Does pork pose a higher risk of Shiga toxin-producing *Escherichia coli* compared to meat of other ungulates? A review

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Abstract

Fresh pork meat and pork products can be a vehicle for the transmission of Shiga toxin-producing *E. coli* (STEC) to humans. The aim of this review article is to provide up-to-date information on the occurrence of STEC on pig farms around the world, the level of contamination of pork meat, and the ensuing risks for humans. The prevalence of STEC in finishing pigs ranges from 0.2 to 86.3% depending on the category of sample, the detection method and the hygiene conditions at the slaughterhouse. The incidence of *stx*-positive pork samples on the retail network worldwide varies between 1.1 and 80.0%. Confirmed detection of *E. coli* serogroup O157 ranges from 1.2 to 23.2% and that of serogroup non-O157 from 0.1 to 14.7%. Most isolates from the pig-farming environment or obtained from slaughterhouses contain the stx_{2e} gene which is associated with porcine oedema disease. STEC isolates obtained from pork meat belong to the low-to-moderate risk category, though they do have the potential to cause illness in humans. The most effective prevention is the perfect cooking of meat.

Foodborne disease, virulence factors, stx genes, finishing pigs, serogroups

On November 5, 1982, the US Centers for Disease Control and Prevention (CDC) published an article on the isolation of *Escherichia coli* O157:H7 from sporadic cases of bloody diarrhoea in the USA (CDC 1982). From that year, the history of Shiga toxin-producing *E. coli* (STEC) began.

In 1982, there were 2 outbreaks in the USA (in the states of Oregon and Michigan) with serious cases of disease including haemolytic uremic syndrome (HUS) (Kim et al. 2020). Hamburgers were found to be the vehicle for the transmission of the pathogen (Wale et al. 2021). In Anglo-Saxon literature, STECs were thus nicknamed 'the burger bug' (Byrne et al. 2020). During the 30 years (1982–2012) since the first occurrence, a total of 740 outbreaks caused by STEC serotypes O157:H7 and O157:NM (NM - non-motile, i.e. without H flagella antigen) were recorded in the USA. A total of 13,526 cases of the disease were confirmed within the outbreaks, 2,765 patients (20%) required hospitalization, 653 (4.8%) developed HUS, and 73 cases (0.5%) died (Kim et al. 2020). The largest outbreak recorded so far was the outbreak in Sakai City, Japan, where, according to some sources, over 12,000 people were affected and several hundred were hospitalized (Reiland et al. 2014; Lee et al. 2021). Mostly schoolchildren were affected and the source of STEC was sprouted radish seeds (Reiland et al. 2014). The global incidence of STEC disease is estimated at approximately 2.5 million acute cases per year, of which around 50% are attributable to contaminated food or water (Soare et al. 2021). In addition to undercooked ground beef dishes (hamburgers), sausages, unpasteurized milk, salad, cantaloupe melons, and apple juice (Kaper et al. 2004) but also vegetables (Govindarajan et al. 2020) have been described as a vehicle for the transmission of STEC. Cattle are considered the primary reservoir of STEC (Ikeuchi et al. 2024). The most common cause of human infections

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with serovar O157:H7 is food or water contaminated with cattle faeces (Koudelka et al. 2018). Cattle show no clinical signs of STEC infection, although naturally infected individuals excrete 10^5–10^6 bacteria/g in their faeces; after experimental colonization, this can be as high as 10^8 bacteria/g (Smith et al. 2002). Of the 187 known *E. coli* serogroups, 158 are associated with the STEC pathotype (Remfry et al. 2021). More than 400 STEC serotypes are associated with human infections worldwide (Nong et al. 2021). STEC is a zoonotic gastrointestinal pathogen capable of causing moderate to severe diarrhoeal disease with life-threatening complications including haemolytic uremic syndrome (Egervärn and Flink 2024).

The aim of this review article was to provide up-to-date information on the occurrence of STEC on domestic pig farms around the world, the level of contamination of pork meat, and the resulting risks for humans.

Current occurrence of STEC in the EU

The EFSA recorded 6,084 confirmed human cases of STEC infection in EU member states in 2021 (EFSA 2022), with the number of recorded cases rising to 7,117 in 2022 (Table 1) (EFSA 2023). Human infection caused by STEC has thereby become the fourth most common infectious foodborne disease in the EU in recent years. Its incidence ('notification rate') reached an average of 2.1 cases per 100,000 inhabitants (EFSA 2022, 2023). Compared to the period 2017–2019 (before the Covid-19 pandemic), the incidence increased by 14.2% (after taking Brexit into account). Nevertheless, the overall trend of STEC infections showed no statistically significant increase in the period 2017–2021, though neither did it decrease (EFSA 2023). In the USA, STEC is responsible for more than 265,000 cases of foodborne diseases and more than 3,600 hospitalisations every year (Walker et al. 2023). STECs have been under the spotlight for epidemiologists since their discovery in the 1980s. This is not because of the frequency of the disease, which does not amount to the number of cases of salmonellosis or infections caused by *Campylobacter* spp., but rather due to the severity of the symptoms, the low infectious dose, and the potential consequences (Nastasijevic et al. 2021).

