7-9-2022

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International Journal of Food Microbiology 377 (2022) 109832

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Review

A review of Shiga-toxin producing *Escherichia coli* (STEC) contamination in the raw pork production chain

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ARTICLE INFO

Keywords:
STEC
Raw pork
Prevalence
Processing
Retail

ABSTRACT

Epidemiological evidence of Shiga toxin-producing *Escherichia coli* (STEC) infections associated with the consumption of contaminated pork highlight the need for increased awareness of STEC as an emerging pathogen in the pork supply chain. The objective of this review is to contribute to our understanding of raw pork products as potential carriers of STEC into the food supply. We summarize and critically analyze primary literature reporting the prevalence of STEC in the raw pork production chain. The reported prevalence rate of *stx*-positive *E. coli* isolates in live swine, slaughtered swine, and retail pork samples around the world ranged from 4.4 % (22/500) to 68.3 % (82/120), 22 % (309/1395) to 86.3 % (69/80), and 0.10 % (1/1167) to 80 % (32/40), respectively, depending upon the sample categories, detection methods, and the hygiene condition of the slaughterhouses and retail markets. In retail pork, serogroup O26 was prevalent in the U.S., Europe, and Africa. Serogroup O121 was only reported in the U.S. Furthermore, serogroup O91 was reported in the U.S., Asia, and South American retail pork samples. The most common virulence gene combination in retail pork around the globe were as follows: the U.S.: serogroup O157 + *stx*, non-O157 + *stx*, unknown serogroups+*stx* + *eae*; Europe: unknown serogroups+(*stx* + *eae*, stx_2 + *eae*, or stx_1 + stx_2 + *eae)*; Asia: O157 + stx_1 + stx_2 + *ehxA*, Unknown+stx_1 + *eaeA* + *ehxA*, or only *eae*; Africa: O157 + stx_2 + *eae* + *ehxA*. STEC strains derived from retail pork in the U.S. fall under low to moderate risk categories capable of causing human disease, thus indicating the need for adequate cooking and prevention of cross contamination to minimize infection risk in humans.

1. Introduction

Fresh pork is a highly perishable food commodity with a water activity between 0.985 and 0.995. Additionally, normal pork meat reaches a pH between 5.6 and 5.7 within three to five hours of harvest (Iacumin and Carballo, 2017). In suitable conditions of temperature, relative humidity, gaseous atmosphere, etc., these intrinsic characteristics support the growth of a variety of foodborne pathogens, including *Arcobacter butzleri* and *A. cryaerophila*, *Campylobacter coli* and *C. jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, *Yersinia enterocolitica*, and *Y. pseudotuberculosis* (ICMSF, 2018). Furthermore, pork may serve as a vehicle for the transmission of helminths such as *Taenia solium* (cysticercosis) and *Trichinella spiralis,* and protozoan parasites such as *Toxoplasma gondii* (ICMSF, 2018) when humans consume muscle tissue containing encysted larvae or oocysts, respectively (USDA-FSIS, 2018).

While *Salmonella* and some parasites are well-established biological hazards in pork and pork products, little is known about the growth and potential survival of Shiga toxin-producing *E. coli* (STEC) in raw pork products. Consumption of contaminated pork products has been arguably linked to STEC outbreaks, in particular STEC O157 (Alberta Health Services, 2018; Honish et al., 2017). *Escherichia coli* O157:H7 outbreaks linked to the consumption of contaminated comminuted and intact cuts of pork were reported in Canada in 2014 and 2018 (Alberta Health Services, 2018, Honish et al., 2017), demonstrating the potential of STEC as contaminants in the pork supply. In the 2014 outbreak, 119 laboratory-confirmed cases were reported, of which 19 % (23/119) of patients were hospitalized, six of whom later developed hemolytic uremic syndrome (HUS). Environmental contamination along with mishandling of products over slaughter, processing, retail, and food service operations were collectively responsible for product contamination (Honish et al., 2017). Four years later, in the other pork-associated STEC outbreak, 13 of 42 laboratory-confirmed cases were hospitalized, and subsequently, one person died (Alberta Health

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https://doi.org/10.1016/j.ijfoodmicro.2022.109832
Received 4 February 2022; Received in revised form 2 July 2022; Accepted 4 July 2022
Available online 9 July 2022

Services, 2018). Twenty-one of the laboratory-confirmed cases were linked to food served at restaurants. Epidemiological investigations pointed towards several raw pork products (ground, ribs, leg, loin, feet, and hocks) as the most likely source of *E. coli* O157:H7 (Alberta Health Services, 2018). These outbreaks highlight the need for increased awareness of pork as a potential source of STEC infection. In many studies, STEC isolates recovered from retail pork products belonged to one or more of the top seven serogroups (O26, O45, O103, O111, O121, O145, O157) that are regulated and considered adulterants in raw, non-intact beef products in the U.S. (USDA-FSIS, 2011). These U.S. regulated serogroups as well as some non-regulated serotypes (e.g., O91:H4), often harbor virulence genes (*stx*$_2$, *eae*, and *ehx*) implicated in human disease.

