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Shiga toxin-producing *Escherichia coli* in swine: the public health perspective

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) strains are food-borne pathogens that are an important public health concern. STEC infection is associated with severe clinical diseases in human beings, including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which can lead to kidney failure and death. Cattle are the most important STEC reservoir. However, a number of STEC outbreaks and HUS cases have been attributed to pork products. In swine, STEC strains are known to be associated with edema disease. Nevertheless, the relationship between STEC of swine origin and human illness has yet to be determined. This review critically summarizes epidemiologic and biological studies of swine STEC. Several epidemiologic studies conducted in multiple regions of the world have demonstrated that domestic swine can carry and shed STEC. Moreover, animal studies have demonstrated that swine are susceptible to STEC O157:H7 infection and can shed the bacterium for 2 months. A limited number of molecular epidemiologic studies, however, have provided conflicting evidence regarding the relationship between swine STEC and human illness. The role that swine play in STEC transmission to people and the contribution to human disease frequency requires further evaluation.

Keywords

shiga toxin-producing *Escherichia coli* (STEC); swine; food safety

Introduction

Infection with Shiga toxin-producing *Escherichia coli* (STEC) is a critical public health concern. STEC infections are associated with outbreaks and sporadic cases of diarrhea and severe clinical diseases in human beings, including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Karmali *et al.*, 1985; Nataro and Kaper, 1998). HUS is a life-threatening thrombotic disorder characterized by acute renal failure, thrombocytopenia and

microangiopathic hemolytic anemia (Karmali *et al.*, 1985; Karmali 1989; Tarr *et al.*, 2005). It is one of the leading causes of acute renal failure in young children worldwide (Williams *et al.*, 2002; Tarr *et al.*, 2005; Karmali *et al.*, 2010). Every year in the USA, more than 170,000 human illnesses are attributed to STEC (Scallan *et al.*, 2011), and food-borne STEC illnesses represent an estimated economic burden of 280 million dollars (Hoffmann *et al.*, 2012).

STEC represent a subset of *E. coli* that produce a cytotoxin known as the Shiga toxin (Stx), or verotoxin. Various STEC transmission routes have been identified, although STEC infections are most frequently associated with consumption of contaminated food (meat, dairy products, produce, and others) and water (Rangel *et al.*, 2005; Doyle *et al.*, 2006; Kaspar *et al.*, 2010). Other sources of infection include animal contact (Durso *et al.*, 2005; Keen *et al.*, 2006, 2007), person-to-person contact (Spika *et al.*, 1986; Carter *et al.*, 1987; Rowe *et al.*, 1993), and airborne transmission (Varma *et al.*, 2003). Cattle have been suggested to be the important animal reservoir of STEC, and they do not typically present with any STEC-associated clinical symptoms (Griffin and Tauxe, 1991; Rangel *et al.*, 2005; Gyles, 2007), except in calves under experimental conditions (Dean-Nystrom *et al.*, 1997). Nevertheless, many other food products, including pork products, have been confirmed as vehicles of STEC transmission (CDC, 1995a, b; Paton *et al.*, 1996; Williams *et al.*, 2000; MacDonald *et al.*, 2004; Conedera *et al.*, 2007; Trotz-Williams *et al.*, 2012). Feral swine, for example, were found to be a risk factor for STEC O157:H7 contamination of the spinach implicated in a 2006 North American outbreak (Jay *et al.*, 2007). In pork-associated outbreaks and cases, it is unknown whether the contamination of pork products occurs during swine processing or via cross-contamination from other foodstuffs (Williams *et al.*, 2000; MacDonald *et al.*, 2004; Conedera *et al.*, 2007; Trotz-Williams *et al.*, 2012). Although swine-associated outbreaks have been reported less frequently than cattle-associated outbreaks, the likelihood that swine represent an important source of STEC infections in human beings cannot be overlooked.

The role that swine play in STEC transmission to human beings needs to be further evaluated to determine whether swine-derived STEC strains are an important public health concern. The severe symptoms associated with STEC infections and the increasing frequency of infections caused by a large variety of STEC serotypes highlight the fact that more research is needed to better understand these pathogens to aid in developing prevention and control strategies. In this literature review, previous studies focusing on STEC of swine origin will be critically summarized.

Food-borne outbreaks associated with pork products

Although only in a few instances, pork has been reported as a potential vehicle involved in outbreaks of STEC infections (CDC, 1995a, b; Paton *et al.*, 1996; Williams *et al.*, 2000; MacDonald *et al.*, 2004; Conedera *et al.*, 2007; Trotz-Williams *et al.*, 2012). Table 1 summarizes the food products involved in these outbreaks and the serotype and virulence gene characteristics of the STEC strains isolated in these outbreaks. Mixed meat products were involved in most of these reported outbreaks. Thus, it was difficult to attribute the source of contamination to any of the animal species. Notably, the most recent outbreaks

were associated with salami containing only pork (Conedera *et al.*, 2007) and large cuts of pork from a whole roasted pig (Trotz-Williams *et al.*, 2012). It is impossible to eliminate the possibility that cross-contamination from different foodstuffs took place during processing. However, pigs were suggested to be the source of STEC O157:H7 infection in the most recent outbreak (Trotz-Williams *et al.*, 2012). Although the responsible source of STEC was not conclusive in these outbreaks, pork products cannot be excluded as a potential vehicle of STEC transmission.

