The Effect of Commensal Microbial Communities on the Fecal Shedding of Shiga Toxin-Producing *E. coli* (STEC) in Beef Cattle

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Summary

This ongoing study compares the gut microbial community composition between shedding steers high in shiga toxin-producing E. coli (STEC) counts and low-shedding steers. Shedders were identified among 170 beef animals over three time periods using selective microbiological culture media. The isolated bacterial cultures were confirmed to be STEC using PCR, 16s rRNA sequencing and a shiga toxin immunoassay. The most abundant strains found in the cattle feces were those belonging to the serogroups O111 (40.3%) and O157:H7 (37.3%), with O103 (8.3%), O26 (6.0%), O83 (4.5%), and O55 (3.0%) being detected in much lower numbers . Out of the 52 animals which were identified as super-shedders of STECs which were selected for microbial community analysis, 61.54% shed STEC in at least two of the three sampling time points. Currently, work is being carried out to evaluate the microbial community composition of the identified STEC high-shedding and low-shedding cattle populations using 454-pyrosequencing.

Introduction

Shiga toxin-producing *E. coli* (STEC) have been a major public health concern in recent times because of their association with foodborne disease outbreaks. This has resulted in many recalls that have been costly to the beef industry and has also impacted consumer confidence.

The major serotype of public health significance within the STEC group is *E. coli* O157:H7 and much effort has been expended to understand the prevalence, transmission, and disease-causing traits of this organism. However, the other strains of STEC, such as O111, O103, O145, O45, O26, O121 and many more, commonly referred to as the non-O157 STEC, have not received nearly as much attention and as a consequence, relatively little is known about their ecology in cattle and disease-causing capacity in humans. In the backdrop of last year's outbreak of hemolytic uremic syndrome (HUS) and hemorrhagic colitis in Germany, caused by *E. coli* O104:H4 the importance of detecting and reducing non-O157:H7 STEC shedding is critical.

Reducing the load of pathogenic STEC at pre-harvest can lead to significant improvements in the safety of beef. It has been shown that the prevalence of STEC among cattle in a pen varies widely (*2001 Nebraska Beef Cattle Report*, p. 81). The exact reason(s) why certain animals within a pen shed high numbers of STEC whilst others that comingle with them within the same pen do not shed is currently unknown. To this end, we hypothesize that competition for nutrients, colonization space, etc. between STEC and native microbial communities within the intestinal tract of cattle may play a role to determine whether a steer sheds or not and whether the animal becomes a high-shedder or low-shedder. Using high throughput genome-sequencing technologies we aim to characterize the microbial communities of STEC high-shedding and low-shedding cattle populations to identify particular microbial populations which may have a protective effect against the colonization of cattle by STEC.

Procedure

During August and September 2011, rectal grab samples of feces were collected weekly for three weeks from 170 animals housed at the UNL research facility Agricultural Research and Development Center (ARDC) near Mead, Neb. Each of these fecal samples was subjected to microbiological analysis to detect and quantify Shiga toxin-producing E. coli (STEC) in feces. Briefly, for each fecal sample 10 g was diluted in 90 ml of phosphate-buffered tryptic soy broth and was blended with a stomacher (AES Laboratoire, France) at 200 rpm, for one minute. From each fecal suspension, a 1-ml aliquot was removed and 50 microliters was plated on the surface of a CHROMagar^{TM} STEC plate (CHROMagar, Paris, France) using a spiral plater (IUL Instruments, Barcelona, Spain) and incubated overnight at 42^{o}C. Characteristic colonies (mauve color) were counted and the number of STEC/g of feces was calculated. Based on these results, candidate STEC high-shedding and low-shedding animals were identified. Four characteristic STEC colonies were selected from each high-shedding fecal sample and cultured in Tryptic Soy Broth. These cultures were then archived until further analysis to confirm shiga toxin production. To determine the serotype(s) of STEC, DNA was extracted from the archived bacterial cultures of the 52 highest-shedding steers using the Quick Extract^{TM} bacterial DNA extraction solution (Epicenter Biotechnologies, Madison, Wisc.) according to manufacturer's protocol. The full length 16s rRNA gene was amplified with the polymerase chain reaction (PCR) using universal 16s rRNA primers (Park et al., 2003) and sequenced after puri-

