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Feces of Finisher Pigs Have a Low Prevalence of Shiga Toxin-Producing *Escherichia coli* that are of Public Health Importance

R. G. Amachawadi

Kansas State University, Manhattan, agraghav@vet.k-state.edu

X. Shi

Kansas State University, xshi@k-state.edu

J. Bai

Kansas State University, jbai@k-state.edu

See next page for additional authors

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Feces of Finisher Pigs Have a Low Prevalence of Shiga Toxin-Producing *Escherichia coli* that are of Public Health Importance

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are major food pathogens that cause mild to bloody diarrhea, including complications of kidney damage and even death, particularly in children and elderly. Seven serogroups of STEC, O26, O45, O103, O111, O121, O145, and O157, called top-7 STEC, are responsible for the majority of STEC infections in the US. Shiga toxins, which are proteins secreted by the bacteria, are major virulence factors contributing to the disease. There are two Shiga toxin types, 1 and 2, encoded *stx1* and *stx2* gene, respectively and each type has several subtypes. Another major virulence factor, intimin, a protein on the bacterial cell surface encoded by the *eae* gene, mediates attachment of the bacterial cell to the intestinal epithelial cells. The severity of STEC infections in humans is dependent on the Shiga toxin type and subtype. Cattle are a major reservoir of STEC and carry the bacteria in the hindgut and shed them in the feces, which is a source of contamination of food and water. Swine have also been shown to harbor STEC in the gut and shed in the feces, and a few outbreaks of STEC infections in humans have been linked to pork and pork products. The STEC does not cause infections in cattle, but in swine, particularly in weaned piglets, it causes edema disease. We conducted a study that utilized molecular (polymerase chain reaction [PCR]) and culture methods to determine prevalence and characteristics of top-7 STEC in the feces of finisher pigs collected from ten pig flows in eight states. A total of 598 fecal samples were collected and analyzed. The overall prevalence of Shiga toxin genes, *stx1* or *stx2*, was 70.1%, and *eae* was detected in 66.7% of the samples. Based on the PCR method, among the top-7 STEC, O26 (14.4%), O121 (22.9%) and O157 (18.5%) were the predominant serogroups detected. None of the *E. coli* O157 isolated, a serogroup implicated in pork-linked outbreaks, contained Shiga toxin genes. Although a number of fecal samples were positive for the top-7 STEC serogroups, culture method identified one strain each of *stx1*-positive O26 (0.2%) and O103 (0.2%), and 23 strains of *stx2*-positive O121 (3.9%). Serogroups O26 and O103 possessed *stx1a* subtype and *eae*, which have the potential to cause serious infections in humans. Serogroup O121 carried the *stx2e* subtype, which is involved in causing edema disease in swine and rarely implicated in human infections. Our results indicated that finisher pig feces contain a high prevalence of top-7 *E. coli* serogroups, but prevalence of top-7 serogroups that have the ability to produce Shiga toxins was low. In conclusion, a majority of STEC shed in the feces of swine are not of major public health importance.

Keywords

E. coli serogroups, real-time PCR, culture approach, finisher pigs

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Authors

R. G. Amachawadi, X. Shi, J. Bai, M. D. Tokach, J. C. Woodworth, S. S. Dritz, J. M. DeRouche, R. D. Goodband, and T. G. Nagaraja

Feces of Finisher Pigs Have a Low Prevalence of Shiga Toxin-Producing *Escherichia coli* that are of Public Health Importance

Raghavendra G. Amachawadi,¹ Xiaorong Shi,² Jianfa Bai,^{2,3} Mike D. Tokach, Jason C. Woodworth, Steve S. Dritz,² Joel M. DeRouchey, Robert D. Goodband, and T.G. Nagaraja²