STEC prevalence in swine populations

Although outbreaks have been rarely reported, the incidence of human STEC outbreaks associated with pork products has raised questions about the sources of STEC in the food chain, from pork products, to pigs at slaughter facilities, to the most upstream origin: on-farm swine. The presence of STEC in pork or pork-associated products has been reported in some epidemiologic studies (Samadpour *et al.*, 1994; Brooks *et al.*, 2001; Jo *et al.*, 2004; Oteiza *et al.*, 2006; Xia *et al.*, 2010; Ateba and Mbewe, 2011; Li *et al.*, 2011). These data were sparse, and the numbers related to STEC prevalence in pork or pork-associated products were not consistent. The discrepancies are potentially attributable to multiple factors. For example, different isolation protocols and different types of pork products were used in these studies. In short, these positive findings of STEC in pork and pork-associated products indicate that STEC are present on retail pork products. The possibility that swine play a role in the contamination of pork products cannot be excluded.

The prevalence of STEC in clinically healthy swine populations has been reported in numerous studies in multiple regions of the world. In the USA, STEC detection in the swine population has been reported. A study was published in which colon samples were obtained from pigs at a US slaughter facility to detect the presence of STEC O157 (Feder *et al.*, 2003). Six (1.9%) of the 305 colon samples were positive for STEC O157:H7. Interestingly, no STEC O157:H7 isolate was recovered in the National Animal Health Monitoring System's (NAHMS) Swine 1995 study (Bush, 1997) and the later NAHMS 2000 study (Feder *et al.*, 2007). However, it is impossible to directly compare the number of STEC O157:H7 positive samples in these studies due to different study designs and sample collection methods. In short, these reports provided evidence that swine in the USA carry STEC serotype O157:H7, although at a much lower rate compared with cattle.

Besides STEC O157:H7, the NAHMS 2000 study also recovered and characterized non-O157 STEC isolates (Fratamico *et al.*, 2004, 2008). At least one STEC isolate was recovered from 196 (28.5%) of 687 fecal samples. A total of 219 STEC isolates were recovered, and they were categorized into various non-O157 O serogroups. Most importantly, some O serogroups had previously been associated with human clinical cases, namely O9, O20, O91, O101, and O121. The results of this study indicated that clinically healthy pigs from multiple states in the USA shed non-O157 STEC at some point during the late finishing period.

A recent study reported no significant effect of antimicrobials (chlorotetracycline and bacitracin) in feed on fecal shedding of non-O157 STEC in finishing swine (Wells *et al.*,

2012). Notably, STEC belonging to important O serogroups, namely O26, O103, and O145, were isolated from 6.9, 2.4, and 4.8% of all the swine rectal swab samples. However, this study targeted a selected group of non-O157 STEC for detection in the samples (O26, O103, O111, O121, and O145). In spite of this approach, the high prevalence of the Shiga toxin gene (*stx*) in enriched samples (58% prior to slaughter) and the detection of non-O157 STEC belonging to important O serogroups highlight the need to further investigate STEC shed by clinically healthy swine.

Besides the USA, studies in other regions of the world reported wide ranging estimates of STEC prevalence from 0% (Oporto *et al.*, 2008; Pinaka *et al.*, 2013) to 68.3% (Borie *et al.*, 1997). The highest prevalence, 68.3%, was reported from one study in Chile (Borie *et al.*, 1997). Rectal swab samples were collected during the evisceration process from swine and cattle at slaughter facilities. Surprisingly, the STEC prevalence in swine samples was higher than that in cattle (28.7%). In addition to the strains identified as non-typable by O serogrouping, O157 was the most prevalent O serogroup among the swine STEC isolates. Other important STEC serogroups, such as O26 and O111, have also been reported (Rios *et al.*, 1999). In one state in Brazil, the prevalence of STEC was 1.4% (one STEC-positive sample) from 74 fecal samples obtained aseptically from swine intestines at slaughter facilities (Martins *et al.*, 2011). A later study performed in another state in Brazil reported one (0.4%) STEC O103 isolate recovered from 215 sponge-rubbed samples from swine carcasses at three slaughter facilities (Borges *et al.*, 2012).

In a South African study, *E. coli* O157:H7 was more frequently found in swine fecal samples and in pork (67.7%) than in cattle fecal samples and beef (27.7%) (Ateba and Mbewe, 2011). In this study, only a selected subset of *E. coli* O157:H7 isolates was analyzed for the presence of *stx*. Therefore, it is difficult to determine the number of STEC O157:H7 positive samples in this study. Nevertheless, this study was in agreement with the previous Chilean studies (Borie *et al.*, 1997; Rios *et al.*, 1999) showing that swine are able to shed STEC at a relatively high rate and are potentially important STEC reservoirs in some places in the world.

