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Modeling variability associated with estimating concentrations of six non-O157 E. coli serogroups in cattle feces based on a multiplex quantitative PCR.

Pius S. Ekong, Michael W. Sanderson, Pragathi B. Shridhar, Jianfa Bai, T. G. Nagaraja, Kansas State University, Manhattan, KS, Contact: pekong@k-state.edu

Purpose
The concentration of non-O157 STEC in the cattle production system is necessary for quantitative microbial risk assessment, but values are difficult to obtain. In order to provide an estimate of the concentration of non-O157 STEC detected in cattle feces and the variability around the concentration estimate, we developed a Monte Carlo simulation model in @RISK based on multiplex quantitative PCR (mqPCR) cycle threshold (C_T) values.
Methods
We utilized mqPCR assays that targeted serogroup-specific genes to detect and quantify the six major non-O157 STEC in cattle feces. A linear equation was fit based on data from post enrichment C_T values and pre-enrichment colony counts (CFU/g) in fecal samples spiked with ten-fold serial dilutions of pooled pure cultures of O26, O103 and O111; and O45, O121 and O145 serogroups. Distributions were fitted to intercept and slope parameters of the linear equations and using a uniform distribution of C_T values, estimates of variability around the calculated concentrations were generated. Distributions of C_T values for each serogroup obtained from feces of 576 naturally-shedding cattle (observed C_T 20.57 - 38.04) were used in the resulting model to predict the field distribution of fecal concentrations and their variability estimates.
Results
Variability around the concentration estimate was similar across the range of positive C_T values. At a C_T of 25, the models predicted a log count of 5.33 CFU/g for O26, O103 and O111 serogroups, (95% prediction interval ±0.66 log), and for O45, O121 and O145 serogroups predicted log count was 5.12 CFU/g, ±0.88 log. Estimated mean log concentration for O26, O103 and O111 in positive field samples was 4.55 (range: 2.31-7.32). For O45, O121 and O145, it was 4.59 (range: 1.98-6.79). Model concentration results were validated against spiral plating counts on field data. Further work will estimate the full distribution of concentrations in field fecal samples accounting for PCR false negative samples.
Conclusions and Relevance
These results provide a method to estimate concentration of STEC in cattle feces along with their measures of uncertainty in PCR positive samples for use in quantitative microbial risk analysis.