ACM/1191

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

Shiga Toxin Producing *E. coli* (STEC) in food

ISSUE

Risk assessment relating to Shiga toxin-producing *Escherichia coli* (STEC) in food can involve many challenges. In particular, there may be uncertainty in terms of the pathogenicity of different strains and the effect of handling practices (e.g. on the destruction of STEC and cross-contamination).

This paper seeks views from the Committee on the risk from STEC in food to support decision making regarding the safety of these foods, including those that are ready-to-eat, raw or where the effectiveness of measures such as heat treatment in destroying STEC or washing of produce to remove STEC is unclear.

Guidance is needed from the Committee to inform the FSA's risk assessment approach when handling incidents involving STEC and to underpin the development of FSA's policy on this topic. For example, it will inform FSA's input into the development by the European Commission (EC) of a 'guidance document on the application of Article 14 of Regulation EC 178/2002 as regards food contaminated with STEC'.

BACKGROUND

Hazard identification and characterisation

1) STEC are a group of *E. coli* characterised by the ability to produce toxins, designated Shiga toxins (*stx1* and *stx2* or their variants) because of their similarity with the toxin produced by *Shigella dysenteriae*. Shiga toxins are also known as verocytotoxins and the terms STEC and VTEC are synonymous.

2) Annex 1 shows data reported in the most recent edition of the European Union (EU) 'Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks' on the number of confirmed STEC cases from 2009-2013 (EU 2015). In 2013, 1,164 confirmed cases of STEC were reported in the UK and a total of 6,043 cases were confirmed in the EU. Disease multipliers for STEC O157 and non-O157 in the EU have been estimated at 51.2 and 209.6 respectively, and a disease multiplier for STEC O157 in the UK has been estimated at 34 (EFSA, 2013a).

3) The symptoms of STEC infection in humans vary from mild to bloody diarrhoea, which is often accompanied by abdominal cramps, usually without fever, and can involve haemolytic-uraemic syndrome (HUS). HUS is characterised by acute renal failure, anaemia and lowered platelet counts.

HUS develops in approximately 10% of patients infected with STEC O157 and is the leading cause of acute renal failure in young children (EU 2014).

4) Data on the reported hospitalisation and case-fatality rates due to zoonoses, including STEC, in confirmed human cases in the EU in 2013 are shown in Annex 2. Of the 6,043 cases confirmed in the EU for which the relevant data was available, 922 (i.e. 37.1%) were hospitalised and 13 (i.e. 0.36%) died (EU 2015). Although the yearly number of STEC cases is lower than the number of reported cases of salmonellosis and campylobacteriosis in the EU, STEC infections are more severe, and it has previously been estimated that the burden per case (in Disability Adjusted Life Years (DALY)) was approximately 3-fold higher for STEC compared to salmonellosis and campylobacteriosis (Havelaar *et el* 2012).

5) The following information on STEC food-borne outbreaks is based on the European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015). In 2013, a total of 73 food-borne outbreaks caused by STEC were reported. This represented 1.4 % of the total number of reported food-borne outbreaks in the EU during that year. Only 12 of the reported outbreaks were supported by strong evidence regarding their source. The main food vehicle for the 12 strong evidence outbreaks was 'bovine meat and products thereof' (4 outbreaks), followed by 'vegetables and juices and other products thereof' (3 outbreaks) and cheese (2 outbreaks). Each of the remaining three outbreaks was associated with fish and fishery products, herbs and spices, and other foods (EU 2015).

6) Information on the setting was provided in all of the 12 strong-evidence outbreaks, although for three outbreaks the setting was reported as 'Others'. Three outbreaks were associated with 'Household' and with 'Restaurant, café, pub, bar, hotel, catering service', while one outbreak was linked to 'School or kindergarten'. Contributing factors were unprocessed contaminated ingredients in four outbreaks and storage time/temperature abuse in one outbreak. For seven outbreaks, the contributing factors were not reported, unknown or not specified ('Other') (EU 2015).

7) The importance of bovine meat as a source of human STEC infections in humans was also illustrated by the foodborne outbreak data from 2012 (EU 2014), in which twelve STEC outbreaks were reported in the EU. Nine outbreaks were due to STEC O157, one to STEC O113:H4, one to 'other' STEC serogroups, and one to a non-grouped *E. coli* positive for heat-labile enterotoxin (LT genes). Half (six out of 12) of the STEC outbreaks, in which information on the implicated food vehicle was provided, were linked to bovine meat and products thereof.

8) The information provided in the EU trends and sources reports (EU 2014 and EU 2015) therefore indicates that there can be a significant degree of uncertainty in attributing foodborne STEC outbreaks to particular sources and in identifying contributing factors, although when evidence has been obtained key sources that have been identified were bovine meat and products thereof.

Current understanding of pathogenic STEC characteristics: serogroups and virulence determinants

9) A large number of *E. coli* serogroups have been recognised as Shiga toxin producers. Data on the distribution of reported confirmed cases of human STEC infections in the EU/EEA (2011–2013) by the 20 most frequent serogroups is shown in Annex 3. The most commonly reported serogroups in 2013 after O157 were O26, O103, O145, O91, O111 and O146 (EU 2015).

10) Detection of STEC is highly dependent on the methods applied. Traditionally, analytical methods have involved culturing faecal specimens and food samples on agar that is selective for STEC O157. Few non-O157 STEC serogroups grow on the selective agar used to grow STEC O157 and so culture of non-O157 STECs using this approach is difficult. This has led to historical underestimation of non-O157 STEC in terms of their true incidence in human illness and prevalence in food and food-producing animals.

11) However, there has been a recent increase in the use of analytical methods capable of detecting both O157 and non-O157 STEC by clinical and food-testing laboratories in the UK and in other countries, in particular the use of genetic (PCR) assays which target the Shiga Toxin-encoding *stx* gene(s) characteristic of all STEC serogroups. For example, this approach is taken in ISO/TS 1316:2012 (ISO 2012). This allows STEC O157 and non-O157 serogroups to be detected and has led to an increase in the number of non-O157 STEC isolates from clinical and food samples.