Several European studies have reported STEC prevalence in swine populations, and most of the earlier studies focused on STEC serogroup O157. A study conducted in the UK reported the recovery of six (0.3%) STEC O157 isolates from over 2000 swine fecal samples at slaughter facilities (Milnes *et al.*, 2009). Similarly, in Ireland, the prevalence of STEC O157:H7 was 0.6% (3 of 480) in fecal samples of pigs at slaughter facilities (Lenahan *et al.*, 2009a). In a study conducted in the Netherlands, STEC O157 was isolated from 1 (0.7%) of 145 fecal samples from pigs at one slaughter facility (Heuvelink *et al.*, 1999). A low proportion (0.7%, 1 of 150) of STEC O157-positive fecal samples was reported from pigs at slaughter facilities in Northern Italy (Bonardi *et al.*, 2003). In Norway, STEC O157:H7 was found in 2 (0.1%) samples of intestinal contents from 1,976 pigs, and one of the two pigs was housed on a farm that also reared cattle. However, no STEC O157:H7 isolate was recovered from samples of cattle on the same farm (Johnsen *et al.*, 2001). Interestingly, other studies also reported isolation of STEC O157:H7 strains from pigs that were kept with cattle or other ruminants on the same farm. For instance, STEC O157:H7 was recovered from 2 (0.08%) of 2,446 fecal samples of slaughtered pigs that were housed with ruminants

on the same farm in a Swedish study (Eriksson *et al.*, 2003). In general, the prevalence of STEC O157:H7 in swine appeared to be low in these studies. However, the presence of ruminants on the same farm that reared swine suggests that horizontal transmission of STEC may occur between different animal species.

Several studies investigated the prevalence of both STEC O157 and non-O157 in clinically healthy swine populations. A study in Belgium reported that non-O157 STEC strains were isolated from 8 (7.9%) out of 101 swine colon fecal samples at a slaughter facility (Botteldoorn *et al.*, 2003). In Switzerland, the *stx* gene was detected in 138 (22%) of the 630 swine fecal samples at a slaughter facility. Subsequently, forty-five non-O157 STEC isolates were recovered from 45 randomly selected *stx*-gene-positive samples (Kaufmann *et al.*, 2006). It was difficult to estimate the true prevalence of STEC in this study because not every *stx*-positive sample was analyzed for STEC isolates. These reports suggest that swine may be potential reservoirs of non-O157 STEC. In these studies, relatively low numbers of STEC isolates were recovered even though the numbers of *stx*-positive samples were high. This fact reflects the challenges of non-O157 STEC isolation. In contrast to the above positive findings, one study conducted in Northern Spain analyzed pooled rectal fecal samples from a total of 510 pigs, and the test results were negative for both O157 and non-O157 STEC (Oporto *et al.*, 2008). Negative isolation of STEC in 106 swine fecal samples was also reported by a study conducted in Central Greece (Pinaka *et al.*, 2013).

The presence of STEC in swine populations has also been documented in Asia. In Japan, the prevalence of STEC O157:H7 in clinically healthy on-farm swine was 1.4%, and this number was similar to the prevalence in Japanese cattle around the same time period (Nakazawa and Akiba, 1999). A later Japanese national surveillance report stated that STEC isolates were recovered from 32 (14%) of 179 swine fecal samples. Some strains belonged to the serotypes frequently associated with human diseases (Kijima-Tanaka *et al.*, 2005). In Korea, only reports of STEC O157:H7 in swine have been published. One (0.3%) STEC O157:H7 isolate was recovered from 345 fecal samples from pigs at slaughter facilities and on farms (Jo *et al.*, 2004). Two STEC O157:H7 isolates were recovered from fecal samples of pigs at slaughter facilities in another study (Kim *et al.*, 2005). However, the prevalence was unknown because the total number of samples from pigs was not provided. In a recent report from Korea, one (0.3%) STEC O157 isolate was recovered from 291 swine fecal samples (Keum *et al.*, 2010). In China, one study reported STEC isolates recovered from 10 (2.1%) of 487 rectal swabs from pigs at a slaughter facility in Hong Kong. The pigs were from a number of provinces in mainland China (Leung *et al.*, 2001). Another study reported the prevalence of STEC in one swine breeding farm in mainland China. STEC O157 was detected in 8 (1.1%) of 720 fecal samples, while non-O157 STEC was isolated from 33 (4.6%) samples (Yan *et al.*, 2011). STEC strains were isolated from 10 (6%) of 169 swine fecal samples obtained from a number of swine farms in one province in Vietnam (Kobayashi *et al.*, 2003).

In addition to domestic swine, feral swine and wild boars have been suggested to play a role in STEC transmission. The most prominent evidence was obtained from a study that took place in the USA in 2006. Feral swine were suggested to be involved in the contamination of spinach, which was associated with a nationwide outbreak caused by STEC O157:H7. The

outbreak strain was isolated from feral swine feces, which were collected directly on the spinach fields (Jay *et al.*, 2007). Moreover, reports in other countries also indicated the presence of STEC in wild boars. STEC O157:H7 was found in 1 (1.4%) of 68 wild boars in Sweden (Wahlstrom *et al.*, 2003). One Spanish study investigated the presence of STEC O157:H7 and non-O157 STEC in wild boars. STEC O157:H7 was detected in 7 (3.3%) and non-O157 STEC was detected in 11 (5.2%) fecal samples from 212 wild boars (Sánchez *et al.*, 2010).

