Prevalence of Shiga-Toxin Producing *Escherichia Coli* in Two Cohorts of Beef Cattle is Associated with Diversity of Microflora and Animal Age

Raies A. Mir[1,2], Sarah M. Markland[1,2], Mauricio Elzo[1], Soohyoun Ahn[3], Nicolas DiLorenzo[1], G. Cliff Lamb[1], Jerry Wasdin[1], Danny Driver[1], K. Casey Jeong[1,2]

Synopsis

In the first cohort of cattle, with ages ranging from 0 to 12 months, Shiga-toxin producing Escherichia Coli (STEC) shedding was greater in young calves of 1-3 months age and as the microflora of calves developed, STEC shedding decreased. In the second cohort of cattle, aged 1 to 11 years, where STEC prevalence peaked at 2 years of age then decreased as animals became older.

Summary

Many of the factors that modulate the colonization and persistence of Shiga-toxin producing Escherichia Coli (STEC) in cattle remain unknown thus causing a challenge for reducing STEC in this host. The objectives of this study were to better understand the effects of animal age and role of natural microflora present in the cattle intestinal tract on the prevalence of STEC in beef cattle. The prevalence of STEC in calves 1-3 months of age was 60%, which was significantly greater than calves older than 4 months of age. We detected greater STEC prevalence and lower microflora diversity in early age, and microflora of STEC shedding animals was different from the non-STEC animals. During sample collection in the second cohort, beginning after 1 year of age, heifers had significantly lower STEC prevalence than cows (37.5% vs. 70%). After 2 years of age, STEC prevalence peaked and tended to decrease as animals became older.

Introduction

The Centers for Disease Control and Prevention (CDC) has estimated that pathogenic Shiga-toxin producing *Escherichia coli* (STEC) cause about 269,000 cases of illness (including approximately 3,700 hospitalizations and 30 deaths) in the United States every year (Scallan et al., 2011). Among STEC strains the *E. coli* O157:H7 serogroup is the best-known and can cause symptoms in affected individuals that include hemorrhagic colitis, bloody diarrhea, and hemolytic uremic syndrome (HUS) in humans (Kaper et al., 2004). Non-O157 STEC serogroups, such as O26, O45, O103, O111, O121, and O145, commonly called the "Big Six" serogroups, accounted for about 71% of non-O157 STEC isolates between 1983 and 2002 and have also been associated with human disease outbreaks in the United States (Brooks et al., 2005). In 2012, the Big Six non-O157 STEC were declared to be food adulterants by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA, 2012).

Controlling the prevalence of STEC in cattle to reduce outbreaks of this pathogen in humans has proved to be a challenge because of the multiple factors that modulate the colonization and persistence of STEC in cattle. Reducing the prevalence of STEC in cattle at the pre-harvest level has been recently highlighted as an intervention point (Arthur et al., 2011; Jeong et al., 2011), and it has been suggested that lowering the STEC prevalence on farms may reduce the total number of *E. coli* O157:H7 outbreaks in humans (Matthews et al., 2013). Several studies indicated that diet and management practices may affect the prevalence of STEC, especially *E. coli* O157:H7 in beef cattle (Fox et al., 2007; Jacob et al., 2010; Zhao et al., 2014). For example, it has been reported that cattle being fed a sorghum-based diet have a high prevalence of *E. coli* O157:H7, and fecal shedding of *E. coli* O157:H7 associated with the inclusion of distiller's grain in the feed (Fox et al., 2007; Jacob et al., 2010). However, information at the pre-harvest level, including animal factors and management practices that may modulate the non-O157 STEC prevalence is limited.

Procedure

Animal management and sample collection

Calves and heifers

Fecal samples were collected from the recto-anal junction (RAJ) of calves and heifers using sterile cotton swabs in March (n=259), June (n=263), August (n=261) and December (n=193), representing the time at which calves were 1-3, 4-6, 7-9, and 10-12 months of age, respectively. Due to culling of calves for reasons unrelated to the study only 193 calves were available for the December sample. The sampling scheme resulted in 188 animals that had four matched samples, which were used to assess colonization dynamics. All samples were transported on ice and processed the same day using the protocol described below.