STEC may be transferred from swine to retail pork via contaminated carcasses during fabrication (Nastasijevic et al., 2020) or during retail and food service handling (Montville et al., 2001; Wachtel et al., 2003; Wilson et al., 2018). Comprehensive microbial source-tracking of STEC transmission along the pork production chain in Argentina concluded that STEC contamination of pork products (as indicated by the presence of the Shiga toxin gene, *stx*) originates on the farm and transfers from pigs through the slaughter and processing steps into the pork supply (Colello et al., 2016). A recent U.S. study tracked presumptive STEC through two large pork harvest and processing facilities and found that 85 % (1310/1536) of market hogs entering the plants had presumptive STEC on their skin, while 5.4 % (83/1536) of finished carcasses in the sales cooler were positive for presumptive STEC after processing (Nastasijevic et al., 2020). This study found a seasonal effect on presumptive STEC prevalence in pork, with lower recovery rates during winter, and concluded that the skin of hogs may be a significant source of STEC in pork meat (Nastasijevic et al., 2020). Overall, consumption of undercooked pork products as well as cross-contamination during food service and domestic food preparation may lead to potential human STEC infections.

2. Review methodology and data scoping approach

The aim of this review is to contribute to our understanding of raw pork products as potential carriers of STEC into the food supply. We have summarized and critically analyzed primary literature reporting the prevalence of STEC in the raw pork production chain. Searches for primary literature were conducted on Scopus®, ScIELO® and PubMed, and relevant articles were retrieved in full through the University of Nebraska-Lincoln library system. Inquiries were conducted using the descriptors "STEC/VTEC AND Swine AND Prevalence OR Occurrence", "STEC/VTEC AND Pork AND Retail" to search article titles, abstracts, and keywords. In total, 77 articles were found with the descriptors "STEC AND Swine AND Prevalence OR Occurrence" and 71 with "STEC AND Pork AND Retail". Criteria for inclusion were defined as any article published in English that reports the occurrence, presence, or prevalence of STEC (or VTEC) in swine, hogs, or pork. All abstracts were read and only those articles that met the inclusion criteria were used for the next phase. After elimination of duplicates, 16 full-text scientific publications were selected for STEC in live swine and 21 for STEC in retail pork. The following information was identified from each publication: meat matrix, geographical location, prevalence, virulence genes, method of isolation, and serogroup/serotype of STEC. No article was found that reported STEC prevalence in swine or pork before 1999. Thus, publication date ranged from 1999 to 2021, indicating that this is an emerging field of study.

For this review, STEC were defined as any *E. coli* that possess Shiga toxin gene(s), *stx*, markers regardless of serogroup denomination or markers for the presence of the intimin (*eae*) gene. Swine (skin, fecal, cecal) or pork (meat) samples that have tested positive in a PCR screening are denoted as "presumptive STEC" instead of "presumptive positive" and sample having *stx* genes in a PCR and then confirmed as *E. coli* isolates through culture are denoted as "culture-confirmed STEC". The relative risk ranking of STEC for causing human disease was determined based on U.S. National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2019) recommendations. This Committee suggests that STEC require both *stx* and intestinal attachment genes (such as *eae*) for causing severe human illnesses. The relative risk was categorized from 1 to 4 denoted highest to lowest risk. The *E. coli* harboring *stx*$_{2a}$ virulence genes with *aggR* (a genetic marker for entero-aggregative *E. coli* [EAEC]) was considered highest risk (1). The U.S.-regulated serogroup O157 and non—O157, along with *stx* and *eae*, were considered relatively high-risk (2). The U.S.-regulated serogroup having *stx* but not *eae* and U.S. unregulated or unknown serogroup having both *stx* and *eae* gene was considered in the moderate (3) risk category. Lastly, the U.S. unregulated or unknown serogroup having only *stx* were considered low risk (4) for lacking the *eae* gene (NACMCF, 2019). We also considered other virulence-encoding genes, including *stx* subtypes, *ehxA* (plasmid-encoded enterohemolysin), *agn43* (a phage variable outer membrane attaching protein), *espP* (a plasmid-encoded serine protease), *katP* (plasmid-encoded virulence genes), *ecpA* (adhesin and *E. coli* common pilus), and *iha* (chromosomal iron-regulated gene for adhesion). Additional information on STEC virulence factors can be found in these studies (EFSA BIOHAZ Panel et al., 2020; FAO/WHO STEC Expert Group, 2018; FAO/WHO, 2018). The proteins encoded by these genes could mediate bacterial adhesion to human and animal intestinal epithelia, plants, and abiotic surfaces. Virulence factors such as *ehxA* can be a marker for the large plasmid found in many Enterohemorrhagic *Escherichia coli* (EHEC) (NACMCF, 2019). However, toxins other than the Shiga toxin expressed by O157 and other STEC are not proven virulence factors (NACMCF, 2019). Thus, none of the virulence factors other than adhesins (e.g., *ecpA*) were considered for additional virulence potential in the risk characterization.

One thing to note is that the primary literature often uses *hlyA* as a misnomer for *ehxA*. Therefore, we only use *ehxA* even if not reported as such in the primary source. The *hlyA* designation is for the structural gene of *E. coli* α-hemolysin (Felmlee et al., 1985). It is a common mistake to call the large plasmid-born enterohemolysin of *E coli* O157:H7 *hlyA* when it is properly called *ehxA*. The hemolysin associated with STEC virulence is encoded by the *ehxA* gene and not by the *hlyA* gene. Presumably, the confusion is because of the earlier published designation of *ehxA* as an EHEC *hlyA*.

As this review presents results, differences in methodologies will be noted when they may impact comparisons. Furthermore, efforts were taken to present similarly collected data (sample area location/size, detection method, etc.) to make relevant comparisons and contrasts among the variable STEC findings described along the pork production chain.