12) As well as the historical paucity of information on the non-O157 serogroups involved in human illness and the difficulty of designating individual serogroups as pathogens, a key challenge in identifying harmful STEC strains is that the genetic elements involved in pathogenicity are mobile and not fully understood. In addition to the *stx* gene(s), human pathogenic STEC usually harbour other virulence factors which are important in the development of disease. The plasticity of the genome, resulting in the acquisition of virulence or adherence properties from other organisms, normally by means of translocation on phages, means that new and unexpected strains are likely to appear in an unpredictable way over time.

13) In April 2013, the European Food Safety Authority (EFSA) published a scientific opinion (EFSA 2013a) on ‘STEC-seropathotype and scientific criteria regarding pathogenicity assessment’. The approach adopted in the development of the opinion involved a summary of the types of pathogenic *E. coli* which have been associated with cases of human disease, and the putative virulence factors therein; the use of data from the European Surveillance System (TESSy data) as provided by the ECDC (European Centre for Disease Prevention and Control) and data available in the EU Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011 for assessing the current situation regarding human infections with STEC in the EU; a review of methods for the isolation and identification of STEC, including detection of virulence factors and

characterisation and typing of STEC strains and virulence genes therein; hazard characterisation, including illnesses associated with STEC and identification of predictive factors for STEC that may contribute to human disease; evaluation of the seropathotype concept using the Karmali approach, a modification of the Karmali approach based on the health outcome of reported confirmed human STEC cases in the EU during 2007-2010, and a molecular approach based on the identification of known or putative colonization genes and additional toxins; and finally exposure assessment, including EU monitoring data on occurrence of STEC in RTE food.

14) The opinion (EFSA 2013a) describes the genes that are currently known to be involved in STEC pathogenicity. Enterohaemorrhagic *E. coli* (EHEC) are a subset of STEC and are typically isolated from cases of severe disease. In addition to the *stx* gene(s), EHEC usually carry the *eae* (*e*nterocyte *a*ttaching and *e*ffacing) gene which encodes the adhesin intimin. Intimin is involved in causing attaching and effacing lesions in infected cells and is therefore associated with intimate attachment of the bacteria to the host gut mucosa. The opinion (EFSA 2013a) indicated that strains which are positive for both *stx2* and *eae* are associated with a high risk of more serious illness (Annex 4) although other virulence gene combinations and/or serotypes may also be associated with serious disease, including HUS. Further analysis by Public Health England (PHE) highlighted the importance of the *stx2a* subtype in the development of severe illness in UK cases (Annex 5).

15) Pathogenic *E. coli* can belong to more than one pathotype (for example they can potentially have features that combine those of both STEC and enteroaggregative *E. coli* (EAEC)). Stx-producing EAEC can be detected by methods that identify the *stx* gene(s) although additional tests are required to identify the genes associated with EAEC. According to the EFSA opinion, for epidemiological purposes, the scheme for strain characterisation should also include additional genes beside the *eae* gene and serogroup-associated genes specific for this pathotype; genes associated with enteroaggregative adhesion (AA) appear to represent the most appropriate choice (EFSA 2013a). The gene encoding the regulator AggR is considered a good target for detecting EAEC and has long been used for this purpose since it regulates the aggregative adherence fimbriae pathogenicity island (AAF PAI), governing the enteroaggregative adhesion along with those specifying other AA-associated plasmid-encoded factors. Because *aagR* is plasmid-located and may not be detected in the event of plasmid loss, the concomitant detection of the chromosomal gene *aaiC,* encoding a secreted protein of EAEC has been proposed to circumvent this possibility (EFSA 2013a).

16) The major outbreak of *E. coli* O104:H4 in 2011 was associated with sprouted seeds, which involved 4,321 confirmed STEC infections and 852 cases of HUS, with 54 deaths in Germany and 15 other countries (EFSA 2013a). This is an example of the genomic variability referred to above. The outbreak strain possessed the *aaiC* and *aggR* genes but lacked *eae* and therefore demonstrated the difficulty in predicting the emergence of ‘new’ pathogenic STEC by screening only for the *eae* gene or by focusing on a restricted panel of serogroups.

17) The opinion (EFSA 2013a) concluded that the Karmali seropathotype classification (i.e. the limitation to 'relevant' serotypes to identify pathogenic STEC, based on their reported frequency in human disease/outbreaks and the severity of the health outcome) does not define pathogenic STEC nor does it provide an exhaustive list of pathogenic serotypes.

18) The opinion (EFSA 2013a) also concluded that it is still not possible to fully define human pathogenic STEC or to identify factors for STEC that absolutely predict the potential to cause human disease, and that there is no single or combination of marker(s) that defines a 'pathogenic' STEC.

19) In addition, the opinion (EFSA 2013a) indicated that the detection of Shiga toxins alone, or of genes encoding for such toxins, is not a sound scientific basis for assessing the disease risk to the consumer. According to the opinion, the isolation of an STEC strain is needed to confirm the presence of *stx* gene(s) in addition to relevant virulence factors in the same live cell whilst excluding the presence of free DNA or free *stx* phages in the enrichment culture.

20) Based on current analytical methods, isolation of *E. coli* from food samples may take 3-5 days, whereas PCR results for the virulence genes may be available on day 2 of the analysis. There may therefore be practical considerations in obtaining confirmation of the presence of *E. coli* from food samples in which *stx* has been detected, given the short shelf life of many products and the need to make timely decisions to protect public health when dealing with incidents. In addition, the need to confirm the presence of *E. coli* adds financial cost and complexity to the analysis.

21) Nevertheless, the opinion (EFSA 2013a) proposed a provisional molecular approach, utilising genes in addition to the *stx* genes, that could be used to assist risk assessment in relation to STEC (Annex 6) whereby;

- any ready-to-eat (RTE) product contaminated with an isolate of one of the STEC serogroups most frequently associated with human illness (i.e. O157, O26, O103, O145, O111, O104) in combination with *stx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS.

- for any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS.

- in the absence of these genes, current available data do not allow any inference regarding potential risks.

