RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of Shiga toxin-producing *Escherichia coli* infection associated with haemolytic uraemic syndrome

5 April 2016

Main conclusions and options for response

A multi-country outbreak of Shiga toxin-producing Escherichia coli (STEC) infection associated with haemolytic uraemic syndrome (HUS) and affecting mostly young children has been reported in the last two months in Romania. Italy reported one related HUS case through the Early Warning and Response System (EWRS) on 21 March 2016. Overall, 25 cases were identified as associated with this outbreak, of which 19 developed HUS and three died. Twelve cases had microbiological and/or serological evidence of STEC O26 infection; 13 additional cases met the probable case definition by developing HUS, testing positive for another STEC O serogroup (O157) or by testing positive by PCR for *stx1* and/or *stx2* and *eae* genes.

Information collected from patients pointed towards a milk processing establishment (the Romanian operator) as a possible source of infection. The implicated milk processing establishment exported a particular type of cheese to four EU countries (Belgium, Germany, Italy and Spain) and one Italian importer further distributed this product to France and Germany. In Romania, one fresh cheese product made of cow's milk produced by the Romanian operator tested positive for *E. coli* O26 not possessing *stx* virulence genes. Other dairy products produced by the Romanian operator tested positive for *E. coli* virulence genes *stx*1, *stx*2 and *eae*. In Italy, a STEC O26 strain (positive for *stx1*, *stx2* and *eae*) was isolated from a sample of the cheese that had been produced by the Romanian operator, imported from Romania and consumed by the Italian child that later developed HUS (stool samples negative for STEC but serum samples positive for the presence of antibodies against the LPS of *E.coli* O26).

The PFGE analysis suggests the potential involvement of multiple strains from a common source or from multiple sources. Multi-strain STEC outbreaks have been reported in the past [1]. Therefore, it cannot be excluded that the cases belong to a single outbreak associated with a source contaminated by different strains. The epidemiological evidence linking some of the Romanian cases and the Italian case to the Romanian operator, in addition to the microbiological findings, are consistent with the hypothesis of a multi-strain outbreak.

The last Romanian case associated with this outbreak had onset of symptoms on 14 March. The cheese consumed by the Italian case was imported in Italy from Romania on 1 March and had been produced on 18 February 2016 with an expiry date of 18 April 2016. A recall of the company products was undertaken in Romania as of 5 March and in Italy as of 15 March. Other Member States subsequently undertook recalls. According to information available at the time of conducting this assessment, the withdrawal of potentially contaminated dairy products following a recall has been limited in Member States, with the exception of Romania. It is therefore possible that affected products are still present in households, and detection of new cases cannot be excluded.

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In order to minimise the spread of the infection and investigate possible new cases in a timely manner, Romania, Italy and other Member States that are possibly involved could consider enhancing surveillance for HUS and STEC cases. Continued enhanced surveillance for HUS in children on a routine basis in Romania could provide further early warning of ongoing or new contamination events. The questionnaire used to interview some of the Romanian cases and the Italian case is available in English from ECDC upon request.

If new cases are identified, these should be reported to the Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD). In such situations public health authorities could also consider conducting an epidemiological analytical study and including further food and environmental sampling in suspect premises to identify the vehicle of infection.

Whole genome sequencing (WGS) on isolates detected so far should be carried out to provide more detailed information about possible links between cases.

The investigation of the European dimension of this cross-border foodborne outbreak has demonstrated the added value of collaboration between Romanian and Italian public health and food authorities as well as ECDC and EFSA in enabling appropriate risk assessment and response.

Source and date of request

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) decision, 21 March 2016

Public health issue

This document assesses the risk to human health posed by a multi-country foodborne outbreak of Shiga toxin-producing *Escherichia coli* (STEC) infections associated with haemolytic uraemic syndrome taking place in the European Union (EU).

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The external reviewers submitted declarations of interest that revealed no conflicts of interest.

Disease background information

Shiga toxin-producing *Escherichia coli* (STEC) O26 isolations in humans

Shiga toxin-producing *Escherichia coli* (STEC) is a group of pathogenic *E. coli* strains capable of producing Shiga toxins that can cause severe enteric and systemic disease in humans. The full serogroup designation of *E.coli* is usually defined by determining both O and H antigens. STEC include around 200 different *E. coli* O serogroups producing Shiga toxin, of which over 100 have been associated with human disease. Two major Shiga toxin types (*stx*1 and *stx*2) have been associated with strains causing human disease [2]. Strains producing *stx*2 are most commonly associated with severe illness such as bloody diarrhoea and haemolytic uremic syndrome (HUS).