The highest incidence rates among EU member states were reported in Ireland (17.6/100,000), Malta (15.0/100,000) or Sweden (8.2/100,000) (EFSA 2023). At the same time, the notification rate in Ireland or Malta practically did not change in 2021 and 2022 (EFSA 2022, 2023). There were 18 deaths associated with STEC infections in the EU in 2021 (EFSA 2022), but the number of fatal cases increased to 28 in 2022 (EFSA 2023). Deaths were mostly reported in the 65+ age group (42.9%) with and without HUS and

Table 1. STEC as an agent of foodborne disease in the EU and its incidence in pigs and pork.

		2018	2019	2020	2021	2022
Number of reported cases		8,161	7,775	4,446	6,084	7,117
Notification rate per 100,000 population		2.0	1.9	1.5	2.1	2.1
Number of STEC outbreaks		48	42	34	31	71
STEC in finishing pigs	number of units sampled	22	104	85	51	NS
	% positive samples	9.1	5.8	42.4	11.8	NS
STEC in fresh pork meat	number of units sampled	330	119	91	604	444
	% positive samples	4.8	6.7	7.7	16.6	2.0
Source		(EFSA 2019)	(EFSA 2021a)	(EFSA 2021b)	(EFSA 2022)	(EFSA 2023)

STEC - Shiga toxin-producing *E. coli*; NS - not specified

followed by the 0–4 years age group (25.0%). In the youngest age group, all deaths were linked to HUS (EFSA 2023). In 2021, death occurred most often in patients in the age group 0–4 years (27.8%), followed by the age group 85 years and older (16.7%) (EFSA 2022).

Cattle are a well-known reservoir of STEC. The bacteria can colonise the distal region of the intestinal tract at the site of the recto-anal junction (Haque et al. 2022). Small ruminants (EFSA 2022; Egervärn and Flink 2024) and cloven-footed game are also an important reservoir of STEC. These well-known facts lead to increased sampling and analysis of beef meat for the presence of STEC. Of the 23,659 food units sampled for the presence of STEC in 22 EU member countries in 2021, fresh and unprocessed beef meat accounted for 5,095 sampled units, i.e. 21.5% (EFSA 2022). Products made of raw pork can also be a vehicle for the transmission of STEC to humans (Haque et al. 2024), for which reason the role played by pigs in human STEC infection requires further research directed at understanding their contribution to the spread of alimentary infections and risk mitigation (Haque et al. 2022).

STEC characteristics that pose a risk to human health

The main virulence factor of STEC is the production of Shiga toxin (Stx) encoded by *stx* genes (Table 2). Stx is one of the most potent bacterial toxins. Stx was first identified and described in the bacterium *Shigella dysenteriae* in the nineteenth century. Around 80 years later, the same toxin was found in a group of *E. coli* isolates. It was designated Stx1 to distinguish it from the Shigella toxin. Stx2, an extremely closely-related toxin that has the same mechanism of action but is immunologically different, was discovered later (Melton-Celsa 2014).

Table 2. STEC virulence factors.

Virulence factor	Gene/operon for the production	Reference
Shiga toxin Stx1/Stx2	stx_{1a}, stx_{1c}, stx_{1d} stx_{2a}, stx_{2b}, stx_{2c}, stx_{2d}, stx_{2e}, stx_{2f}, stx_{2g}, stx_{2h}, stx_{2i}	(Smith et al. 2002; Koudelka et al. 2018 Lee et al. 2021)
Fimbria	*sfm*A, *sfm*C, *sfm*D, *sfm*H, *sfm*F, *hcp*ABC, *ecp*EDCBAR, *lpf agg*R	(Croxen and Finlay 2010; Moeinirad et al. 2021; Reiland et al. 2014; Szczerba-Turek et al. 2019)
Bacterial effector proteins	*esp*	(Reiland et al. 2014)
Outer membrane attaching protein	*agn*43	(Haque et al. 2022)
Intimin	*eae*	(Smith et al. 2002; Reiland et al. 2014)
STEC autoagglutinating adhesin	*saa*	(Amézquita-López et al. 2018; Moeinirad et al. 2021)
Tir	*tir*	(Reiland et al. 2014)
TccP	*tcc*P	(Reiland et al. 2014)
Enterohaemolysin	*ehx*A, *hly*A, *she*A	(Amézquita-López et al. 2018; Moeinirad et al. 2021; Haque et al. 2022)
Serine protease	*esp*P	(Reiland et al. 2014)
Acyltransferase	*ehx*C	(Reiland et al. 2014)
Subtilase cytotoxin	*sub*A	(Amézquita-López et al. 2018)

