500 samples from egg-content). All analyses by cultural method have been completed. Seventy pools of egg-shells were positive for *Salmonella* spp., of which 66 were positive for *S.* Enteritidis. Two pools of egg-content were positive for *S.* Enteritidis (in both pools, the pools of the corresponding shells were positive too, indicating a possible cross-contamination from the shells). MLVA typing of all *S.* Enteritidis isolates is ongoing. The first typing results indicated that eight out of the nine isolates currently tested have MLVA pattern 2-9-7-3-2. WGS analyses have been performed for the eight isolates with MLVA type 2-9-7-3-2 and results show that three isolates belongs to WGS cluster 1 and five isolates belongs to WGS cluster 2. The remaining isolate has MLVA type 2-10-8-3-2, which is not related to this outbreak.

According to additional information on tracing-forward investigation of the eggs provided by the Netherlands, the eggs were further distributed (directly or indirectly) to the following countries: the Netherlands, Belgium, France, Germany and the United Kingdom.

## Belgium

At the end of September 2016, the Federal Agency for the Safety of the Food Chain (FASFC) in Belgium investigated two butchers associated with a cluster of cases reporting purchasing meat in those locations. In total, 51 food samples were analysed for both implicated butchers, of which 20 were positive for *S.* Enteritidis MLVA 2-9-7-3-2 profile including minced beef/pork (containing eggs), herbs, spaghetti sauce (with minced meat), vole au vent (contains raw eggs), pork chop and filet americain (with minced meat). Seven food isolates with the MLVA 2-9-7-3-2 profile were selected and are currently being sequenced using WGS. Via tracing-back of the eggs, it was found that two different wholesalers (wholesalers F and G) sold the eggs to the two butchers, and that a third wholesaler (wholesaler H) did a common delivery of eggs to the previous two wholesalers (F and G). Traceability of eggs from the wholesaler H indicates that these eggs came indirectly (from another wholesaler I in Belgium) from the Polish packing centre B (Figure 5). Investigation is still going on.

# Croatia

On Tuesday 11 of October, the Central Competent Authority (CCA) in Croatia was informed by the public health service about an infection with *S.* Enteritidis. Five members of the same family were infected, of which one 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 in a retail chain. During the investigation the remaining eggs from the family house were sampled and the content was analysed (without egg-shell): out of 22 eggs, 2 were positive for *S.* Enteritidis. The final typing of *S.* Enteritidis isolates is still in progress. The investigations identified a specific batch of eggs originating from the packing centre B in Poland as the one possibly linked with the cases. The CCA sampled eggs from the same batch and the same company at the retail outlet where the eggs were bought by the family; the samples (including both the egg-shell and the egg-content) tested negative. Via tracing-back of the sampled eggs, a wholesaler was identified in Croatia (wholesaler J) that sold the eggs of the suspected batch to the retail chain on 3 October; the suspected eggs originated from the Polish packing centre B (Figure 5). At the premises of this wholesaler, 1 070 samples of egg-shell and egg-content were taken and the analysis is ongoing. Another two batches of eggs from the same Polish packing centre B are under investigation. In addition, other samples were taken in the house of the family from food leftovers (cooked rice and peas, and leftovers of meat), as well as from kitchen surfaces and utensils and some of them (cooked rice and peas, kitchen utensils) tested *Salmonella* positive.

# Norway

At the beginning of October, Norway reported two non-human isolates of *S.* Enteritidis MLVA type 2-9-7-3-2, identified in a company's own check sampling at a line for the production of homogenised liquid egg, prior to pasteurisation. The sampling took place in May and November 2015 and sequence analysis showed that the isolates belong to WGS cluster 2 (Annex 2a). The tracing investigation showed that the isolates originated from unpasteurised liquid eggs made from industrial fresh whole eggs of class B imported to Norway from the Polish packing centre B.

# Poland

In Poland, an official investigation was carried out at the Polish packing centre B, which was identified as the possible source of the contaminated eggs. The Local Veterinary Inspector (LVI) took swabs from surfaces in the production area of the Polish packing centre B and analyses are still ongoing. The tracing-back investigation showed that the Polish egg packing centre B receives eggs from 10 poultry farms. The LVI in Poland took stool samples at the farms and samples of eggs originating from the farms that provide eggs to the mentioned Polish packing centre B. Analyses are still ongoing. The farms provided information on previous analytical tests performed: samples of faeces, eggs and swabs were collected in the context of the company's own checks in the period June 2015-March 2016. All samples tested *Salmonella* negative. Out of these 10 farms, two were mentioned as the possible source of infection; they tested *Salmonella* negative in the context of the sampling carried out by the operators as part of *Salmonella* national control programmes (Regulation (EU) No 517/2011[*]).