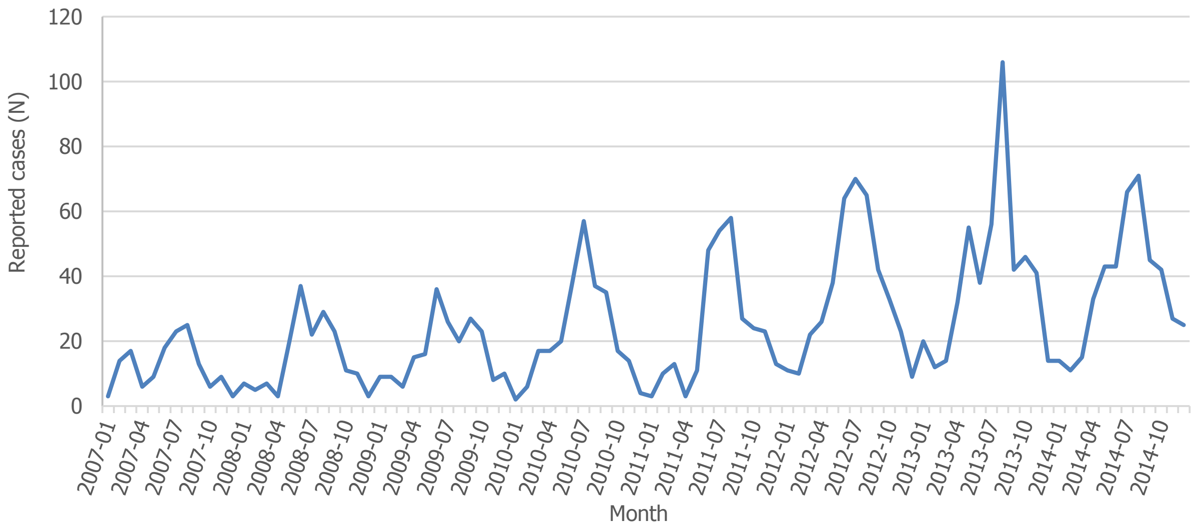
HUS is a consumptive coagulopathy characterised by microangiopathic haemolytic anaemia and thrombocytopenia, with a particular predilection for the kidney and the central nervous system. HUS has a number of aetiologies, but the diarrhoea-associated form of HUS is more common in children and associated with Shiga toxin-producing *Escherichia coli* (STEC) [3].

Among STEC, serogroup O26 (STEC O26) is the second most commonly reported in Europe after O157. In the period 2010–2014, 19 EU/EEA countries (Austria, Belgium, the Czech Republic, Denmark, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Romania, Slovenia, Spain, Sweden and the United Kingdom) reported 2 356 confirmed cases of STEC O26 (mean of 379 reported cases per year, ranging from 267 to 476) with most cases during this period being reported from Ireland (32%) followed by Germany (15%) and Sweden (12%).

These cases were predominantly in young children; the median age for all cases with information available was two years (interquartile range [IQR] 1–14), 973 of cases with available information on gender (52%) were female. Symptom onset for STEC O26 cases was distributed over the year with a peak during the period June-September. Eighty-eight percent of the cases (1 271) with available information on importation were acquired within the reporting country. An increase in STEC O26 cases has been reported from 2012 onwards (Figure 1), which is most likely partly due to the increased use of methods capable of identifying serogroups other than O157 [4]. As of 23 March 2016, 463 cases of STEC O26 have been reported to the European Surveillance System (TESSy) for 2015.

During the period 2010–2014, 254 cases (12%) of STEC O26 with information available on the toxin profiles were positive for *stx*1, *stx*2 and *eae* genes. The median age for the cases was three years (IQR 1–15) and there was no gender difference among cases. HUS was reported in 28 of these cases (11%), with 24 (86%) of these cases being children under four years of age. In 2015 (quarterly data Q1–Q4), six countries reported 26 *stx*1, *stx*2 and *eae* positive STEC O26 cases to TESSy.

Figure 1. Distribution of confirmed cases of Shiga toxin-producing *Escherichia coli* (STEC) O26 by month of notification in EU/EEA, TESSy data, 2007–2014 [5]



STEC O26 has been associated with community-wide outbreaks. Two outbreaks, in Germany [6,7] and Denmark [8], were caused by *stx*1-producing strains and were not associated with the occurrence of HUS cases. Conversely, HUS was a common feature in outbreaks due to *stx*2-producing STEC O26, such as those that occurred in France in 2005 [9,10] and in Italy in 2013 [9]. In both episodes, the consumption of dairy products was confirmed (French outbreak) or suspected (Italian outbreak).

Shiga toxin-producing *Escherichia coli* (STEC) molecular typing data in TESSy

Since November 2012, ECDC has been collecting molecular typing data (Pulsed-Field Gel Electrophoresis – PFGE) for STEC isolates from Member States on a voluntary basis. As of 23 March 2016, 1 610 STEC isolates have been submitted to TESSy, 167 of them reported to be of serogroup O26. Of these, 130 isolates from eight Member States have approved and curated PFGE profiles, involving 106 PFGE pulsotypes, 13 of which are represented in the database by more than one isolate. Two of these 13 patterns include isolates from more than one country.

Shiga toxin-producing *Escherichia coli* (STEC) isolations in animals and food

Similar to the data for human infections, the STEC serogroup O26 was the second most frequently reported serogroup in food and animal samples in Europe from 2005, with an increasing trend between 2011 and 2014 [4]. Overall, a wide range of STEC serogroups was reported, with STEC O157 being the most frequent in food and animal samples. However, many of the Member States' surveillance and monitoring programmes are focused on STEC O157, potentially biasing the estimates of the frequency of non-O157 STEC serogroups. An analysis of the 2014 STEC data indicated that serogroups O26 and O103 were reported more frequently than O157 in the food samples (mainly from bovine meat, raw milk and dairy products) tested using the ISO/TS 13136:2012 standard method [10], which is able to detect STEC regardless of its serogroup [4].

Information on the number of STEC O26 isolates in food and animals reported by EU Member States and non-Member States from 2005 to 2014 in accordance with Directive 2003/99/EC is presented in Table 1.

From 2005 to 2014, 12 EU Member States and Norway reported 166 isolates of STEC O26 from animals and food. The majority of the isolates (123) were reported in animals, mainly from cattle (64), followed by pigs (17), dogs (14), sheep (9), cats (7), fowl (2), domestic horses (2), zoo animals (2), goats (1), deer (1), mink (1), wild boar (1), birds (1) and other animals (1). The remaining 43 isolates of STEC O26 were from meat from bovine animals (16), cheese (12), milk (6), meat from other animals (4), butter (1), other processed food products and prepared dishes (2) and other or unspecified food (2).