STEC - Shiga toxin-producing *E. coli*; Tir - translocated intimin receptor; TccP - Tir-cytoskeleton coupling protein

Shiga toxins (previously known as verotoxins or Shiga-like toxins) represent a group of bacterial protein toxins (of a size of around 70 kDa) that inhibit protein synthesis in susceptible eukaryotic cells. Stx1 and Stx2 are now referred to as Stx1a and Stx2a, and a number of other Stx subtypes have also been described. There are two immunologically distinct variants of Stx1a, specifically Stx1c and Stx1d, though these are only rarely isolated from patients with STEC infections, and when they are this is only in cases with mild symptoms of the disease. The Stx1a type is associated with severe STEC infections (Auvray et al. 2023). Stx2a has the variants Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g. Stx2a, Stx2c and Stx2d are associated with serious symptoms of the disease. Type Stx2e occurs in pigs, in which it is an agent of oedema disease. The stx_{2e} gene is considered a key virulence factor for endothelial cell damage in pigs (Haque et al. 2022). STEC strains with Stx2f were initially isolated from pigeons (Melton-Celsa 2014). STEC strains can express one or a combination of several *stx* subtypes (Auvray et al. 2023).

STEC possessing the Shiga toxin Stx2a in combination with the attaching protein intimin (encoded by the *eae* gene) can cause severe cases of disease, including bloody diarrhoea and HUS (Egervärn and Flink 2024). STEC strains with the *eae* gene for intimin production are referred to as enterohaemorrhagic *E. coli* (EHEC). According to the authors Nastasijevic et al. (2021), EHEC are responsible for more than 75% of severe cases of disease induced by STEC.

To date, more than 200 STEC serogroups that have been the cause of foodborne diseases in the form of sporadic cases or foodborne outbreaks (FBO) have been identified (Walker et al. 2023). Epidemiological studies have shown that STECs belonging among the 'top seven' serogroups, namely serogroups O26, O45, O103, O111, O121, O145 and O157, were more often involved in severe cases of disease and FBO (Koudelka et al. 2018). Meanwhile, O157 causes almost two-thirds of STEC infections in the USA. The remaining six 'non-O157' serogroups among the 'top seven' cause the majority (71%) of illnesses, hospitalisations and deaths caused by non-O157 STEC (Walker et al. 2023). For this reason, the 'top seven' serogroups are regulated and considered undesirable in raw intact beef products in the USA (Reiland et al. 2014). In Europe, meanwhile, the most common non-O157 serogroups associated with human illness include O26, O91, O103, O145 and O146 (Egervärn and Flink 2024). The most common serogroup in the EU in 2021 was O157 (15.2% cases of illness in which serogroups were reported), followed closely by O26 (14.8%) (EFSA 2022). Similarly, in 2022, the most frequently reported serogroups in the EU were O157 (22.3%) and O26 (20.0%) (EFSA 2023). In Australia, the most frequent STEC serogroups between 2001 and 2010 were O157 (58.0%), O111 (13.7%), O26 (11.1%), O113 (3.6%), O55 (1.3%), and O86 (1.0%) (Vally et al. 2012). Similarly, in Japan, the major STEC serogroups were O157 (54%), O26 (16%), O103 (5.8%), and O111 (5.7%) (Ikeuchi et al. 2024). A study by Korean authors Yun et al. (2021) analysed STEC strains isolated from patients with diarrheal disease between 2009 and 2018. The annual incidence of STEC shows an increasing trend in Korea (0.12 cases/100,000 population in 2009 and 0.23 cases/100,000 population in 2018). The incidence of STEC infections increased from May and was at its highest in the summer period (June-August), but decreased again in the winter. The highest proportion of infected people was in the age group ≤ 4 years (38.1%), followed by the categories 10–19 years (13.6%) and 5–9 years (13.1%). A proportion of 42% of HUS was found in the age group ≤ 4 years. The findings were consistent with the results of the study by Oporto et al. (2020). Also, in the USA in the years 1996–2011, STEC O157 was most often isolated in the group of children 1–4 years old, followed by the age group 5–9 years (Sodha et al. 2015). Yun et al. (2021) further studied 418 STEC strains belonging to 57 serogroups. The most common serogroup in Korea is O157 (20.3%), followed by O103 (13.6%), O26 (7.7%), O111 (5.5%), O91 (4.3%), O108 (2, 4%) and O8 (2.2%). In 418 STEC strains, 45.4% of isolates carried only stx_1 genes, 29.2% of strains contained stx_1/stx_2 and 25.4% of strains only stx_2.

