

# Microbial Source Tracking of *E. coli* and Land Use Data Correlations for the Lamoille River Basin, Vermont

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## Introduction

Microbiological impairment of water systems is assessed by observing the levels of fecal indicator bacteria such as fecal coliforms and enterococci (EPA, 2005). One of these fecal indicator bacteria, *Escherichia coli* (*E. coli*) is found in the lower digestive tract of warm-blooded animals. Therefore, potential sources include waste from humans, domestic animals, farm animals, and wildlife (Duris, et al, 2011). Surface runoff during rain events in urban, forested and agricultural areas may transport possible harmful pathogens into available public water and recreational areas (EPA, 2005). These fecal indicator bacteria are used to test water quality and possible presence of harmful pathogens that could cause illness to people exposed to them.

*E. coli* samples from 19 sites in the Lamoille River Basin were filtered and isolated. Once the *E. coli* were isolated, they were subjected to genetic comparison through ribotyping. Ribotyping is a method of analysis used by many environmental water quality laboratories to fingerprint microbial source tracking (Carson et al, 2001). With the ribotyping data retrieved, and GIS information of the Lamoille River Basin land use, we could then compare in IBM SPSS Statistical Analysis software where correlations were present.

## Materials and Methods

*E. coli* was collected from streams during two days in mid-June from 2008 to 2014. Those samples were taken at 19 different stream sites, 3 samples per site, during high and low flood events. Samples were kept on ice, and then filtered onto membrane filter paper with MI broth and kept at 35 degrees Celsius in an incubator for 20-24 hours.

Once *E. coli* colonies were isolated, they were cultured on McConkey II selective media (BD Medical, Franklin, NJ), and isolated three times to get a pure strains. Once isolated, the *E. coli* was ribotyped on an automated riboprinter (DuPont-Qualicon, Wilmington, Delaware) using EcoRI preselective amplification primers. The genetic barcodes were compared using similarity dendrograms and if above a threshold of 92% they were considered a match.

Colilert data was also collected using 100ml of water at stream water dilutions 100% 10% and 1%. IDEXX Colilert-18 reagent was added to the respective stream sample, poured into foil sealed IDEXX Colilert Quanti-Trays, and incubated over night at 35 degrees Celsius. Referring to the Quanti-Tray/2000 MPN table, the large and small positive wells were counted for cell density.

Using the Colilert data for cell density per 100ml, and proportional abundances from ribotyped single-species, multi-species, and unmatched isolate point sourced matches from samples in the library at Johnson State College, we could then use software to find correlations between bacterial density and amounts of land use practices. The size of correlation effects and understanding *r* were interpreted through the effect sizes described by Cohen (1988) with  $r=0.30$  as medium Effect Size and  $r=0.50$  as large Effect Size (Cohen, 1988)