Summary

Shiga toxin-producing *Escherichia coli* (STEC) are major food pathogens that cause mild to bloody diarrhea, including complications of kidney damage and even death, particularly in children and elderly. Seven serogroups of STEC, O26, O45, O103, O111, O121, O145, and O157, called top-7 STEC, are responsible for the majority of STEC infections in the US. Shiga toxins, which are proteins secreted by the bacteria, are major virulence factors contributing to the disease. There are two Shiga toxin types, 1 and 2, encoded *stx1* and *stx2* gene, respectively and each type has several subtypes. Another major virulence factor, intimin, a protein on the bacterial cell surface encoded by the *eae* gene, mediates attachment of the bacterial cell to the intestinal epithelial cells. The severity of STEC infections in humans is dependent on the Shiga toxin type and subtype. Cattle are a major reservoir of STEC and carry the bacteria in the hindgut and shed them in the feces, which is a source of contamination of food and water. Swine have also been shown to harbor STEC in the gut and shed in the feces, and a few outbreaks of STEC infections in humans have been linked to pork and pork products. The STEC does not cause infections in cattle, but in swine, particularly in weaned piglets, it causes edema disease. We conducted a study that utilized molecular (polymerase chain reaction [PCR]) and culture methods to determine prevalence and characteristics of top-7 STEC in the feces of finisher pigs collected from ten pig flows in eight states. A total of 598 fecal samples were collected and analyzed. The overall prevalence of Shiga toxin genes, *stx1* or *stx2*, was 70.1%, and *eae* was detected in 66.7% of the samples. Based on the PCR method, among the top-7 STEC, O26 (14.4%), O121 (22.9%) and O157 (18.5%) were the predominant serogroups detected. None of the *E. coli* O157 isolated, a serogroup implicated in pork-linked outbreaks, contained Shiga toxin genes. Although a number of fecal samples were positive for the top-7 STEC serogroups, culture method identified one strain each of *stx1*-positive O26 (0.2%) and O103 (0.2%), and 23 strains of *stx2*-positive O121 (3.9%). Serogroups O26 and O103

¹ Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University.

² Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

³ Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University.

possessed *stx1a* subtype and *eae*, which have the potential to cause serious infections in humans. Serogroup O121 carried the *stx2e* subtype, which is involved in causing edema disease in swine and rarely implicated in human infections. Our results indicated that finisher pig feces contain a high prevalence of top-7 *E. coli* serogroups, but prevalence of top-7 serogroups that have the ability to produce Shiga toxins was low. In conclusion, a majority of STEC shed in the feces of swine are not of major public health importance.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens and seven serogroups, O26, O45, O103, O111, O121, O145, and O157 account for the majority of the STEC-associated illnesses in humans.⁴ The STEC causes mild (colitis) to bloody diarrhea (hemorrhagic colitis), and in children, the infection could lead to hemolytic uremic syndrome (HUS) because of renal failure, and even death. Shiga toxins, which are proteins secreted by the bacteria, are major factors responsible for the disease. There are two types of Shiga toxins, 1 and 2, and within each type there are several subtypes. Another protein, intimin, encoded by the *eae* gene, which mediates attachment of *E. coli* to intestinal cells, is also an important factor for infection. The severity of infection is dependent on the type and subtype of Shiga toxin. Serotype *E. coli* O157:H7 is the frequent cause of several major outbreaks, which are estimated to cause approximately \$405 million in losses every year.⁵ Similar to cattle, swine also harbor STEC in the gut and shed them in the feces, which can be a source of food contaminations. A few outbreaks of STEC infections have been linked to pork and pork products. In cattle, STEC do not cause infection; however, in pigs, STEC causes edema disease, particularly in weaned piglets. The disease in pigs is caused by STEC that produce a subtype of Shiga toxin, called 2e. Information on the prevalence of STEC in swine feces is limited. Therefore, our objective was to utilize PCR and culture methods to determine prevalence, isolate STEC strains of top-7 serogroups, and subtype Shiga toxin genes to assess public health importance.

Procedures

A multi-site field study that included ten pig flows in eight swine-producing states (Iowa, Minnesota, South Dakota, Nebraska, North Carolina, Oklahoma, Kansas, and Ohio) was conducted. In each pig flow, six finishing sites were randomly selected and fecal samples from ten finisher pigs at each site were collected 2-3 weeks before pigs were shipped for slaughter. A total of 598 fecal samples were collected and transported in coolers with ice to the Kansas State University Pre-Harvest Food Safety laboratory. The fecal samples were enriched with *E. coli* broth and then subjected to a real-time (RT) PCR assay that targeted *stx1*, *stx2*, and *eae* genes. Samples positive for either *stx1* or *stx2* were tested by a multiplex PCR assay that targeted top-7 (O26, O45, O103, O111, O121, O145, and O157) serogroups. Samples positive for the top-7 serogroups were subjected to a culture method that involved immunomagnetic capture and plating

⁴ Centers for Disease Control and Prevention (CDC). 2019. Reports of selected *E. coli* outbreak investigations. <https://www.cdc.gov/ecoli/outbreaks.html>. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED).

