E. coli Strain Demographics and Transmission in Cattle

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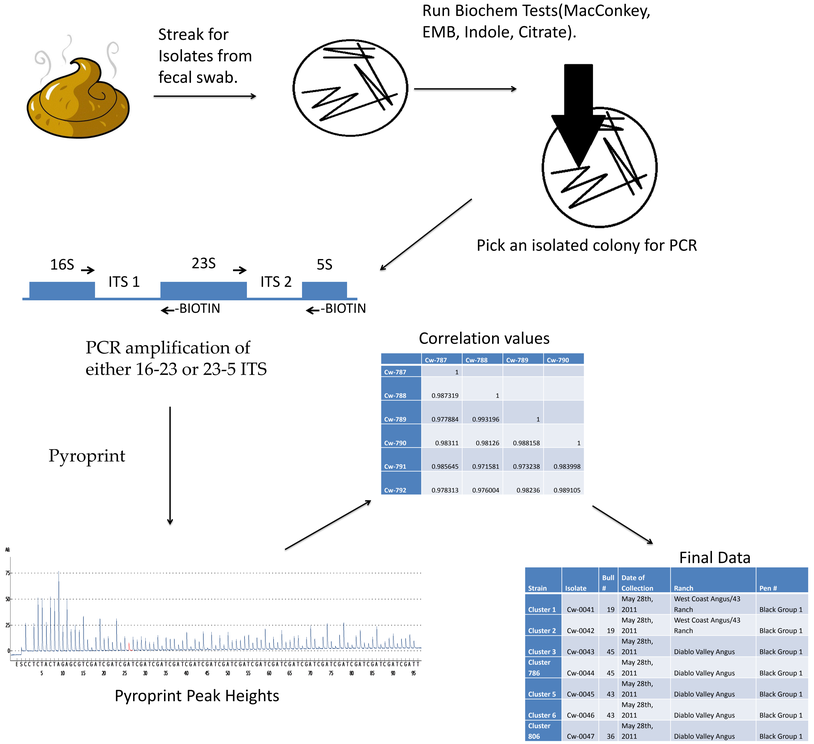
Abstract

In the United States, symptoms caused by pathogenic strains of *E. coli* are on the rise. A major source of these pathogenic stains is the bovine digestive tract. The purpose of this project is to determine if *E. coli* are transferred among cohabitating animals and if dominant bovine strains exist. In this project *E. coli* are grouped into strains through the creation of molecular fingerprints taken from two distinct polymorphic loci within the rRNA operon. Strain-specific pyroprints are generated by amplifying the intergenic transcribed spacer regions between the 16S-23S (ITS-1) and 23S-5S (ITS-2), and pyrosequencing the products.

Our working hypothesis is that the environment plays a significant role in the microbial flora of cattle. We expect bulls from the same farm will be more likely to share *E. coli* strains with one another than those of different farms. Furthermore, we predict that when bulls from different farms cohabitate, the *E. coli* strain demographics will shift so that the pen will be a more significant predictor of the strain isolated from bulls than the farm from which they originated. *E. coli* were collected from bulls that arrived at Cal Poly from farms across the state. Two isolates were taken from bulls when they first arrived, then another two isolates from the same bulls after cohabitating for four months. A total of 380 bulls were sampled in this manner over the summers of 2011 and 2012. Preliminary results support our hypotheses. *E. coli* isolated from bulls when they first arrived showed many common strains from the same farm and very few from different farms. Conversely, *E. coli* isolated after bulls had cohabited for 4 months showed more strains in common across farms, common to the pens these bulls inhabited.

Figure 1. The Pyroprinting Method



Introduction

Escherichia coli, a species in the *Enterobacteriaceae* family, is usually a non-pathogenic bacterium found in the intestines of many animals. *E. coli* can be categorized further into individual strains where each strain is the same species but because of slight differences in its genetic structure strains have separate attributes. Most *E. coli* strains are commensal but, there are a few strains of *E. coli* that can be pathogenic. Illnesses derived from pathogenic strains of *E. coli* are a problem in the United States and throughout the world. Pathogenic strains of *E. coli* produce serious infections and sometimes death. One major reservoir of *E. coli* is the bovine intestinal tract. Bovine contamination by pathogenic strains of *E. coli* is a concern among ranchers but not much is known about the transmission of *E. coli* from one animal to another. This project aims to shed some light on the transmission and demographics of different *E. coli* strains in cattle. Using this knowledge we can help reduce the possibility of contamination worldwide.

This project uses a new method known as pyroprinting to distinguish between closely related strains of *E. coli* (Figure 1). Pyrosequencing all copies of the Intergenic Transcribed Spacer (ITS) regions between either the 23 and 5 ribosomal subunit genes (ITS1) or the 16 and 23 ribosomal subunit genes a molecular fingerprint. Combining the two fingerprints results in a very discriminating way to separate strains of *E. coli*.