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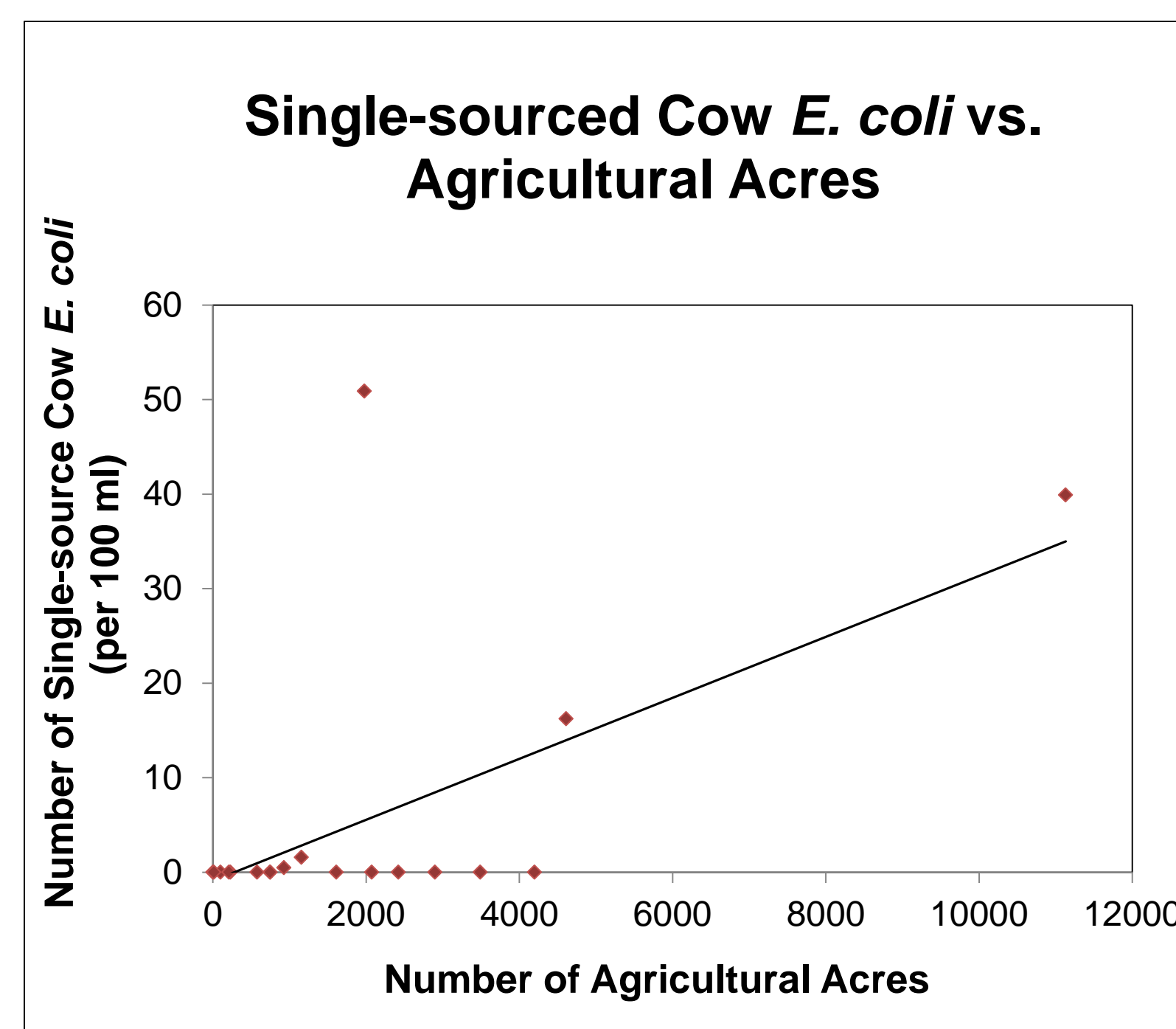