In the period 2005–2010, Austria, Germany, Latvia, the United Kingdom and Norway reported 58 isolates of STEC O26 in animals, mainly from cattle (26), followed by pigs (9), dogs (9), sheep (5), cats (5) and birds, goats, minks and zoo animals with one isolate each. During the same period (2005–2010), France, Germany and Norway reported 10 isolates in food, mainly bovine meat (7).

In 2011, only nine isolates of STEC O26 were reported by two Member States. Latvia reported seven isolates in animals (cattle, cats, dogs, fowl and sheep) and Ireland reported two isolates in food (bovine meat).

In 2012, overall 22 STEC O26 isolates were reported by Austria, Germany, Latvia and Ireland. The majority of the isolates (20) was reported in animals: cattle (10), pigs (7) and deer, dogs and wild boars with one isolate each. Most of the animal isolates were reported by Germany, followed by Latvia and Austria. No isolations from ruminants other than bovine animals were reported in 2012. The only two isolates reported in food were detected by Ireland in 'other processed food products and prepared dishes'. No isolates from bovine meat were reported in 2012.

In 2013, seven Member States (Austria, Germany, Estonia, France, Italy, Latvia and Sweden) reported 30 STEC O26 isolates in both animals (16) and food (14). The isolates reported were obtained from cattle (12) and dogs, fowl and pigs with one isolate each. Isolations in food occurred in: 'cheese made from unspecified milk or other animal milk' (7), bovine meat (2), 'meat from other animal species or not specified' (2), milk (2) and butter (1). Similar to 2012, no isolations from ruminants other than bovine animals were reported in 2013.

In 2014, eight Member States (Austria, Belgium, Estonia, France, Germany, Italy, the Netherlands and Spain) reported 37 STEC O26 isolations in animals and food. The majority of isolates (22) were reported from animals, mainly from cattle (14), sheep (3), domestic horses (2) and deer, dogs and wild boars with one isolate each. Out of 22 animal isolates, 17 were reported by Germany, while the remaining animal isolates originated from Austria and Italy. Seven Member States reported 15 STEC O26 isolates from food, mainly bovine meat (5), different types of cheese (5), milk (4) and other/unspecified food (1).

Table 1. Reported isolations of STEC O26 from animals and food in EU Member States and other reporting countries, 2005–2014[a]

Source	No. of isolations in 2005-2010	No. of isolations in 2011	No. of isolations in 2012	No. of isolations in 2013	No. of isolations in 2014	Total no. of isolations
Animals						
Birds	1					1
Cattle (bovine animals)	26	2	10	12	14	64
Cats	5	2				7
Deer					1	1
Dogs	9	1	2	1	1	14
Horses, domestic					2	2
Gallus gallus (fowl)		1		1		2
Goats	1					1
Minks	1					1
Pigs	9		7	1		17
Sheep	5	1			3	9
Wild boars					1	1
Zoo animals	1			1		2
Other animals			1			1
Total animals	**58**	**7**	**20**	**16**	**22**	**123**
Food						
Butter				1		1
Cheeses made from cows' milk (soft and semi-soft)					1	1
Cheeses made from sheep's milk (fresh)					1	1
Cheeses, made from unspecified milk or other animal milk				7	3	10
Meat from bovine animals	7	2		2	5	16
Meat from other animal species or not specified				2		2
Meat from wild game - land mammals	1					1
Meat, red meat	1					1
Milk from cows' (raw milk)				1	4	5
Milk from other animal species or unspecified				1		1
Other processed food products and prepared dishes			2			2
Other food or unspecified food	1				1	2
Total food	**10**	**2**	**2**	**14**	**15**	**43**
Total (animals and food)	**68**	**9**	**22**	**30**	**37**	**166**

(a) Data reported to EFSA in accordance with Directive 2003/99/EC. During the period 2005–2014, 12 EU Member States (Austria, Belgium, Estonia, France, Germany, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, and United Kingdom) and Norway reported information on STEC O26 isolates.

Note: 'Cheeses, made from unspecified milk or other animal milk': 3 isolates of STEC O26 from curd cheese and 7 isolates from unspecified cheese. 'Meat from bovine animals': 5 isolates of STEC O26 from carcase, 3 isolates from fresh meat, 6 from minced meat and 2 isolates from unspecified meat. 'Meat from other animal species or not specified': 1 isolate of STEC O26 from fresh meat and 1 isolate from minced meat. 'Meat, red meat' includes: meat from bovines, pigs, goats, sheep, horses, donkeys, bison and water buffalos.

Event background information

Epidemiological investigation

On 9 and 10 February 2016, the National Institute of Public Health of Romania (INSP) was alerted to 12 paediatric cases of HUS admitted to the Children's Emergency Hospital 'M.S. Curie', in Bucharest. The children, mainly from Arges district in southern Romania, had developed diarrhoea (some bloody diarrhoea) between 25 January and 9 February [11].

The confirmation of the outbreak was based on retrospective data on HUS in Romania actively collected from the four regional hospitals (in Bucharest, Cluj, Iasi and Timisoara), which are referral centres for management of HUS cases. Between 2010 and 2015, 101 HUS cases were diagnosed in Romania, with an average of annually reported cases of 16, a minimum of five cases in 2010 and a maximum of 25 in 2015. The ten cases initially identified in February 2016 (as of 10 February 2016) represented a two-fold increase compared with the monthly maximum of five HUS diagnoses observed during the six previous years.

