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Shiga toxin-producing *Escherichia coli* (STEC) in meat and leafy greens available in the Swedish retail market – Occurrence and diversity of *stx* subtypes and serotypes

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) is a major cause of foodborne illness, ranging from mild diarrhea to permanent kidney failure. This study summarizes the results of four surveys performed at different time periods, which investigated the occurrence and characteristics of STEC in beef, lamb and leafy greens available in the Swedish retail market. Such data is required when assessing the public health risk of varying types of STEC in different foods, and for establishing risk management measures. Samples from domestic and imported products were collected based on their availability in the retail market. The occurrence of STEC was investigated in 477 samples of beef, 330 samples of lamb and 630 samples of leafy greens. The detection of virulence genes (stx_1, stx_2, *eae*) was performed using real-time PCR followed by the isolation of bacteria from *stx*-positive enriched samples using immunomagnetic separation or an immunoblotting method. All STEC isolated from the food samples was further characterised in terms of *stx* subtyping and serotyping through whole genome sequencing. STEC was isolated from 2 to 14 % of beef samples and 20 to 61 % of lamb samples, depending on the region of origin. STEC was not isolated from samples of leafy greens, although *stx* genes were detected in 11 (2 %) of the samples tested. In total, 5 of the 151 sequenced STEC isolates from meat contained *stx2* and *eae*, of which 4 such combinations had the stx_{2a} subtype. The stx_2 gene, stx_{2a} in particular, is strongly associated with serious disease in humans, especially in combination with the *eae* gene. The isolates belonged to 20 different serotypes. Two isolates from beef and one from lamb belonged to the serotype O157:H7 and contained genes for stx_2 and *eae*. Overall, several combinations of *stx* subtypes were found in isolates from beef, whereas stx_{1c}, either alone or together with stx_{2b}, was the dominant combination found in STEC from lamb. In conclusion, STEC was rare in whole meat samples of domestic beef in the Swedish retail market, whereas such bacteria were frequently found in minced meat and whole meat samples of imported beef and domestic and imported lamb. Although the number of isolates containing genes linked to an increased risk of severe disease was low, beef and lamb in the Swedish retail market is a common source of human exposure to potentially pathogenic STEC.

1. Introduction

Infection by Shiga toxin-producing *E. coli* (STEC) represents a significant health problem because it can cause severe disease in humans and can be especially dangerous for small children. STEC is a zoonotic gastrointestinal pathogen capable of causing mild to severe diarrhea with a risk of potentially life-threatening complications, including haemolytic-uraemic syndrome (HUS) (EFSA, 2020; FAO and WHO, 2019; Tozzoli and Scheutz, 2014). Cattle are regarded as the main reservoir of STEC, but other ruminants, such as sheep and goats, are recognized as notable contributors to the dissemination of STEC, though to a lesser extent. STEC can colonize the gut in ruminants asymptomatically (Söderlund et al., 2012) and may be transmitted to humans through consumption of contaminated food or water, via cattle manure, through direct contact with animals, from person-to-person contact, and from the use of contaminated recreational water. Measures to control the contamination of food with STEC include the application of good agricultural practice, good hygiene and good production practices along the food chain (FAO and WHO, 2019, 2022). The main virulence factor for STEC is the production of Shiga toxins (Stx; encoded by *stx* genes), which has two major forms, Stx1 and Stx2. They are divided into various subtypes based on a standardized taxonomy, with novel, additional

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subtypes recently reported (EFSA, 2020; Gill et al., 2022). Some subtypes, especially Stx2a in combination with an adhesin protein (intimin; encoded by the *eae* gene), are strongly linked to severe illness such as bloody diarrhea and HUS. The most common STEC serogroup associated with human illness is O157, and the most common non-O157 serogroups associated with human illness in Europe include O26, O103, O91, O146 and O145. However, all strains are considered to have the potential to cause disease and pose a health risk (EFSA, 2020; FAO and WHO, 2019). Foods that have been implicated in outbreaks are often foods of animal origin (meat, milk, milk products), but also drinking water or vegetables (sprouts, lettuce). In some outbreaks with fresh produce, the origin of contamination was suspected to be contaminated irrigation water and access by farm animals to the immediate cultivation environment. In most outbreaks, however, the dissemination route remains unknown (FAO and WHO, 2019; Kintz et al., 2019; Tack et al., 2021).