⁵ Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M., Davies, R., et al. (2020). Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA J.* 18:e05967. doi: 10.2903/j.efsa.2020.5967.

on selective media for detection and isolation of top-7 serogroups of STEC. Shiga toxin genes of isolated STEC strains were subtyped. Data were considered multilevel in nature, with site nested within each pig flow. Bivariate descriptive statistics of prevalence of *stx1*, *stx2*, *eae*, and serogroup-specific genes were assessed by pig flow prior to model building. The cumulative prevalence along with their 95% confidence intervals were calculated at the sample level across all 10 pig flows in eight states as the proportion of samples that were positive for each virulence gene and serogroup divided by the total number of samples tested (n = 598).

Results and Discussion

Based on the RT-PCR assay, 70.1% (419/598) of fecal samples were positive for either *stx1* or *stx2* and 66.7% (398/598) were positive for intimin gene. The prevalence of *stx2* was higher than *stx1* (65.1 vs. 25.8%). The prevalence of Shiga toxin genes ranged from low in fecal samples collected from Kansas (35%), Oklahoma (30%) and North Carolina (20.3%) to high (>70%) in the states of Minnesota, Iowa, Ohio, South Dakota, and Nebraska.

A total of 225 (49.1%) fecal samples were positive for one or more top-7 serogroups by the mPCR assay. Of the top-7 STEC, O121 (17.6%, 95% CI: 14.5-20.6) was the predominant serogroup, followed by O157 (14.1%, 95% CI: 11.3-16.8), and O26 (11%, 95% CI: 8.5-13.5) (Table 1). All ten pig flows from eight states had fecal samples positive for serogroups O26 and O157 (Table 1). Only two fecal samples, one from Iowa and another from South Dakota, were positive for serogroup O111. A majority of the PCR-positive samples (n=225) had one (64.0%) or two (25.3%) STEC serogroups, and none had more than five serogroups in a sample. The seven STEC serogroups, clinically relevant to human infections, were present in all 10 pig flows in eight states at varying proportions, and the prevalence of serogroup O121 was the highest (17.6%). *E. coli* O157 was the second most prevalent top-7 serogroup in this study. Interestingly, none of the O157 isolates obtained were positive for Shiga toxin gene, which was surprising because O157 from cattle feces are almost always positive for Shiga toxin genes. Interestingly, the reported outbreaks of STEC infection associated with pork products were more often with O157 than other serogroups.^{6,7}

The culture method identified a total of 190 isolates positive for one of the seven serogroups. The four predominant serogroups isolated were O26 (38/598; 6.4%), O45 (24/598; 4.0%), O121 (62/598; 10.4%), and O157 (24/598; 4.0%). None of the fecal samples yielded the O111 serogroup. Of the 190 isolates, only 25 (4.2%) were positive for the Shiga toxin gene, which included one strain each of O26 and O103 and 23 strains of O121 (Table 2). None of the O45 (n=24), O145 (n=8) and O157 (24) isolates contained the *stx* or *eae* gene.

⁶ Trotz-Williams LA, Mercer NJ, Walters JM, Maki AM, Johnson RP. Pork implicated in a Shiga toxin-producing *Escherichia coli* O157:H7 outbreak in Ontario, Canada. *Can. J. Publ. Hlth.* 2012; 103:322-326.

⁷ Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrar JA, Lau DK, O'Connell J, Millington A, Asmundson RV, Atwill ER, Mandrell RE. *Escherichia coli* O157: H7 in feral swine near spinach fields and cattle, central California coast. *Emerging Infectious Diseases.* 2007; 13:1908-1911.

Public Health Importance of Swine STEC Isolates

Although *E. coli* belonging to the top-7 serogroups implicated in human infections were isolated, only a small proportion (25/190; 13.1%) of the isolates—belonging to O26, O103, and O121—carried the genes that produce Shiga toxins, which are the major virulence factors. Both O26 and O103 were positive for *stx1* and *eae* genes, and the 23 O121 strains were positive for *stx2* and negative for *eae*. Of the two Shiga toxin types, Shiga toxin 2 is more toxic to cells and more commonly associated with severe and serious complications of human STEC illnesses than Shiga toxin 1. Subtyping of the Shiga toxin gene indicated that *stx1* of O26 and O103 was of 1a subtype, which has the potential to cause severe infections—particularly in association with intimin (*eae*) gene. All strains of serogroup O121 (n=23) carried the *stx2e* gene, which is the subtype most commonly detected in swine feces and involved in causing edema disease. In contrast to swine, Stx2e-producing *E. coli* is rarely isolated from other animals or humans. Shiga toxin 2e-producing *E. coli* have been isolated from sporadic cases of mild diarrhea in humans, therefore, are not a major threat to cause human infections.