Human-animal contact is an important risk factor for STEC transmission, thus the presence of STEC in swine in zoos and petting zoos has also been investigated. No STEC O157 was detected in 45 pig fecal samples collected at US institutions accredited by the Association of Zoos and Aquariums (Keen *et al.*, 2007). In contrast, STEC O157:H7 was recovered in 13 (1.2%) of 1,102 fecal samples from pigs in 19 county fairs in two states in the USA (Keen *et al.*, 2006). In the UK, an epidemiologic study was conducted to detect the presence of STEC O157 in 'open farms' in which there is close contact with animals to attract the general public (Pritchard *et al.*, 2009). STEC O157 was isolated in thirty (17.9%) of 168 swine fecal samples. However, this number may not reflect the true prevalence at one time point as it represents a collated number from samples collected from 22 different farms within a 10-year period. Moreover, it was not clear whether the pigs were housed together or in close contact with other animal species. Although the prevalence of STEC O157:H7 in these reports was low, pigs at facilities where direct contact with human beings may occur are another potential risk factor of STEC transmission.

In general, these data indicated that STEC strains in swine are geographically widely distributed given the fact that reports were from many regions in different continents. Although the prevalence numbers are wide ranging, these data provide epidemiologic evidence that STEC strains are prevalent in swine. Multiple factors may account for the discrepancies in the data among these studies, such as differences in farm management systems, sample sources, sampling methods, isolation protocols, and diagnostic tests used.

A number of reports have described detection of STEC O157 in swine fecal samples, and various non-O157 STEC serotypes were also detected in swine (Botteldoorn *et al.*, 2003; Kaufmann *et al.*, 2006; Fratamico *et al.*, 2008). STEC belonging to various serogroups of which some that have been associated with human illness were identified in swine (Kaufmann *et al.*, 2006; Fratamico *et al.*, 2008), and, surprisingly, none of the serogroups were those commonly associated with swine edema disease. In addition, most of the above epidemiologic studies were cross-sectional studies, and they are unable to determine if the prevalence of STEC in swine changes with time (Dohoo *et al.*, 2010). More research needs to be done to understand STEC shed by clinically healthy swine.

STEC in swine: edema disease

Besides isolation from clinically healthy swine, STEC can cause edema disease, which affects post-weaning pigs and young finishing pigs. Some STEC strains, in particular edema disease-associated STEC, also play a role in fatal shock, that occurs in pre- and post-weaning pigs (Fairbrother and Gyles, 2012). STEC strains belonging to certain serogroups,

including O138, O139, O141, and O147 are more frequently associated with edema disease (Fairbrother and Gyles, 2012). These STEC strains colonize in the small intestine and typically produce the variant Shiga toxin 2e (Stx2e). The toxin enters the bloodstream and binds to the specific receptor, globotetrao-sylceramide (Gb4), which is located on epithelial and endothelial cells (DeGrandis *et al.*, 1989). The toxin then impairs blood vessels, leading to edema, ataxia, and death (Fairbrother and Gyles, 2012). In general, the clinical presentations of swine edema disease are somewhat different from those associated with human STEC diseases.

Besides Stx2e, STEC strains associated with edema disease have been found to possess virulence factors different from those found in STEC strains isolated from human clinical cases. For example, F18 fimbriae are absent in most human-derived STEC (Sonntag *et al.*, 2005), but are essential for adherence to swine epithelial cells (Bertschinger and Gyles, 1994), and therefore are likely to play a role in specificity and adaptation to the host. There are risk factors, including dietary changes and the introduction of pigs to new herds, suggested to be important for edema disease onset (Fairbrother and Gyles, 2012).

Human strains of Stx2e-producing *E. coli*

Interestingly, in addition to their isolation from pigs, Stx2e-producing *E. coli* strains were recovered from samples of wastewater treatment plants in France (Vernozy-Rozand *et al.*, 2004), and from meat (pork, beef, wildlife) and milk in Germany (Beutin *et al.*, 2007). Notably, although rarely documented, Stx2e-producing *E. coli* strains have been isolated from human patients with HUS (Thomas *et al.*, 1994) and uncomplicated diarrhea (Pierard *et al.*, 1991; Muniesa *et al.*, 2000; Friedrich *et al.*, 2002; Beutin *et al.*, 2004; Sonntag *et al.*, 2005). Moreover, Stx2e-producing *E. coli* strains have been recovered from stool samples of asymptomatic human beings (Friedrich *et al.*, 2002; Sonntag *et al.*, 2005). The etiologic role of Stx2e-producing *E. coli* strains in these human cases has not been determined. No particular source of infection has been identified in these human cases associated with Stx2e-producing *E. coli*.

