

Title: Shiga toxin-producing *Escherichia coli* in swine feces in commercial production systems and assessment of public health implications – NPB #18-057

revised

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Scientific Abstract:

Shiga toxin-producing *E. coli* (STEC) are major food borne pathogens in humans and cause diarrhea ranging from mild to bloody (enteritis to hemorrhagic enteritis), occasionally leading to complications of renal failure (hemolytic uremic syndrome; HUS) in children and elderly. Shiga toxins 1 and 2, secreted proteins encoded by *stx1* and *stx2* genes, respectively, and intimin protein encoded by *eae*, are major virulence factors responsible for STEC infections. Shiga toxin 2 is associated with more severe infections than Shiga toxin 1. Each Shiga toxin has several subtypes, and a few subtypes are associated with more serious infections, including HUS, than other subtypes. As many as 140 serogroups of *E. coli* are known to produce Shiga toxins. Seven serogroups, considered top-7 STEC, O26, O45, O103, O111, O121, O145 and O157, are responsible for the majority of human STEC infections. Ruminants, particularly cattle, are a major reservoir of STEC. The organisms colonize the hindgut and are shed in the feces, which serves as a major source of contamination of food and water. There are reports of STEC in feces of a number of other animals, domestic and wild, including swine. STEC do not cause any disease in ruminants, but cause edema disease in swine, particularly in weaned piglets. A few foodborne STEC infections in humans traced to pork and pork products have been reported. Studies on fecal prevalence of STEC, particularly of the top-7 STEC, in pigs are limited. Therefore, objectives of this study were to determine prevalence and isolate and identify STEC in feces of finisher pigs. Fecal samples (n=598) from finisher pigs in commercial production systems from ten pig flows located in eight states were collected. Samples were enriched in *E. coli* broth and tested by real time PCR to detect Shiga toxin genes, *stx1* and *stx2*. Fecal samples positive for *stx1* and or *stx2* genes were then subjected to conventional PCR to detect the top-7

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STEC serogroups. Fecal samples positive for one or more of the top-7 serogroups were then cultured by immunomagnetic bead separation and plating on selective media for isolation and identification STEC. A conventional 8 plex-PCR assay targeting major serogroups previously reported in swine feces (O8, O26, O137, O59, O86, O91, O100, O120, and O174) was developed and validated. Fecal samples directly plated onto nonselective media were used to identify serogroups other than the top-7 STEC.

The overall prevalence of either *stx1* or *stx2* was 70.1%, and *stx2* was the predominant Shiga toxin gene (65.1%). Based on the conventional PCR assay, among the top-7 STEC, O26 (14.4%), O121 (22.9%) and O157 (18.5%) were the predominant serogroups. Although a number of fecal samples were positive for the top-7 STEC serogroups, culture method identified only O26 (0.2%), O103 (0.2%), and O121 (3.9%) to carry Shiga toxin gene. The 8-plex PCR assay indicated that serogroups of O8, O86, O174, O100, and O91 as dominant serogroups, with the overall prevalence of 88.6%, 35.5%, 24.1%, 20.2%, and 15.6%, respectively. The culture method identified O8, O86, and O76 as the dominant non-top-7 STEC in cattle feces. A majority of STEC strains isolated contained *stx2*, and only four strains (O26, O103 and two strains of O51) carried *eae*, which codes for intimin protein that mediates attachment of STEC onto epithelial cells. The subtyping of *stx* gene indicated that O26 and O103 strains isolated contained *stx1a*, which is the subtype associated with many of the top non-O157 strains that cause human infections. The serogroup O121, which was the dominant top-7 STEC isolated from finisher pigs in this study, and all the non-top-7 STEC, including the dominant serogroups of O8, O86, and O76, contained *stx2e*, a subtype that is associated with the edema disease. In conclusion, PCR and culture methods identified that feces of finisher pigs contain high prevalence of STEC serogroups, but prevalence of top-7 serogroups carrying Shiga toxin gene was low. A majority of STEC strains isolated from feces contained *stx2e*, a subtype that is associated with edema disease and is generally not involved in STEC infections in humans. Therefore, a majority of STEC shed in the feces of swine are not of major public health importance.