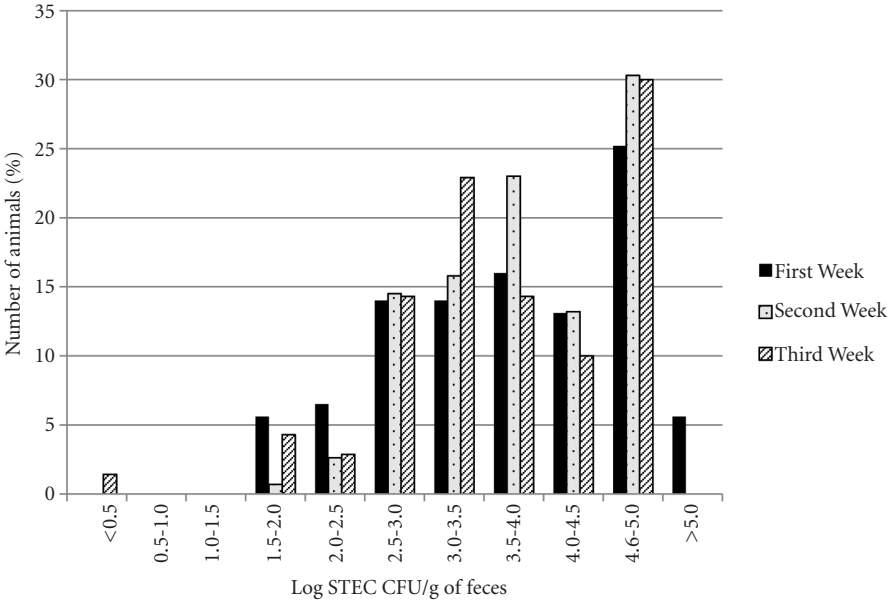

Figure 1. Number of beef animals associated with different levels of STEC shedding during all three sampling time points.

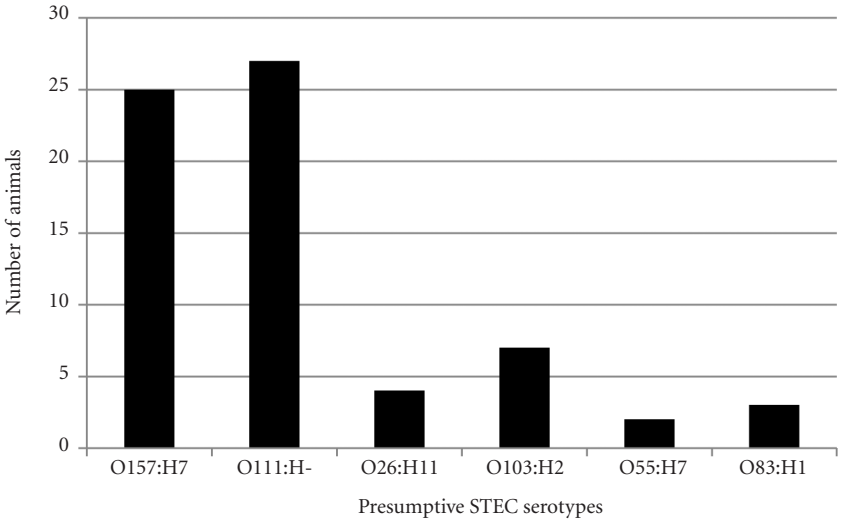

Figure 2. Number of animals shedding different STEC serotypes based on sequence matches to known sequences available in RDP Database.

fication using shrimp alkaline phosphatase and exonuclease I treatment (Sambrook, Fritsch, and Maniatis, 1989). The 16s rRNA sequences that were generated were compared to known 16s rRNA sequences of bacteria using the Ribosomal Database Project (*http://rdp.cme.msu.edu/*) and sequences available at the National Center for Biological Information (NCBI). The bacterial cultures were also screened for the presence of shiga toxin genes (*stx* 1 and *stx* 2) using PCR as described previously (Paton and Paton, 1998) serotype specific primers and also for the production of shiga toxin(s) using the ProSpecT Shiga Toxin *E. coli* Microplate Assay (Remel, Lenexa, Kansas) to further confirm that the particular isolates were STEC.

Results

Figure 1 presents the number of cattle associated with different levels of STEC shedding during the three sampling time points. Animals shedding >4 log cfu/g of feces were considered to be high shedders and those shedding ≤2.5 log cfu/g were considered to be low-shedders. Among the 52 high shedding animals selected for microbial community analysis, 16 (30.77%) shed STEC at levels >4 log cfu/g of feces during all three sampling time points, and 32 (61.54%) shed STEC at levels >4 log cfu/g of feces in at least two of the three sampling time points. However, three animals that started off as low-shedders during the first week were shedding STEC at high levels by the third week and one animal which started as a high-shedder in the first week was shedding low levels of STEC in the third week. In the first week of sampling, 6 animals shed STEC in excess of 5 log cfu/g of feces but during the second and third week none of the animals shed STEC at such high levels.

Figure 2 represents the number of beef steers shedding different serotypes of STEC based on 16s rRNA sequencing results, serotype specific PCR and Latex agglutination tests. The most common serogroups found in the cattle feces were those belonging to the serogroups O111 (40.3%) and O157 (37.3%), with O103 (8.3%), O26 (6.0%), O83 (4.5%), and O55 (3.0%) being encountered in much lower numbers.

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