

# Shiga toxin-producing *Escherichia coli* in pork, are they really only a problem for beef?

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## Background

Shiga toxin-producing *Escherichia coli* (STEC) can be the cause of severe life-threatening disease, outbreaks, and recalls. The most common STEC that causes disease is O157:H7, but about half of STEC infections are caused by non-O157 STEC, with most of those infections caused by six serogroups (O26, O45, O103, O111, O121, and O145). Since cattle are a natural reservoir for STEC, beef products can be contaminated during harvest and processing which has led the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to declare these seven STEC as adulterants and monitor their presence in raw beef products. Other less common non-O157 STEC that are equally as pathogenic are present but not identified by screening tests. Swine and pork products are not recognized as a source of STEC and their presence in pork is not regulated. However, screening tests have indicated that 82.3% of swine entering harvest carry STEC on their skin, with 7.8% of post-scald carcasses and 1.7% of finished carcasses still contaminated by a STEC. This level of STEC on finished carcasses implies there likely could be STEC in final pork products and that the STEC may be present in the processing and/or fabrication environment. The data presented here describe the results of a survey of STEC in raw pork products and an evaluation of pork processing environments for STEC.

## Materials and Methods

Randomly selected regulatory raw pork enrichment broths (n=1,477) were obtained from FSIS laboratories and screened for STEC using Bio-Rad iQCheck (VirX and SerO) test kits according to package inserts. Broths that screened positive for a STEC were cultured to confirm their presence by immunomagnetic concentration (IMS) for indicated serogroups and direct plating to STEC selective (ChromAgar STEC and Rainbow agar) and non-selective (BCIG-SMAC and washed sheep blood agar) media. If no serogroup was indicated by the screening test, only direct plating was performed. Suspect colonies were picked and characterized for Shiga toxin (*stx*) subtypes intimin (*eae*), and the presence of 16 nle (non-locus of enterocyte effacement) and 17 other virulence factors by PCR; as well as serogroup using a combination of PCR and serology. Further, broths that had screened positive for STEC (*stx*<sup>+</sup> and *eae*<sup>+</sup>) were examined with digital droplet PCR (ddPCR) (Bio-Rad ddCheck STEC) to help resolve the presence of STEC from interfering background bacteria that led to positive screening results. Pork processing environments (n=156) were represented by samples collected from floor drains at 2 pork processors over periods of 15 and 16 weeks. Drains were located in coolers and along ham, butt, and loin processing lines. A cellulose sponge was used to swab the interior of opened drains to collect organisms from the inner surfaces. Drain samples were screened and cultured for STEC as described above. Further, drain samples were examined for biofilm forming ability on polystyrene surfaces, and ability to protect a co-inoculated STEC from treatment with 300ppm quaternary ammonium sanitizer (QAC).

## Results

Amongst the raw pork samples 22.1% (327/1477) screened positive for the presence of STEC. Culture isolation only confirmed 5 (0.34%) with an isolate.

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Four of the isolates were of beef regulated serogroups O103 (n=3) and O121 (n=1), while another was of a less common serogroup (O49). All were isolated from non-intact or comminuted raw pork samples. The STEC O103 all possessed *stx1*, and the STEC O121 and O49 possessed *stx2*. However, the *stx2* of the O49 was subtype 2e, which is not commonly associated with severe disease causing STEC. The STEC O121 possessed 10 of the *nle* virulence factors and 4 of the other virulence factors, while the STEC O103 possessed 6 to 7 and 3 to 5 of the *nle* and other virulence factors, respectively. The STEC O49 had fewer *nle* (4) but more other (7) virulence factors. The ddPCR identified 265 (82.3%) of the 322 STEC screen positive samples, that could not be culture confirmed, as negative or having non-linked *stx* and *eae*, indicating these samples contained mixed cultures of interfering bacteria and not STEC. Three (1.9%) of the drain samples were found to have a STEC present by the screening PCR test, however no STEC were isolated. Biofilms formed by drain sample organisms varied in the strength of biofilms formed and their ability to protect co-inoculated STEC from QAC treatment. Cooler sample formed biofilms provided greater protection of STEC (20 to 100% protected) than those formed by processing ham, and butt lines (0-60% and 5 to 85% protection respectively).

### **Conclusions**

Raw pork had a STEC prevalence of 0.3%. In comparison, FSIS reported 0.34% non-O157 STEC in manufacturing beef trim during 2021-22. Further, pork processing environments were found to support STEC if present and some samples were able to form biofilms that protected STEC from sanitization. Screening pork for STEC however identifies 4 to 5 times as many samples as potential positive than is found in beef due to the common presence of interfering organisms, as identified by ddPCR. Considering these results, pork processors should consider ensuring their safety systems can control STEC.