Identification of Shiga toxin-Producing *Escherichia coli*

A combination of culture-based and nucleic acid-based methods for the detection and enumeration of Shiga toxin-producing *Escherichia coli* (STEC) were used. We used MacConkey agar (Becton Dickinson Company, MD, USA) to culture Gram-negative enteric bacteria in fecal samples. Purified isolates were subjected to colony PCR for the detection of *stx1*and *stx2* genes.

Metagenomic Analyses

To understand the association of microflora with animal age and its effect on the STEC dynamics, we did metagenomic analyses of the fecal samples from the claves using the latest technology of 454 pyrosequencing (Macrogen, South Korea).

Statistical Analyses of the Prevalence and Concentration of STEC

Statistical analysis of the microbiological findings with animal factors was conducted using the STATA software package (STATA® MP 11.2, StataCorp, College Station, Texas, USA) with a significance threshold of $\alpha < 0.05$.

Results

Association between Animal Factors and the STEC Prevalence in Beef Calves

The main finding from this study is that beef calves shed STEC at an early age and the shedding of STEC decreases as the animal ages until it reaches a low level of ~20% in the beef herd at 12 months of age (Figure 1). The concentration of STEC (log CFU/swab) also decreased as calves matured. Our results indicate that females shed higher STEC when they are ~12 months old. There was no consistent pattern of STEC shedding across calf breed groups over the four samplings during their first year of life. This study found that STEC shedding was greater in young calves 1-3 months of age and that STEC shedding decreased as the microflora of calves developed. The microflora of STEC shedding animals was shown to be different from the non-shedding animals (Figure 2). Further studies are needed to determine the transmission routes and factors affecting early STEC colonization in calves.

Association between Animal Factors and the STEC Prevalence in Beef Heifers and Cows

In this study we showed that STEC shedding is less prevalent in heifers than in cows (Figure 3A). The lower concentration of normal microflora (*Enterobacteriaceae*) leads to greater STEC concentrations (Figure 3B). It is also shown that animal age is a critical factor for the prevalence and dynamics of STEC in cattle. Heifers younger than two years of age without any previous live births had significantly lower STEC prevalence compared to cows that had previously given birth.

Association between Microflora and the STEC Shedding

Our results indicate that Shannon index is linearly correlated with F:B ratio and we speculate that older calves have diverse microflora which reduces STEC prevalence and concentration of STEC shed by the calves. These findings have clear implications for the development of on-farm mitigation strategies. By segregating animals with the greatest risk of infection and greatest rates of shedding, it could be possible

to lower the concentrations of STEC at the pre-harvest level and prevent transmission from cattle to humans. This information could also be used to develop dynamic transmission models that use different rates of colonization with respect to age to simulate the prevalence in herds over time and evaluate the efficacy of potential interventions. Since findings here indicated that when animals became two years old they were more likely to be colonized and were more likely to transmit STEC to other animals through increased shedding, further understanding of factors that increase the STEC prevalence at this stage of development would be critically important to provide interventions to reduce STEC levels in animals.

Acknowledgements
This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2014-67021-21597.

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Figures

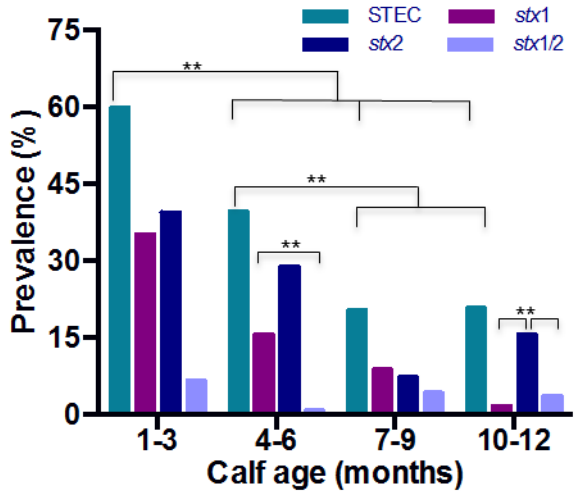

Figure 1: Prevalence of STEC in beef calves.

