Microbial Source Tracking as it Relates to Land Use in the Lamoille Valley, VT

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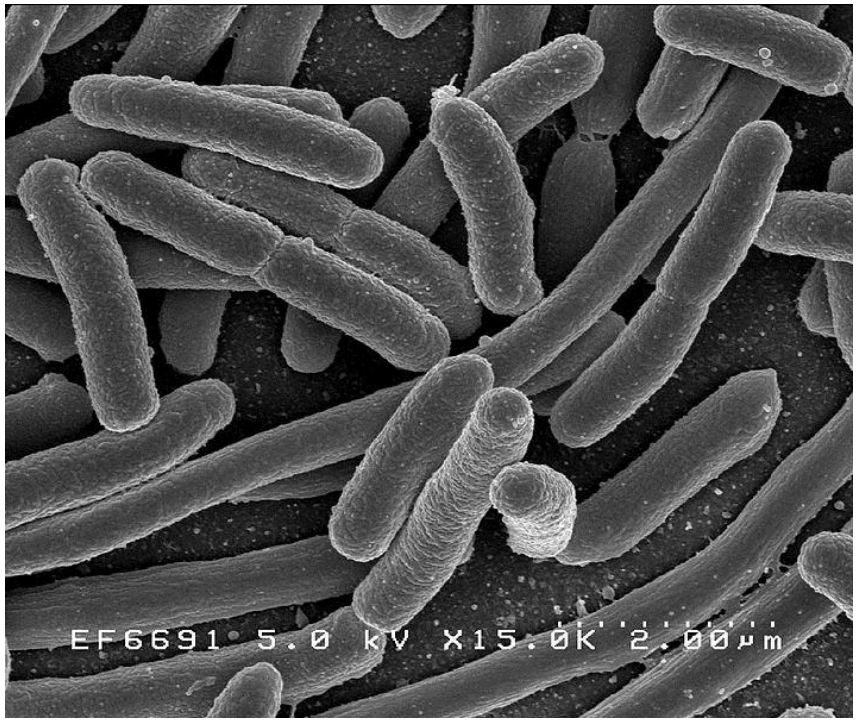
Escherichia coli

- Gram-negative, rod-shaped bacterium
- Associated with fecal material from humans and other warm-blooded animals
- Its presence suggests potential human health risk
- Significant economic loss from beach closing and cancelled harvesting of shellfish



http://en.wikipedia.org/wiki/File:EscherichiaColi_NIAID.jpg

The Concept

- Hypothesis
  - *E. coli* are not randomly distributed in streams of the Lamoille River basin
- Prediction
  - The non-random distribution is related to land use practices