3. Live swine as a reservoir for STEC

STEC can be transmitted to humans through person-to-person contact, direct contact with animals, or ingestion of contaminated food or water (Caprioli et al., 2014; Smith et al., 2014). Beef cattle are well-established reservoirs for STEC and some STEC have been demonstrated to colonize the distal region of the recto anal junction in bovines (Arthur et al., 2010; Moxley and Acuff, 2014). In swine, STEC play an important role in the pathogenesis of edema disease, an infectious illness that affects post-weaning piglets and young finishing-age pigs (Tseng et al., 2014). Studies have found that swine are susceptible to STEC O157:H7 infection and can shed the bacterium for up to two months (Cha et al., 2018; Tseng et al., 2014). In fact, Shiga toxin subtype 2e (*stx*$_{2e}$) is considered a key virulence factor for the damage of swine endothelial cells (Ercoli et al., 2015).

Longitudinal cohort studies of swine STEC carriage by Tseng et al. (2015) and Cha et al. (2018) identified swine as a significant reservoir of STEC in the U.S. Tseng et al. (2015) reported that 65.3 % (98/150) of finishing pigs aged 10- to 24- weeks from two different sites of a commercial farm in the Midwestern U.S. tested positive for STEC by culture isolation. Similarly, Cha et al. (2018) estimated 68.3 % (82/120) STEC-

positive pigs sampled from a commercial farm located in Ohio between June 2014 and April 2015. The prevalence rate varied by sampling site (50 to 97.5 %), cohort (15 to 100 %), and seasonality, with a higher average prevalence in autumn (36 %, 81/224). The researchers collected a total of 898 fecal samples from 120 pigs (20 pigs × 6 cohorts) of the same ages as previously described, of which 44.2 % (397/898) tested positive for at least one *stx* gene marker (Cha et al., 2018). Isolates were obtained by conventional culture methods with multiple serotypes not linked to human diseases such as O59:H21 (*stx*$_{2e}$), but also the clinically important O157:H7 (*stx*$_{2c}$, *eae*) and O26:H11 (*stx*$_{1a}$, *eae*) were recovered at a similar frequency (1.7 %, 2/120), indicating that commercial pigs may act as sources of human STEC infections (Cha et al., 2018).

In a study by Nastasijevic et al. (2020), 1536 hogs arriving at slaughter over the course of a year (384 per season) were examined for STEC by PCR and culture isolation. The culture-confirmed STEC prevalence was 26.4 % (414/1536) with a higher prevalence rate in the spring (29 %, 120/414) and summer (32.6 %, 135/414) months compare to winter (16.2 %, 67/414) and automn (22.2 %, 92/414) samples (Nastasijevic et al., 2020). In this study, the most commonly present *stx* subtypes in the isolates were *stx*$_{1a}$, *stx*$_{2a}$, *stx*$_{2e}$, and/or *stx*$_{2c}$. In a previous retrospective U.S. study, Baranzoni et al. (2016) showed that swine might carry *stx*$_{1a}$-, *stx*$_{2d}$-, *or stx*$_{2e}$-producing *E. coli* with virulence gene profiles linked to human infections, consistent with the observations of Nastasijevic et al. (2020).

Studies from around the world have demonstrated a high prevalence of human clinically relevant STEC in commercial swine populations. In Italy, Ercoli et al. (2016) found a 38.6 % (81/210) *stx*-positive and subsequently a 12.4 % (26/210) culture-positive STEC in pig fecal samples collected from the Umbria and Marche regions during a one-year period. On the other hand, a comparatively lower frequency of STEC (5.6 %, 23/409) was estimated in pig fecal samples in Ibadan, Nigeria (Ojo et al., 2010). Isolation via conventional methods and serological confirmation followed by molecular screening identified serogroups O111, O128, and O157 in the positive samples (Ojo et al., 2010). A similar prevalence (4.4 %, 22/500) of stx$_2$ positive STEC was detected in the fecal samples of healthy adult pigs collected from two commercial farms located in the Eastern Cape province, South Africa between April and July 2014 (Iwu et al., 2016). The authors examined for serogroups O26, O45, O103, O111, O121, O145 and O157 within the 22 STEC and found that seve were O26 while the others 15 were of an untargeted serogroup (Iwu et al., 2016). Lastly, an isolation rate of 12.8 % (16/125) of *E. coli* O157:H7 was reported in tonsil swabs of 4- to 6-week-old clinically healthy pigs collected from different herds of five intensive pig farms (25 sample/farm) located in the vicinity of Hubei province, China (Khan et al., 2018), indicating one of the higher prevalence values among live swine.

Overall, the prevalence of *stx*-positive *E. coli* isolates from swine ranged from 4.4 to 68.3 % around the world (Table 1). Variation in results may be explained by multiple factors, including methodological differences. For instance, studies that used modified Rainbow Agar supplemented with tellurite instead of MacConkey agar showed a higher STEC recovery rate (Cha et al., 2018; Ercoli et al., 2016). Similarly, sorbitol MacConkey agar supplemented with cefixime increases the recovery rate of *E. coli* O157:H7 while suppressing growth of some non-O157 serogroups (Iwu et al., 2016; Ojo et al., 2010). Other studies used washed sheep blood agar with Mytomicin C, which identifies STEC through the enterohemolyic phenotype (Nastasijevic et al., 2020; Sugiyama et al., 2001). This approach allows the relative abundance of enterohemorrhagic *E. coli* (EHEC) to be identified among other types of STEC but can bias serotypes to those expressing the *ehx* gene.