Data on the occurrence and characteristics of STEC is scarce for both domestic and imported foods in the Swedish retail market, and data in other countries is limited and usually only focuses on the presence of serogroup O157 (EFSA and ECDC, 2022). Such data is required when assessing and managing public health risks from STEC in food. The aim of the present study was to summarize the results of four surveys performed at different time periods, which investigated the occurrence and characteristics of STEC in domestic and imported beef, lamb and leafy greens products available in the Swedish retail market.

2. Material and methods

2.1. Sampling

2.1.1. Beef and lamb

Sampling of beef and lamb available at retail in Sweden was carried out in three different sampling periods (Table 1), with the intention of distributing samples of the imported meat in relation to the respective import volume of each type of meat based on statistics from the Swedish Board of Agriculture (2009, 2017). Samples of fresh meat from cattle or sheep were collected at retail by official inspectors or personnel at the Swedish Food Agency or the National Veterinary Institute (SVA). Beef samples were collected at stores and outlets in ten major cities in Sweden and samples of lamb were collected in the fourth largest city (Uppsala) from retail chains accounting for 94 % of the retail market share in Sweden (DELFI et al., 2017). Samples of domestic meat and meat imported from other EU-countries and countries outside of the EU were collected based on their availability at retail during the respective sampling period (Table 1). Samples of whole meat (sirloin, tenderloin, entrecôte, etc) and minced meat were collected chilled or frozen, except in the case of Swedish beef, which was collected as chilled whole meat. Each sample represented a unique batch or single production date.

2.1.2. Leafy greens

Samples of fresh leafy greens (lettuce, spinach, and cabbage) from which the leaves are eaten, including lettuce mixtures, were collected at retail stores, outlets and markets in ten major cities in Sweden by official inspectors or personnel from the Swedish Food Agency. The collection of domestic and imported products was based on their availability at retail during the sampling period (Table 1). Samplers were encouraged to sample the same types of product on multiple occasions to capture seasonal variation. Samples were collected unwrapped or packaged in air or a modified atmosphere. Each sample obtained from a packaged product represented a unique batch or single production date. Potted products and composite foods, such as lunch salads, were not included.

2.2. Sample preparation for detection of STEC

All analyses of the different surveys were performed at the Swedish Food Agency, except for the sample preparation for the domestic beef, which was performed by the SVA. A 25 g sample, from the surface of the whole meat or from the mixed minced meat, was placed aseptically in a stomacher bag with 225 mL of buffered peptone water (lamb and domestic beef) or tryptic soy broth (leafy vegetables and imported beef) and homogenized. The broths were incubated at 37 ± 1 °C (lamb and domestic beef) or 41.5 °C (leafy vegetables and imported beef) for 18–24 h. The enriched broths for the domestic beef were kept at −70 °C with 20 % glycerol until screening for Shiga toxin genes.

2.3. Screening for Shiga toxin genes and serogroups

Genomic DNA was extracted from 200 µL of enriched broth using an automated nucleic acid purification system (BioRobot EZ1, Qiagen) and an EZ1 DNA Tissue kit (Qiagen) according to the manufacturer's instructions. The extracted genomic DNA was eluted in a 100 µL elution buffer (Qiagen). For lamb and domestic beef, detection of the genes *stx*$_1$ and *stx*$_2$ was performed using the real-time PCR method described in ISO/TS 13136:2012 with some minor modifications (details can be found in Appendix A, Table A.1). For leafy greens and imported beef, detection of *stx* genes was performed using the real-time PCR method described in Nielsen and Andersen (2003) (Appendix A, Table A.1). Upon detection of one or both *stx* genes (leafy greens and imported beef only), real-time PCR for the genes of the five serogroups (O157, O26, O103, O111, and O145) was performed (Perelle et al., 2004, 2005) (Appendix A, Table A.1.). All PCR amplifications were done using a real-time PCR instrument cfx96 c1000 (BioRad) or an ABI 7500 (Applied Biosystem) and by maintaining a final volume of 20 µL, including 5 µL of DNA template. Each run included positive and negative template controls.

2.4. Isolation of STEC

2.4.1. Immunomagnetic separation

In the event of detection of *stx* genes and any of the serogroups (O157, O26, O103, O111, or O145) in samples from leafy greens and

Table 1
Description of food samples in the different surveys and the STEC and *stx* genes detected.