Exposure assessment

22) Information on STEC in food and animals from the European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015) is provided in Annex 7 although this does not include UK data. Key findings from the discussion section on STEC in this report are as follows. Contaminated bovine meat is considered to be a major source of food-borne STEC infections in humans in the EU. In 2013, 12 Member States (MS) reported data on STEC in fresh bovine meat and low proportions of single samples were positive for STEC and for STEC O157. A wide range of different STEC serogroups, including the ones reported from human isolates, was reported from both cattle and small ruminants and their meat, indicating that both animal species can be the reservoirs of a diverse range of STEC strains that are virulent to humans. Small ruminants were reported to be positive for non-O157 STEC strains in high proportions by two MS. This is consistent with sheep and goats being considered an important source of STEC strains that are virulent to humans. STEC has been considered a hazard of high public health relevance for sheep and goat meat inspection (EFSA, 2013b). There were a few reports of STEC in fresh ovine meat but not in fresh pig meat. According to the Scientific Opinion of the Panel on Biological Hazards on monitoring of STEC (EFSA, 2007), pigs have not been identified to be major sources of human STEC infection in Europe (EU 2015).

Proposed approach taking into account strain severity and exposure assessment

23) Article 14 of Regulation (EC) No 178/2002 prohibits food being placed on the market if it is unsafe. Food is deemed to be unsafe if it is considered to be injurious to health or unfit for human consumption. Once a hazard is identified in food which might make it injurious to health, an assessment of the associated risk should be carried out, taking into account the potential exposure of consumers to this hazard. This exposure assessment should consider the normal conditions of use of the food, such as cooking, and the particular health sensitivities of specific categories of consumers where food is intended for that category of consumers.

24) As well as considering the potential severity of illness that particular STEC strains detected in food may cause, it is also important to consider how likely it is that STEC will be directly ingested by consumers, taking into account the intended use of the food. As different types of food are associated with different levels of risk for humans to become infected by STEC, information on the final destination or intended use of the food needs to be taken into account in the exposure part of the risk assessment. The effectiveness of interventions by consumers and FBOs such as caterers to eliminate or reduce risk to an acceptable level will need to be considered (e.g. the washing of fresh produce or the cooking of raw food) and whether handling practices may lead to a risk of cross-contamination.

25) The EC is currently drafting a guidance document which aims to assist competent authorities of MS when they are confronted with food with positive STEC results. The draft guidance document emphasizes that it does not intend to provide guidance on how STEC surveillance or monitoring should be conducted by MS or FBOs.

26) An approach proposed in the draft EC guidance is that when the laboratory results have confirmed the presence of the hazard (i.e. presence in an isolated *E. coli* strain of an *stx* gene), the contaminated food may be classified, for the ease of convenience, according to two risk profiles: **food profile 1** and **food profile 2**.

27) **Food profile 1** would include contaminated RTE (food category "a") or non-RTE food frequently or usually consumed without a sufficient treatment able to eliminate or reduce to an acceptable level the risk of infection by STEC (food category "b"). In order to help classify food in this latter category, the domestic consumption habits in the particular MS should be taken into account (e.g. minced beef steak is often consumed undercooked or even rare in certain MS). Food profile 1 should be considered as the riskiest food as regards the possibility of human infection.

28) **Food profile 2** would include only contaminated food very likely to be consumed with the appropriate treatment able to eliminate or reduce to an acceptable level the risk of infection by STEC (e.g. food intended to be thoroughly cooked before consumption) and for which clear information is provided to the consumers, including information on the label, and possible other information generally available to consumers concerning the avoidance of specific adverse health effects from a particular food or category of foods (food category "c").

29) The draft EC guidance acknowledges that under certain circumstances, risk assessors may be confronted with contaminated food which is not yet at retail level and has different intended end uses (e.g. beef carcasses). In this case, risk assessors cannot easily classify it in one of the two risk profiles and should base their classification decision on the FBO's ability to demonstrate, to the satisfaction of the competent authority, that the concerned product will be correctly labelled to inform successive FBOs and final consumers that thorough cooking is needed before consumption. If this FBO's capacity cannot be demonstrated, the food concerned should be considered as a food with a risk profile 1 following a precautionary approach.

30) The approach proposed in the draft EC guidance is therefore that:

- Food with a **risk profile 1**, should be considered as the riskiest category as regards the possibility of human infection and should be considered unsafe (and corrective actions triggered) as soon as the hazard (i.e. the presence of *stx* in an isolated *E. coli* strain) has been confirmed.

- For food with a **risk profile 2**, only the detection of STEC strains belonging to the serogroups most frequently associated with severe

illnesses (i.e. O157, O26, O103, O145, O111, O104) with the relevant virulence markers should be considered unsafe (and corrective actions be triggered) because of the risk of subsequent cross-contamination to RTE foods at retail or at the domestic kitchen level.

31) According to the draft EC guidance, the corrective action would eliminate or reduce to an acceptable level the risk of infection by STEC, such as withdrawal/recall from the market or submitting the product for further processing by a treatment sufficient to eliminate the STEC hazard (e.g. an appropriate heat treatment).

32) Flow charts summarising the approach proposed in the draft EC guidance are shown in Annexes 8 and 9.

33) The FSA's current view is that the confirmed presence of STEC in RTE food (i.e. *stx* in an isolated *E.coli* strain) is an unacceptable risk to public health and that it is appropriate to take action to remove contaminated food from the market. This would also apply to food that would not receive sufficient treatment to eliminate STEC. It is recognised that not all STEC strains will be pathogenic but the uncertainty in the evidence base, in particular the plasticity of the genome and the potential for the unpredictable appearance of new pathogenic strains, justifies a precautionary approach.

34) For foods in profile 2, the guidance suggests that interventions would be appropriate following the detection of strains of particular serogroups with specific virulence markers. The FSA has supported this approach but recognises that based on available evidence it may be difficult to assess the impact on the public health risk of the interventions proposed in the guidance. It is also unclear whether currently available evidence allows an accurate assessment of the public health risk associated with presence of STEC in foods in profile 2 and particularly the ability of hygiene controls applied by consumers and FBOs such as caterers to reduce the risks to an acceptable degree.

35) The Committee should be aware that whilst this paper aims to cover the key points and information relating to STEC in food, additional data sources may be available.

NEXT STEPS

36) The Committee is invited to assess the strength of the evidence relating to the risks associated with STEC in food. In particular we would like to seek the Committee's views on the following:

    1) Whether it is appropriate to consider the presence of *stx* in an isolated *E. coli* strain ("presence of STEC") in RTE food (and foods that will not receive sufficient treatment to eliminate STEC) to present an unacceptable risk to health.