A study was conducted to analyze Stx2e-producing *E. coli* strains from asymptomatic people, people with uncomplicated diarrhea, and diseased pigs to compare their virulence gene profiles and adherence to human and swine intestinal epithelial cells (Sonntag *et al.*, 2005). Virulence genes commonly found in STEC strains associated with HC and HUS, such as the gene, *eae*, that encodes for intimin, an important attachment protein, were not detected in both the human- and swine-derived Stx2e-producing *E. coli* strains. This fact may suggest that unknown virulence factors are involved in the pathogenicity of human Stx2e-producing *E. coli* strains. Additionally, the human-derived Stx2e-producing *E. coli* strains adhered to human epithelial cells but not swine epithelial cells. In contrast, swine-derived Stx2e-producing *E. coli* strains lysed human epithelial cells and adhered to swine epithelial cells. This study only analyzed Stx2e-producing *E. coli* strains from diseased pigs, and thus, Stx2e-producing *E. coli* strains from healthy pigs need further examination.

Beutin *et al.*, however, have characterized Stx2e-producing *E. coli* from people with uncomplicated diarrhea, people with no clinical symptoms, diseased pigs, and healthy pigs

at slaughter facilities to determine their serotypes, distribution of virulence genes, and Stx2e production (Beutin *et al.*, 2008). They found that Stx2e-producing *E. coli* strains from different sources were heterogeneous with regard to serotypes and virulence genes. In agreement with the results of Sonntag *et al.*, (2005), virulence genes commonly detected in STEC strains associated with HC and HUS, such as *eae* and *ehxA*, the latter of which encodes an enterohemolysin, were not detected by PCR in both the human- and swine-derived Stx2e-producing strains analyzed in this study. Additionally, the authors used an enzyme immunoassay to find that human-derived strains produced significantly higher amounts of Stx2e than strains from diseased pigs. Interestingly, the toxin-production level did not significantly differ between strains from human subjects with uncomplicated diarrhea and those with no clinical symptoms. However, different environments (in hosts or under experimental conditions) may contribute to the differences of toxin production. Another likely explanation is that there are other unidentified virulence factors in the pathogenic Stx2e-producing *E. coli* strains.

In all of these reports, the primary conclusion was that there was a lack of evidence to suggest that Stx2e-producing *E. coli* strains are a critical public health concern. Nevertheless, these results also suggest that unknown virulence factors may contribute to the pathogenesis of Stx2e-producing *E. coli* strains in human hosts. Additionally, Stx2e-producing *E. coli* strains have been mostly isolated from human patients with uncomplicated diarrhea. Patients with uncomplicated diarrhea may not seek medical attention, which may contribute to the low reporting frequencies of Stx2e-producing *E. coli* infections in human patients. Some commercial serological tests do not detect the toxin Stx2e (Feng *et al.*, 2011). The limitation of diagnostic tests may contribute to missed detection of Stx2e-producing *E. coli* infections. On the other hand, the picture remains unclear regarding the source of Stx2e-producing *E. coli* in human infections. More research is warranted to address whether there is an association between human Stx2e-producing *E. coli* strains and strains from pork, pigs, or the associated swine environment.

Molecular epidemiology of swine STEC

The presence of virulence genes other than *stx* also plays a role in the capability of STEC strains to cause disease (Bugarel *et al.*, 2011). Therefore, examining the presence or absence of the selected virulence genes (virulence gene profile) is essential in assessing the public health risk of STEC strains (Cobbold and Desmarchelier, 2000; Slanec *et al.*, 2009). A considerable challenge for understanding virulence gene profiles of swine STEC is that the profile of virulence genes targeted varies in each report. The most common virulence genes evaluated are *stx1*, *stx2*, *eae*, and *ehxA*. Different *stx1*, *stx2*, *eae*, and *ehxA* gene combinations have been detected in swine STEC strains (Borie *et al.*, 1997; Feder *et al.*, 2003). In the NAHMS 2000 study in the USA, although a majority of swine STEC strains carried the *stx2e* gene, 6% of the strains carried other *stx2* variants. The *eae* gene was not detected in any of the swine isolates in this study (Fratamico *et al.*, 2008), and this was in agreement with other studies (Leung *et al.*, 2001; Kijima-Tanaka *et al.*, 2005). In contrast, many studies have identified the *eae* gene in STEC strains from clinically healthy swine (Borie *et al.*, 1997; Heuvelink *et al.*, 1999; Nakazawa and Akiba, 1999; Johnsen *et al.*, 2001; Bonardi *et al.*, 2003; Botteldoorn *et al.*, 2003; Feder *et al.*, 2003; Jo *et al.*, 2004; Kim *et al.*,

2005; Kaufmann *et al.*, 2006; Keen *et al.*, 2006; Lenahan *et al.*, 2009a; Ateba and Mbewe, 2011). Meanwhile, detection of the *ehxA* gene in swine STEC isolates was reported in numerous studies (Heuvelink *et al.*, 1999; Botteldoorn *et al.*, 2003; Kim *et al.*, 2005; Chapman *et al.*, 2006; Kaufmann *et al.*, 2006; Fratamico *et al.*, 2008; Lenahan *et al.*, 2009a; Sánchez *et al.*, 2010; Ateba and Mbewe, 2011). A limited number of studies described testing for different subsets of STEC virulence genes in swine STEC strains (Chapman *et al.*, 2006; Zweifel *et al.*, 2006; Fratamico *et al.*, 2008; Lenahan *et al.*, 2009a). For instance, some *eae*-negative swine STEC strains carried the *saa* gene (STEC autoagglutinating adhesion), and other adhesins (Chapman *et al.*, 2006). The above studies suggest that swine STEC strains carry various combinations of virulence genes. A subset of swine STEC strains does carry important virulence genes in human pathogenesis, such as *eae*. More extensive examination is needed because the presence or absence of many other STEC virulence genes has not been examined in swine STEC strains.