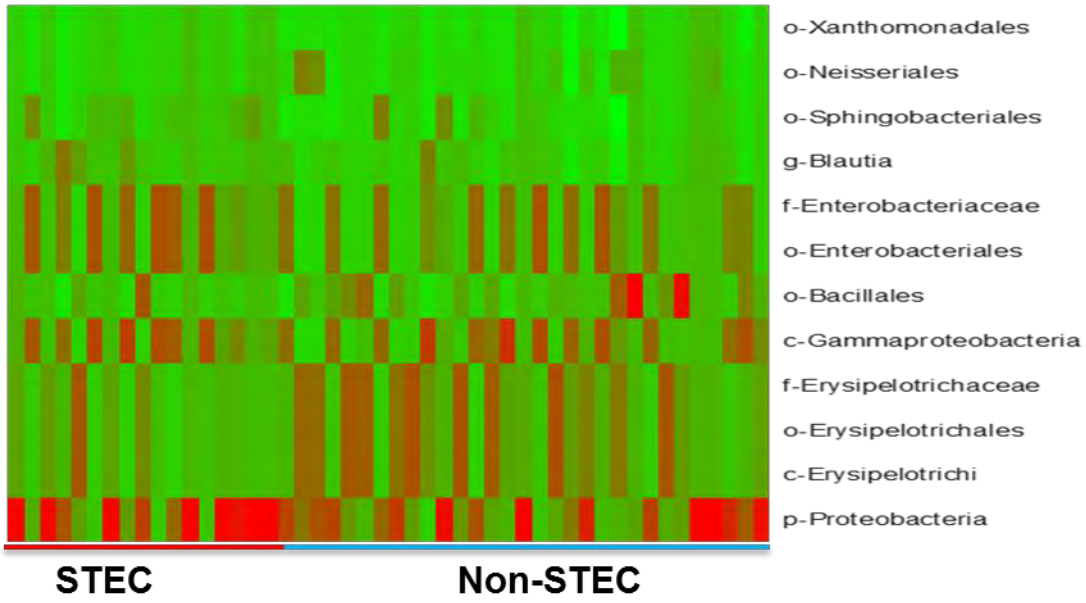

Figure 2: Difference in microflora of STEC versus non-STEC animal.

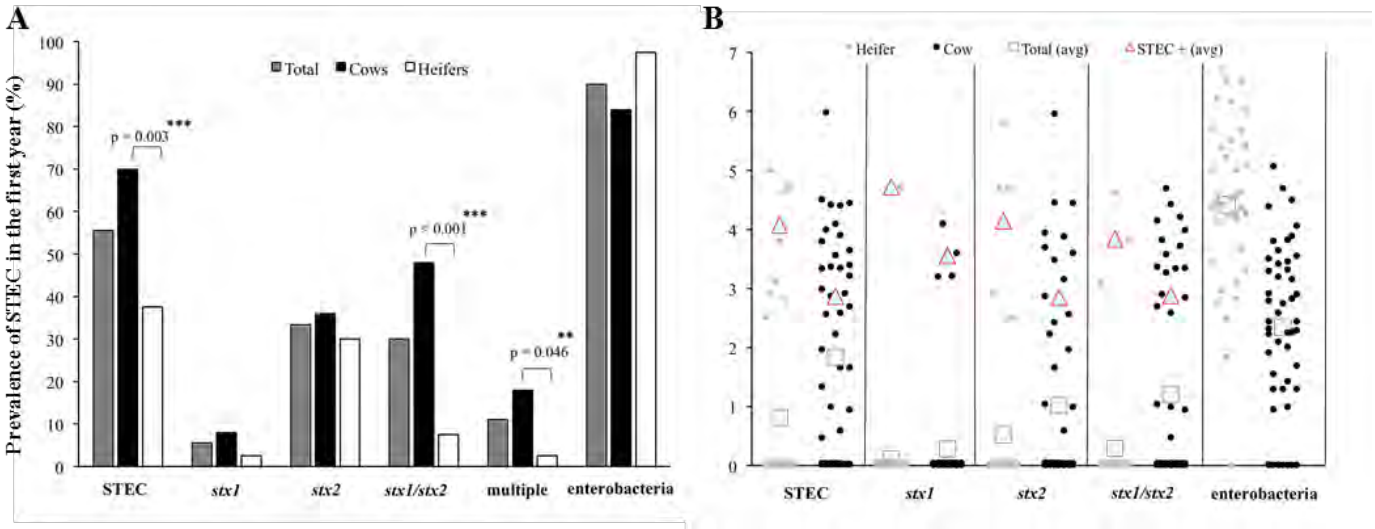

Figure 3: Prevalence and concentration (log CFU/swab) of STEC in heifers and cows.