Food category	Region of origin	Sampling period	Samples, n	*stx* gene-positive samples, n (%)	STEC-positive samples, n (%)	STEC isolates, n
Beef	Domestic (Sweden)	2015–2016	300	7 (2)	6 (2)	6
	Imported (EU)	2010–2011	135	36 (27)	17 (13)	18
	Imported (South America)	2010–2011	42	10 (24)	6 (14)	9
Lamb	Domestic (Sweden)	2017–2018	95	54 (57)	41 (43)	42
	Imported (EU)	2017–2018	59	50 (85)	36 (61)	47
	Imported[a] (Oceania, South America)	2017–2018	149	40 (27)	30 (20)	34
Leafy greens	Domestic (Sweden)	2012–2013	147	2 (1)	0 (0)	0
	Imported (EU)	2012–2013	365	5 (1)	0 (0)	0
	Imported (Asia, Africa)	2012–2013	10	0 (0)	0 (0)	0
	Mixed origin[b] (EU)	2012–2013	108	4 (4)	0 (0)	0

[a] New Zealand ($n = 144$), Chile ($n = 5$).
[b] Mixture of ingredients originating from two or more EU-countries, including Sweden.

imported beef, an attempt to isolate STEC was performed using manual immunomagnetic separation (IMS) (Dynal Biotech) according to the manufacturer's instructions. Isolation of STEC O157 was performed directly on the enriched samples in case of positive PCR-screening to enable determination of risk management options, whereas isolation of O26, O103, O111, and O145 was performed on frozen enrichment broth. The enrichment broth was frozen with 20 % glycerol at −70 °C and thawed at room temperature prior to isolation. After IMS, the supernatant from each sample, as well as positive and negative controls, were plated onto SMAC (Difco 279100) and CT-SMAC (Difco 279100, Supplement SR 172 E) for the isolation of STEC O157, RMAC (Difco 281810, Alfa Aesar A16166) and CT-RMAC (Difco 281810, Alfa Aesar A16166, Supplement SR 172 E) for the isolation of O26, and MAC (Difco 281810) and Selective differential media based on a chromogenic compound for the isolation of O103, O111 and O145 (Possé et al., 2008).

2.4.2. Immunoblotting

In the event of detection of *stx* genes in samples from domestic beef and lamb, an attempt was made to isolate STEC by using immunoblotting, according to Atalla et al. (2000). Immunoblotting was also used for those samples from imported beef and leafy greens that were not successfully isolated by using IMS and for the remaining *stx*-positive isolates belonging to other serogroups than O157, O26, O103, O111, and O145. Immunoblotting was performed on frozen enrichment broth (20 % glycerol at −70 °C). The enriched beef samples were thawed at room temperature and those from lamb were thawed by rapidly heating the broth at 50 °C and then allowing it to stand about an hour at room temperature. In brief, the capture membrane (82 mm nitrocellulose membranes, pore size 0.2 μm; VWR International) was precoated with rabbit anti-Stx anti-bodies (2 μg/mL) and blocked with a wash buffer containing 1 % gelatin. The enrichment broth was thawed and diluted 10-fold in peptone water (Oxoid) containing 1 % NaCl (Merck). The capture membrane was positioned on TSA plates (Oxoid) containing 25 ng/mL Mitomycin C (Sigma-Aldrich). Above the capture membrane, a second uncoated membrane (82 mm cellulose acetate, pre size 0.45 μm; Satorius Group) was positioned. A volume of 100 μL from selected dilutions was spread onto the membranes and incubated at 37 °C for 18–24 h. The membranes were marked for later reorientation. The capture membrane was then removed, and the upper membrane was replaced on the TSA plates and stored at 4 °C for later use. A mixture of monoclonal antibodies for Stx1, Stx2a/c, Stx2e, and Stx2d-variants (2 μg/mL) was used for the capture membrane as the secondary antibody, followed by alkaline phosphatase-labeled rabbit anti-mouse IgG (0.1 μg/mL; Jackson Immuno-research). Subsequently, BCIP/NBT (Seracare) was used for detection. Presumptive STEC colonies from the immunoblotting were streaked on NA (Oxoid CM0003).

