

# Dynamics of Shiga Toxin-Producing *Escherichia coli* (STEC) Shedding in Beef cattle

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## Abstract

**Introduction:** Even with advancements in pre- and post-harvest food safety, Shiga toxin-producing *Escherichia coli* (STEC) still present challenges to human health. Since cattle are the primary reservoir for STEC, lowering the prevalence of this pathogen in farm animals may reduce STEC outbreaks in humans. However, many factors that modulate the colonization and persistence of STEC in cattle remain unknown.

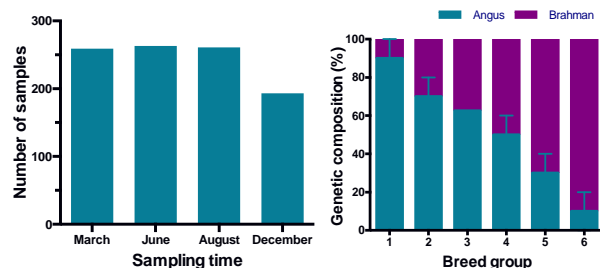
**Purpose:** This study reveals the role of animal age and environmental factors influencing the shedding of Shiga toxin-producing *E. coli* (STEC) in beef cattle.

**Methods:** Several animal factors like breed, age, sex were investigated to determine their influence on STEC shedding. A cohort of 300 beef calves from a multi-breed beef calf population derived from Brahman and Angus cattle was sampled four times every three months after birth. This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration of Shiga toxin-producing *Escherichia coli* (STEC) from the fecal samples. Data were analyzed by regression methods and McNemar's test for matched pairs using STATA software.

**Results:** The herd prevalence of STEC in March was 59.8%, which was significantly higher compared to any of the other sampling times (39.5% in June, 20.3% in August and 20.7% in December). The *stx2* genotype was predominant in the herd, whereas *stx1/stx2* was the lowest in all samplings. There was no significant association between breed group, sex of the calf and average weight gain with the STEC shedding. However, we observed STEC shedding was significantly affected by animal age. Furthermore, seasonal variation of STEC shedding was observed.

**Significance:** This study provides insight that animal age is a significant factor that influences the prevalence of STEC; therefore this finding has implications for the development of on-farm mitigation strategies.

## Materials and Methods



### Sample collection and cultural characterization

Swabs samples were collected from the rectal anal junction of ~ 260 beef calves four times at different age groups of the growing season. Calves belonged to a multi breed group ranging from Black Angus to Brahman. Fecal samples were immediately transferred to the laboratory and plated on MacConkey agar for initial detection of Gram negative Enterobacteriaceae members. Colonies grown on MacConkey agar were confirmed as STEC by multiplex colony PCR. Statistical Analysis was performed with SAS 9.3 software package.

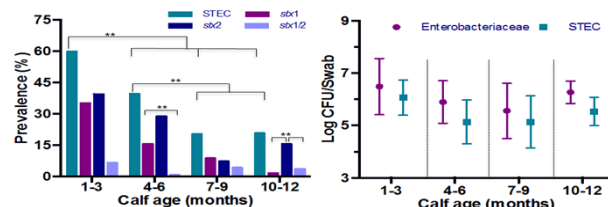
### Primers for PCR detection of the STEC

*Stx1*: KCP 011(TGTCGCATAGTGAACCTCA) and KCP 012(TGCGCACTGAGAAGAAGAGA)  
*Stx2*: KCP 013(CCATGACAACGGACAGAGTT) and KCP 014(TGTCGCGAGTTATCTGACATTC)  
 Multiplex colony PCR was conducted (Jeon et al., 2014). PCR products were visualized on 1.5 % agarose gel in Tris-EDTA buffer after electrophoresis and ethidium staining

### Next Gen Sequencing and Metagenomic analysis of microflora

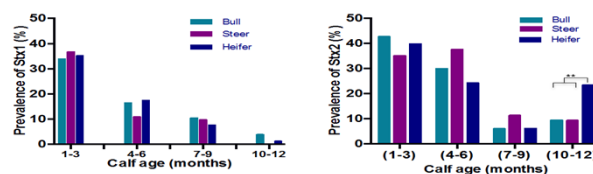
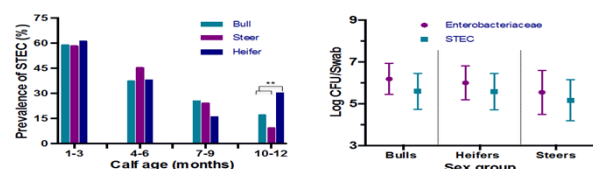
DNA from 48 fecal samples from all four age groups and including both STEC positive and negative samples was isolated by Power Soil Kit (MoBio Inc). The samples were sequenced using 454 FLX pyrosequencing technology (Macrogen, South Korea). The association of microflora with STEC was determined by statistically analysis using SAS 9.3 and Prism. The heat maps and dendrograms were generated using R-program