Trace-forward investigation was carried out by competent authorities to identify the distribution of the suspected batch of eggs put on the market, based on information provided by the involved Member States. The General Veterinary Inspector (GVI) informed that the suspected batch was distributed to Croatia, Belgium and the Netherlands.

On 20 October 2016, the Polish Competent Authority issued an administrative decision which banned placing on the market eggs from the Polish packing centre B. This decision was addressed to Polish packing centre B and all farms providing eggs to the packing centre B. This decision didn't refer to eggs used in production of products sufficiently treated (heat treated).

On 24 October, Poland provided information on the following list of countries to which eggs were distributed (directly or indirectly): Belgium, Bulgaria, Croatia, the Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, the Netherlands, Norway, Romania and the United Kingdom.

[*] Commission Regulation (EU) No 517/2011 of 25 May 2011 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain Salmonella serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010 Text with EEA relevance

# Scotland

An Incident Management Team led by Health Protection Scotland and including membership from Food Standards Scotland and the Food Standards Agency (London) was convened in the United Kingdom to investigate a national outbreak of *S.* Enteritidis PT 8 MLVA 2-9-7-3-2 (WGS clusters 1 and 2) that occurred between July 2015 and February 2016. As part of that investigation, interviews of cases in Scotland identified that the majority (18/24, 75%) reported eating out in the week before onset of symptoms. This included two small restaurant clusters (7 cases in total). 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.

Egg supply chain investigations carried out at 12 of the restaurants in Scotland where cases had reported eating showed that these restaurants utilised multiple food business operators to source their eggs 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 originating from the Polish packing centre B. Approximately, 1 000 eggs were sampled from the common wholesaler K based in Scotland. These related to six different suppliers and all tested negative for *Salmonella*. Investigation of poultry supply chains into the restaurants did not identify a common supply chain.

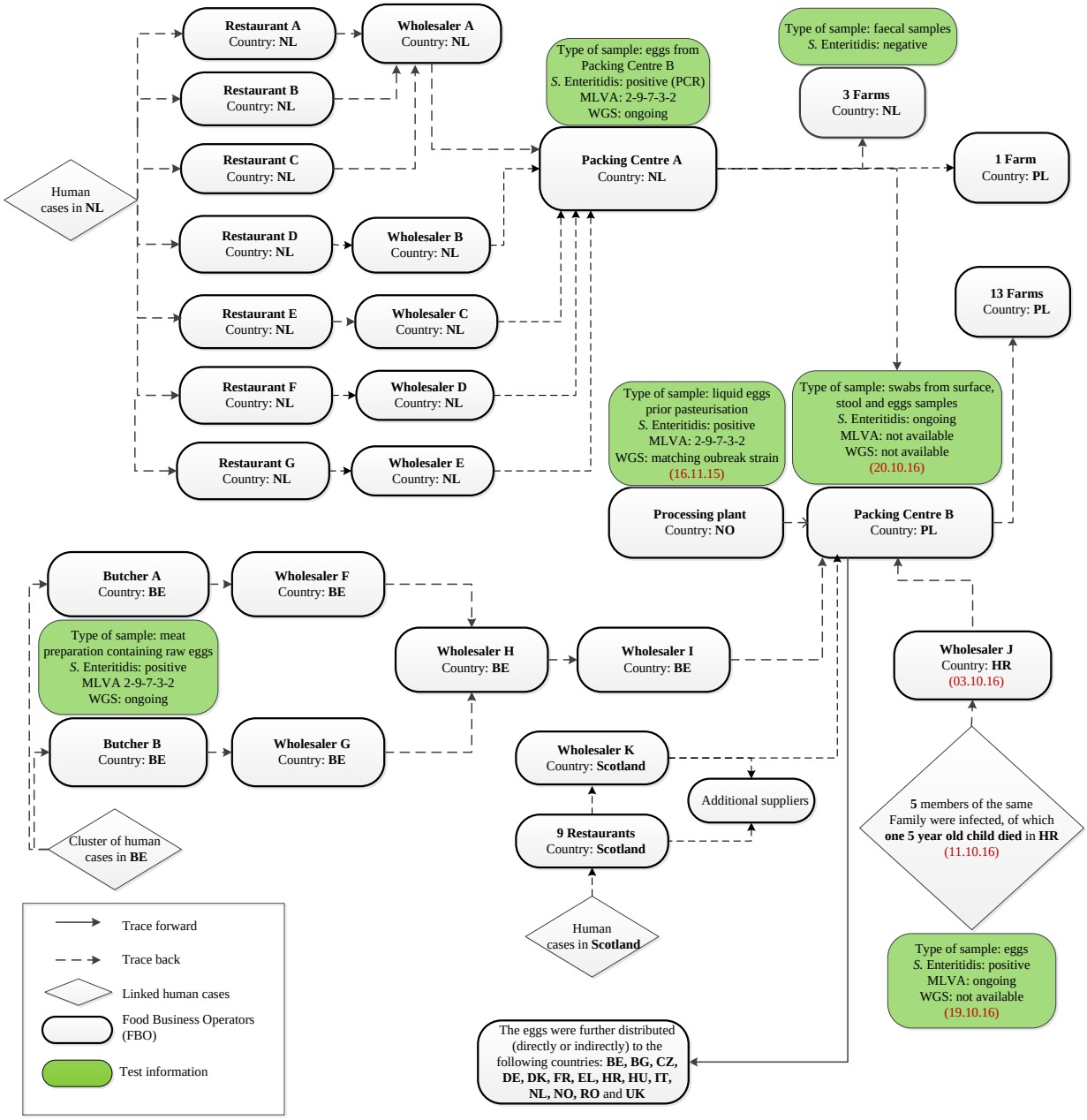
Since July 2016 there have been four small restaurant clusters (10 cases in total) of *S.* Enteritidis PT8 MLVA 2-9-7-3-2 or 2-9-6-3-2 reported in Scotland. Food Standards Scotland is currently investigating egg supply chains into these four restaurants.