Although comprehensive studies of swine STEC carriage and on-farm shedding are limited, there is scientific evidence to show that swine may serve as a reservoir for STEC strains potentially pathogenic to humans, including those expressing *stx*$_{2e}$ and that may possess *eae* and *ehx* genes. The most common serogroups identified are O8 and O121, followed by O55, O86, O91, O108, O138, O139, O141, O147, and O149 (Fratamico et al., 2004; Ju et al., 2012; Remfry et al., 2020; Tseng et al., 2014).

4. Prevalence of STEC in slaughtered swine and pork processing

STEC can be transmitted from the skins and gastrointestinal contents of pigs to their carcasses during skinning, evisceration, and further processing (Khan et al., 2018). However, very few studies have reported the prevalence of STEC in cecal, organs, or fecal samples of slaughtered swine. In Italy, Arancia et al. (2019) reported that 52.1 % (122/234) of slaughtered swine cecal samples collected from abattoirs during 2015 were *stx*-positive. Samples were screened for *stx*$_1$, *stx*$_2$, *and eae* gene markers followed by an isolation step and subsequent characterization of the isolates. Of 66 *stx*$_2$-positive isolates, 74 % ($n = 49$) possessed the *stx*$_{2a}$ gene subtype, in some cases along with *stx*$_{2b}$ (4.5 %, 3/66) or *stx*$_{2c}$ (12 %, 8/66). The remaining 17 isolates (26 %) harbored the *stx*$_{2e}$

Table 1
Prevalence of STEC in the live and slaughtered swine around the world.

Origin	Country	Sample	Prevalence (%)	Identification	Reference
Farm	Nigeria	Fecal	5.6 (23/409)	Culture	Ojo et al. (2010)
	USA	Fecal	65.3 (98/150)	Culture	Tseng et al. (2015)
	Italy	Fecal	38.6 (81/210)	PCR	Ercoli et al. (2016)
			12.4 (26/210)	Culture	
	South Africa	Fecal	4.4 (22/500)	Culture	Iwu et al. (2016)
	USA	Fecal	68.3 (82/120)	Culture	Cha et al. (2018)
		Fecal	44.2 (397/898)	PCR	
	China	Tonsil swab	12.8 (16/125)	Culture	Khan et al. (2018)
	USA	Skin	85.3 (1310/1536)	Culture	Nastasijevic et al. (2020)
Slaughterhouse	France	Carcass swab	50 (75/175)	PCR	Bouvet et al. (2001)
	Belgium	Carcass swab	12.8 (17/122)	PCR	Botteldoorn et al. (2003)
	Switzerland	Fecal	22 (138/630)	PCR	Kaufmann et al. (2006)
	Brazil	Intestine	1.4 (1/74)	Culture	Martins et al. (2011)
	Canada	Carcass	4.8 (51/1067)	Culture	Bohaychuk et al. (2011)
	Argentina	Carcass	4.1 (6/147)	Culture	Colello et al. (2016)
	Italy	Carcass swab	13.8 (29/210)	PCR	Ercoli et al. (2016)
			1.9 (4/210)	Culture	
	China	Intestine, liver, kidney, and meat	86.3 (69/80)	Culture	Khan et al. (2018)
	Italy	Cecal	52.1 (122/234)	PCR	Arancia et al. (2019)
			28.2 (66/234)	Culture	
Processing plant	USA	Raw intact and nonintact pork	22.1 (309/1395)	PCR	Scott et al. (2020)
			0.2 (3/1395)	Culture	

PCR: STEC identified by PCR without isolation; Culture: STEC confirmed by isolation.

subtype, while none of the isolates carried *eae* (Arancia et al., 2019).

A study conducted in China reported an 86.3 % (69/80) prevalence of *E. coli* O157:H7 in pork intestine, liver, kidney, and meat samples obtained from two different slaughterhouses in the vicinity of Hubei province (Khan et al., 2018). In contrast, a Brazilian study reported a much lower culture-confirmed STEC occurrence (1.4 %, 1/74) in slaughtered swine intestinal samples (Martins et al., 2011). In Switzerland, 22 % (138/630) of swine fecal samples collected at slaughter tested positive for *stx* by PCR (Kaufmann et al., 2006). Among the isolated non-O157 serogroups, one O103:H2 STEC strain harbored a combination of virulence genes (*stx*$_1$, *eae*, and *ehxA*) that has pathogenic characteristics for humans. However, one *E. coli* O157:H7 isolated via immunomagnetic separation (IMS) lacked *stx* genes (Kaufmann et al., 2006).

STEC prevalence on carcass samples also varies across studies. Bohaychuk et al. (2011) estimated a 4.8 % (51/1067) STEC prevalence in pork carcasses swabs collected from provincially inspected hog slaughter facilities in Alberta, Canada between 2006 and 2007. The prevalence in their study was defined by a culture-isolated STEC from a sample that screened positive for *stx* via PCR (Bohaychuk et al., 2011). In Argentina, a comparable prevalence rate (4.1 %, 6/147) of STEC was estimated from slaughtered swine carcass (Colello et al., 2016). Multiple STEC of different serotypes were isolated, including O1:H9 (*stx*$_{2e}$, *ehaA*), O91:H21 (*stx*$_1$, *agn43, ehaA, iha*), ONT:H29 (*stx*$_1$, *stx*$_2$), ONT:HNM (*stx*$_2$), and O8:HNM (*stx*$_{2e}$, *ehaA*). It should be noted that serogroup O91 has a history of causing HUS or bloody diarrhea in humans (Feng et al., 2017; Mellmann et al., 2009).