2.5. Characterization of STEC isolates

Presumptive STEC colonies from IMS and the immunoblotting were confirmed through real-time PCR for the *stx* genes as described above. The total DNA of the STEC isolates was extracted using an automated nucleic acid purification system (BioRobot EZ1, Qiagen) and an EZ1 DNA Tissue kit (Qiagen) according to the manufacturer's instructions. The DNA was quantified using the Qubit dsDNA kit (Thermo Fisher Scientific) and controlled for purity using NanoDrop ND1000 (Saween & Werner current Thermo Fisher Scientific). STEC isolated from domestic and imported beef was sent to the Public Health Agency of Sweden for whole genome sequencing. Ion Torrent 400 base-pair chemistry was used, together with Library Builder™ and the Ion Torrent platform (Thermo Fischer). Serotyping in silico, *stx*-subtyping and virulence gene detection (stx_1, stx_2, *eae*) of the sequenced isolates from beef were conducted by the Swedish Public Health Agency. The isolates from lamb were submitted for whole genome sequencing as paired-end 2 × 150 bp with a Nextera library preparation using an Illumina NovaSeq at the SciLifeLab Clinical Genomics facility, Solna, Sweden (www.scilifelab.se). Serotyping in silico, *stx*-subtyping and virulence gene detection (stx_1, stx_2, *eae*) of the sequenced isolates from lamb were conducted using tools on the Galaxy public server ARIES (Istituto Superiore di Sanità, www.iss.it/site/aries).

3. Results

3.1. Occurrence of STEC in meat

STEC was isolated from 6 (2.0 %) of the 300 samples from Swedish beef, 17 (13 %) of the 135 samples of beef from other EU-countries, and 6 (14 %) of the 42 samples of beef from countries outside of the EU (Table 1). STEC O157 was found in 0 (0 %), 1 (0.7 %) and 1 (2.4 %) of the corresponding samples. Three samples from imported beef contained 2 or 3 variants of STEC-isolates, resulting in a total of 33 isolates in samples from beef. The stx_1 and/or stx_2 genes were detected in 7 enrichment broth cultures from Swedish beef and 46 broths from imported beef (Table 1). The rate of isolation of the *stx*-positive samples was 86 % (6/7) and 50 % (23/46), respectively.

STEC was isolated from 41 (43 %) of the 95 samples from Swedish lamb, 36 (61 %) of the 59 samples of lamb from other EU-countries, and 30 (20 %) of the 149 samples of lamb from countries outside of the EU (Table 1). STEC O157 was found in 0 (0 %), 1 (1.7 %) and 0 (0 %) of the corresponding samples. In total, 123 STEC were isolated from lamb, because 16 of the samples contained 2 different STEC isolates. One or both *stx* genes were detected in 144 enrichment broth cultures from lamb (Table 1), generating an STEC isolation rate of 74 % (107/144) for lamb samples.

3.2. Occurrence of STEC in leafy greens

No STEC was isolated from the 630 samples of leafy greens (Table 1). A total of 11 (1.7 %) of the enriched samples tested positive for stx_1 and/or stx_2. Those 11 presumptive STEC was detected in enriched samples from domestic products and products from other EU-countries, but not in leafy greens from outside of the EU (Table 1).

3.3. Characteristics of STEC in meat

For the 33 isolates from beef, PCR-screening revealed that 1 (3 %) contained stx_1 gene, 23 (70 %) contained stx_2 gene and 9 (27 %) contained both stx_1 and stx_2 genes. Of the total of 28 isolates that were sequenced, the gene *eae* was found in 5 (18 %) isolates, 4 of which also contained stx_2; stx_{2a} and/or stx_{2c} (Table 2). The isolates contained 16 combinations of *stx* subtypes. The subtype stx_{2a} was present in various combinations in 16 (57 %) isolates, of which 3 (11 %) also contained *eae*. The subtype stx_{2d} was found in 11 isolates from beef, either alone or together with other stx_1 or stx_2 subtypes. The subtypes stx_{1c} and stx_{1d} were not found in isolates from beef. The isolates belonged to 20 different serotypes, of which O22:H8, O26:H11, O157:H7, O171:H2 and ONT:H19 were found in 2 or more isolates (Table 2). The serotype O157:H7, which was found in two isolates from beef imported from the EU or South America, contained stx_{1a} + stx_{2c} + *eae* or stx_{2a} + stx_{2c} + *eae* (Table 2). The three remaining *eae*-positive isolates that were sequenced belonged to O26:H11 and contained stx_{1a} or stx_{1a} + stx_{2a} (Table 2).