In order to identify additional cases possibly associated with this outbreak, a national HUS and severe diarrhoea surveillance system was set up on 15 February 2016 in Romania. As of 23 March, 14 additional cases had been identified as associated with this outbreak in previously and newly-affected districts in Romania.

On 21 March 2016, Italy published a message in the Early Warning Response System (EWRS) reporting a HUS case in a 14 month-old patient resident in Tuscany, Italy. The child had developed gastroenteritis symptoms on 6 March, was admitted to the hospital on 9 March with severe bloody diarrhoea and abdominal pain, developing HUS on 12 March 2016. The health authorities of the Tuscany region started the epidemiological investigation and applied standard control measures. A link with the Romanian outbreak was suspected based on the history of food consumption.

Based on the epidemiological and microbiological findings in Romania and Italy, the European outbreak case definition formulated for this outbreak was based on STEC laboratory confirmation, HUS diagnosis [12] and date of illness onset (Annex 1).

Overall 25 cases were identified as associated with this outbreak in Romania (24) and in Italy (1), 12 cases were laboratory confirmed (11 in Romania and one in Italy) and 13 cases fulfilled the definition for a probable case (all in Romania). The median age of confirmed cases was 12 months (range 6–38 months). The median age was 12.5 months (range 5 months–33 years) among the 12 probable cases with available information. Twenty cases were under two years of age. Fifteen of the 25 cases were females, giving a female to male ratio of 1.5. Onset dates ranged from 25 January 2016 to 14 March 2016 and three probable cases were asymptomatic (Figure 2). Nineteen cases (nine confirmed and 10 probable) developed HUS and three of these died during this outbreak on 4, 15 and 16 February respectively. These cases (one confirmed and two probable) were in children from Romania under two years of age.

The 24 cases from Romania (11 confirmed, 13 probable) resided in seven different districts, mostly in the southern part of the country. No travel outside Romania before onset of symptoms was reported by the Romanian cases. The confirmed case from Italy was from Tuscany region and did not travel to Romania in the weeks before onset of symptoms (Figure 3).

A harmonised questionnaire was prepared to interview the cases or their relatives on relevant exposures and generate hypotheses on the cause of this outbreak. The questionnaire was designed using the standard questionnaire adopted by the Italian Registry for HUS (www.iss.it/seu) with additional questions from questionnaires used to investigate cases with STEC infection in other EU Member States. The questionnaire was written in English and in Romanian. Parents of young cases were asked about their child's food and water exposure in the ten days before symptom onset and about episodes of diarrhoea among other family members. As of 23 March 2016, relatives of 15 Romanian cases and the single Italian case were interviewed.

The information collected from the first eight interviews showed that the only common exposures were related to food consumption. Most of the cases had eaten fruits, vegetables, meat and dairy products, in particular fresh cheese and yoghurt made from pasteurised cow's milk. Fruits and vegetables had been bought either from small local chains or supermarkets with large distribution areas. Vegetables and meat were reported to have been thoroughly cooked before being eaten. Dairy products had been bought from several shops. One retail food chain distributing food only in Arges district was mentioned in three interviews in relation to the consumption of fresh cheese and yoghurt. The analysis of 15 questionnaires from 14 cases in Romania and one case in Italy indicated consumption of dairy products by all cases and consumption of fresh cheese made from pasteurised cow's milk for 12 cases.

Figure 2. Distribution of cases by diarrhoea onset, O26 confirmation and area of residence, Romania and Italy, January to March 2016, as of 23 March 2016 (n=22; three asymptomatic cases not included)