A relatively higher frequency (13.8 %, 29/210) of STEC indicated by PCR was found in carcass swabs obtained from Italian pig slaughter facilities in the Umbria and Marche regions (Ercoli et al., 2016) where swabs covered half the carcass rather than 100 cm^2 like other survey studies (Bohaychuk et al., 2011; Colello et al., 2016). Of 29 *stx*-positive samples, 26 harbored *stx*$_2$ and *eae*, two possessed only *stx*$_2$, and one carried both *stx*$_1$ and *eae* genes (Ercoli et al., 2016). However, only 1.9 % (4/210) of carcasses were culture-positive and none of the STEC were top seven or harbored the *eae* gene (Ercoli et al., 2016). In Belgium, of 122 carcass swabs from slaughterhouses, 17 (12.8 %) tested positive for the *stx* gene marker via VTEC/EHEC multiplex PCR (Botteldoorn et al., 2003). A much higher PCR positivity rate (50 %, 75/175) was reported in French slaughtered swine carcasses; however, none of the isolates belonged to serotype O157:H7 (Bouvet et al., 2001). A study conducted in the U.S. reported a relatively lower STEC prevalence rate in raw pork samples collected from processing facilities. Only three culture positive samples (0.2 %, 3/1395) were found and belonged to serogroup O103 and O157 (Scott et al., 2020). Importantly, 22.1 % (309/1395) of the samples screened positive for both *stx* and *eae* genes but did not confirm positive for any of the top seven STEC serotypes, indicating that STEC serogroups different from the ones regulated by the USDA-FSIS may be circulating in raw pork.

In brief, presumptive STEC prevalence in swine slaughter ranged from 0.2 to 86.3 % depending upon sample categories, detection method, and the hygiene condition of the slaughterhouses (Table 1). Relatively higher occurrence was reported in cecal samples followed by carcass and fecal samples irrespective of geographical location. Cross-contamination, mishandling of the carcasses, and the unhygienic environmental condition of the facilities may be contributing factors positively associated with higher prevalence rates (Khan et al., 2018; Ojo et al., 2010). Overall, the researchers concluded that slaughtered swine may contribute to STEC transmission. Even though many isolates did not test positive for virulence factors implicated in severe illness cases in humans, the role of swine in STEC human infections needs to be further investigated for source attribution and risk mitigation (Scott et al., 2020; Tseng et al., 2015).

5. Prevalence and virulence factors of STEC in retail pork products

Xia et al. (2010) found a very low prevalence (0.1 %, 1/1167) of culture-confirmed STEC in pork chops that were purchased from different retail markets in the U.S. states of Georgia, Maryland, Oregon, and Tennessee between 2002 and 2007. A higher culture-confirmed prevalence rate (5.2 %, 12/231) was reported for ground pork samples purchased at chain grocery stores in the Washington D.C. area between March 2009 and 2010 (Ju et al., 2012); however, those STEC isolates lacked *eae* and were not regulated serogroups, unlike the Xia et al. (2010) and Scott et al. (2020) studies. The observation of non-regulated STEC types in pork products is common. In a study by Ju et al. (2012), eight of 16 STEC isolates were serogroup O91 and the remaining eight belonged to unknown serogroups. This O91 serogroup exhibited high cytotoxicity for Vero cells, indicating its potential as a human pathogen. Serogroup O91 has been previously associated with clinical cases of HUS in France and Germany (Bonnet et al., 1998; Mellmann et al., 2009). Additionally, Jung et al. (2019) found culture-confirmed U.S. non-regulated STEC serogroups that harbored both *stx* and *eae* gene at a 2.5 % (13/514) prevalence rate among samples of ground or non-intact and intact pork collected in the U.S. states of Delaware, New Jersey, and Pennsylvania between July and December 2017, and like Xia et al. (2010) and Scott et al. (2020) found a very low prevalence (0.8 %; 4/514) of culture-confirmed STEC. Recently, Zhang et al. (2021) reported on the prevalence of culture-confirmed STEC O157 (0.1 %, 1/789) and non-O157 (2.2 %; 13/580) in ground pork samples collected between 2014 and 2016 from 11 large cities across Canada. The non-O157 STEC isolates carried single *stx* genes (*stx*$_{1a}$, *stx*$_{2e}$, or *stx*$_{2a}$) while the STEC O157 carried a virulence gene combination (*stx*$_{2a}$, *eae*, and *ehxA*) with high potential for human disease (Zhang et al., 2021).

In Argentina, a contamination rate of 4.6 % (4/87) was observed in minced pork samples (Colello et al., 2016). The isolates were identified as STEC of serotypes O91:H21, O91:HNM, and ONT:HNM. Interestingly, the STEC O91 isolated from retail pork in this study and in the U.S. (Ju et al., 2012) were also found in carcass samples (Colello et al., 2016; Nastasijevic et al., 2020), suggesting transmission of the same STEC along the production and retail chain as expected. However, this transmission was not verified by a subsequent confirmatory whole genome sequence study.