Figure 1.: Single-sourced Cow *E. coli* vs. Number of Agricultural Acres

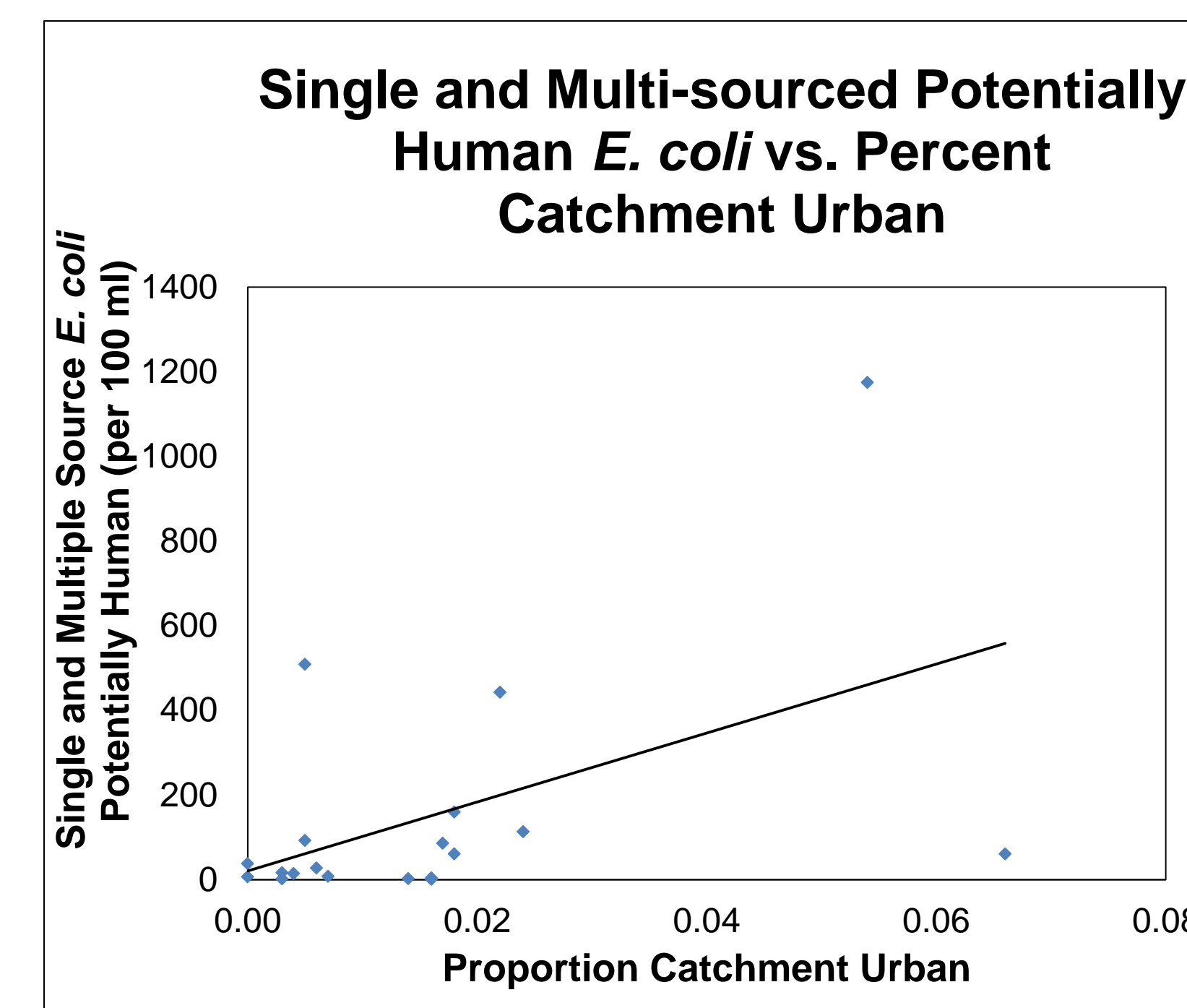


Figure 2.: Single and Multi-sourced Potentially Human *E. coli* vs. Percent Catchment Urban