In conclusion, our study demonstrates that feces of finisher pigs contain high prevalence of Shiga toxin genes. The prevalence of the top-7 serogroups responsible for a majority of human STEC infections was low in the feces of finisher pigs sampled in the study. This suggests that most Shiga toxin genes were associated with serogroups that are not involved in human infections. A majority of STEC strains isolated in the study belonged to O121 with Shiga toxin 2e, a subtype that is associated with the edema disease in swine, but rarely implicated in human infections. Therefore, a majority of STEC shed in the feces of swine are not of major public health importance.

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Table 1. Prevalence of serogroups O26, O45, O103, O111, O121, O145, and O157 and three major virulence genes, *stx1*, *stx2*, and *eae* of Shiga toxin-producing *Escherichia coli* in swine feces (n = 598) based on a multiplex-PCR assay

STEC serogroups	No. of samples positive (%)									
			Iowa				South Dakota			
	Kansas (n = 60)	Minnesota (n = 60)	Pig flow 1 (n = 60)	Pig flow 2 (n = 60)	Oklahoma (n = 60)	Ohio (n = 59)	Pig flow 1 (n = 60)	Pig flow 2 (n = 60)	Nebraska (n = 60)	North Carolina (n = 59)
O26	3 (5)	11 (18.3)	15 (25.0)	3 (5.0)	4 (6.7)	10 (16.9)	3 (5.0)	1 (1.7)	15 (25.0)	1 (1.7)
O45	1 (1.7)	5 (8.3)	2 (3.3)	4 (6.7)	0	3 (5.1)	0	1 (1.7)	12 (20.0)	0
O103	3 (5)	4 (6.7)	3 (5.0)	4 (6.7)	1 (1.7)	3 (5.1)	2 (3.3)	0	1 (1.7)	0
O111	0	0	1 (1.7)	0	0	0	1 (1.7)	0	0	0
O121	3 (5.0)	13 (21.7)	32 (53.3)	5 (8.3)	0	10 (16.9)	2 (3.3)	7 (11.7)	23 (38.3)	10 (16.9)
O145	4 (6.6)	3 (5.0)	2 (3.3)	2 (3.3)	4 (6.7)	4 (6.8)	1 (1.7)	0	9 (15.0)	1 (1.7)
O157	27 (45)	6 (10.0)	17 (28.3)	7 (11.7)	6 (10.0)	6 (10.2)	2 (3.3)	1 (1.7)	11 (18.3)	1 (1.7)

Table 2. Prevalence of top-7 serogroups of Shiga toxin-producing *Escherichia coli* (STEC) in swine feces (n = 598) based on the culture method

STEC serogroups	No. of <i>stx</i> -positive isolates/total no. of serogroup-positive isolates										
			Iowa				South Dakota				Total
	Kansas (n = 60)	Minnesota (n = 60)	Pig flow 1 (n = 60)	Pig flow 2 (n = 60)	Oklahoma (n = 60)	Ohio (n = 59)	Pig flow 1 (n = 60)	Pig flow 2 (n = 60)	Nebraska (n = 60)	Carolina (n = 60)	(n = 598)
O26	0/1	0/6	0/9	0	0/4	1/8	0/3	0/1	0/6	0	1/38
O45	0/1	0/5	0/1	0/3	0	0/1	0	0/1	0/12	0	0/24
O103	1/1	0/2	0/3	0	0	0/2	0/1	0	0	0	1/9
O111	0	0	0	0	0	0	0	0	0	0	0
O121	3/3	4/6	2/17	0/4	0	0/8	1/1	5/6	1/9	7/8	23/62
O145	0	0/1	0	0/1	0/4	0/2	0	0	0	0	0/8
O157	0/11	0/1	0/2	0	0/4	0	0/1	0	0/4	0/1	0/24
Total (%)^a	4/17 (6.7)	4/21 (6.7)	2/40 (3.3)	0/10 (0)	0/12 (0)	1/25 (1.7)	1/6 (1.7)	5/8 (8.3)	1/42 (1.7)	7/9 (11.9)	25/190 (4.2)

^aPercent prevalence in fecal samples.