Unlike North and South America, some European studies have reported relatively high STEC prevalence rates in pork sausages and non-ready-to-eat (NRTE) pork. In Italy, a 19 % (41/213) presumptive STEC prevalence rate with one culture-confirmed was estimated in fresh pork sausages collected from retail outlets in the Emilia Romagna Region between 2012 and 2013 (Bardasi et al., 2015). A similar rate (14 %, 65/465) of presumptive STEC with a higher culture-positive rate (10.8, 7/65) was reported in retail NRTE pork samples collected in the same region between January 2014 and August 2016 by the same research group (Bardasi et al., 2017). Of note, 42 of the 65 STEC isolates possessed *eae*, suggesting greater potential virulence. Another Italian study reported a comparable culture-confirmed STEC isolation rate (10.3 %, 13/126) in fresh pork sausages obtained from butcher shops of Napoli and Salerno provinces (Villani et al., 2005). Nearly half (46 %, 11/24) of the STEC were suspected O157:H7 and possessed *eae* with *stx*$_1$ and/or *stx*$_2$ genes. Yet, another Italian study reported a low prevalence rate (2.8 %; 19/675) of presumptive STEC (2.8 %; 19/675) in fresh retail pork sausages collected from the Umbria and Marche regions (Ercoli et al., 2016). However, no isolates were recovered from the presumptive-positive samples (Ercoli et al., 2016).

In Switzerland, Fantelli (2001) found a 1.1 % (2/189) isolation rate for culture-confirmed non-O157 STEC belonging to serogroups O20:H7 and O82:H8 in minced pork samples collected from Swiss butchers shop between January and June 2000. Both strains harbored the *stx*$_2$ gene and lacked *eae*, with the O82:H8 isolate possessing additional virulence

genes, *ehxA*, and *espP* (Fantelli, 2001). In the Netherlands, STEC O157:H7 was isolated from two of 262 samples (0.8 %) of fresh pork collected from different butcher shops (Heuvelink et al., 1999). The researchers did not examine the samples for other STEC, so the actual prevalence may be underestimated. In summary, 0.8 to 1.1 % of retail pork was contaminated by culture-confirmed STEC in European countries, where the contamination rate was higher in Italian pork products; and both O157:H7 and non-O157 STEC serogroups/serotypes were found.

Studies in Asia reported higher potential STEC contamination of pork than those from the Americas and Europe; however, researchers in Asia conducted culture confirmation mostly for *E. coli* O157:H7. A study from Wuhan, China reported that 37.8 % (67/177) of raw pork samples sold at retail markets between July 2011 and September 2013 contained *stx* gene markers (Li et al., 2016). Forty-one of the *stx*-positive samples also had the *rfb* O157 gene (encoding the O-antigen specific for *E. coli* O157:H7), and an unspecified number of those were cultured with only one yielding an *E. coli* O157:H7 (Li et al., 2016). Another Chinese study reported *E. coli* O157:H7 in 40.8 % (49/120) of pork meat and liver samples collected from six different wet markets and supermarkets located in the vicinity of Hubei province (Khan et al., 2018). Wet market samples had higher *E. coli* O157:H7 contamination than those at supermarkets (53.3 % vs. 28.3 %) likely due to the poorer hygienic conditions and handling of raw products. However, a study that collected meat samples from supermarkets and farmer markets located in Zigong and Beijing area reported a prevalence rate of 8.2 % (26/318) *stx*-positive retail raw pork samples (Bai et al., 2015). Another study from Southern China also found that only 1.4 % (2/145) of retail raw pork samples were contaminated with *E. coli* O157:H7 (Zhang et al., 2015), possibly indicating adherence to stricter hygienic practices.

A Korean study that examined pork samples for non-O157 STEC reported a low prevalence (1.4 %, 3/217) of STEC in retail packaged pork obtained between 2006 and 2012 (Lee et al., 2018). The three STEC isolates were one serotype O91:H14 and two O121:H10. The STEC O121:H10 harbored stx_{2e} and *ecp*A, whereas the O91:H14 carried stx_1 along with *ecp*A and the plasmid-encoded virulence genes *ehx*A, *esp*P, and *kat*P. Interestingly, a pork-derived O91:H14 strain from this study along with two beef-derived isolates were the same multi-locus sequence type (MLST; ST33) as 13 human O91:H14 isolated from diarrheal patients, suggesting a potential epidemiological link (Lee et al., 2018). In short, in Asian retail pork samples, the STEC prevalence based on *stx* genes was as high as 53.3 % while confirmed STEC identified by culture isolation was rare.

In South Africa, a high recovery rate (80 %, 32/40) of *E. coli* O157:H7 in retail pork purchased from supermarkets was reported by Ateba and Mbewe (2011). The isolates were characterized for stx_1, stx_2, *eae,* and *ehxA* and only three had the typical *E. coli* O157:H7 genotype; the other 29 were atypical O157:H7 lacking *stx* and/or *eae* genes. In a Nigerian study, a 4 % culture-confirmed prevalence (8/200) was found in retail pork (Ojo et al., 2010). Of the eight positive isolates, six belonged to serogroup O157, and the other two were O26 and O111, but it is not clear from the report whether the isolates carried typical virulence factors of severe disease-causing STEC. Lastly, a study conducted in New Zealand reported the STEC prevalence in raw retail pork at 4 % (1/35). This lone STEC was serotype O156:H- and possessed the stx_2 gene (Brooks et al., 2001).