Ribotyping – Microbial Source Tracking

Cultivation Dependent

Library Dependent

Genotypic Analysis

Extract Nucleic Acids

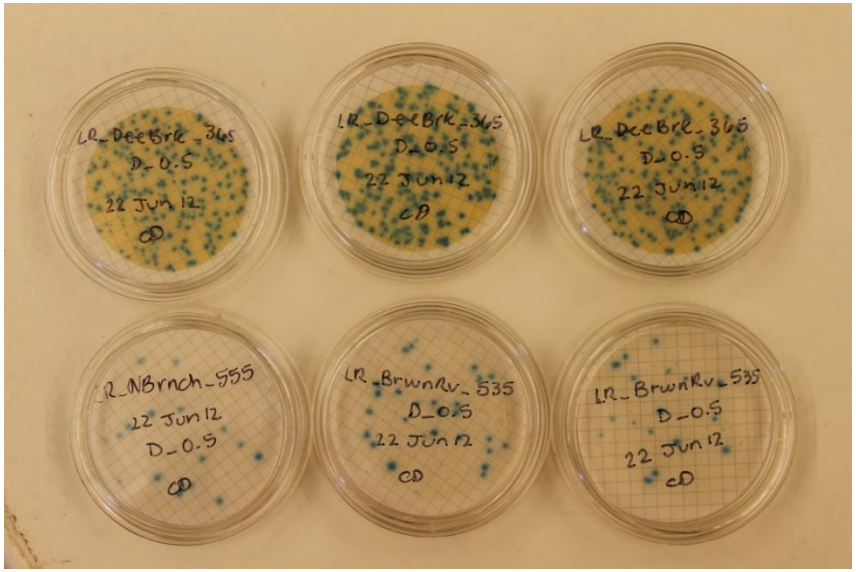
Restriction Enzyme Digestion

Blot & Hybridize with rRNA gene probe

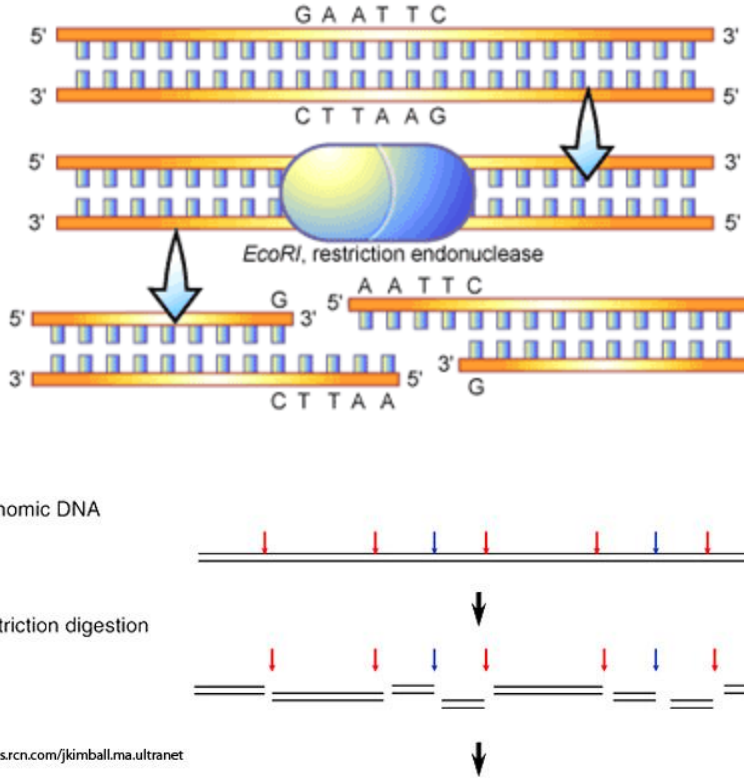