The relative risk to humans from STEC has been categorised by the U.S. National Advisory Committee on Microbiological Criteria for Foods on a scale from 1 to 4, with 1 being associated with the greatest risk and 4 being the lowest risk (NACMCF 2019). Strains of *E. coli* carrying virulence genes stx_{2a} with *agg*R (a genetic marker for enteroaggregative *E. coli*, EAEC) are considered to pose the greatest risk (grade 1). Serogroup O157 and non-O157 serogroups with *stx* and *eae* genes (EHEC) pose a relatively high risk (grade 2). Serogroups regulated in the USA with *stx* genes but no *eae* genes and unregulated or unknown serogroups with both *stx* genes pose an intermediate risk (grade 3). Unregulated or unknown serogroups having the *stx* gene but not the *eae* gene belong to the low-risk category (grade 4).

There are also other genes encoding STEC virulence factors: *ehx*A (plasmid-encoded enterohaemolysin), *agn*43 (outer-membrane attachment protein), *esp*P (plasmid-encoded protease), *iha* (chromosomal iron-regulated adhesion gene), etc. (Haque et al. 2022). According to the NACMCF (2019), the following virulence gene patterns in STEC strains have the potential to cause human disease (from high to low risk): stx_{2a}, *agg*R, *eae* (serogroup O157), the presence of *stx* in non-O157 serogroups from the 'big six' group, and the presence of *stx* and *eae* in other serogroups. Furthermore, the presence of the *ehx*A gene can increase the virulence of STEC, and occasionally even intimin alone can lead to diarrhoeal disease due to lesions known as 'attaching' and 'effacing' (A/E lesions) (Haque et al. 2022). Nevertheless, according to the authors Egervärn and Flink (2024), all STEC strains are considered to have the potential to cause disease and thereby pose a health risk to consumers.

Current STEC occurrence in domestic ungulates and food

In 2021, a total of 3,746 sampled livestock units (individual animals, herds or flocks) were analysed in seven EU member states (EFSA 2022). The most tested animal category was cattle (3,316 samples in five countries), with the proportion of positive samples being 6.1%. While 17.4% of the 322 sampled units collected on farms were positive, the proportion of positive samples among the 2,994 samples collected at slaughterhouses was considerably lower (4.6%).

In 2021, 22 member states reported a total of 23,659 sampled food units analysed for the presence of STEC, of which 3.6% were positive (EFSA 2022). A large number (5,095) of the sampled units tested were fresh and unprocessed beef. A total of 2,280 units of samples were collected during processing (in 9 member states), of which 7.5% were positive. These were largely samples taken directly at slaughterhouses (9.1%). A total of 2,815 sampled units (in 12 countries) were taken in the distribution chain, with a positive rate of 4.1% (EFSA 2022). Ikeuchi et al. (2024) investigated the contamination of beef carcasses with STEC in slaughterhouses to assess the potential risks of STEC. In total, 524 gauze samples were collected from the surfaces of beef carcasses at 12 domestic slaughterhouses from November 2020 to February 2023. The STEC O157:H7 isolation rate was low (0.6%), but the isolation of stx-positive *E. coli* of minor O-serogroups from samples of eight (72.7%) facilities indicated the potential risk of minor STEC foodborne infections.

Essendoubi et al. (2019) collected 803 samples from the surface of bovine carcasses at approved slaughterhouses in Alberta, Canada, of which 401 were after hide removal and before evisceration, 402 after evisceration before the start of chilling. *Escherichia coli* O157:H7 were isolated from 7.4% of pre-evisceration samples and 5.2% of post-evisceration samples. Non-O157 strains were found in 3.2% of carcasses before evisceration and in 3.9% of carcass samples after evisceration. The differences in the proportions of positive samples before and after evisceration were non-significant ($P > 0.05$). Most of the positive results were recorded in the warmer months of the year (June-November).