In total, 45 (37 %) of the 123 sequenced STEC isolates from lamb were positive for stx_1, 10 (8 %) were positive for stx_2 and 68 (55 %) were positive for both *stx* genes (Table 2). The gene *eae* was found in 3 (2 %) of the 123 sequenced isolates, of which 1 (1 %) was in combination with stx_2; stx_{2a} + stx_{2c}. The isolates contained 12 combinations of *stx* subtypes. The subtype stx_{2a} was present in 3 (2 %) isolates, 1 (1 %) of which also had *eae*. The most common combination of *stx* subtypes in STEC from lamb was stx_{1c} + stx_{2b} (36 %), followed by stx_{1c} (35 %) and stx_{1a} + stx_{2b} (20 %). The subtype stx_{2d} was found in one isolate from lamb. The isolates belonged to 28 different serotypes, of which O91:H14 was the most common followed by O128:H2 and O174:H8 (Table 2). The most

Table 2
Characterization of sequenced STEC-isolates.

stx subtype	*eae* gene	Number of isolates	Serotypes in lamb (n)	Serotypes in beef (n)
stx_{1a}	eae	2	O145:H28 (1)	O26:H11 (1)
stx_{1a}, stx_{2a}	eae	2		O26:H11 (2)
stx_{1a}, stx_{2a}		3		O113:H21 (1); O185:H28 (1); ONT:H25 (1)
stx_{1a}, stx_{2b}		24	O91:H14 (23)	O91:H14 (1)
stx_{1a}, stx_{2a}, stx_{2d}		1		O8:H16 (1)
stx_{1a}, stx_{2c}	eae	1		O157:H7 (1)
stx_{1a}, stx_{2d}		2		O183:H18 (1); ONT:H19 (1)
stx_{1c}	eae	1	O145:H28 (1)	
stx_{1c}		42	O174:H8 (4); O128ab/ac:H2 (1); O146:H21 (4); O76:H19 (8); O15:H27 (1); O6:H10 (5); O153/O178:H7 (5); O104:H7 (4); O166:H28 (3); O38:H26 (2); O136:H20 (2); O78:H4 (1); ONT:H16 (2)	
stx_{1c}, stx_{2b}		44	O174:H8 (9); O128ab/ac:H2 (12); O146:H21 (6); O15:H27 (4); O166:H28 (1); O38:H26 (2); O5:H19 (2); O113:H4 (1); O75:H8 (1); O76:H19 (1); O123/O186:H10 (5)	
stx_{1c}, stx_{2b}, stx_{2d}		1	O176:H4 (1)	
stx_{1d}		1	O149:H1 (1)	
stx_{2a}		6	O113:H21 (1); O130:H11 (1)	O22:H8 (2); O88:H8 (1); O179:H8 (1)
stx_{2a}, stx_{2c}	eae	2	O157:H7 (1)	O157:H7 (1)
stx_{2a}, stx_{2c}		2		ONT:H19 (1); O22:H8 (1)
stx_{2a}, stx_{2d}		3		O116:H48 (1); O163:H19 (2)
stx_{2b}		5	O87:H16 (3); ONT:H14 (2)	
stx_{2b}, stx_{2c}, stx_{2d}		1		O22:H16 (1)
stx_{2b}, stx_{2d}		1		ONT:H29 (1)
stx_{2c}		3	O174:H21 (1)	O185:H7 (1); O171:H2 (1)
stx_{2c}, stx_{2d}		1		O171:H2 (1)
stx_{2d}		2		O113:H4 (1); O174:H25 (1)
stx_{2e}		1	O8:H19 (1)	

common combination of *stx* types and serotype was stx_{1a} + stx_{2b} and O91:H14. One isolate, from lamb imported from EU, belonged to O157:H7 and harboured genes for stx_{2a}, stx_{2c} and *eae*. The two remaining *eae*-positive isolates belonged to O145:H28 and contained stx_{1a} or stx_{1c} (Table 2).

4. Discussion

The need for data on the prevalence and characteristics of STEC in food in order to assess and manage public health risks has been highlighted by the FAO and WHO (2019), and the EFSA (2020). In the present study investigating meat and leafy greens available in the Swedish retail market, STEC was rare in whole meat samples of domestic beef, whereas such bacteria were frequently found in minced meat and whole meat samples of imported beef and domestic and imported lamb. However, due to differences in methodology and sampling periods between surveys of the various foods, differences in the occurrence of STEC are indicative but do not allow for generalised conclusions. For example, domestic beef was only collected as whole meat, which may have affected the occurrence of STEC in such samples. *Salmonella* spp. was not found in any of the meat samples, neither beef nor lamb (Egervärn et al., 2014; Flink, 2019), suggesting that STEC is more common than *Salmonella* spp. in such meat at retail.