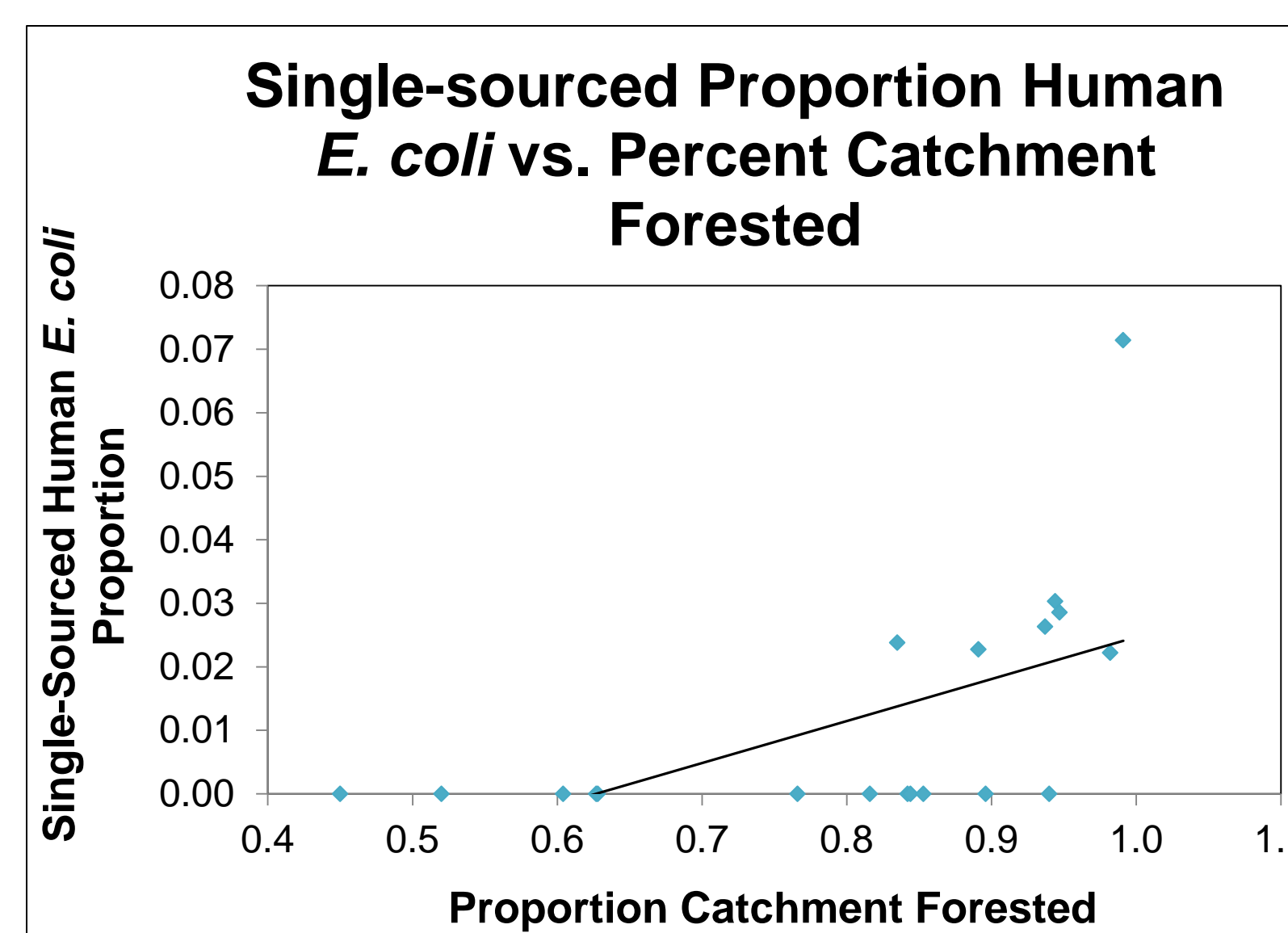


Figure 3.: Single-sourced Proportion Human *E. coli* vs. Percent Catchment Forested