Arthur et al. (2004) sampled two commercial slaughterhouses in the USA during three visits. Carcass surfaces were sampled at 5 locations on the line: 1) beef hide (after hide opening but before hide removal), 2) from the body surface after hide removal before evisceration, 3) post evisceration and final inspection before final washes, 4) in the chill cooler after all antimicrobial interventions (between sampling points 2 and 3, the carcasses were steam vacuumed, followed by treatment with 2–3% lactic acid; between points 3 and 4, washing with hot water 90 °C and then with peroxyacetic acid, at the end of the steam treatment) and finally 5) chilled carcass. STEC O157 contamination was detected in 218 skin samples (75.7%) with a range of 50.0–93.8% within 6 sampling days. A significant difference was found between the two plants ($P < 0.0001$; 88.2% plant A, 63.2% plant B). From sample point 2, i.e. the carcass before evisceration, there were positive results in 14.7% (range 2.1–25.0%); even in this case the proportion of positive samples was higher in plant A ($P < 0.0001$). After treating the carcasses with lactic acid, there was a drop in positive samples to 3.8% (in the range of 0.0–10.4%). At this point of sampling, no difference was detected between the two plants ($P > 0.05$). From sample point 4, the proportion of positive samples was 0.3% (only 1 positive sample), *E. coli* O157 could not be isolated from any carcass after chilling.

Chinese authors Dong et al. (2020) published a study in which they also demonstrated a decreasing frequency of STEC isolation during slaughter processing of cattle. In two beef slaughterhouses (capacity 30–50 animals/h) in China, the cited authors took a total of 600 samples for the presence of STEC. Six points at the slaughter were sampled: faeces of slaughtered animals, their hide, carcasses after hide removal before evisceration, carcasses after evisceration and washing with water, carcasses after chilling (approximately 24 h after slaughter), and meat after cutting. Five samples were taken from each point during a visit to the plant, each plant was visited 10 times. For the presence of stx_1 and/or stx_2 genes, 45% of faeces samples, 31% of hide samples, 14% of carcass samples before evisceration, 13% of carcass samples before chilling, 9% of carcass after chilling and 18% of cuts were positive. STEC was isolated from 20% of the positive samples. Sixteen serogroups were identified, most often O39 (23%), O76 (15%), O157 was detected only once. Also, Dong et al. (2015) found a low frequency of positive STEC O157 samples in Chinese beef slaughterhouses. In the period July–September, 510 samples were taken from the points of slaughtered cattle in four beef slaughterhouses in China, which coincided with the publication of Dong et al. (2020). Analysis revealed 0.59% of samples positive for STEC O157:H7. From these three positive samples, six STEC O157:H7 isolates were obtained. Positive samples came from two plants, specifically, in plant A there was a positive sample of faeces and one chilled carcass, in plant B one hide sample (Dong et al. 2015).

From 816 samples of raw meat (beef, pork, mutton) collected in Southeast China in retail between 2010–2016, 49 STEC isolates were obtained and further analysed. Of these, 14.3% (n = 7) belonged to serogroup O157. Among the 42 non-O157 isolates, there were three each from serogroups O26 and O128, two isolates each from serogroups O103, O121, O111, O8, and O166, the remaining 26 serogroups were represented by one isolate each. Of the 49 STEC isolates, 51.0% contained the stx_2 gene, 20.4% both stx_1 and stx_2 genes, and 28.6% carried only the stx_1 gene. Gen *eae* comprised 34.7% of STEC isolates. All strains of serogroup O157 were positive for *eae* (Nong et al. 2021).

In Brazil, the authors de Assis et al. (2021) performed a meta-analysis to determine the STEC contamination rate of beef. A total of 4,286 samples described in 25 articles were included in the study. The result of the study was that the overall contamination rate of beef in Brazil is extremely low (1%) but with a fairly large variance from 0% to 27%. Most of the included samples were from pre-chilled carcasses (n = 1,719), which also had the highest average finding of positive samples (n = 129; 8%).

Small ruminants are a significant reservoir of STEC. Samples of mutton were taken in four member countries (505 sampled units) in 2021, of which 9.7% were found to be positive; 4.6% sampled units from the slaughterhouse were positive and 14.3% samples taken in the distribution chain were positive. Goat meat was sampled in two states only, with negative results. Three member countries reported the results of analyses of fresh deer meat, with 16.8% of 101 samples being positive. Deer hunting is risky from the viewpoint of STEC, as it may be associated with perforation of the intestine and subsequent contamination of the meat. In 2021, seven countries reported the results of analyses of pork meat, with 16.6% of 604 samples testing positive. Other animal species tested in seven countries involved samples of poultry, horse meat, rabbit meat and venison and the meat of farmed game, with positive findings in 6.5% of samples (EFSA 2022).

In the period of January–February 2017, the authors of the study by Baschera et al. (2019) collected samples of faeces from 163 healthy camels from one farm in Kenya. The animals were raised extensively and shared pastures with cattle and game. Tests for the detection of *stx* genes using real-time PCR revealed 53 positive samples (32.5%). STEC was isolated from 20 of them (12.3% of all samples collected). The most frequently detected serotype (n = 9; 45% of all isolates) was STEC O156:H25, three isolates were assigned to serotype O43:H2. The stx_{2a} gene was detected in 11 isolates. The intimin gene *eae* was detected in 9 isolates. All strains of serotype O156:H25 possessed stx_{2a} and *eae* genes, which indicates their potential risk.