Overall, the prevalence rate of *stx*-positive retail pork samples around the globe ranged from 1.1 (2/189) to 80 % (32/40). The reported prevalence rate of confirmed *E. coli* O157 and non-O157 ranged from 1.2 (8/675) to 23.2 % (41/177) and 0.1 (1/1167) to 14.7 % (26/177), respectively, in studies targeting multiple STEC serogroups/serotypes. STEC O157 was more prevalent than non-O157 serogroups in each continent but this is likely biased because many studies focus solely on *E. coli* O157:H7. Among the most common severe disease-causing non-O157 serogroups, O145 was prevalent in the U.S., Europe, and Asia. Similarly, serogroup O26 was found in the U.S., Europe, and Africa but not reported in Asia. Serogroup O121 was only reported in the U.S. and O45 only in Asia. The common serogroup reported in the U.S. and European studies was O103. Other than the U.S. regulated serogroups, O91 was isolated in the U.S., Asia, and South American retail pork samples. The most common virulence gene combination around the globe are described next. For U.S. studies: serogroup O157 plus *stx*, big-6 non-O157 plus *stx*, and unknown serogroup plus *stx* and *eae*. European studies: unknown serogroups plus *stx* and *eae,* stx_2 plus *eae*, and stx_1 plus stx_2 and *eae*. Asia: O157 serogroup plus stx_1, stx_2, *and ehxA*; unknown plus stx_1, *eae*, and *ehxA*; or only *eae*. Africa: O157 plus stx_2 plus *eae* and *ehxA*.

6. Difficulties in comparing prevalence studies

Some of the variability in the results presented in this review likely derives from differences in experimental design; geographical and climatological conditions; production, husbandry, and processing practices; as well as STEC definition and detection methods within individual studies. For example, carcass analytical sampling areas varied from 100 cm^2 to 4000 cm^2. Some studies relied on PCR for *stx* markers, often including *eae* for the interpretation of STEC presence. However, some primary sources reported prevalence based on culture isolation only. In some cases, particular serogroups (O26, O45, O103, O111, O121, O145 and O157) were examined by PCR in a sample, while other sources targeted these serogroups with IMS methods. The depth of characterization of isolates varied as well, from sole confirmation to extensive virulence factor profiling.

Comparing prevalence studies is challenging due to the different collection methods, sample sizes and origins, geographic location, and isolation protocols. For example, the low frequency of STEC seen in an African study (Ojo et al., 2010) may be due to the use of enrichment culture without an IMS step, which is known to enhance STEC recovery of targeted serogroups for which magnetic beads exist. Addition of novobiocin to the enrichment culture medium and the high background microflora of retail samples such as ground pork could contribute to the inhibition of certain non-O157 serogroups and subsequently report lower prevalence (Ercoli et al., 2016).

Other than isolation protocol, the detection marker could contribute to differences in prevalence estimates. In the U.S., many of the studies were based on serogroup markers that targeted only the seven most common severe disease-causing STEC. However, NACMCF (2019) recommends using virulence markers such as *stx* or *eae* genes rather than serogroup or serotype to identify pathotype. This is because a serogroup marker such as an O antigen itself is not a true virulence factor, unlike Shiga toxin and intimin. Keeping this in mind, any serotype of *E. coli* that can produce Shiga toxin and adhere to the human intestinal epithelial cells has the potential to cause human disease. In the U.S., *stx* and *eae* genes were identified in retail pork that lacked all of the seven most common serogroup genes (Jung et al., 2019). Furthermore, a recent U.S. baseline study reported that 22.1 % (309/1395) of pork harbored both *stx* and *eae* genes but culture confirmation aimed at the seven most common STEC serogroups only recovered three isolates (Scott et al., 2020). In both studies, the samples that were not culture-confirmed for a common STEC may still harbored a potentially dangerous strain (possessing *stx* and *eae*) but the narrowly directed culture efforts failed to identify them. Therefore, STEC prevalence in pork may be underestimated in the U.S. because testing often targeted only the beef regulated STEC serogroups without considering other serogroups that may possess virulence gene markers associated with human illness.

Serotypes that have not been associated with human disease may harbor *stx* and *eae* or *aggR* and pose a high risk to humans. According to NACMCF (2019), STEC strains that contain any of the following patterns of virulence gene factors have the potential to cause human disease, from high to low risk: stx_{2a}, *aggR, eae,* O157 serogroup; *stx* and"big six" non—O157; *stx*, *eae*, and other serogroups; and *stx* plus *eae*. Furthermore, the presence of the *ehxA* gene may enhance the virulence potential of STEC and occasionally intimin alone can lead to diarrhea in

humans by an attaching and effacing mechanism (Ercoli et al., 2016; Werber et al., 2008). Interestingly, a significant number of the prevalence studies conducted in Europe, Asia, Africa, and the Americas have detected one or more of these virulence gene combinations in swine and pork products. From the primary data summarized in this review, it is apparent that STEC strains derived from retail pork fall under low to moderate risk categories and thus can cause human disease. Table 2 summarizes the virulence profile of retail pork isolates from around the world and qualitatively categorizes the risk associated with the corresponding virulence profiles. Additional investigation is warranted on the genetic relatedness between pork and human clinical STEC isolates.