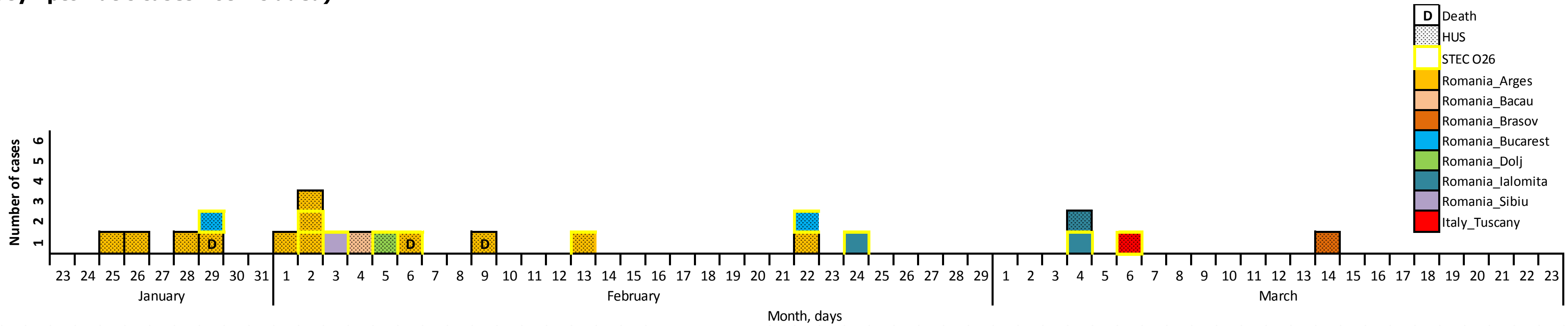
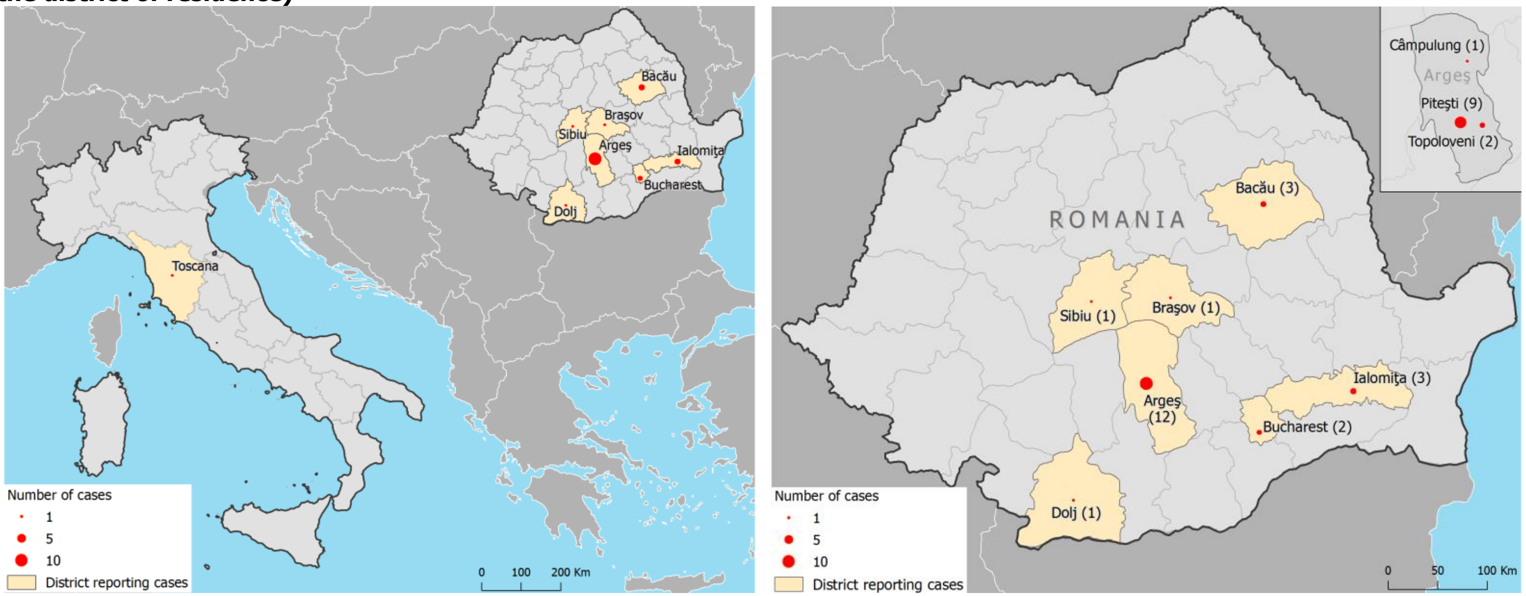


Figure 3. Distribution of outbreak cases by area of residence, Romania and Italy, January to March 2016, as of 23 March 2016 (n= 24, one case missing information on the district of residence)



Microbiological investigation in human samples

The microbiological investigation has been compiled from results of tests of stool and serum samples from outbreak cases carried out between January and March 2016. The tests were performed using different methods by Romanian district hospitals, the Romanian National Reference Laboratory (NRL) from Cantacuzino Institute in Bucharest, and the Italian National Reference Laboratory for *E. coli* at the Istituto Superiore di Sanità (ISS) in Rome, Italy.

No stool samples were available for the initial cases whose serum samples were tested for antibodies to the lipopolysaccharide (LPS) of the major STEC serogroups (O157, O26, O103, O111, O145 and O55) by ELISA [11]. For later cases, culture enrichments of stool samples were tested by PCR to detect the presence of Shiga toxin-encoding (*stx*1 and *stx*2) and intimin-encoding (*eae*) genes [13]. PCR-positive samples were streaked onto agar plates and colonies resembling *E. coli* were tested for the presence of *stx*1, *stx*2 and *eae* genes by PCR. Strains that were PCR-positive for at least one of these genes were then tested with O antisera against the same main STEC serogroups [13] by slide agglutination.

Eleven of 25 confirmed and probable cases associated with this outbreak in Romania and Italy have tested positive for O26, one case has tested positive for both O26 and O157 and one case has tested positive for O157 (Table 2).

Table 2. Results of the O serogroup testing in serum or stool samples for 25 human cases, Romania and Italy 2016

O serogroup testing	O26	O157	Negative to all tested O serogroups	Total
Slide agglutination	6*	1	6	13
LPS antibody testing	6**	1**	6	12**
TOTAL	12**	2**	12	25

** Samples from one case were positive at both LPS antibody testing and slide agglutination.*

*** Serum samples from one case tested positive both for O26 and for O157 by LPS antibody testing.*

Table 3 shows the results of the PCR-based testing for the presence of the *stx*1, *stx*2 and *eae* genes combined with the results of the O serogroup testing.