Molecular genotyping methods, such as pulsedfield gel electrophoresis (PFGE), have been frequently utilized to evaluate the pathogenic potential of STEC strains and provide suggestions for future strategies in control and prevention of STEC transmission (Cobbold and Desmarchelier, 2001; Lanier *et al.*, 2009). For example, these methods are used to identify the source of STEC during food-borne outbreaks and to determine the genetic relatedness of STEC strains (Barrett *et al.*, 1994; Samadpour, 1995; Diekema *et al.*, 1997; Izumiya *et al.*, 1997; Singer *et al.*, 2004). A limited number of studies have incorporated a small number of swine STEC strains to examine their genetic relatedness with strains from human subjects with diarrhea, HC, HUS, or without clinical symptoms (Franke *et al.*, 1995; Rios *et al.*, 1999; Johnsen *et al.*, 2001; Lenahan *et al.*, 2009b). Most of these studies focused on STEC O157:H7 strains, and only one study investigated STEC O101 strains (Franke *et al.*, 1995). The results from these studies were inconsistent. One Chilean study indicated that the swine STEC O157 strains were categorized into the same cluster with strains from human HUS cases in the same geographic area by analyzing the PFGE patterns. However, no further epidemiologic relationship could be drawn between the swine isolates and human cases (Rios *et al.*, 1999). One study conducted in Norway reported similarities of PFGE patterns among swine STEC O157:H7 strains and those isolated from human cases. However, there was no other epidemiologic information provided about the human STEC cases (Johnsen *et al.*, 2001). In contrast, in an Irish study the investigators stated that swine STEC O157:H7 strains were not genetically related to human strains at the PFGE pattern similarity criterion of 80% (Lenahan *et al.*, 2009b).

One study implemented repetitive element sequencebased PCR (rep-PCR) to examine the genetic relatedness of STEC O101 strains from swine and a human case of diarrhea. Interestingly, the result of the analysis indicated that swine STEC O101 strains showed a high degree of relatedness with the human STEC O101 strain. Moreover, the *stx2e* gene sequences from STEC O101 strains of two different origins shared a high degree of homology, and none of the STEC O101 strains carried the *eae* or *ehxA* genes (Franke *et al.*, 1995). A direct relationship cannot be ascertained between the swine and human STEC since the strains were from two different countries and isolated in different time periods.

However, the molecular evidence highlights that swine may be a potential STEC reservoir and a source of human infections.

A number of studies have examined the genetic relatedness of swine STEC strains to strains isolated from other animal reservoirs. The PFGE patterns indicated that swine STEC strains were not closely related to STEC strains of bovine origin (Rios *et al.*, 1999; Lenahan *et al.*, 2009b), ovine origin (Lenahan *et al.*, 2009b) and from turkeys (Heuvelink *et al.*, 1999). A Korean study demonstrated that swine STEC O157:H7 strains had distinct randomly amplified polymorphic DNA (RAPD) patterns compared with strains from cattle at a similarity criterion of 63% (Kim *et al.*, 2005). Another study showed that swine STEC O157:H7 strains demonstrated some similarity in PFGE patterns to strains from cattle (Johnsen *et al.*, 2001). A Polish study reported that one swine STEC O157 strain clustered with STEC O157 strains from cattle at a similarity criterion of 80% based on PFGE patterns. The swine STEC O157 strain was from a weaned pig with diarrhea, and no other epidemiologic relationship was described between the swine and cattle STEC strains (Osek and Gallien, 2002).

In the epidemiologic investigation following a spinach-associated outbreak of STEC O157:H7 in 2006 in the USA, PFGE and multilocus variable number tandem repeat analysis (MLVA) were used to analyze STEC isolates from different sources (Jay *et al.*, 2007). Indistinguishable PFGE patterns from STEC isolates from cattle, feral swine, surface water, and soil were identified. MLVA also categorized the STEC isolates with indistinguishable PFGE patterns together with the outbreak STEC strain as one cluster. This evidence led the authors to suggest that feral swine may serve as a risk factor for STEC contamination of the spinach fields (Jay *et al.*, 2007). Inspired by this outbreak, a Spanish study used PFGE to examine the genetic relatedness of STEC isolates from wild boar and a human patient with diarrhea (Sánchez *et al.*, 2010). Surprisingly, a STEC isolate from a wild boar shared an indistinguishable PFGE pattern with a STEC isolate from a human patient with diarrhea in the same geographic area. Although there were no epidemiologic data available to establish a relationship between the human case and wild boar, the molecular genotyping results indicated that wild boars are a potential source of STEC contamination (Sánchez *et al.*, 2010).