This is the first study reporting the occurrence of STEC in beef and lamb available in the Swedish retail market. The occurrence of STEC in Swedish retail beef sampled in 2015–2016 (2.0 %; only whole meat tested) was somewhat lower than the proportion of positive samples (5.3 %) obtained in a baseline study of carcasses of Swedish cattle in 2006–2007 (personal communication M. Lindblad). STEC O157, frequently implicated in STEC-related foodborne outbreaks in Sweden, was not found in the domestic beef samples tested. However, the prevalence of STEC O157 among Swedish cattle, based on faecal samples taken at slaughter, has been in the range of 2.2–3.5 % since 2005 (SVA, 2019). Besides the differences in methodology and sampling period, the relatively high occurrence of STEC in Swedish lamb (43 %) sampled in 2017–2018 may be partly explained by the differing slaughter hygiene practices for cattle and sheep. For example, carcass contamination of shorn sheep has been found to be less than that of unshorn sheep (Alvseike et al., 2019). On the other hand, STEC O157 was not present in Swedish lamb, which is in slight contrast with the result obtained in a study of faeces from Swedish slaughter sheep in 2007–2008, in which 1.8 % of the animals tested positive for STEC O157 (SVA, 2019).

The occurrence of STEC in bovine meat imported from other EU countries from 2010 to 2011 (13 %) was approximately ten times higher than the occurrence in European meat (1.4 %) reported in the EU zoonosis report 2011 (EFSA and ECDC, 2013). Similarly, the occurrence in lamb imported from other EU countries from 2017 to 2018 (61 %) was six times higher than reported in the EU during the same time period (11 %) (EFSA and ECDC, 2019). In the latest EU zoonosis reporting, the occurrence of STEC was 5.7 % in bovine meat and 10 % in lamb meat (EFSA and ECDC, 2022). However, the results are not directly comparable with the surveys of the present study because samples have been taken at different stages of the food chain and, in particular, not all member states have analysed for all serogroups. The higher occurrence obtained in the present study for both beef and lamb may be partly due to the use of immunoblotting as an analytical method of isolating *E. coli* which produces Shiga toxin and not only STEC of a certain serogroup. The use of immunoblotting is also a contributing factor to the high isolation frequency of STEC (50 % for beef samples and 74 % for lamb samples) from *stx*-positive enrichment broths. This is in contrast to previous studies, in which isolation frequencies of 20–37 % have been reported for similar meat matrices (Brusa et al., 2013; Hoang Minh et al., 2015; Toro et al., 2018). Immunoblotting is not included as an option in the ISO/TS 13136:2012, but is particularly useful if there are many samples which are collected and analysed simultaneously. In contrast, the use of IMS is not preferred because it is not available for all STEC serogroups. In addition, low recovery rates have been observed with IMS for some targeted serogroups (Hallewell et al., 2017; Kraft et al., 2017). The result on the occurrence of STEC O157 is more in line with the data from contemporary reports by EFSA and ECDC (2013, 2019); 0.7 % versus 0.3 % in beef and 1.7 % versus 0.6 % in lamb. At the time of the survey of imported beef, the most widely analytical method used internationally aimed to specifically detect and isolate STEC O157 (EFSA and ECDC, 2013). In 2021, nine years after the introduction of the ISO/TS 13136:2012 method, 3.8 % of food samples and 42 % of animal

samples were still being assessed using methods targeting only STEC O157 (EFSA and ECDC, 2022).

The proportion of STEC positive samples from beef imported from other EU countries was in the same order of magnitude as in beef imported from non-EU countries, 13 % and 14 %, respectively; South American countries imported the most beef to Sweden at the time of the survey of imported beef (Swedish Board of Agriculture, 2009). The presence of STEC in South American beef was in contrast with the results of similar surveys performed in that area, in which STEC was isolated from 23 % (Llorente et al., 2014) and 27 % (Brusa et al., 2013) of beef samples, respectively. In contrast to beef, the occurrence of STEC in lamb was lowest in samples from non-EU imported meat. The occurrence of STEC in samples from New Zealand lamb, which constituted 97 % of all such samples, was 19 % (data not shown). This is in agreement with a similar New Zealand study of domestic lamb (Brooks et al., 2001).