Santo-Domingo et al. 2005

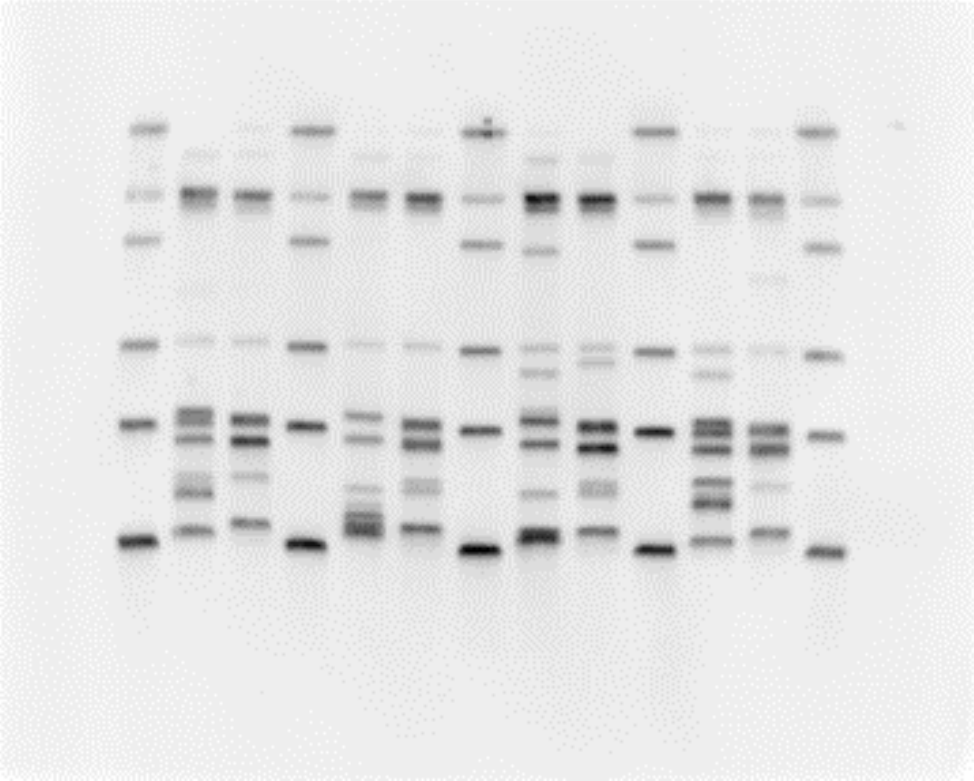
Collect and Filter for *E. coli*

Purify Cultures & Confirm IDs

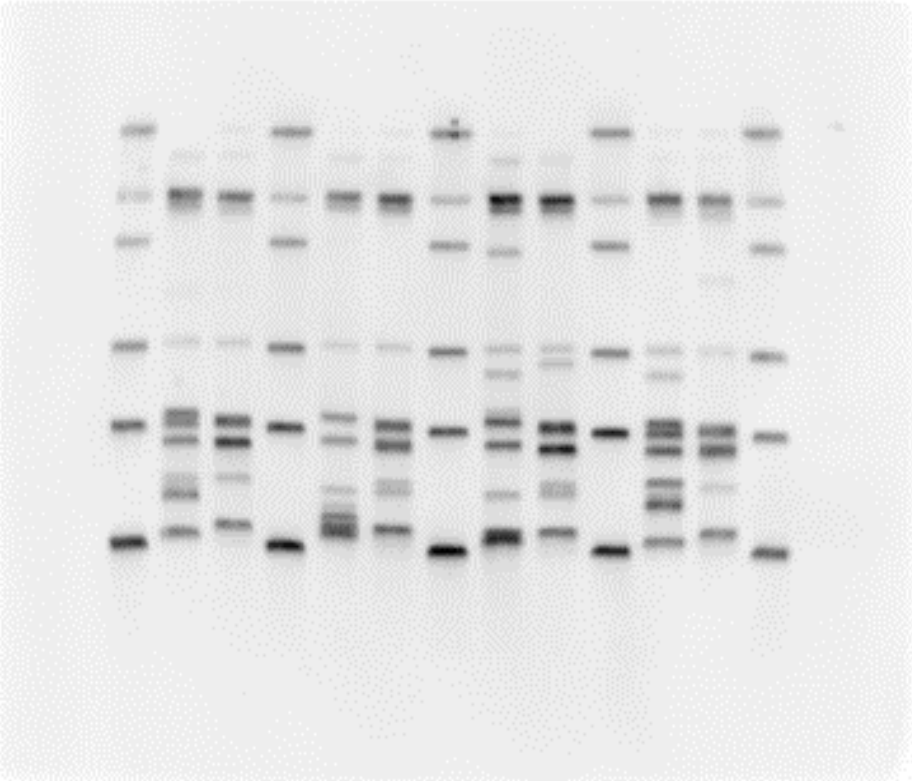


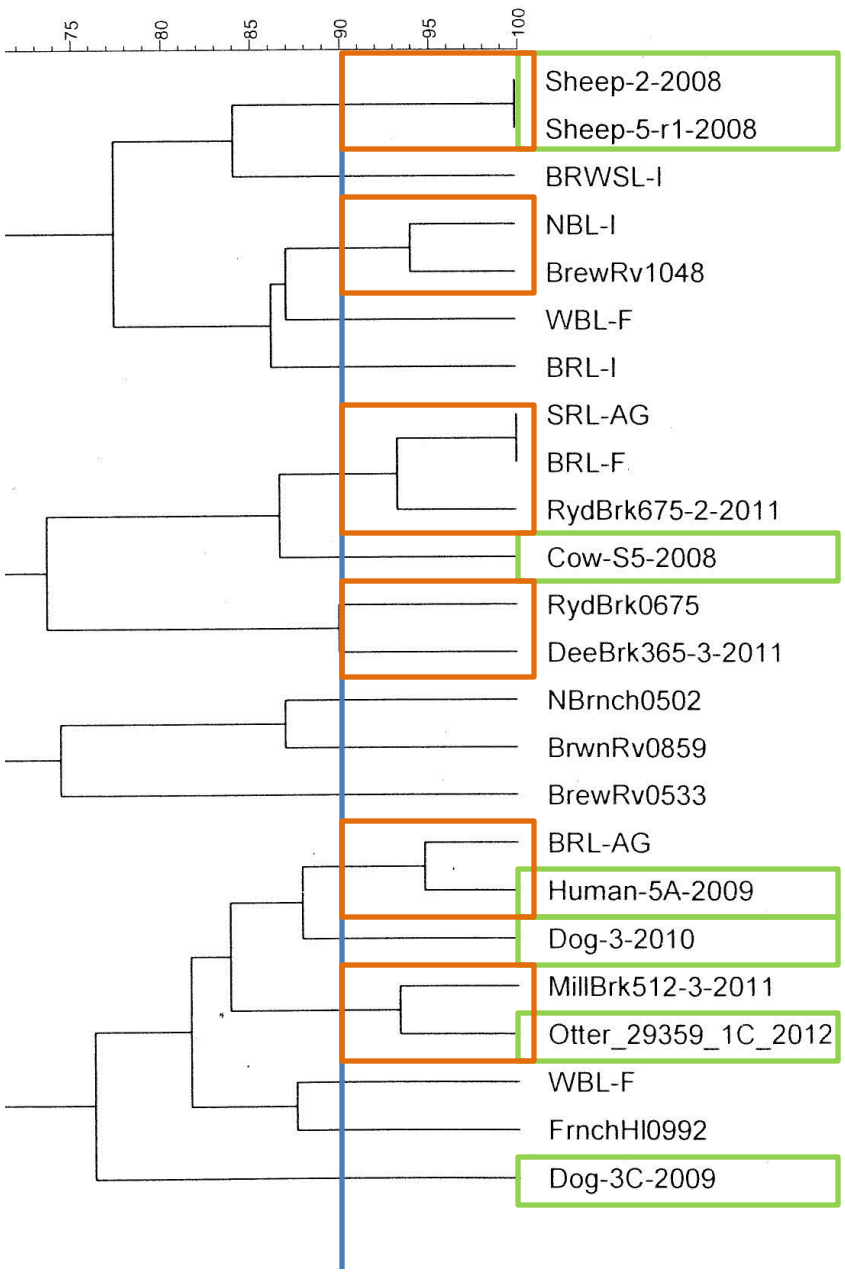
Ribotyping


G A A T T C