# Analyses to be performed by the European Union Reference Laboratory for *Salmonella* on the food isolates

Following the request to national RASFF focal points and the findings of the national investigations, the European Union Reference Laboratory (EURL) for *Salmonella* received six isolates of *S.* Enteritidis from the Czech Republic. MLVA analysis of these six isolates revealed no match with the MLVA profiles of the outbreak so that no further WGS analysis will be done. One non-human isolate of S. Enteritidis MLVA type 2-9-7-3-2, three isolates MLVA type 2-10-7-3-2, two isolates MLVA type 2-10-6-3-2 and four S. Enteritidis without MLVA type identified through this investigation are being sequenced in Denmark. Finally, seven non-human isolates of *S.* Enteritidis MLVA type 2-9-7-3-2 are currently being sequenced in Belgium. Sequencing results will be provided to the EURL for *Salmonella* for analysis.

To ensure compatibility with the WGS analysis of the isolates from humans, the data from anonymised non-human isolates will also be analysed at Public Health England (PHE). Two French *S.* Enteritidis isolates have been sent to EURL *Salmonella* for further analyses.

**Figure 5. Graphical representation of traceability information available in RASFF (25.10.16)**




# ECDC and EFSA threat assessment for the EU

A multi-country outbreak of *S.* Enteritidis has been confirmed by WGS analysis on isolates belonging to MLVA type 2-9-7-3-2 and 2-9-6-3-2. The outbreak is still ongoing with cases reported from seven EU/EEA Member States.

By WGS, the human isolates split into two distinct genetic clusters using PHE nomenclature as 1.2.3.175.175.175.% (cluster 1) and 1.2.3.18.359.360.% (cluster 2). The first isolates belonging to one of the WGS clusters associated with this outbreak were identified in 2012. The number of confirmed and probable cases has increased steadily since May 2016. Taking into account the reporting delay, the outbreak may not have peaked yet. New cases are expected to be reported, particularly in Belgium and the Netherlands where the majority of the cases were identified in the recent weeks. Only 5% of probable cases were found to be unrelated to the two WGS outbreak clusters and excluded through WGS analysis. The majority of the remaining probable cases is therefore likely to be associated with this outbreak. In addition, all EU/EEA countries to which confirmed cases reported travel should be considered as affected by this outbreak (i.e. Poland and Hungary).

Epidemiological investigations in the Netherlands and Scotland linked cases to food establishments. However, in both countries it was not possible to show evidence of an association with specific food items, highlighting the possibility of a contaminated food item used in the preparation of different dishes. The epidemiological investigation in Belgium linked cases to consumption of meat products containing eggs from two butchers.

