BEEF INDUSTRY SAFETY SUMMIT
March 3-5, 2015
Dallas, TX

Project Title: **PCR- and Culture-based Methods to Detect and Quantify Six Major Non-O157 Serogroups of Shiga Toxin-producing *Escherichia coli* in Cattle Feces**

Presenters: **T. G. Nagaraja**, Pragathi Shridhar, Lance Noll, Diana Dewsbury, Xiaorong Shi, Jianfa Bai, Natalia Cernicchiaro, and David G. Renter
E-mail address: tnagaraj@vet.k-state.edu
Mailing address: University Distinguished Professor, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine
Kansas State University
Manhattan, KS, 66506-5606

Category: Pre-harvest research

Published: Unpublished to date

Objectives: Shiga toxin-producing *E. coli* (STEC) belonging to six O serogroups, O26, O111, O103, O121, O45, and O145 account for the majority of non-O157 STEC illnesses in the US. We have developed and validated conventional PCR (cPCR), multiplex quantitative PCR (mqPCR), and culture methods to detect, quantify, and isolate the six non-O157 serogroups of STEC in cattle feces. Our objective was to compare the three methods to detect and quantify non-O157 serogroups of STEC in cattle feces collected from a commercial feedlot.

Experimental Design & Analysis:
A total of 576 fecal samples were collected in the summer of 2013. Fecal samples were suspended and enriched in *E. coli* broth. Fecal suspensions after enrichment were subjected to three detection methods: cPCR that targeted the six serogroups and four virulence genes (*stx*1, *stx*2, *eae*, and *ehxA*), mqPCR assays that targeted the six serogroups, and a culture method that involved immunomagnetic separation with serogroup-specific beads and plating bead suspension onto a selective medium, followed by PCR confirmation of presumptive chromogenic colonies. Fecal suspensions before enrichment were subjected two quantification methods: mqPCR method and spiral plate culture method.

Key Results:
Of the 576 fecal samples, 548 (95%), 447 (77.6%) and 428 (74.3%) were positive for at least one of the six serogroups by mqPCR, cPCR, and culture method, respectively. All samples that were positive by cPCR were positive by mqPCR. However, 101 (17.5%) and 126 (21.9%) samples that were positive by mqPCR were negative by cPCR and culture method, respectively. Interestingly, of the 28 samples negative by mqPCR or cPCR, six (21.4%) samples were positive by the culture method. Regardless of the method of detection, O103 was the predominant serogroup (>

55%), followed by O26, O45, O121, O145, and O111. Of all the non-O157 serogroup-positive isolates obtained in the study (n=640), only a small number (23/640; 3.6%) of O103 (n=10), O26 (n=7), and O145 (n=6) isolates carried Shiga toxin (*stx*1 only) and intimin genes. Of the 576 fecal samples, 127 (22%) and 146 (25.3%) samples were quantifiable (≥ log 3 CFU/g of feces) by mqPCR and spiral plating method, respectively. In conclusion, more samples were detected as positive for one or more of the six non-O157 serogroups by mqPCR than the cPCR or culture method. However, a few (6/28) of the samples negative by mqPCR or cPCR assay were positive by the culture method.

Industry Application:

Only a few studies have reported on preharvest prevalence and concentrations of non-O157 STEC in cattle. One of the reasons for the limited data is the lack of validated and standardized methods. We have developed, validated, and determined applicability of both PCR and culture-based methods to detect and quantify the six serogroups of non-O157 *E. coli* in cattle feces. The methods also should be applicable to hide and carcass swab samples. Moreover, these detection protocols can be used to generate baseline fecal, hide, and carcass prevalence and concentration data, which are needed to identify factors associated with the prevalence and persistence of non-O157 STEC and to assess efficacy of preharvest or postharvest mitigation strategies.