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Project Title: Identification of Novel Biomarkers for Rapid Screening of Pathogenic STEC (*stx* and *eae*- positive strains) in Beef Samples

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Objective: Current methods for screening Enterohemorrhagic *Escherichia coli* (EHEC) O157 and non-O157 in beef enrichments typically rely on the molecular detection of *stx*, *eae*, and top7 serogroup-specific *wzx* or *wzy* gene fragments. As these genetic markers can also be found in some non-EHEC strains, a number of 'false positive' results are obtained. The objective of our study was to explore the suitability of using additional biomarkers, along with *stx* and *eae*, to allow a significant reduction in the number "presumptive positive" beef samples that need to be further cultured for isolation and confirmation of the top7 regulated EHEC.

Experimental Design & Analysis: A very large panel of *E. coli* strains (n = 1,100), comprising EHEC (n = 340), enteropathogenic *E. coli* (EPEC) (n = 392), STEC (n = 193), and apathogenic *E. coli* strains (n = 175) were tested by high throughput qPCR for selection of genetic markers which dominate in *stx*- and *eae*-positive *E. coli* (typical EHEC). We explored the suitability of these novel molecular markers as candidates for a more accurate screening of EHEC strains in 1,739 beef enrichments and measured the reduction of the number of 'presumptive positive' beef enrichments by including these biomarkers along with *stx* and *eae*.

Key Results: High throughput qPCR allowed selection of genetic markers (*espK*, *espV*) which dominate in *stx*- and *eae* positive *E. coli* (typical EHEC) whereas they are less represented in other pathogroups (EPEC, STEC) and non-pathogenic *E. coli*. Hence, 98% of the Top7 EHEC serogroups regulated in the US were tested positive for *espK* and/or *espV*. The only few EHEC O26:H11 strains that tested negative for *espK* and *espV* were associated with a new highly virulent clone, which is positive for *stx2* only. Such strains can be detected via specific CRISPR sequences. Of the 1,739 beef enrichments tested, 180 were positive for both *stx* and *eae* genes. Ninety (50%) of these tested negative for *espK*, *espV* but twelve out of these negative samples were positive for the $CRISPR_{O26:H11}$ gene marker specific for a newly emerging virulent EHEC O26:H11 clone. We show that screening for *stx*, *eae*, *espK*, and *espV*, in association with the $CRISPR_{O26:H11}$ marker is a better approach to narrow down the EHEC screening step in beef

enrichments. The number of potentially positive samples was reduced by 48.88% by means of this alternative strategy compared to the classical reference methods, thus substantially improving the discriminatory power of EHEC screening systems.

How can this be applied in the industry? Novel biomarkers that unambiguously identify typical EHEC strains (*stx*-positive and *eae*-positive *E. coli* strains) in complex samples are a desirable goal. We identified a novel approach combining the detection of *stx*, *eae* and few additional biomarkers, including dedicated CRISPR targets/sequences, to narrow down the EHEC screening steps. This method provides a significant reduction of the presumptive positives that should be submitted to serogroup testing and the time-consuming and laborious isolation step of confirmation.