Between February 2010 and March 2013, Frank et al. (2019) collected a total of 124 faecal samples and rectal swabs from 77 roe deer living in Bavaria. Of these, 90 samples were positive for the presence of *stx* genes. A total of 143 STEC isolates were obtained within the study, of which 122 were positive for stx_2. None of the isolates contained the *eae* gene. The authors concluded that the strains in the roe deer population have only a low pathogenic potential for humans. Long-term excretion of STEC in roe deer was also observed.

Meat products and meat preparations were analysed for STEC in 11 EU member countries. Of the 2,485 sampled units, 2.3% were positive (EFSA 2022). Of the 2,985 samples of milk and dairy products analysed in eight EU states, 1.8% were positive. Of these, STEC was confirmed in 1.4% of 503 samples of raw cow's milk. This was a lower proportion than in samples of cheese, in which 1.7% of 1,930 samples collected in eight countries were positive.

STEC was confirmed in only 0.3% of 3,110 samples of fruit and vegetables, while only two out of 1,214 sampled units of spices and herbs, salads and seed sprouts (0.2%) were positive. A total of 865 samples were taken and tested in other food categories (bread and bread products, ready meals, fish and fish products, etc.), of which 39 were positive (of which 37 were in the category bread and bread products). The finding of 6.3% of positive samples in the category bakery products is interesting. Such a high level of contamination may be due to flour contamination. Flour has been identified as a cause of STEC FBO in the USA and Canada (Zhang et al. 2021). Kindle et al. (2019) tested 70 samples of flour from a Swiss retail chain. Two isolates of serogroup O11 possessed the stx_{1c}/stx_{1d} genes, two belonged to serogroup O146 with stx_{2b}, and one isolate of O103 contained the stx_{1a} and *eae* genes. Zhang et al. (2021) also obtained an isolate of serogroup O103 with the same genetic make-up when they analysed 347 samples of packaged wheat flour in Canada. The habit of tasting raw dough or handling raw dough may contribute to human infection (EFSA 2022).

STEC in finishing pigs and pork meat

STEC has the potential to contaminate pork in the supply chain. Isolates were obtained from pork meat products that belonged to the aforementioned 'top seven' serogroups (O26, O45, O103, O111, O121, O145, O157) that are monitored in raw beef products in the USA

(Haque et al. 2022). Scott et al. (2020) analysed 1,395 samples of pork meat (individual parts including cuts, as well as samples of minced meat) for the presence of STEC as part of the activities of the FSIS (Food Safety Inspection Service) in the USA. STEC could be proven by culturing in only three samples (0.2%) (serogroup O103 in two samples and serogroup O157 in one sample), though 309 samples were positive for the presence of the genes *stx* and *eae*.

The incidence of *stx*-positive samples of pork meat on the retail network around the world fluctuates between 1.1 and 80.0%. The confirmed incidence of *E. coli* O157 ranges from 1.2 to 23.2% and that of non-O157 between 0.1 and 14.7% (Haque et al. 2022).

The contamination of pork meat with STEC occurs at slaughterhouses. Nastasijevic et al. (2021) analysed pig carcasses at two slaughterhouses in the USA (daily capacity 11,000–17,000 pigs) during the course of the year. Samples were taken from the surface of the carcasses during three stages of slaughter processing using cellulose sponges: 1) from carcasses after bleeding and before scalding (area 1,500 cm^2/carcass), 2) after scalding (60 °C) and before evisceration (area 4,000 cm^2/carcass), and 3) after 24 h of chilling (cold room 4 °C, area 4,000 cm^2/carcass). The warm carcasses were treated with a 2% lactic acid solution (room temperature/10–30 s) when they entered the cold store. A total of 1,536 pig carcasses were sampled. PCR was used to monitor the occurrence of the genes *stx* (STEC) and *stx* and *eae* (EHEC), and positive samples were subjected to culture examination. The occurrence of *stx* genes was detected in 85.3% of carcass skin samples, in 17.5% of samples from the carcass surface before evisceration, and in just 5.4% of carcasses after chilling. The *stx* and *eae* genes together were detected less frequently (82.3%, 7.8%, and 1.7%, respectively). A total of 368 STEC isolates were obtained from the samples by culturing, of which 46 were EHEC isolates. The most frequently detected STEC serogroups were O121 (63.0% of all STEC isolates), O8 (6.7%) and O91 (6.0%). The most frequent EHEC serotype was O157:H7 (63% of all EHEC). The scalding of pig carcasses was able to reduce the surface-contaminating microbiota by three log cfu/cm^2. Nevertheless, the study showed that the pig skin may be an important source of STEC in pork meat.