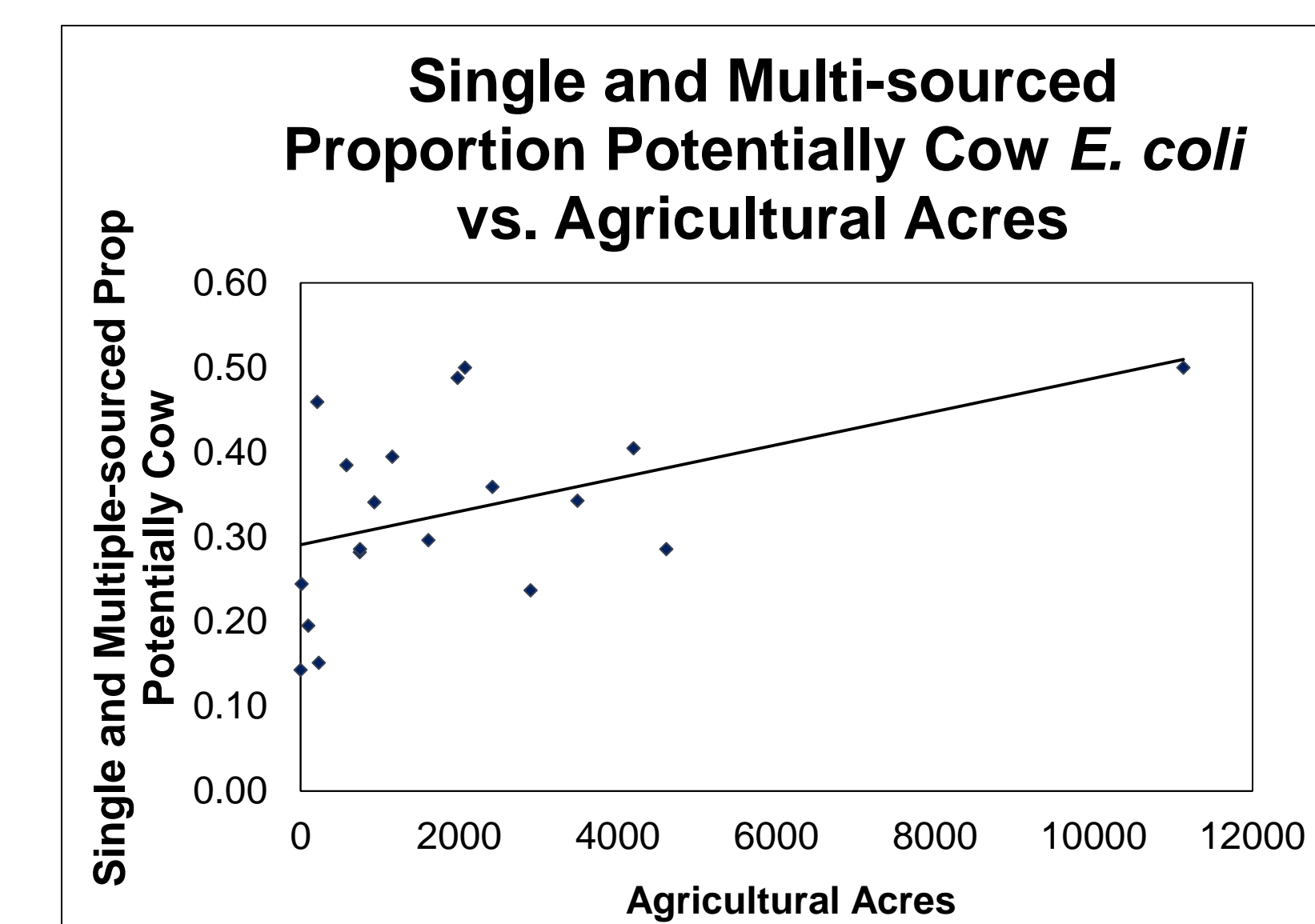


Figure 4.: Single and Multi-sourced Proportion Potentially Cow vs. Number of Agricultural Acres