2) If there is sufficient evidence to determine whether for food in profile 2, the presence of *stx* in an isolated *E. coli* strain of serogroup O157, O26, O103, O145, O111, O104 with [1] *eae* or [2] *aaiC* and *aggR* presents an unacceptable risk to health particularly taking into account control measures by consumers and FBOs such as caterers.

3) Confirmation of an isolated *E. coli* strain in food samples that are positive for *stx* can involve the practical issues outlined in paragraph 20. If analytical results are only available for the genetic results without confirming their presence in an isolated *E. coli* strain, would the Committee consider it possible to assess the potential risk to public health?

37) If there are any areas arising from these questions which the Committee feels they are currently not in a position to answer, then consideration could be given to establishing a short life working group to address them in more detail including reflection on what further information might be needed.

Secretariat
October 2015

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Annex 1

Reported cases and notification rates per 100,000 of human STEC infections in the EU/EEA, 2009–2013

	2013			2012		2011		2010		2009	
	Total Cases	Confirmed Cases & Rates		Confirmed Cases & Rates		Confirmed Cases & Rates		Confirmed Cases & Rates		Confirmed Cases & Rates	
		Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Austria	130	130	1.54	130	1.55	120	1.43	88	1.05	91	1.09
Belgium[(b)]	117	117	-	105	-	100	-	84	-	96	-
Bulgaria	1	1	0.01	0	0.00	1	0.01	0	0.00	0	0.00
Croatia[(c)]	2	-	-	-	-	-	-	-	-	-	-
Cyprus	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Czech Republic[(d)]	17	17	0.16	9	0.09	7	0.07	-	-	-	-
Denmark	199	191	3.41	199	3.57	215	3.87	178	3.22	160	2.90
Estonia	8	8	0.61	3	0.23	4	0.30	5	0.38	4	0.30
Finland	98	98	1.81	32	0.59	27	0.50	20	0.37	29	0.54
France[(e)]	218	218	-	208	-	221	-	103	-	93	-
Germany	1673	1639	2.00	1573	1.93	5558	6.82	955	1.17	887	1.08
Greece	2	2	0.02	0	0.00	1	0.01	1	0.01	0	0.00
Hungary	13	13	0.13	3	0.03	11	0.11	7	0.07	1	0.01
Ireland	581	564	12.29	412	8.99	275	6.02	197	4.33	237	5.24
Italy[(b)]	70	65	-	50	-	51	-	33	-	51	-
Latvia	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Lithuania	6	6	0.20	2	0.07	0	0.00	1	0.03	0	0.00
Luxembourg	10	10	1.86	21	4.00	14	2.74	7	1.39	5	1.01
Malta	2	2	0.48	1	0.24	2	0.48	1	0.24	8	1.95
Netherlands	1184	1184	7.06	1049	6.27	845	5.07	478	2.88	314	1.91
Poland	8	5	0.01	3	0.01	5	0.01	4	0.01	0	0.00
Portugal[(f)]	-	-	-	-	-	-	-	-	-	-	-
Romania	6	6	0.03	1	0.01	2	0.01	2	0.01	0	0.00
Slovakia	7	7	0.13	9	0.17	5	0.09	10	0.19	14	0.26
Slovenia	17	17	0.83	29	1.41	25	1.22	20	0.98	12	0.59
Spain	28	28	0.06	32	0.07	20	0.04	18	0.04	14	0.03
Sweden	551	551	5.77	472	4.98	477	5.07	334	3.58	228	2.46
United Kingdom	**1164**	**1164**	**1.82**	**1337**	**2.11**	**1501**	**2.40**	**1110**	**1.79**	**1336**	**2.17**
EU Total	**6112**	**6043**	**1.59**	**5680**	**1.50**	**9487**	**2.58**	**3656**	**1.00**	**3580**	**0.98**
Iceland	3	3	0.93	1	0.31	2	0.63	2	0.63	8	2.51
Liechtenstein	-	-	-	-	-	-	-	-	-	-	-
Norway	103	103	2.04	75	1.50	47	0.96	52	1.07	108	2.25
Switzerland[(g)]	80	80	1.00	63	0.79	76	0.97	34	0.44	58	0.75

(a): Y: Yes; N: No; A: Aggregated data; C: Case-based data;-: No report.
(b): Sentinel surveillance; no information on estimated coverage. Thus notification rate cannot be estimated.

(c) : All cases of unknown case classification.

(d): Mandatory notification of VTEC in 2008 and reported to ECDC from 2011.

(e): Sentinel surveillance; only cases with HUS are notified.

(f): No surveillance system.
(g): Switzerland provided data directly to EFSA.

Source: European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015).

Annex 2

Reported hospitalisation and case-fatality rates due to zoonoses in confirmed human cases in the EU, 2013

Disease	Number of confirmed[a] human cases	Hospitalisation				Deaths			
		Confirmed cases covered[a],[b] (%)	Number of reporting MS[c]	Reported hospitalised cases	Hospitalisation rate (%)	Confirmed cases covered[a],[b] (%)	Number of reporting MS[c]	Reported deaths	Case-fatality rate (%)
Campylobacteriosis	214,779	12.7	13	11,922	43.6	52.9	14	56	0.05
Salmonellosis	82,694	26.4	12	7,841	36.0	49.6	14	59	0.14
Yersiniosis	6,471	15.3	12	481	48.4	62.4	14	2	0.05
VTEC infections	6,043	41.1	16	922	37.1	59.3	18	13	0.36
Listeriosis	1,763	42.1	15	735	99.1	69.7	19	191	15.6
Echinococcosis	794	22.7	12	127	70.6	28.5	13	2	0.88
Q fever	648	NA	NA	NA	NA	51.2	11	2	0.61
Brucellosis	357	55.2	9	139	70.6	28.3	11	1	0.99
Tularaemia	279	26.9	8	39	52.0	46.2	9	0	0
West Nile fever[a]	250	20.8	3	52	91.7	90.8	6	16	3.4
Trichinellosis	217	74.7	7	106	65.4	82.5	8	1	0.56
Rabies	1	100	1	1	100	100	1	1	100

NA: not applicable as the information is not collected for this disease.
(a): For West Nile fever the total number of cases were included.
(b): The proportion (%) of confirmed cases for which the information on hospitalisation or death was available.
(c): Not all countries observed cases for all diseases.

