**FS19: Animal Colonization patterns of STEC O157 Strains**

**Motivation:** Shiga toxigenic *Escherichia coli* (STEC) from different food products including beef from cattle and produce are associated with disease outbreaks in humans. Although cattle feces is a presumed source for the contamination of produce, it is unclear if isolates from lettuce or spinach outbreaks can colonize and shed in the feces of the cattle at a similar rate to cattle isolates. Additionally, STEC serotype O157 (O157) have been divided into three different chromosomal lineages (Lineages I, I/II, and II), which may confound proper conclusions due to genetic differences. To date, O157 strains have been studied with *in vitro*, *in vivo*, and *in silico* models, but a majority of conclusions are from strains belonging to lineage I. Therefore, the objective of this study is to compare O157 isolates from a variety of sources and lineages to a well-characterized lineage I O157 outbreak strain (EDL933) to assess if there are differences between O157 strains when examining shedding and colonization.

**Objectives:**

1. **To assess if O157 strains have preferential niches of colonization within the gastrointestinal tract.**
2. **To assess the variation in shedding between STEC strains.**

**Materials and Methods**

This project will run in approximately September-November, 2019 at NCAH building 7.

***Animals, housing and experimental design***

A total of 32 Jersey-specific dairy breed calves will be used, with the calves being split into two groups of 16 each with two different dates of arrival. Upon arrival, calves will be separated into groups of 4 and separated into 4 treatment rooms.

Calves will be vaccinated with Alpha for Clostridium and Inforce and/or Vista for bovine respiratory disease prior to shipment. Calves will also have been treated with de-wormer approximately five days prior to pick up. Calves will not have received Endovac, unless documented and administered at least two months prior to pick up. Calves will acclimate in their treatment room for approximately 12-14 days prior to the inoculation. Calves will be inoculated with 1010 CFU of one of the four O157 strains: EDL933, RM6067, TW14588, and FRIK1989. Calves (n=4) housed together will receive the same O157 strain. Calves will be given ad libitum access to water and feed for the duration of the experiment.

The treatments include:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **Isolation Source** | **Chromosomal Lineage** | **First Set of Calves (n=16) Inoculation Group\*** | **Second set of Calves (n=16) Inoculation Group\*** | **Room** |
| **EDL933** | **Human** | **I** | **A** | **D** | **5109** |
| **RM6067** | **Spinach** | **I/II** | **A** | **D** | **5111** |
| **TW14588** | **Lettuce** | **I** | **B** | **C** | **5113** |
| **FRIK1989** | **Cow feces** | **II** | **B** | **C** | **5115** |

\* These groups correspond to the Timeline section.

***Sampling and data collection***

Fecal samples and RAJ swabs (RAMS) for each animal will be collected seven days prior to inoculation, upon inoculation, every day after inoculation for six days, and every two days thereafter.

Blood samples will be collected via jugular venipuncture on days -7, 0, 1, 8, and 13/14 of the experiment. Fecal grab samples and RAJ swabs will be collected at day -7, 0, 1-6, 8, 10, 12, and 13/14. All calves will be euthanized via intravenous administration of sodium pentobarbital under the directive of a veterinarian. The Kudva protocol for bacterial culture of fecal, RAMS and tissue content samples for O157 will be followed. All collected tissues at necropsy will be (1) cultured, (2) frozen, (3) formalin fixed, and (4) preserved in RNAlater.

Tissues to be collected at necropsy:

* RAJ
* Proximal ileum
* Distal ileum
* Ileo-cecal valve
* Cecum
* Spiral colon
* Distal colon

***Timeline:***

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Day** | **-12/14** | **-6** | **0** | **1** | **2-5** | **7, 9, 11** | **14** |
|  | **Task** | Animal arrival | Fecal, RAMS, Blood | Fecal, RAMS, Blood, Challenge | Fecal, RAMS,  Blood | Fecal, RAMS | Fecal, RAMS, | Fecal, RAMS,  Blood,  Necropsy, |
| **I** | **Group A** | 9/26 | 10/01 | 10/07 | 10/08 | 10/09-10/12 | 10/14 (Blood), 10/16, 10/18 | 10/21 |
| **Group B** | 9/26 | 10/03 | 10/10 | 10/11 | 10/12-10/15 | 10/17 (Blood), 10/19,  10/21 | 10/24 |
| **II** | **Group C** | 10/24 | 10/29 | 11/04 | 11/05 | 11/06-11/09 | 11/11 (Blood), 11/13, 11/15 | 11/18 |
| **Group D** | 10/24 | 11/01 | 11/07 | 11/08 | 11/09-11/12 | 11/14 (Blood),  11/16,  11/18 | 11/21 |