The prevalence of STEC at pig slaughter ranges from 0.2 to 86.3% depending on the category of sample, the detection method, and the hygiene conditions at the slaughterhouse (Haque et al. 2022). A relatively higher incidence has been reported in caecal samples, followed by carcass and faecal samples. Cross-contamination, inappropriate carcass handling, and unsanitary slaughterhouse conditions may contribute to a higher prevalence of STEC. Remfry et al. (2021) tested 598 faecal samples from finishing pigs in the USA. They obtained 178 isolates belonging to 23 serogroups by culturing, of which the dominant serogroups were O8, O86, and O121. Twenty-six strains contained the stx_{1a} gene, while 152 strains possessed the stx_{2e} gene.

Ercoli et al. (2016) collected and analysed samples of colon content (n = 210) and swabs from the carcass surface (n = 210) from 210 finishing pigs. They also collected 675 samples of fresh pork meat and uncooked pork products (five samples of each of 135 types) from the main processing plants in the Umbria and Marche regions of Italy. They did not detect *stx* genes in 129 of the 210 samples of colon content, although they found 105 samples to be positive for *eae* genes. The genes stx_2 and *eae* were detected in 75 of 81 *stx*-positive samples, while six samples were positive only for stx_2. The stx_1 gene was not detected in this category of samples. STEC isolates of 28 strains were obtained from 26 samples. All were positive for stx_2, but did not have *eae*. A total of 27 strains were positive for stx_{2e}. In total, 181 of 210 carcass samples (86.2%) were negative for *stx* genes; 144 samples were positive for *eae*. STEC isolates were obtained from only four carcass samples, and genes encoding intimin were not demonstrated in any of these. Three strains were positive for stx_{2e} and one for stx_{2d}; 97.2% of 675 samples of fresh pork meat or fermented products were negative for *stx* genes. Nineteen samples of meat preparations were positive; STEC could not be isolated from any of them.

Kaufmann et al. (2006) tested 630 faecal samples obtained from healthy pigs at one Swiss slaughterhouse. They isolated 31 O157 strains and 45 STEC strains. The O157 isolates were negative for *stx*, though three were positive for *eae*, one for *ehx*A (haemolysin production) and two strains carried the *paa* gene (known as porcine A/E-associated protein). Due to the absence of the *stx* gene, the O157 strains were assigned to the enteropathogenic *E. coli* (EPEC) pathotype. These strains have the ability to form A/E lesions in the colon, but do not have the genetic information to produce Shiga toxins. A total of 98% of STEC isolates contained stx_2 genes, specifically 42 stx_{2e} strains, one stx_2 and stx_{2e} combination strain, and one stx_{2e} strain together with stx_{2e}. According to the authors of the study, a high prevalence of STEC was found in finishing pigs, though the virulence factors showed that most of the isolated strains had low virulence for humans.

Escherichia coli possessing the Stx2e toxin together with F18 fimbriae (encoded by the *fed*A gene) cause an infectious disease known as oedema disease (ED) in pigs (Casanova et al. 2018; Berger et al. 2023). The agents of this disease belong to the STEC pathotype and form a specific subgroup known as EDEC (oedema disease *E. coli*). Oedema disease occurs most often in young pigs in the first two weeks after weaning. F18 fimbriae allow EDEC to adhere to enterocytes in the intestine. Stx2e is absorbed into the blood, damaging the walls of blood vessels and as a result, the blood fluid permeates into the tissues leading to swelling (oedemas) in various organs. Most EDECs also produce α-haemolysin (Berger et al. 2023). In Germany, 99 farms were tested (2,405 faecal samples, 479 swabs from pen floors, 185 samples of piglet saliva obtained using cotton ropes and 231 samples from these ropes) for the presence of EDEC. On 37.4% of these farms, stx_{2e} genes together with *fed*A were confirmed, with the presence of *E. coli* with the stx_{2e} gene being found on 53.5% of farms (Berger et al. 2023). The emergence of ED in wild boars in France was caused by atypical hybrid of STEC and enterotoxigenic *E. coli* (ETEC) O139:H1 (Perrat et al. 2022). Analysis of STEC strains isolated from wild boars in France during 2013–2019 confirmed serotype O139:H1 positive for both Stx2e and F18 fimbriae. However, in contrast to classical STEC O139:H1 strains circulating in pigs, the isolates from wild boars also possessed enterotoxin genes *sta1* and *stb*, typical for ETEC (Perrat et al. 2022).