Source: European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015).

Annex 3

Distribution of reported confirmed cases of human VTEC infections in the EU/EEA, 2011–2013, by the 20 most frequent serogroups in 2013

Serogroup	2011			2012			2013		
	Cases	MS	%	Cases	MS	%	Cases	MS	%
O157	2201	21	41.0	1981	19	54.9	1799	23	48.1
O26	289	17	5.4	417	17	11.6	477	17	12.8
O103	808	12	15.0	231	13	6.4	160	12	4.3
NT (non typeable)	148	15	2.8	136	11	3.8	298	10	8.0
O145	80	12	1.5	112	11	3.1	96	11	2.6
O91	116	8	2.2	131	8	3.6	94	11	2.5
O111	52	9	1.0	66	10	1.8	78	13	2.1
O146	48	8	0.9	59	9	1.6	75	9	2.0
O128	54	9	1.0	37	8	1.0	41	8	1.1
Orough	28	4	0.5	24	5	0.7	41	5	1.1
Non-O157	16	1	0.3	21	3	0.6	36	3	1.0
O57	0	0	0.0	0	0	0.0	29	1	0.8
O113	34	8	0.6	24	8	0.7	27	6	0.7
O117	17	5	0.3	22	6	0.6	24	8	0.6
O121	27	7	0.5	27	4	0.7	23	7	0.6
O177	18	5	0.3	4	3	0.1	22	7	0.6
O76	21	6	0.4	22	7	0.6	20	9	0.5
O63	26	2	0.5	12	2	0.3	18	3	0.5
O182	1	1	0.0	1	1	0.0	15	5	0.4
O5	22	5	0.4	7	4	0.2	15	5	0.4
Other	1363	-	25.4	274	-	7.6	350	-	9.4
Total	**5369**	**24**	**100.0**	**3608**	**22**	**100.0**	**3738**	**24**	**100.0**

Source: 22 MS and two non-MS: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom.

Source: European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015).

Annex 4

Virulence characteristics of reported confirmed VTEC cases in 2007-2010 including all cases, hospitalised cases only and haemolytic uraemic syndrome (HUS) cases only (based on TESSy data as provided by ECDC)

Cases	Virulence[a] characteristics					
	eae,*vtx*1	*eae*,*vtx*2	*eae*,*vtx*1,*vtx*2	*vtx*1	*vtx*2	*vtx*1,*vtx*2
All[b] (n = 7 278)	612 (8.4)	4 254 (58.5)	1 642 (22.6)	295 (4.1)	287 (3.9)	188 (2.6)
Hospitalised[b],[c] (n = 313)	22 (7.0)	185 (59.1)	85 (27.2)	4 (1.3)	10 (3.2)	7 (2.2)
HUS[b] (n = 371)	10 (2.7)	294 (79.2)	37 (10.0)	2 (0.5)	24 (6.5)	4 (1.1)

(a): *eae* = intimin-coding gene, *vtx*1 = verocytotoxin 1 gene, *vtx*2 = verocytotoxin 2 gene.
(b): The percentage (between brackets) is calculated using the corresponding total number of cases (either 7 218, 313 or 371) as denominator.
(c): Data on hospitalisation have only been collected for the last two years (2009 and 2010).

Source: EFSA Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA 2013a).

Annex 5

Shiga-toxin types and development of HUS among non-O157 cases in England: 2009-2013

Shiga toxin type or subtype	*eae*+				*eae*−			
	No. of isolates*	No. of isolates associated with bloody diarrhoea (%)	No. isolates of associated with hospitalization (%)	No of isolates associated with HUS (%)†	No. of isolates*	No. of isolates associated with bloody diarrhoea (%)	No. of isolates associated with hospitalization (%)	No of isolates associated with HUS (%)†
stx1 only	11	6 (54.5)	2 (18.2)	0	11	3 (27.3)	1 (9.1)	0
stx2a	32	17 (53.1)	21 (65.6)	17 (53.1)	7	6 (100.0)	4 (66.7)	3 (50.0)
stx2a, stx2c	4	2 (50.0)	2 (50.0)	1 (25.0)	1	0	0	0
stx2b	0	0	0	0	10	1 (10.0)	0	0
stx2c	0	0	0	0	1	1 (100.0)	0	0
stx2d	0	0	0	0	1	0	0	0
stx2g	0	0	0	0	1	0	0	0
stx2, no *stx2* subtype	3	1 (33.3 %)	0	0	6	2 (33.3)	1 (16.7)	1 (16.7)
Total	50	26 (52.0)	24 (48.0)	18 (36.0)	38	13 (34.2)	6 (15.8)	4 (10.5)

*Excludes nine isolates detected in the infectious intestinal disease II study (Tam *et al.* 2012) as these clinical data are unavailable.
†Two HUS cases had multiple strains of non-O157 isolated.

Source: Byrne *et al* (2014)

Annex 6

Proposed [(a)] molecular approach for the categorisation of STEC (*stx* present)

Group	Genes[(b)]	Serogroups	Potential risk[(c)]	
			Diarrhoea	HUS/HC[(d)]
I	*eae*-positive or (*aaiC* and *aggR*)-positive	O157, O26, O103, O145, O111, O104	High	High
II	*eae*-positive or (*aaiC* and *aggR*)-positive	Any other	High	Unknown
III	*eae*-negative and (*aaiC* plus *aggR*)-negative	Any other	Unknown	Unknown

(a): As yet this proposed molecular approach must be regarded as provisional. This is because screening VTEC for the presence of *eae*, *aaiC* and *aggR* genes is not routinely undertaken by all laboratories reporting data to TESSy.
(b): Additional to the presence of *vtx* genes. *eae* = intimin-coding gene, *aaiC* = chromosomally-encoded gene encoding secreted protein of EAEC, *aggR* = plasmid-encoded regulator gene.
(c): Needs epidemiological studies for confirmation.
(d): HUS = haemolytic uraemic syndrome, HC = haemorrhagic colitis.

STEC strains falling under group I should be regarded as representing a higher risk.

For STEC that would fall under group II there is still uncertainty whether or not they are able to cause HUS due to as yet unknown additional virulence mechanisms.