## STEC shedding is higher in early life



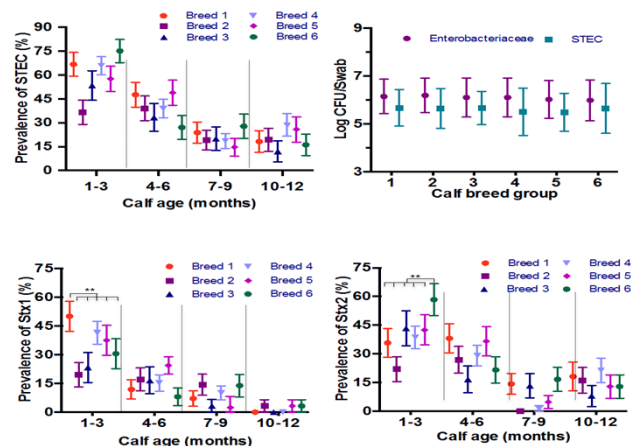
- Among STEC positive, animals the concentration of STEC shedding was not different

## Heifers shed higher STEC than bulls



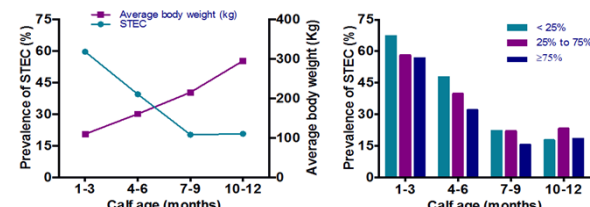
- At 12 months age, heifers shed higher STEC than bulls
- Concentration of STEC shedding was not different

## Breed group affects STEC prevalence in early age



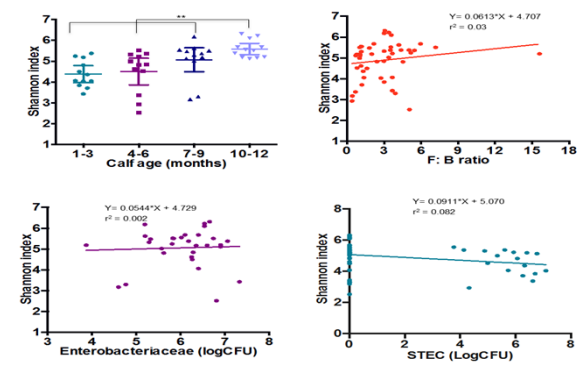
- Breed 1 shed higher Stx1 in early age, while as breed 6 shed higher Stx2; overall there was not a significant association between breed group and STEC shedding

## Effect of weight gain on STEC shedding



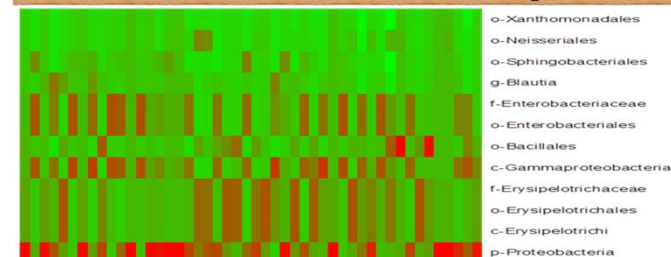
- Body weight increased with age, but ADG didn't affect STEC prevalence

## Diversified microflora reduced the STEC shedding



- Higher Shannon index means diverse microflora and better animal health

## Microflora affects the STEC shedding



- STEC positive had higher Proteobacteria, Non-STEC had higher Erysipelotrichi

## Conclusions

1. Calves shed STEC even in early age and shedding reduces as the animals mature
2. Heifers shed more STEC at 12 month age which maybe due to physiological stress
3. Gut microflora diversity increases with age
4. The STEC shedding significantly decreases with increasing diversity of microflora

## Acknowledgements

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