The food safety authorities in Belgium, Croatia, the Netherlands and Scotland carried out extensive environmental and food investigations to identify the source of this outbreak. Since *S.* Enteritidis is mostly associated with chickens, the investigations focused, among other food items, on the tracing of eggs and chicken meat supply chains into food establishments where outbreak cases had reported eating or buying their food. These investigations showed that a number of food establishments (the Netherlands and Scotland), food shops (Belgium) or a retail chain (Croatia) received eggs supplies from the packing centre B in Poland. In the Netherlands, Belgium and Croatia some of the sampled eggs were found to be positive for *S.* Enteritidis. In the Netherlands, molecular typing results on isolates from eggs intended for direct human consumption sampled in October 2016 indicated that eight out of the nine isolates tested have MLVA pattern 2-9-7-3-2. Based on the WGS analyses, three isolates belong to the WGS cluster 1 and five belong to the WGS cluster 2.

Two food isolates from Norway from May and November 2015, identified through a company's own check controls, were demonstrated to be part of the WGS cluster 2. The isolates originated from unpasteurised liquid eggs made from fresh whole eggs imported to Norway from the Polish packing centre B. The first food isolate sampled in November 2015, shared a high degree of similarity to Dutch and Scottish isolates from 2015, and more recent Scottish isolates. The second food isolate sequenced, sampled in May 2015, was also shown to belong to WGS cluster 2.

The sampling time between the different food isolates belonging to the WGS clusters associated with this outbreak detected in the Netherlands and Norway demonstrates that products contaminated with the outbreak strain from the Packing centre B in Poland have been distributed to different EU countries on multiple occasions in a period of at least seventeen months between May 2015 and October 2016.

To date, only cases from countries performing typing of *S.* Enteritidis isolates have been identified. Since typing is performed in a minority 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 possible that this multi-country outbreak is associated with one or more common sources persisting in the EU/EEA since at least 2012.

Additional non-human *S.* Enteritidis isolates from Belgium, the Czech Republic and Denmark, some with the MLVA profile associated with this outbreak, are being analysed by WGS. Additional environmental investigations are currently ongoing on the origin and contamination of the food products from which the non-human isolates were detected. Additional results from the WGS analysis and the epidemiological and food investigations might bring information on possible additional vehicles, and possibly sources, of infection associated with this outbreak.

Competent authorities in Poland and affected countries where eggs from the packing centre B were distributed already introduced restrictive measures to withdraw and stop placing the implicated eggs on the market and investigations are ongoing to eliminate the source. ECDC, EFSA and countries public health and food safety authorities will continue monitoring the reporting of the outbreak strains in humans and food to assess the impact of these measures.

ECDC and EFSA are liaising with relevant authorities in Member States and at the EU level to facilitate the coordination of investigation and response measures.

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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, 2004–2014**