For STEC that would fall under group III there is uncertainty whether or not they are able to cause disease and we are unable to make a scientific judgement based on current knowledge of virulence characteristics.

Source: EFSA Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA 2013a).

Annex 7

Information on STEC in food and animals from the European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 (EU 2015)

Data on STEC detected in food and animals are reported annually on a mandatory basis by EU Member States to the EC and EFSA, based on Directive 2003/99/EC. The data are published in the annual 'Summary Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks'. To improve the quality of the data from STEC monitoring in the EU, EFSA issued technical specifications for the monitoring and reporting of STEC in animals and food in 2009 (EFSA, 2009). These guidelines were developed to facilitate the generation of data which would enable a more thorough analysis of STEC in food and animals in the future. The specifications encourage MS to monitor and report data on serogroups defined by the BIOHAZ Panel as the most important regarding human pathogenicity. Only results for the most important animal species and foods that might serve as a source for human infection in the EU are presented in the annual reports.

The following paragraphs on STEC in food and animals provide the key findings from the 2013 report (EU 2015):

STEC serogroup O157 was primarily detected in ruminants (cattle, sheep and goats) and meat thereof. The proportion of STEC found in sheep and goats, and ovine meat reported by the MS was higher than the proportion found in cattle and in bovine meat, although only few MS provided data. The main reported STEC serogroups in food were O157, O26, O103, O121 and O55. The human pathogenic STEC serogroups isolated from the bovine meat and cattle samples included STEC O157, O26, O87, O103 and O113, whereas O145 and O111 were also detected from milk samples. In 2013, more than twenty different serogroups were reported from cattle, and the most frequently reported were; O157, O26, O174, O103, O91, O185 and O22. Besides serogroup O157, a range of serogroups were detected in sheep: O76, O146, O113, O103: O112, O121, O149 and others.

STEC in food

Bovine meat and unpasteurized (raw) milk

Contaminated bovine meat is considered to be a major source of food-borne VTEC infections in humans. In 2013, twelve MS reported data on STEC in fresh bovine meat; all from surveillance and monitoring programmes. A total of 3,898 samples (all single) were tested, and of these low proportions, respectively, 2.5 % and 1.3 % were positive for STEC and for STEC O157. Positive findings of serogroup O103 (Belgium and Slovenia), O26 (France), O87 and O113 (both Germany) in bovine meat were also reported. MS reported STEC information by sampling stage (slaughterhouse, processing plant and retail) and those were low to very low for STEC and for STEC O157.

Nine MS tested 860 raw milk samples from bovine animals intended for direct human consumption and 2.3 % were STEC-positive. In addition to three of the serogroups reported from bovine meat (O157, O103 and O26), O145 and O111 were also

detected in milk samples. Eight MS also tested STEC in non-raw milk and non-raw dairy products such as cheeses, and low to very low proportions, respectively 5.0 % and 0.2 % were positive for STEC and for STEC O157.

Ovine meat
Four MS tested in total 67 fresh ovine meat samples and eight (11.9 %) and two (3.0 %) samples tested positive for STEC and STEC O157, respectively. The Netherlands tested 34 samples from retail and found six (17.7 %) to be positive (all non-O157), and Spain tested eight samples and found one (O157) to be positive. Austria and Italy found no STEC-positive samples.

Pig meat
In total, six MS reported testing of 447 fresh pig meat samples from processing plant, retail and slaughterhouse, with no positive findings of VTEC.

Vegetables and sprouted seeds
In 2013, ten MS reported data on STEC in vegetables. In total, 1,895 samples were tested. Only three samples were STEC-positive (0.2 %); Ireland and Slovakia found one O157 positive sample each. Eight MS reported investigations of RTE sprouted seed with no positive findings.

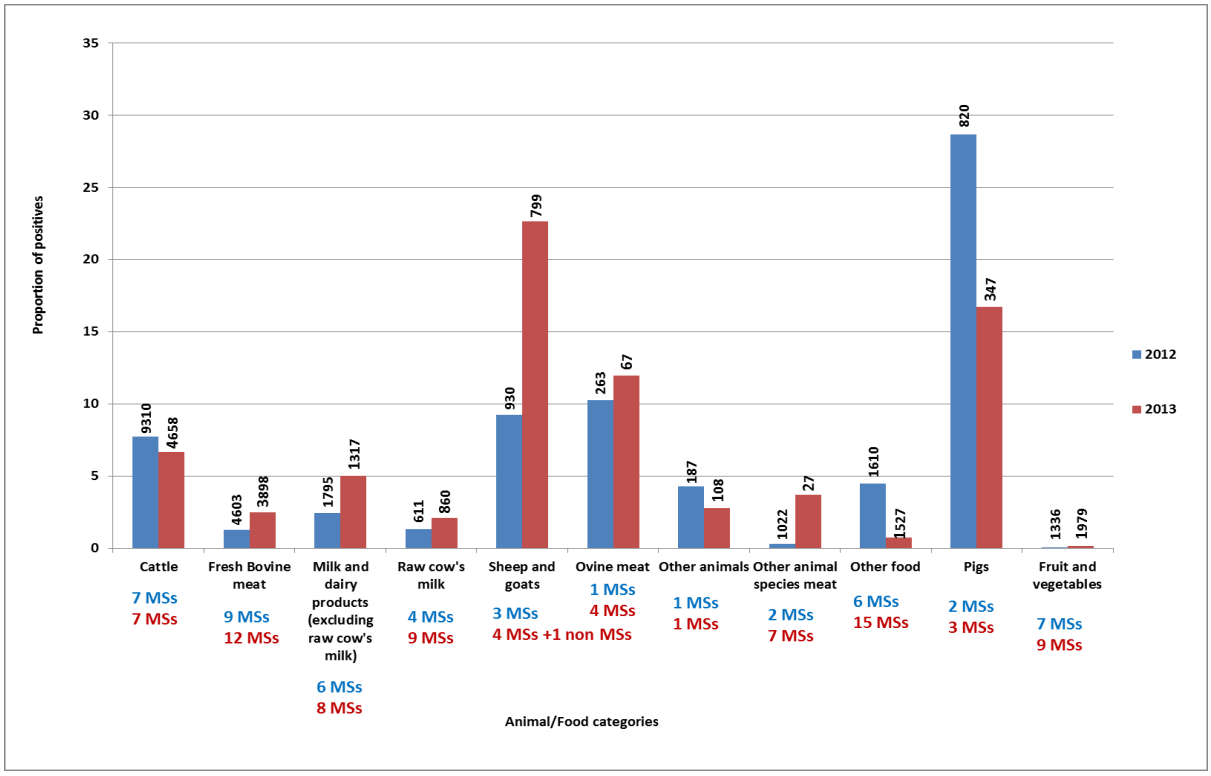
VTEC serogroups in food
In total, 12 MSs provided information on STEC serogroups in 271 isolates. The most frequently reported serogroup was VTEC O157 (49.5 %) and these mainly originated from meat from bovine animals (42.5 %) (fresh meat, minced meat, meat preparations and meat products), meat from pigs (14.9 %) (minced meat, meat preparations and meat products) and mixed meat (13.4 %).