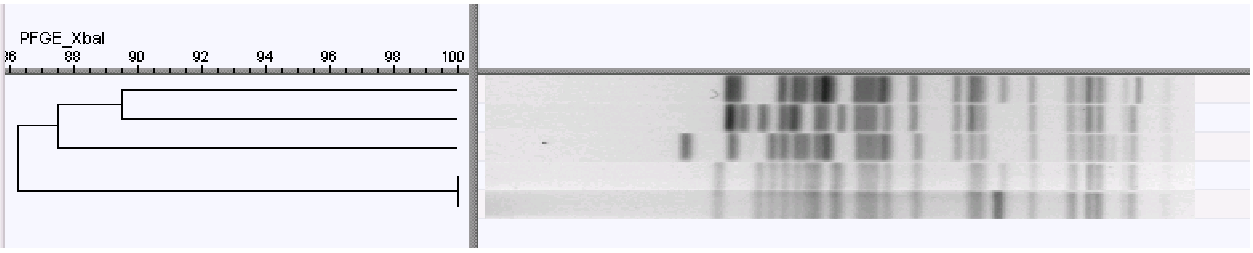
Table 3. Results of the testing for the 25 human cases associated with this outbreak in Romania and Italy 2016

	O serogroup testing by LPS	O serogroup testing by slide agglutination	Total
Confirmed cases (O26)	6	6	12
*stx*1+, *stx*2+, *eae*+		4	4
*stx*1+, (*stx*2-), *eae*+		1	1
(*stx*1-), *stx*2+, *eae*+		1	1
No PCR testing for *stx* / *eae* genes performed	6		6
Probable cases (non-O26)	6	7	13
*stx*1+, *stx*2+, *eae*+		1	1
*stx*1+, (*stx*2-), *eae*+		2	2
(*stx*1-), *stx*2+, *eae*+		2	2
No PCR testing for *stx* / *eae* genes performed	6	2	8
TOTAL	**12**	**13**	**25**

Serum samples of the case from Italy tested positive for antibodies to the LPS of O26. Stool samples from the same case were negative by PCR-based testing for the presence of the *stx*1, *stx*2 and *eae* genes.

PFGE was performed for five STEC O26 isolates obtained from confirmed cases (Figure 4). Two of them (4th and 5th in Figure 4) showed indistinguishable PFGE patterns whereas the other three all showed non-identical PFGE profiles (86–93% similarity). The three cases with non-identical PFGE patterns are from different districts, had onset of symptoms in early to mid-February and all have different virulence gene profiles (one case is PCR-positive to both *stx*1 and *stx*2, one case is PCR positive only to *stx*1 and one case is PCR positive only to *stx*2). The two cases with indistinguishable PFGE patterns are from the same district, had onset of symptoms in late February to early March and share the same virulence gene profile (PCR-positive to both *stx*1 and *stx*2). As of 23 March 2016, there were no cases reported to TESSy from other EU/EEA countries with PFGE profiles indistinguishable from those of the confirmed cases above. PFGE data from more cases would be highly desirable to be able to further characterise the outbreak strain(s).

Figure 4. Cluster analysis of PFGE data available for confirmed STEC O26 cases (n=5).



Further characterisation of the outbreak associated strains will be achieved by whole genome sequencing (WGS) analysis.

Environmental and food investigation reported to the Rapid Alert System for Food and Feed (RASFF)

Following the consideration by the Romanian Ministry of Health of certain foods as the possible infection source of the STEC outbreak, on 25 February 2016, the National Sanitary Veterinary and Food Safety Authority (NSVFSA) in Romania received the list of possible implicated foods as part of the epidemiological investigation. The final list contained 53 different suspect food categories. A total of 574 samples from 141 food items were collected from 24 different premises between 26 February 2016 and 14 March 2016 in five districts: Argeș, Bacau, Dolj, Ialomita and Vrancea. The results showed that from 16 types of food (five dairy products, 10 poultry and one mix beef and pork meat) collected in all the five districts, 86 samples were positive by real-time PCR for one or more of the investigated genes (*stx*1, *stx*2, *eae*) and O26 as displayed in Table 4.

Table 4. Results of the positive food samples collected from premises, as of 23 March 2016

Characterisation of positive findings	Positive samples	Food type
*stx*2+ *eae*+ O26+	25	Cheese from cow's milk
eae+ O26+	21	Poultry
eae+ O26+ isolation+	25	Poultry
*stx*1+ eae+	5	Mix beef and pork meat
eae+ O26+	5	Cow's milk
eae+ O26+	5	Cheese from cow's milk
TOTAL	**86**	

According to the ISO/TS 13136:2012 method used for the analysis of food, presumptive presence of STEC O26 was reported for 25 food samples (25 cheese) because the PCR positive results were not followed by a STEC O26 isolation (*eae*+ O26+). Following the identification of the presumptive presence of *STEC* O26 in a batch of fresh cheese, samples were collected at a milk processing establishment in Argeș district of Romania (hereinafter referred to as the Romanian operator), which produced dairy products from cow's milk. The Romanian operator suspended activities on 28 February 2016.

Of the 574 samples, 45 from 9 different dairy products were collected from the dairy plant belonging to the Romanian operator and analysed for STEC. Five samples from a single dairy product type (cheese from cow's milk) were presumptively positive for STEC O26 and *eae*, but without isolation. Moreover, 25 from the 574 samples from cheese from cow's milk from five different dairy products collected in premises other than the Romanian operator's, but produced by the Romanian operator, were presumptively positive for STEC O26 (*stx*2+ *eae*+ O26 +): 15 samples (stx2+ *eae*+ O26 +) from three batches of dairy product collected at a local supermarket in Arges, 10 samples (stx2+ *eae*+ O26 +) from two batches of different dairy products collected in two local hospitals, both in Arges district.

An additional 44 non-food samples were collected at the premises of the Romanian operator: drinking water, environmental samples, and ingredients (rennet, calcium chloride and salt). Environmental samples were taken from the surface of various pieces of working equipment and components of the processing machinery, as well as from cheese packaging material. All were negative.