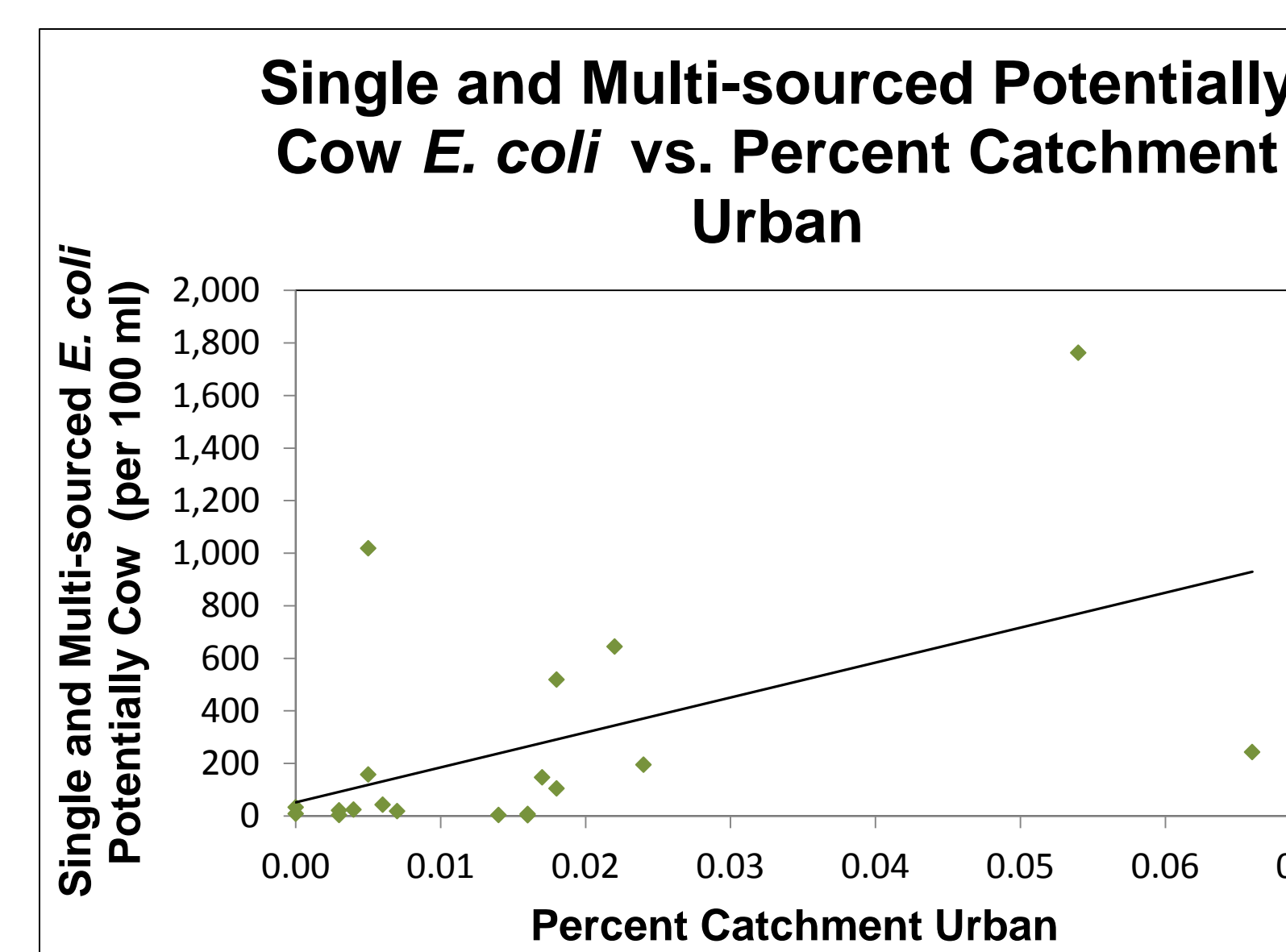


Figure 5.: Single and Multi-sourced Potentially Cow *E. coli* vs. Percent Catchment Urban