STEC in wild boars

Wild boars can also be a source of STEC in the food chain. Sánchez et al. (2010) examined rectal faeces samples collected from 212 wild boars killed in the 2007–2008 hunting season in southwest Spain. Overall, STEC were detected in 17 (8.0%) of the animals sampled. *Escherichia coli* O157:H7 was isolated from 7 (3.3%) animals and non-O157 STEC were isolated from 11 (5.2%); stx_1 genes were found in 4 isolates, stx_2 genes in 12, and 1 isolate contained both stx_1 and stx_2 genes. The *eae* gene was detected in 8 isolates (Sánchez et al. 2010).

In Tuscany, Italy, 200 wild boars were sampled by rectal swabs during the 2018–2019 hunting season. *Escherichia coli* was isolated from 175 animals, 31.4% of the isolates were negative for tested genes encoding virulence factors, 21.7% of the isolates were classified as STEC; 6.3% of isolates as EHEC (Bertelonni et al. 2020). The higher presence of STEC virulence genes was confirmed by Peruzy et al. (2022) in wild boar meat samples in the Campania region, Italy. In 28 wild boar meat samples, *eae* genes were detected in 27 (96.4%) samples, stx_1/stx_2 genes in 12 (42.9%) samples. During two hunting seasons (2020–2021 and 2021–2022) Siddi et al. (2024) sampled 66 wild boars killed in Sardinia. Colon contents (66), mesenteric lymph nodes (66) and carcass surface (49) were sampled from each animal. *Escherichia coli* was detected from each of the 66 wild boars sampled, although not always from all samples collected. STEC pathotype could not be detected from any sample.

Szczerba-Turek et al. (2019) analysed 152 rectal swabs obtained from 152 wild boars killed in autumn–winter 2017/2018 in north-eastern Poland. STEC virulence genes were detected in 64 samples (42.1%), stx_2 genes were detected in 27 isolates, stx_1 genes in 10 isolates and stx_1/stx_2 genes in six isolates. Six isolates belonged to serogroup O157, of which five belonged to serotype O157:H7; 14 isolates belonged to serogroup O103, the same number belonged to serogroup O146, nine isolates to serogroup O145, and six to serogroup O45.

Risks associated with presence of STEC in pork meat

STEC isolates obtained from pork meat belong to the low-to-moderate risk category, though they do have the potential to cause human disease (Haque et al. 2022). Consumption of insufficiently heat-treated pork meat or meat exposed to inappropriate temperature conditions during storage and transport or in gastronomy and households may lead to potential human STEC infections (Haque et al. 2024). Pork meat has been the source of outbreaks of alimentary illness caused by serovar O157:H7, specifically in Canada in 2014, 2016, and 2018 (Amézquita-López et al. 2018).

The aim of the study published by Haque et al. (2024) was to determine and compare the growth parameters of serogroups O157, O91, and other non-O157 strains in ground pork at different temperatures (10–40 °C) and to develop and validate a model of the competition of two microbial groups (STEC versus total microbiota - total microbial count). The minimum growth temperature determined for O157 strains was 5.2 ± 1.0 °C and that for non-O157 strains 4.5 ± 2.5 °C, which was lower than for serogroup O91 (7.0 ± 1.3 °C). Inhibition of STEC by contaminating microbiota (the total microbial count) was dependent on temperature. At 10 and 15 °C, the total microbial count was dominant and affected the maximum specific growth rate (μ_{max}) of STEC, although at 20 or 25 °C the μ_{max} values for the total microbial count were similar to the μ_{max} values for STEC. At 30 °C, STEC became the dominant microbiota, surpassing the μ_{max} for the total microbial count. Neither group was dominant at 40 °C. The maximum bacterial population density was always significantly higher for the total microbial count than for STEC at all tested temperatures. The growth of the natural contaminating microbiota was not suppressed by competition with STEC. Conversely, the growth of STEC in pork meat may be affected by the total microbial count depending on the storage temperature.

Conclusions

The results of numerous studies indicate that pork meat and pork products can be naturally contaminated by a heterogeneous population of STEC strains. These strains are transferred from farms, on which pigs can be asymptomatic carriers, to slaughter facilities and subsequently to cutting plants and other processing plants. Contaminated products can then cause human infections.

Isolates with the stx_{2e} gene are most often detected from samples taken at slaughterhouses from carcasses or from the contents of the caecum. *Escherichia coli* with the Stx2e toxin together with F18 fimbriae causes oedema disease in pigs. STEC isolates obtained from pork meat are in the low-to-moderate risk category, even though they do have the potential to cause human disease.

The most effective prevention is the perfect cooking of meat. According to USDA-FSIS recommendations for effective inactivation of STEC, intact cuts of pork meat should reach a temperature of 60 °C (140 °F), while the figure for ground meat is 71.1 °C (160 °F) (Reiland et al. 2014).

Conflict of interest

The authors declare no conflict of interest.

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