Results presented by these molecular epidemiologic studies are varied, which is potentially due to multiple factors. For example, there is potential selection bias of the isolates included in the genotypic analysis. Limited numbers of swine STEC strains and strains from other origins were included in the above molecular genotypic studies. The analytical results may not reflect the complete picture of genetic relatedness between STEC strains from swine and other origins. Moreover, most of the studies focused on STEC O157:H7. There is a paucity of knowledge on the genetic relatedness of non-O157 STEC from different origins. Conclusively, the genetic relationship of swine STEC strains with human clinical strains is still poorly understood. Filling these current knowledge gaps is relevant to not only better understand swine STEC but also to assess the potential public health risk of swine STEC.

The effect of swine STEC strains on human intestinal cells

Kennedy *et al.* (2010) infected human Caco-2 cells, which is a human colonic epithelial cell line, with STEC O157:H7 strains isolated from human patients or from healthy pigs at slaughter facilities. The human and swine STEC strains possessed the same virulence gene profiles, including *eae* and other virulence genes, published in an earlier study (Lenahan *et al.*, 2009b). Notably, swine STEC O157:H7 strains induced greater loss of monolayer cell integrity than human-source STEC. Moreover, microarray experiments that examined RNA levels transcribed from different STEC genes indicated that swine and human STEC strains had different gene expression profiles. When exposed to swine STEC strains, expression levels of some genes involved in cytoskeleton rearrangement were up-regulated in the human Caco-2 cells (Kennedy *et al.*, 2010). However, more research is needed to further examine the effect of infection with swine STEC strains on human epithelial cells.

STEC infection in swine: animal model studies

Swine have been used as an animal model in studies examining STEC colonization (Jordan *et al.*, 2004), virulence of different STEC strains (Baker *et al.*, 2007; Shringi *et al.*, 2012), and treatment of STEC infections (Sheoran *et al.*, 2005). Interestingly, some studies evaluated the pathogenicity and colonization of intimin-negative STEC strains in swine models. One study found that intimin-negative non-O157 STEC strains colonize and cause similar intestinal lesions and systemic diseases as intimin-positive STEC O157:H7 strains in cesarean-derived colostrum-deprived (CDCD) neonatal pigs (Dean-Nystrom *et al.*, 2003). Moreover, a later study reported that there were no significant differences in bacterial counts and the numbers of tissues infected between intimin-positive and intimin-negative STEC O157:H7 strains in 12-week-old adult pigs (Jordan *et al.*, 2005). Both intimin-positive and intimin-negative experimental strains were recovered in the pigs 38 days after inoculation. Notably, the result of this study in pigs is different from a previous study indicating that intimin aids the persistence of STEC O157:H7 strains in adult cattle and sheep (Cornick *et al.*, 2002). This may suggest that other adhesins contribute to the colonization of STEC in swine. The above evidence demonstrates that swine are biologically capable of carrying STEC, including intimin-positive STEC strains.

To further understand if swine have the potential to become reservoirs of STEC, researchers have pursued experimental challenge models. Booher *et al.* inoculated 3-month-old pigs with mixtures of STEC O157:H7 and other pathogenic *E. coli* strains to observe the level and duration of fecal shedding (Booher *et al.*, 2002). They reported that STEC O157:H7 strains were isolated from swine fecal samples up to 2 months after inoculation, and the experimental strains were recovered in swine intestinal tissues. Shedding of experimental STEC strains was dose-dependent, and strains inoculated at lower levels (10^7 CFU per strain per animal) were not recovered in the feces 2 months after inoculation. The dose-dependent shedding is similar to what was found in cattle under experimental conditions (Cray and Moon, 1995). Interestingly, one of the experimental strains inoculated at the lower dose was recovered in swine cecum tissues at necropsy 2 months after inoculation (Booher *et al.*, 2002). This study suggested that swine could shed STEC O157:H7 for at least 2 months.

Cornick and Helgersen conducted an experimental study to observe the transmission of STEC O157:H7 among pigs (Cornick and Helgersen, 2004). The 3-month-old pigs were inoculated with a STEC O157:H7 strain that originated from a human outbreak, via addition of the organisms added to their feed. Moreover, they were interested in examining the effect of two different housing conditions on STEC O157:H7 shedding level and duration. In this housing experiment, a STEC strain of bovine origin was inoculated in conjunction with the STEC strain of human origin. One group of pigs was housed individually on decks with feed provided from a trough, while the other groups of pigs were housed two per pen with feed given on the cement floors. Transmission of STEC O157:H7 was observed from infected donor pigs to naïve pigs, which were housed in the same pen. Fecal shedding of STEC O157:H7 was observed in exposed naïve pigs for at least two weeks after exposure. In addition, the level and duration of STEC O157:H7 shedding was not significantly affected by different housing conditions.