7. Conclusions

The collective scientific evidence suggests that pork and pork products may be naturally contaminated with a heterogeneous population of STEC strains. For many years, difficulties in detecting and differentiating STEC serogroups led industry and regulatory agencies to focus mostly on serogroup O157. However, it is clear that the prevalence of STEC in pork is underestimated. Recent advancement in the detection of O157 and non-O157 serogroups as well as virulence factor profiling could show the actual scenario of STEC prevalence in the pork production chain. These strains are passed over from the farm, where swine may be asymptomatic carriers, into slaughter and deboning operations, and eventually, the into meat supply, potentially causing infections in humans. The severe symptoms associated with STEC infections, as well as the increasing frequency of infections caused by a large variety of STEC serotypes, highlight the need for additional research to understand the ecology of these pathogens in pork and to aid in the development of prevention and risk mitigation strategies. Due to the frequent detection of STEC in swine and pork products, pork must be cooked according to USDA-FSIS guidelines reaching 60 °C/140 °F for intact pork products and 71.1 °C/160 °F for non-intact pork products to effectively inactivate STEC and lower the potential risk of foodborne illness in humans.

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Table 2
Virulence profile of STEC isolated from retail pork around the world.

Continent	Location	Matrix	Serogroup/serotype		Virulence gene	Relative Risk*	Reference
			Non-O157	O157			
Africa	Nigeria	Raw pork	O26, O111	O157	*stx*$_1$+ *stx*$_2$+ *eae* + *ehxA*	2	Ojo et al. (2010)
	South Africa	Raw pork	NT	O157: H7	*stx*$_2$ + *eae* + *ehxA*	2	Ateba and Mbewe (2011)
Asia	China	Raw meat	NT	O157: H7	*stx*$_1$ *or stx*$_2$	3	Khan et al. (2018)
	China	Raw pork	O45, O145		*stx*$_2$ + *eae*	2	Li et al. (2016)
				O157: H7	*stx*$_1$ + *stx*$_2$ + *eae* +*ehxA*	2	
	China	Raw pork	O91:H4, O57:H21, O98:H30, O121:H10, O141:H29 O8:H19	ND	*stx*	4	Bai et al. (2015)
					stx + *ehxA*	4	
	China	Raw pork	NT	O157	*stx*$_1$ + *eaeA* + *ehxA*	2	Zhang et al. (2015)
				O157: H7	*stx*$_2$ + *eaeA* + *ehxA*	2	
	Korea	Packaged	O121:H10	ND	*stx*$_{2e}$ + *ecpA*	4	Lee et al. (2018)
		Raw pork	O91:H14		*stx*$_1$ + *ehxA* + *espP* +*katP*+ *ecpA*	3	
Australia/New Zealand	New Zealand	Raw pork	O156:H-		*stx*$_2$	4	Brooks et al. (2001)
Europe	Italy	Pork sausage	NT	O157: H7	*stx*$_1$+ *stx*$_2$+ *eae*+ *rfbE*+ *ehxA*	2	Villani et al. (2005)
	Italy	Pork sausage	O104, O145, and O26 O103	O157	*stx*$_1$ *and/or stx*$_2$	3	Bardasi et al. (2015)
					stx$_1$ + *eae*	2	
	Italy	Pork sausage	O145, O103, O26	O157	*stx*$_1$ + *stx*$_2$ + *eae*	2	Ercoli et al. (2016)
	Italy	Pork meat, minced pork, sausages	O145, O26, O103, O104, O111	O157	*stx*$_1$ + *stx*$_2$ + *eae*	2	Bardasi et al. (2017)
	Netherland	Minced pork	NT	O157: H7	*stx*$_2$ + *eae*	2	Heuvelink et al. (1999)
	Switzerland	Minced pork	O20:H7 O82:H8	ND	*stx*$_2$	4	Fantelli (2001)
					stx$_2$+ *ehxA* + *espP*	4	
North America	Canada	Ground pork		O157: H7	*stx*$_{2a}$ + *eae* + *ehxA*	2	Zhang et al. (2021)
	USA	Ground pork	O91 Unknown	NT	*stx*$_{2a}$ + *ehxA*	4	Ju et al. (2012)
					stx$_{2dact}$ + *ehxA*	4	
	USA	Ground pork, pork cuts	O103, O121	O157	*stx*$_1$ *and/or stx*$_2$	3	Magwedere et al. (2013)
	USA	Pork chop	ONT:H51	ND	*stx*$_1$	4	Xia et al. (2010)
	USA	Ground pork	Unknown (Not US regulated serogroup)		*stx* + *eae* + *ehxA*	3	Jung et al. (2019)
South America	Argentina	Raw and minced pork	O9:H21, O9:HNM, ONT:H21 ONT:HNM	ND	*stx*$_2$ + *agn43*	4	Colello et al. (2016)
					stx$_{2e}$ + *agn43*	4	
					stx$_2$ + *stx*$_{2e}$ + *eae* + *agn43*	3	

NT = Not targeted, ND=Not detected. *Relative risk ranking for causing Human disease is determined based on NACMCF (2019).
The following interpretation was applied: 1 = *stx*$_{2a}$ + *aggR;* 2 = *stx* + *eae* + O157 serogroup OR *stx* + *eae* + US regulated non-O157serogroup; 3 = only *stx* + O157:H7 OR *stx* + *eae* or other Adhesin+unknown/other serogroup (not US regulated), 4 = *stx* + unknown/other serogroup (not US regulated) OR *stx* + *ehxA* or other virulence marker+ unknown/other serogroup (not US regulated).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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