The second most reported serogroup was VTEC O145 (7.8 %), which was mainly detected in cheese made from unspecified milk (57.1 %) and milk from cows (28.6 %). Serogroup STEC O103 was mainly reported from bovine meat and cow's milk, and serogroup O26 was mainly reported from cheese made from unspecified milk. Other reported serogroups were VTEC O15, O113, O2, O22, O78, O136, O146, O76, O87 and O178. Non-VTEC O157 was reported in 21.4 % of the isolates.

STEC in animals
In 2013, 12 MS and one non-MS provided data on STEC in animals and the results are provided below.

Cattle
In total, 4,658 samples from farms and slaughterhouses were tested. The overall proportion of positive STEC units found in cattle was low as in 2012 (see Figure 1). In total, in 2013, 6.7 % of the units tested positive for STEC, 4.3 % were positive for non-O157 and 1.4 % was positive for STEC O157. In 2013, more than twenty different serogroups were reported from cattle, where the most frequently reported were O157 (96), O26 (12), O174 (8), O103 (7), O91 (5), O185 (3) and O22 (3).

Figure 1: Proportion of STEC positive samples in animal/food categories in Member States and non-Member States, 2012-2013



Other animals: cats, dogs and *Gallus gallus* (laying hens).
Other meat: meat from pigs and poultry.
Other food: sprouted seed, live bivalve molluscs, juice, other food, spices, herbs and other processed dishes, ready-to-eat food.
Source 2012: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Romania, Slovenia, Spain, and Sweden.
Source 2013: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Netherlands, Norway, Poland, Slovakia and Spain.

Pigs

In 2013, three countries reported data for pigs (Germany, Italy and the Netherlands), but only two of them found STEC-positive results: the Netherlands (15.8 % positive pens) and Germany (23.0 % positive holdings and 17.0 % positive animals). The overall proportion of STEC-positive units was 16.7 % (Figure 1). No positive samples for the O157 serogroup were reported and no further serogroup information was reported. In 2012 the overall proportion of STEC-positive units was 28.7 % (Figure 1).

Sheep and goats

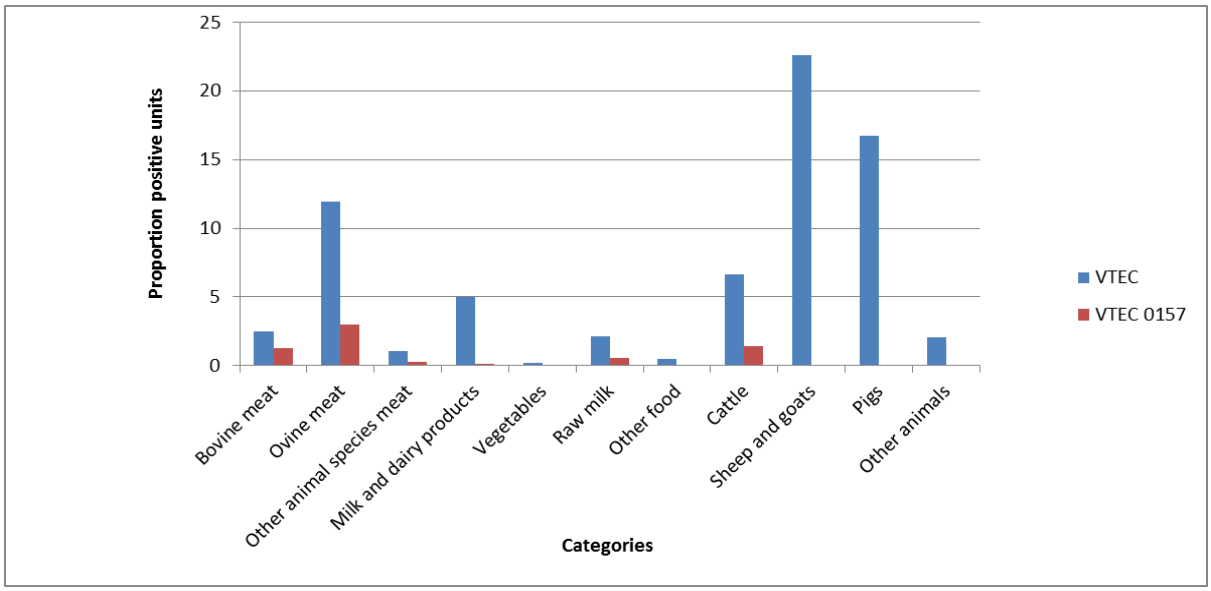
In 2013, in total 799 units were tested and 22.7 % were positive for STEC (none were O157-positive). In 2012, the proportion of positive STEC units was 9.3 %. Extremely high (above 70 %) non-O157 STEC-positive proportions in animals were

reported in 2013 by the Netherlands in sheep and by Germany in goats. Besides serogroup O157, a range of serogroups were detected in sheep: O76, O146, O113, O103: O112, O121, O149 and others.

VTEC serogroups in animals

As the serotype most commonly reported in human cases in the EU is STEC O15, the focus of the MS's surveillance programmes traditionally been on this serotype. In 2013, STEC O157 was most commonly detected in ruminants and meat products thereof (Figure 2).