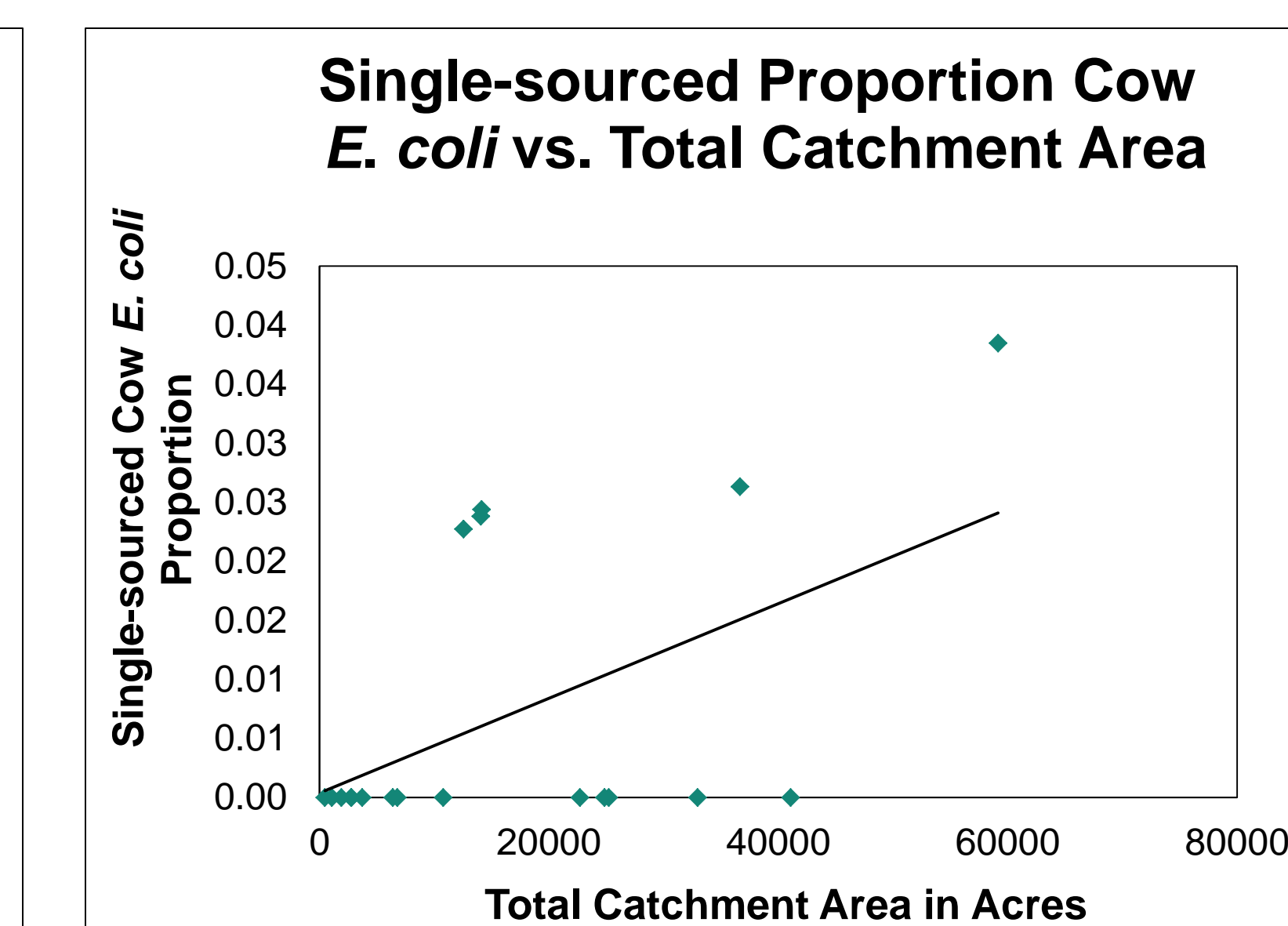


Figure 6.: Single-sourced Proportion Cow *E. coli* vs. Total Catchment Area

## Results

- ◆ *Figure 1:* Single-sourced Cow *E. coli* vs. Agricultural Acres had strong correlation ( $r=0.579$ , significance at the 0.01 level, 2-tailed). Looking at the trendline it appears that as the number of agricultural acres increases, the probability of *E. coli* being single-sourced by cows, more likely in a few samples.
- ◆ *Figure 2:* Single and Multi-sourced Potentially Human *E. coli* vs. Percent Catchment Urban had a moderately strong correlation ( $r=0.495$ , significance at the 0.05 level, 2-tailed). Looking at the trendline it appears that as the urban catchment area increases, the probability of *E. coli* being single or multi-sourced by Humans increases.
- ◆ *Figure 3:* Single-sourced Proportion Human *E. coli* vs. Percent Catchment Forested had a moderately strong correlation ( $r=0.568$ , significance at the 0.05 level, 2-tailed). Looking at the trendline it appears that starting at approximately 63% catchment forested, as forested land increases, the probability of human point-sourced *E. coli* also increases.
- ◆ *Figure 4:* Single and Multi-sourced Proportion Potentially Cow *E. coli* vs. Agricultural Acres had a moderately strong correlation ( $r=0.464$ , significance at the 0.05 level, 2-tailed). Looking at the trendline it appears that as agricultural acres increase, the probability of *E. coli* being single or multi-sourced by cows increases.
- ◆ *Figure 5:* Single and Multi-sourced Potentially Cow *E. coli* vs. Percent Catchment Urban had a moderately strong correlation ( $r=0.511$ , significance at the 0.05 level, 2-tailed). Looking at the trendline it appears that as the size of the catchment that is urban increases, the probability of *E. coli* being single or multi-sourced by cows increases.
- ◆ *Figure 6:* Single-sourced Proportion Cow *E. coli* vs. Total Catchment Area had a moderately strong correlation ( $r=0.513$ , significance at the 0.05 level, 2-tailed). Looking at the trendline it appears that as the total drainage area size increases, the probability of *E. coli* being single-sourced by cows increases.

## Discussion

Warm blooded animal sources of *E. coli* is correlated with land use practices as described by EPA (2005) Kephart, et al (2010), and Meays, et al (2006), among others. Research has found characterizing the source of fecal contaminations in watershed areas is best understood by identifying the spatial distribution, applying microbial source-tracking techniques, and developing multiple lines of evidence. (Kephart, et al, 2010) Further studies of microbial source-tracking correlations with land use in the Lamoille River Basin could help anticipate how current and future land use practices could affect our local water quality, and potentially reduce risks to human health.

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