Stool samples were collected from 19 employees of the Romanian operator: seven were positive for *E. coli*, with three of them positive for *stx* genes, including one for *stx*1, *stx*2 and *eae*, i.e. a probable case.

Two milk suppliers of the Romanian operator were also investigated. Samples from raw milk collected from the milk tank in one of the farms tested presumptively positive for *E. coli* O26 (*eae*+ and O26 +). An additional sample taken afterwards from the same farm was negative. Four additional water samples (mains, water tank, pond and waste water from milking equipment) and one bovine faecal sample were negative for STEC. Samples from raw milk collected at the second farm were negative for STEC.

The Romanian operator exported dairy products to Germany (1 410 kg), Italy (6 517 kg), Spain (10 385 kg) and Belgium (1 108 kg) during February 2016. As a result of the investigation, on 5 March 2016 the Romanian operator initiated the voluntary withdrawal of the dairy products produced in February 2016 and the recall of all products produced in February 2016 and sold to other Member States. At national level, 20 363.87 kg of dairy products have been withdrawn from six Romanian districts and destroyed. The closure of activities by the Romanian operator was enforced by the local veterinary authorities of Arges district on 8 March 2016, following the presumptive detection of *E. coli* O26 of another two batches of fresh cheese and boiled cheese (*eae*+ O26+).

The parents of the HUS case in Italy reported that the child had consumed different types of cheese imported from Romania, including cheese produced by the Romanian operator. The retailer in Tuscany where the family reported having purchased the cheese (a shop selling products from Romania), had been supplied by one importing Italian wholesaler. A remaining portion of the cheese, not wrapped in the original packaging but belonging to the same brand and produced by the Romanian operator was sampled and tested for the presence of STEC with the ISO/TS 13136:2012 method. The sample was positive by real-time PCR for the presence of *stx*1, *stx*2, *eae* and O26 genes (presumptive presence of STEC O26). A STEC O26 strain positive for the presence of *stx*1, *stx*2, and *eae* genes was isolated (presence of STEC O26). This STEC O26 strain has been subjected to PFGE but the results are pending at the time of producing this assessment.

Two additional food samples were collected in two retail shops in Tuscany on 15 March 2016 (where the family of the case bought the cheese) and in a different location in the same area on 17 March 2016. They were of two different brands, both produced by the Romanian operator. Results were not available at the time of producing this assessment.

At the time of producing this assessment, there is only information on 2 400 kg of dairy products withdrawn and destroyed by the Spanish authorities, 1 191.61 kg by the German authorities, a very small quantity by the Belgian authorities (150 kg), and 1 696.4 kg of dairy products withdrawn by four importing Italian wholesalers. One of the Italian wholesalers is responsible for the secondary distribution of some of the imported dairy products to Germany and France. The other importing Italian wholesalers imported and distributed dairy products produced by the Romanian operator within Italy.

ECDC and EFSA threat assessment for the EU

Romania reported an outbreak of STEC infection associated with HUS affecting 11 confirmed and 13 probable cases since January 2016. On 21 March, Italy reported one HUS case epidemiologically linked to the same outbreak. The outbreak was initially detected due to the high number of HUS cases in children less than 2 years of age reported in a short period of time by the main paediatric hospital in Bucharest. The case-fatality ratio was high, particularly at the beginning of the outbreak, with three deaths amongst the 12 initially reported cases. Since no unspecific HUS surveillance was present in Romania, initial confirmation of the outbreak was done comparing the observed HUS cases with the HUS cases referred to in the four main referral hospitals in Romania in the previous six years.

Due to the lack of stool samples and thus isolation of STEC strains from the initial cases, the first evidence of a causative agent of this outbreak was obtained by the detection of circulating antibodies against the LPS of different *E. coli* serogroups (serological test) through collaboration with the Italian National Reference Laboratory for *E. coli* at the ISS in Rome. The collection of more recent patients' stool samples also allowed detection of Shiga toxin-encoding (*stx*1 and *stx*2) and intimin-encoding (*eae*) genes by PCR, and strain characterisation by PFGE for five cases where a STEC strain could be isolated. The PFGE profiles are indistinguishable for two of the cases whereas the other three are non-identical (86–93% similarity). As of 23 March 2016, 12 cases had laboratory confirmation for O26 infection with an additional 13 meeting the probable case definition by developing HUS, testing positive for another STEC O serogroup (O157) or by testing positive by PCR for *stx1* and/or *stx2* and *eae* genes.

Most cases were in children under two years of age, which is the group with the highest risk of HUS, following STEC O26 infection. Since only a proportion of STEC infections progress to HUS, it is highly likely that a number of mild or asymptomatic STEC infections have not been ascertained. Arges in Romania, was the most affected district with 13 cases reported; nonetheless six additional districts, mostly in southern Romania, reported 11 additional cases.

The onset of symptoms in the cases were distributed over an eight-week period, suggesting a persistent common source outbreak, possibly associated with different vehicles of infection and possibly multiple strains contaminating the same or multiple source(s). This could explain the different STEC serogroups, toxin genes and PFGE profiles identified by the microbiological investigation in human and food samples. Notably, blood samples from the same case tested positive for both O26 and O157 by serology. It cannot be excluded, however, that only a portion of the reported cases are part of this outbreak, while the remaining would be unrelated sporadic cases identified because of the enhanced surveillance ongoing in Romania since 15 February 2016. Furthermore, a small proportion of cases may be secondary cases infected through person-to-person transmission within the household. Stool samples from two asymptomatic household contacts of cases were found positive for *stx* encoding genes.