The above experimental study (Cornick and Helgersen, 2004) supported a previous study (Booher *et al.*, 2002), suggesting that shedding of STEC is dose-dependent and that swine are biologically competent hosts for STEC O157:H7. Additionally, results of this study (Cornick and Helgersen, 2004) indicated that STEC O157:H7 infection may be maintained in the swine population and that transmission readily occurs between animals. In another subsequent study by the same research group, STEC O157:H7 was recovered not only from pigs housed close to the infected donor in the adjacent pen (nose-to-nose contact), but also from naïve pigs housed in pens apart from the infected donor (Cornick and Vukhac, 2008). The isolation of the experimental bacterial strain in the air samples provided further evidence to support the hypothesis that contaminated aerosols are a means of indirect STEC O157:H7 transmission among pigs.

Antibiotic-free feed was provided in the above animal model studies. However, in most conventional swine production systems, at least in the USA, feed with antimicrobials is commonly used. Thus, the effect of antimicrobials in feed on STEC O157:H7 shedding was also examined (Cornick, 2010). A number of frequently used antimicrobials for growth promotion in US swine production were selected for this study, namely bacitracin methylene disalicylate, chlortetracycline, and tylosin. Three groups of young adult pigs were given feed with each of the three selected antimicrobials, and another group of control pigs was fed with antimicrobial-free feed. A STEC O157:H7 strain from a human outbreak in Washington State in the USA (Griffin *et al.*, 1988) was orally inoculated at a dose of 10^7 CFU per strain per animal. One month after inoculation, the numbers of pigs that shed STEC O157:H7 and levels of STEC O157:H7 shedding were significantly lower in two groups given feed with tylosin and chlortetracycline compared with the control group. However, no significant differences were found in the infection levels and numbers of pigs that shed STEC O157:H7 between the bacitracin and the control groups. Interestingly, a more recent study by Wells *et al.*, (2012) reported the numbers of pigs shedding STEC O26, O103, and O145 did not significantly differ between antimicrobial and control groups. More research is needed to evaluate the effect of sub-therapeutic doses of antimicrobials in feed on STEC shedding.

Future directions

Swine are not viewed as an important STEC reservoir given the rare known incidence of cases of severe human illness associated with STEC of swine origin. Nevertheless, the association between swine STEC and human illnesses needs to be further investigated. Pork products and feral swine have been associated with a number of STEC outbreaks. STEC causes edema disease in swine. However, STEC can be also isolated from clinically healthy pigs at rates occasionally higher than those for cattle. In addition, animal model studies suggested that swine are biologically competent for colonization and for shedding of STEC for at least 2 months after inoculation.

The increasing surveillance of non-O157 STEC infections has raised the awareness non-O157 STEC (Law, 2000; Bettelheim, 2007; Gyles, 2007; Henderson, 2008; CDC, 2012). In the USA, six non-O157 STEC serogroups, namely O26, O103, O111, O121, O45, and O145, were recently classified as adulterants, similar to O157, in raw, non-intact beef product (FSIS, 2011). Non-O157 serogroups, namely O26, O103, O145, O111, and O91, were also identified as the major public health concerns in Europe (EFSA, 2009). Some of the non-O157 STEC strains were highly virulent and carried virulence gene profiles that were rarely reported in the previous studies. For example, the STEC O104:H4 strain involved in a large-scale outbreak in 2011 in Germany was an enteroaggregative *E. coli* that carried genes typical of this pathogenic *E. coli* group, but that also carried the *stx2* gene (Bielaszewska *et al.*, 2011). Most STEC strains detected in clinically healthy swine were non-O157 STEC (Fratamico *et al.*, 2008). However, there is still very limited information about swine STEC.

Based on the increasing role of non-O157 STEC in human illnesses, the high prevalence and limited epidemiologic investigation of STEC in swine, we recommend further research to elucidate the unclear association between swine STEC and human illness. This will serve public health through determining whether swine are an important reservoir for STEC infection in people. If the evidence indicates swine are an important reservoir, efforts can be directed at control of this pathogen in this species, and, if swine ultimately are determined to not be an important reservoir, public health resources can be directed away from swine and toward further understanding of the epidemiology of STEC, particularly non-O157 serotypes.

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Table 1

Outbreaks associated with pork products

Outbreak location and year	STEC serotype	stx gene subtype	eae gene	Food product	Reference
Washington and California, USA, 1994	O157:H7	Not specified	Not specified	Salami (mixed pork and beef)	(CDC, 1995a)
Australia, 1995	O111:H-	stx1, stx2	Positive	Mettwurst (mixture of raw pork, beef, and lamb)	(CDC, 1995b; Paton <i>et al.</i> , 1996)
Ontario, Canada, 1998	O157:H7	Not specified	Not specified	Salami (mixed pork and beef)	(Williams <i>et al.</i> , 2000)
British Columbia, Canada, 1999	O157:H7	Not specified	Not specified	Salami (mixed pork and beef)	(MacDonald <i>et al.</i> , 2004)
Italy, 2004	O157: non-motile	stx1, stx2	Positive	Salami (pork only)	(Conedera <i>et al.</i> , 2007)
Ontario, Canada, 2011	O157:H7	Not specified	Not specified	Pork from a whole roasted pig	(Troitz-Williams <i>et al.</i> , 2012)