Lettuce and other fresh produce are important sources of contamination in STEC related outbreaks, both in Sweden and internationally (Marshall et al., 2020; SVA, 2019). In 2005, one of the largest national foodborne outbreaks was reported, in which lettuce that had been irrigated with contaminated water from a stream where grazing cattle were located upstream was the probable source (Söderström et al., 2008). In the present study, *stx* genes were detected in less than 2 % of samples from domestic and imported leafy vegetables, but no bacteria were isolated. In contrast, data from the contemporary EU zoonosis surveillance showed that STEC was present in 0.2 % of samples from vegetables (EFSA and ECDC, 2015), compared to 0.5 % in the latest update (EFSA and ECDC, 2022). As with meat, however, our results are not directly comparable to those of EFSA. Furthermore, a meta-analysis of retail vegetables and fruits since 2000 in Europe reported a 0.7–1.4 % pooled prevalence of STEC-positive samples of lettuce, salad and leafy greens (Nunes Silva et al., 2017). *Salmonella* spp. was found in four samples tested (Egervärn et al., 2014, unpublished results) and thus, unlike the beef samples, *Salmonella* spp. was more common than STEC in leafy vegetables.

The stx_2 gene, rather than stx_1, is strongly linked to serious disease in humans, especially when in combination with the *eae* gene (EFSA, 2020; FAO and WHO, 2019). Previous studies have reported that the specific subtype stx_{2a} is most consistently associated with severe disease in humans (Byrne et al., 2018; Dallman et al., 2015; FAO and WHO, 2019). In the present study, stx_2 was the dominant *stx* subtype, and 146 of the 151 sequenced isolates from beef and lamb belonged to one of the 18 variants of *stx*-combinations linked to STEC isolated from humans in Sweden in 2020 (Swedish Public Health Agency, 2021). Furthermore, five isolates from meat contained stx_2 and *eae*, of which four had the stx_{2a} subtype. However, although all but eight of the sequenced isolates from meat lacked the *eae* gene, it is well known that *eae*-negative isolates can also cause disease (EFSA, 2020; Franz et al., 2015; Otero et al., 2017).

Overall, the *stx* subtypes found in STEC isolates from beef were more heterogeneous than those found in lamb, with several different combinations of *stx* subtypes identified. The occurrence of stx_{2a}-positive STEC isolates in meat has rarely been reported previously (Varcasia et al., 2018) but is more commonly reported in cattle and sheep (Jinnerot et al., 2020; McCarthy et al., 2021; Okuno et al., 2021). The subtype stx_{2d}, which is also linked to higher risk of serious disease (FAO and WHO, 2019), also occurred in isolates from both beef and lamb, but was, like stx_{2a}, more common in beef. Similarly, other studies have isolated the subtype stx_{2d} in isolates from both cattle and sheep (Gobius et al., 2003; Tasara et al., 2008). In the present study, none of the stx_{2d}-positive isolates contained *eae*. However, such variants of STEC could still be linked to serious disease, because they have a different adhesin and, possibly, additional virulence factors besides the toxin (FAO and WHO, 2019; Melton-Celsa et al., 2015). The observed absence of stx_{1c} in isolates from beef is in agreement with a previous study of STEC in cattle or beef (Fan et al., 2019). In contrast, stx_{1c}, alone or together with stx_{2b}, was the most common combination of *stx* subtypes found in STEC in samples from lamb. The occurrence of stx_{1c}, with or without stx_{2b}, is frequently reported in STEC from sheep or derived food products (Martin and Beutin, 2011; Otero et al., 2017; Sánchez et al., 2012), as well as from wild ruminants (FAO and WHO, 2019). The stx_{1c} subtype is reported mainly in *eae*-negative strains, causing mild infections (FAO and WHO, 2019; Friedrich et al., 2003; Varcasia et al., 2018).

More than half of the 151 sequenced STEC isolates from beef and lamb belonged to one of the 67 identified serotypes isolated from human cases in Sweden during 2020, of which O26:H11 and O157:H7 were the most common serotypes (Swedish Public Health Agency, 2021). Above all, there is a strong association between STEC O157:H7 and severe complications, such as HUS, making these bacteria a particular public health concern (Söderlund et al., 2014). The most common cause of HUS in Sweden remains infection with the STEC O157:H7 variant known as clade 8, which is endemic in the southeastern part of the country (SVA, 2022). Thus, such HUS cases in Sweden are generally domestically acquired, and Swedish ruminants are considered to be the major reservoir and source of infection (Söderlund et al., 2014). In this study, we found three isolates with serotype O157:H7 from beef and lamb, which also contained stx_2 and *eae*. However, the only clade 8 isolate found, which was from beef, did not belong to the variant that is endemic in the southeast of Sweden (data not shown). Söderlund et al. (2012) have suggested that since highly similar genotypes of STEC O157:H7 is found in both cattle and sheep, pathogenic strains of O157:H7 can circulate freely between both ruminant reservoirs.