Early information from exploratory questionnaires administered to cases pointed at the Romanian operator as a possible source of infection. The confirmed case reported in Italy had also consumed a cheese produced by the Romanian operator and imported in Italy on 1 March. A STEC O26 strain (*stx1*+, *stx2*+ *eae*+) was obtained from the leftover cheese consumed by the patient's family. The Romanian operator closed the production and recalled the distributed dairy products. However, the recall of dairy products distributed within Romania and exported or redistributed to five Member States has had limited impact in terms of product withdrawal outside of Romania. It could be that some batches had already been purchased by consumers. The precise source of contamination has not yet been identified since multiple dairy products produced by the Romanian operator have tested positive for STEC O26. It is also possible that multiple sources of contamination have contributed to this outbreak. HUS cases with STEC O26 infection have been reported in March in Romania and Italy. Therefore, the identification of new cases linked to the possible source(s) of this outbreak should not be unexpected in Romania and possibly in other EU countries where products from the Romanian operator have been distributed.

Conclusions and options for response

A multi-country outbreak of Shiga toxin-producing Escherichia coli (STEC) infection associated with haemolytic uraemic syndrome (HUS) and affecting mostly young children has been reported in the last two months in Romania. Italy reported one related HUS case through EWRS on 21 March 2016. Overall, 25 cases were identified as associated with this outbreak, of which 19 developed HUS and three of these died. Twelve cases had microbiological and/or serological evidence of STEC O26 infection; 13 additional cases met the probable case definition by developing HUS, testing positive for another STEC O serogroup (O157) or by testing positive by PCR for *stx1* and/or *stx2* and *eae* genes.

Information collected from patients pointed towards a milk processing establishment (the Romanian operator) as a possible source of infection. The implicated milk processing establishment exported a particular type of cheese to four EU countries (Belgium, Germany, Italy and Spain) and one Italian importer further distributed this product to France and Germany. In Romania, one fresh cheese product made of cow's milk produced by the Romanian operator tested positive for *E. coli* O26 not possessing *stx* virulence genes. Other dairy products produced by the Romanian operator tested positive for *E. coli* virulence genes *stx*1, *stx*2 and *eae*. In Italy, an STEC O26 strain (positive for *stx1*, *stx2* and *eae*) was isolated from a sample of the cheese that had been produced by the Romanian operator, imported from Romania and consumed by the Italian child that later developed HUS (stool samples were negative for STEC but serum samples positive for the presence of antibodies against the LPS of *E. coli* O26).

The PFGE analysis suggests the potential involvement of multiple strains from a common source or from multiple sources. Multi-strain STEC outbreaks have been reported in the past [1]. Therefore, it cannot be excluded that the cases belong to a single outbreak associated with a source contaminated by different strains. The epidemiological evidence linking some of the Romanian cases and the Italian case to the Romanian operator, in addition to the microbiological findings, are consistent with the hypothesis of a multi-strain outbreak.

The last Romanian case associated with this outbreak had onset of symptoms on 14 March. The cheese consumed by the Italian case was imported to Italy from Romania on 1 March and had been produced on 18 February 2016 with an expiry date of 18 April 2016. A recall of the company products was undertaken in Romania as of 5 March and in Italy as of 15 March. Other Member States undertook recalls subsequently. According to information available at the time of conducting this assessment, the withdrawal of potentially contaminated dairy products following a recall has been limited in Member States, with the exception of Romania. It is therefore possible that affected products are still present in households, and detection of new cases cannot be excluded.

In order to minimise the spread of the infection and investigate possible new cases in a timely manner, Romania, Italy and other possibly involved Member States could consider enhancing surveillance for HUS and STEC cases. Continued enhanced surveillance for HUS in children on a routine basis in Romania could provide further early warning of ongoing or new contamination events. The questionnaire used to interview some of the Romanian cases and the Italian case is available in English from ECDC upon request.

If new cases are identified, these should be reported to the Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD). In such a situation, public health authorities could also consider conducting an epidemiological analytical study and including further food and environmental sampling in suspect premises to identify the vehicle of infection.

Whole genome sequencing (WGS) on isolates detected so far should be carried out to provide more detailed information about possible links between cases.

The investigation of the European dimension of this cross-border foodborne outbreak has demonstrated the added value of collaboration between Romanian and Italian public health and food authorities as well as ECDC and EFSA in enabling appropriate risk assessment and response.

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Annex 1. European outbreak case definition for multi-country outbreak of STEC infection associated with HUS

The European outbreak case definition defines a case as follows:

A **confirmed case** as:

a resident in Romania

OR

a resident in the EU with an epidemiological link to Romania

AND

with any laboratory confirmation for *E. coli* O26 infection after 15 January 2016

A **probable case** as:

a resident in Romania

OR

a resident in the EU with an epidemiological link to Romania

AND

with clinical haemolytic uremic syndrome (HUS) after 15 January 2016

OR

testing positive for the following STEC virulence genes: *stx*1 and/or *stx*2 and *eae* by PCR after 15 January 2016

OR

testing positive for a *E. coli* serogroup other than O26 after 15 January 2016

Exclusion criteria
Travel history: cases are defined as travelled-associated when travelling out of the EU in the two weeks before symptoms onset or before sampling date if asymptomatic

An **epidemiological link** with Romania is defined as:

- Travel history to Romania since 15 January
- Close contact with an individual who has a travel history to Romania since 15 January
- Consumption of dairy products produced in Romania after the 15th January