Figure 2: Proportion of STEC and STEC O157 positive samples in all food/animal categories in Member States and non-Member States, 2013



Other animal species meat: broilers, deer, goats, horses, other animal species unspecified, pigs, poultry, rabbits, turkeys and wild boars. Other food: bakery products, beverages non-alcoholic, cereals, crustaceans, egg and egg products, fish and fishery products, mixed red meat, infant formulae, juice, live bivalve molluscs, molluscan shellfish, mushrooms, nuts and nut products, other food unspecified, processed food and prepared dishes, ready-to-eat salads, sauces and dressings, snails, soups, spices and herbs, water. Milk and dairy products exclude raw milk.

Source 2013: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Netherlands, Norway, Poland, Slovakia and Spain.

In total 13 and 1 non-MSs provided information on STEC serogroups in 377 isolates. The reported STEC isolates, where detailed information was provided on serogroups, originated mainly from cattle and from sheep (173 and 115 isolates, respectively). The most frequent reported serogroup in the reported isolates was STEC O157 (25.1 %), and the majority of the isolates was detected in cattle (98.1 %). Other main serogroups reported from cattle was O26 (11 isolates), O174 (8 isolates), O103 (5 isolates), O91 (5 isolates) and O185 (3 isolates).

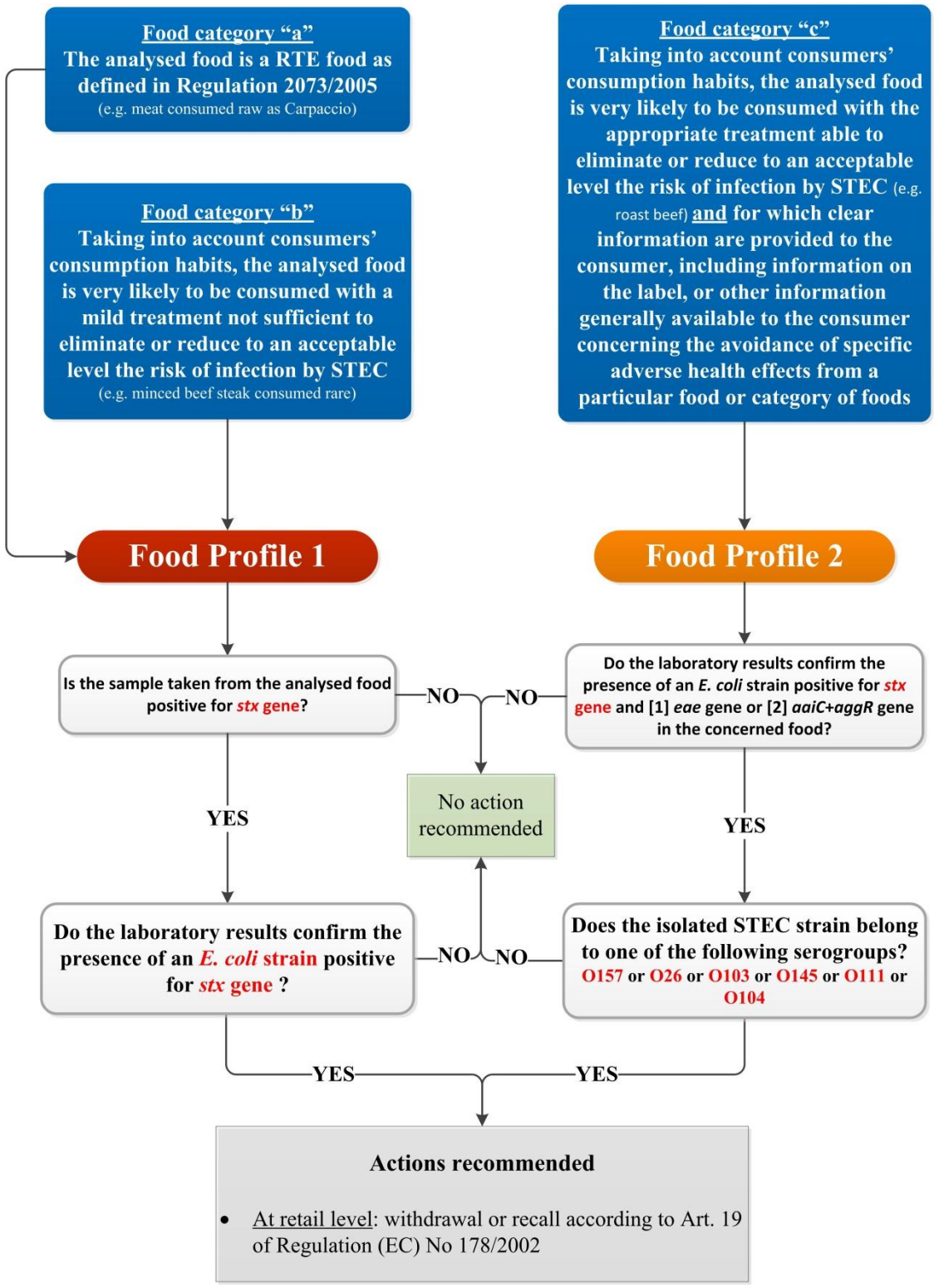
The distribution of serogroups reported from sheep was more diverse; the most frequent serogroups were O145 and O146 (17 isolates each), O5 (14 isolates), O76 and O87 (11 isolates each). Other main findings in sheep were serogroups O166 (8), O113 (7), O75 (4), O91, O128 and O174 (3 each).

Information on serogroups was provided on 48 pig isolates mainly reported by the Netherlands (60.4 %) and Latvia (31.3 %). All isolates were reported as non-O157 with no further information on the serogroup.

Annex 8

Approach proposed in draft EC guidance on the application of article 14 of regulation 178/2002 as regards food contaminated with STEC

Flowchart 1: recommendations for a harmonized application of Article 14 of Regulation (EC) No 178/2002 as regards analysed foodstuffs contaminated by Shiga toxin producing E. coli sampled at retail level.



Annex 9

Approach proposed in draft EC guidance on the application of article 14 of regulation 178/2002 as regards food contaminated with STEC

Flowchart 2: recommendations for a harmonized application of Article 14 of Regulation (EC) No 178/2002 as regards analysed foodstuffs contaminated by Shiga toxin producing E. coli sampled along the food chain (except at retail level).