Of the 28 different serotypes found in lamb, only four were also found in beef (O157:H7, O91:H14, O113:H21 and O113:H4). Several of the serotypes found in lamb are strongly associated with sheep (O91:Hx, O128:H2, O146:H21, and O76:H19) (Barlow et al., 2006; Martin and Beutin, 2011; Otero et al., 2017; Sánchez et al., 2012). Similarly, in a survey of Australian lamb, the most common serogroups were O91 and O128 (Barlow et al., 2006). Furthermore, in a German study by Martin and Beutin (2011) the serotype O128:H2 was significantly more common in lamb than in other types of meat (pork, beef, and meat from wild boar, red deer and hare). The serotypes O146:H21 and O76:H19 were also associated with STEC from lamb, but not as strongly as O128:H2 (Martin and Beutin, 2011).

Risk classification of STEC isolated from food has been a challenge due to a lack of data that determines the ability and extent to which different subtypes of STEC can cause severe disease (Lindqvist et al., 2023; NACMCF, 2019). Thus, data on the characteristics of STEC in food, such as those obtained in the present study, is highly relevant. Previously, STEC risk classification was based on serotypes and classified STEC into seropathotypes (Karmali et al., 2003). Information on serotype could be used in epidemiological investigations and for taxonomic categorization of STEC isolates. However, according to the FAO and WHO (2019), the serotype should not be considered as a virulence criterion, because isolates with the same serotype should not be assumed to carry the same virulence genes and, thus, should not be classified at the same risk level. Instead, the risk classification is best predicted based on virulence factors, including virulence gene combinations and gene expression, as well as dose ingested and the susceptibility of the human host (EFSA, 2020; FAO and WHO, 2019; NACMCF, 2019). What is essential is that all STEC should be considered as potentially pathogenic, capable of causing diarrhea at the very least, and that all *stx* subtypes may be associated with severe illness to varying degrees (EFSA, 2020; FAO and WHO, 2019; NACMCF, 2019). An approach ranking STEC found in food according to the potential risk of severe illness and based on national data is therefore preferred. In light of this, data from the present survey has recently been used in a study by Lindqvist et al. (2023), which compared different models for classifying STEC strains detected in food in the Swedish retail market with the probability of severe clinical outcomes. The study also developed an approach for the ranking and classification of STEC strains based on their potential public health burden (Lindqvist et al., 2023).

In conclusion, STEC was rare in whole meat samples of domestic beef

in the Swedish retail market, whereas such bacteria were frequently found in minced meat and whole meat samples of imported beef and domestic and imported lamb. STEC was not isolated from samples of leafy greens. Although the number of isolates containing genes linked to an increased risk of severe disease was low, beef and lamb in the Swedish retail market is a common source of human exposure to potentially pathogenic STEC.

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CRediT authorship contribution statement

Maria Egervärn: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Catarina Flink:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A

Table A.1
Details of real-time PCR screening applied in the different surveys.

	Primers and probes		Mastermix		Internal positive control	Real-time PCR programme	Real-time PCR instrument
	stx genes	Serogroups (O26, O103, O111, O145, O157)	*stx* genes	Serogroups (O26, O103, O111, O145, O157)			
Imported beef	Nielsen and Andersen, 2003	Perelle et al., 2004 (O26, O111, O145, O157); Perelle et al., 2005 (O103)	TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems®)	TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems®)	None	95 °C 10 min; 45 cycles 95 °C 15 s, 60 °C 1 min	BioRad cfx96 c1000; ABI 7500
Domestic beef	ISO/TS 13136:2012		PerfeCTa PCR ToughMix® (Quanta Biosciences)		TaqMan® Exogenous Internal Positive Control Reagents (Applied Biosystems®)	95 °C 10 min; 45 cycles 95 °C 15 s, 60 °C 1 min	BioRad cfx96 c1000
Leafy greens	Nielsen and Andersen, 2003	Perelle et al., 2004 (O26, O111, O145, O157); Perelle et al., 2005 (O103)	PerfeCTa multiplex supermix (Quanta Biosciences)	TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems®)	None	95 °C 10 min; 45 cycles 95 °C 15 s, 60 °C 1 min	BioRad cfx96 c1000
Lamb	ISO/TS 13136:2012		PerfeCTa PCR ToughMix® (Quanta Biosciences)		TaqMan® Exogenous Internal Positive Control Reagents (Applied Biosystems®)	95 °C 10 min; 45 cycles 95 °C 15 s, 60 °C 1 min	BioRad cfx96 c1000

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