| 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 | Total no. of positive units 2004-2014 |
|---|---|---|---|---|---|---|
| **Food** | | | | | | |
| Eggs[a] | 765 | 25 | 102 | 22 | 23 | 937 |
| Egg products[b] | 94 | 7 | 7 | 4 | 3 | 115 |
| Meat from broilers | 1 451 | 107 | 298 | 499 | 551 | 2 906 |
| Meat from spent hens | 0 | | | 28 | 40 | 68 |
| Meat from turkey | 41 | | 14 | 7 | 8 | 70 |
| Meat from other poultry[c] | 739 | 84 | 37 | 7 | 4 | 871 |
| Meat from pig | 196 | 21 | 22 | 17 | 11 | 267 |
| Meat from bovine animals | 125 | 2 | 4 | 18 | 13 | 162 |
| Meat from other animal species[d] | 109 | 16 | 22 | 17 | 18 | 182 |
| Fish and fishery products[e] | 31 | 9 | 1 | 2 | | 43 |
| Dairy products (including cheeses) and milk | 20 | 1 | 1 | 5 | 3 | 30 |
| Fruits and vegetables[f] | 22 | 2 | 1 | | 4 | 29 |
| Other food[g] | 324 | 26 | 15 | 11 | 15 | 391 |
| **Total isolations in food** | **3 917** | **300** | **524** | **637** | **693** | **6 071** |
| **Animals** | | | | | | |
| Gallus gallus (fowl) | 1 8728 | 1 381 | 1 533 | 902 | 954 | 23 498 |
| Ducks | 284 | 8 | 39 | 32 | 24 | 387 |
| Geese | 296 | 23 | 60 | 63 | 27 | 469 |
| Turkeys | 243 | 34 | 16 | 21 | 15 | 329 |
| Other poultry or gamebirds[h] | 375 | 5 | 96 | 16 | 141 | 633 |
| Other birds[i] | 59 | 7 | 4 | 3 | 2 | 75 |
| Cattle | 792 | 23 | 62 | 60 | 148 | 1 085 |
| Other ruminants[l] | 14 | 3 | 4 | 8 | | 29 |
| Pigs and wild boars | 283 | 18 | 17 | 34 | 20 | 372 |
| Domestic solipeds | 24 | 8 | 4 | 5 | 6 | 47 |
| Cats and dogs[m] | 63 | 8 | 20 | 3 | 2 | 96 |
| Reptiles | 14 | | 8 | 13 | 4 | 39 |
| Other animals[n] | 180 | 50 | 20 | 120 | 45 | 415 |
| **Total isolations in animals** | **21 355** | **1 568** | **1 883** | **1 280** | **1 388** | **27 474** |
| **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 | 31 |
| Feed material of oil seed or fruit origin | 12 | 4 | 1 | 3 | | 20 |
| Feed material of marine animal origin | 13 | | | 5 | | 18 |
| Feed material of cereal grain origin | 12 | 2 | 2 | | | 16 |
| Compound feedingstuffs for pigs | 6 | 1 | | 2 | | 9 |
| Compound feedingstuffs for cattle | 7 | | | | | 7 |
| Other feedingstuffs/feed material or unspecified[o] | 33 | 1 | 6 | 0 | 0 | 40 |
| **Total isolations in feed** | **184** | **11** | **15** | **11** | **21** | **242** |
| **Total isolations (food, animals, feed)** | **25 456** | **1 879** | **2 422** | **1 928** | **2 102** | **33 787** |

*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 (2010-2014): 937 S. Enteritidis isolates distributed as follows: 846 from table eggs, 84 from liquid eggs for egg products, 7 from unspecified eggs. (b) Egg products (2010-2014): 115 S. Enteritidis isolates distributed as follows: 89 from unspecified egg products, 16 from liquid egg products, 7 from ready-to-eat egg products, 3 dried egg products. (c) 'Meat from other poultry' includes: meat from duck, geese, other poultry species and meat from poultry unspecified. (d) 'Meat from other animal species' includes: 'meat from bovine animals and pig', 'meat from deer (venison)', 'meat from rabbit', 'meat from sheep', 'meat from wild boar', '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' include: crustaceans, fish, unspecified fishery products, live bivalve molluscs, molluscan shellfish. (f) 'Fruits and vegetables' include: 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', 'frogs leg', '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' include: ostriches, other poultry, partridges, pheasants, pigeons, unspecified poultry, quails. (i) 'Other birds' include: canary, parrots, psittacidae and unspecified birds. (l) 'Other ruminants' include: deer, goats, sheep, 'sheep and goats'. (m) 'Cats and dogs' include: cats, dogs and the generic animal category 'pet animals, all'. (n) 'Other animals' include: badgers, bears, chinchillas, falcons, finches, fish, foxes, fur animals, guinea pigs, hedgehogs, land game mammals, leopards, lion, lynx, mice, minks, moose, rabbits, raccoon dogs, rodents, squirrels, water buffalos, wind animals, zoo animals and other animals. (o) 'Other feedingstuffs/feed material or unspecified' include: 'compound feedingstuffs for fish', 'compound feedingstuffs for fur animal', unspecified compound feedingstuffs', 'other feed material' and 'other feedingstuffs'.*

# Annex 2a

Annex 2a can be found as a separate PDF accompanying the rapid outbreak assessment at the ECDC website http://ecdc.europa.eu/en/publications/risk_assessment/Pages/default.aspx

Single Nucleotide Polymorphism (SNP)-based phylogenetic trees of *S.* Enteritidis isolates from Denmark, Belgium, Finland, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom. SNP address 1.2.3.175.%.

*Outbreak type is highlighted red.*
*Non-human isolates presented in red font.*
*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*

# Annex 2b

Annex 2b can be found as a separate PDF accompanying the rapid outbreak assessment at the ECDC website http://ecdc.europa.eu/en/publications/risk_assessment/Pages/default.aspx

SNP-based phylogenetic trees of *S.* Enteritidis isolates from Belgium, the Netherlands, Norway, Sweden and the United Kingdom. SNP address 1.2.3.18.360.%.

*Outbreak type is highlighted red.*
*Non-human isolates presented in red font.*
*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*