Cal Poly hosts a "Bull Test" event each year where yearling bulls are transported from across California, arriving at Cal Poly in May. The bulls are tested, vaccinated and housed in 5 separate pens for four months until they are sold at auction in September. We collected two isolates of *E. coli* from each yearling bull when they arrived in May and before they left in September, over two summers in 2011 and 2012. These isolates were then pyroprinted and classified into strains with the same ITS1 and ITS2 pyroprints. We then analyzed the results to look for transmission of *E. coli* strains between the bulls.

In a preliminary study to determine the number of strains a single animal can carry 30 isolates were collected from a single bull fecal sample. These isolates fell into 15 different strains. Clearly cattle carry a large number of different *E. coli* strains in their digestive tract at the same time and since we collected only 2 isolates each time we sampled we could not comprehensively follow the fate of individual strains in our study.

Hypothesis: Cattle will readily exchange *E. coli* when in close proximity.
Prediction 1: Cattle from geographically different areas will harbor different strains of *E. coli*.
Prediction 2: When cattle are housed together they will exchange *E. coli* strains.

Results and Discussion

Table 1. Number of Isolates Collected

Date of Sampling	Number of Bulls Sampled	Number of Strains Pyrosequenced
28-May-11	175	350
3-Sep-11	144	288
12-May-12	180	360
1-Sep-12	139	278

Table 2. Number of Isolates in the Strains Identified

Isolates/Strain	Strain Count
1	490
2	146
3	55
4	22
5	13
6	7
7	8
8	2
9	2
10	1
11	3
Total	749

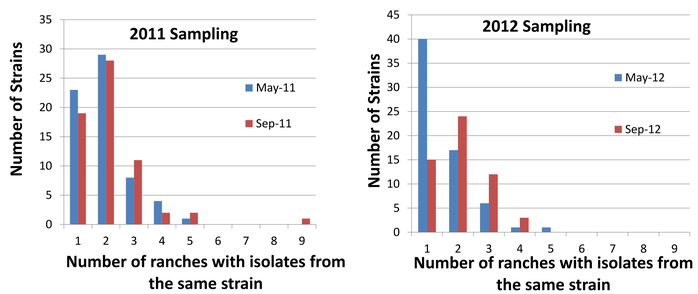
Table 3. Number of strains collected on multiple sample dates

Dates/Strain	Strain Count
1	701
2	48
3	1

Over the 2 year sampling period a total of 1276 *E. coli* isolates were collected from 638 samples (Table 1). These isolates fell into 749 strains with the majority strains comprised of a single isolate (Table 2). Most strains were comprised of isolates taken during a single sampling period with less than 50 strains containing isolates collected on 2 different sampling dates (Table 3). Together these data indicated our analysis should focus on the frequency of strain sharing within a single sampling date.

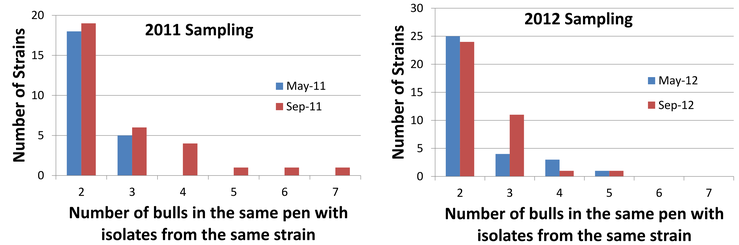
Results

Figure 2. More strains of *E. coli* were shared after 4 months of cohabitation



For *E. coli* strains with more than 1 isolate, these graphs depict the number of strains (y-axis) where isolates came from one or more ranches (x-axis). Sampling in May 2011 took place 2 weeks after bulls first arrived at Cal Poly. Sampling in May 2012 took place as bulls arrived at Cal Poly. In both cases the distribution of strains with isolates from multiple ranches increased after bulls were housed together for 4 months. Similarly, the number of strains with isolates from only one ranch decreased after 4 months of cohabitation.

Figure 3: When Bulls are housed together they will more readily share *E. coli*.



For *E. coli* strains with more than 1 isolate, these graphs depict the number of strains (y-axis) that were shared across a number of bulls inhabiting the same pen (x-axis). In the May 2011 sampling the maximum number of bulls sharing the same strain of *E. coli* within a pen was 3. While in September 2011 a maximum of 7 different bulls shared strains with their pen mates. Similar but less striking results were found in 2012.

Conclusions

- **Prediction 1 is supported**: Initially (May samplings) most strains with more than one isolate contained isolates from bulls originating from the same ranch (Figure 2).
- **Prediction 2 is supported**: After cohabitation (Sept samplings) there was an increase in the number of strains containing isolates collected from bulls originating from different ranches (Figure 2). In addition, there was an increase from May to Sept in the number of bulls sharing isolates from the same strain when the bulls were housed together in the same pen (Figure 3).

Acknowledgements

This study was supported by a grant from the W. M. Keck Foundation, and funds from the Environmental Biotechnology Institute and the College of Science and Mathematics. Thanks to Michael Hall for allowing access to the bulls from the Cal Poly Bull Run. Thanks to Aldrin Montana for his work in developing a clustering program.