5'
3'
3'
5'
C T T A A G
5'
3'
3'
5'
EcoRI, restriction endonuclease
G
5'
3'
A A T T C
5'
3'
5'
3'
C T T A A
G
Total genomic DNA
Restriction digestion
http://users.rcn.com/jkimball.ma.ultranet



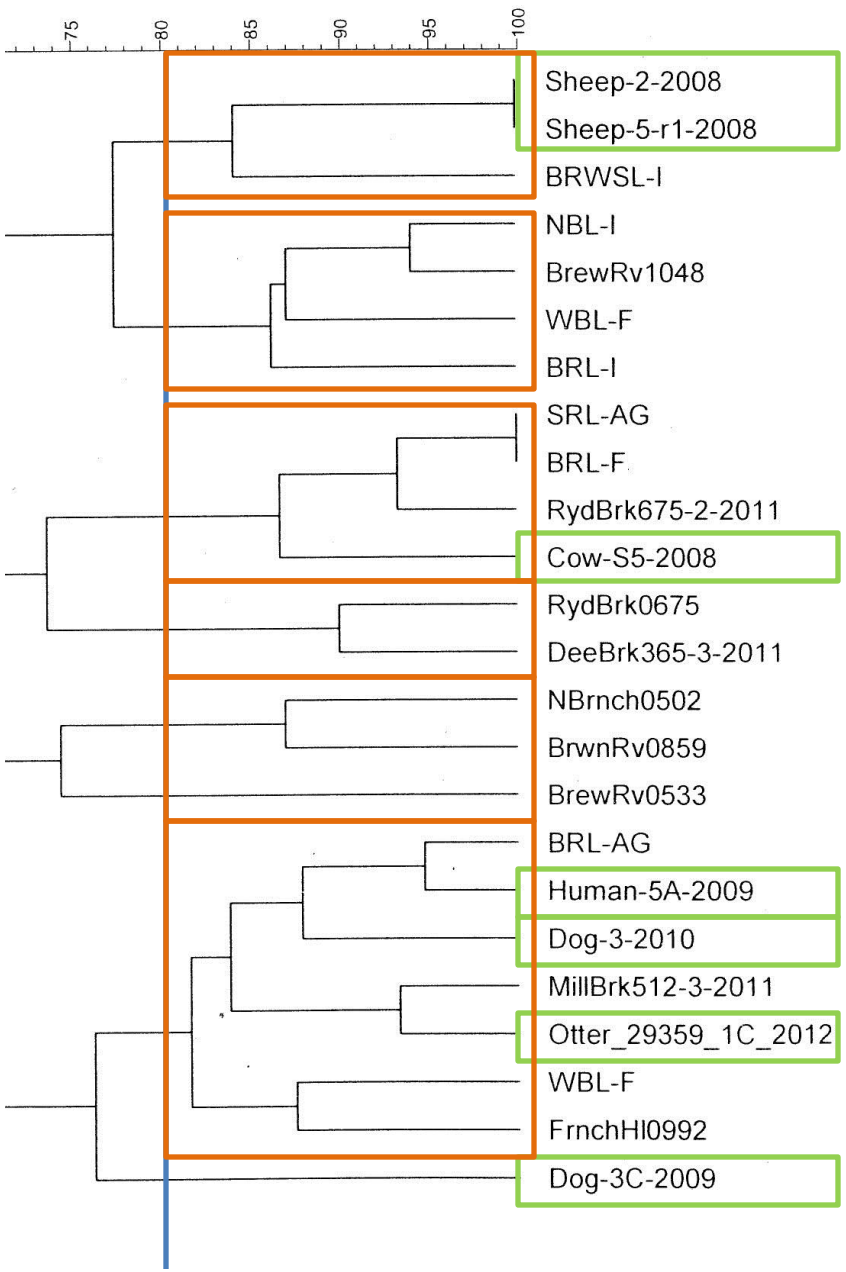
Dice's Similarity





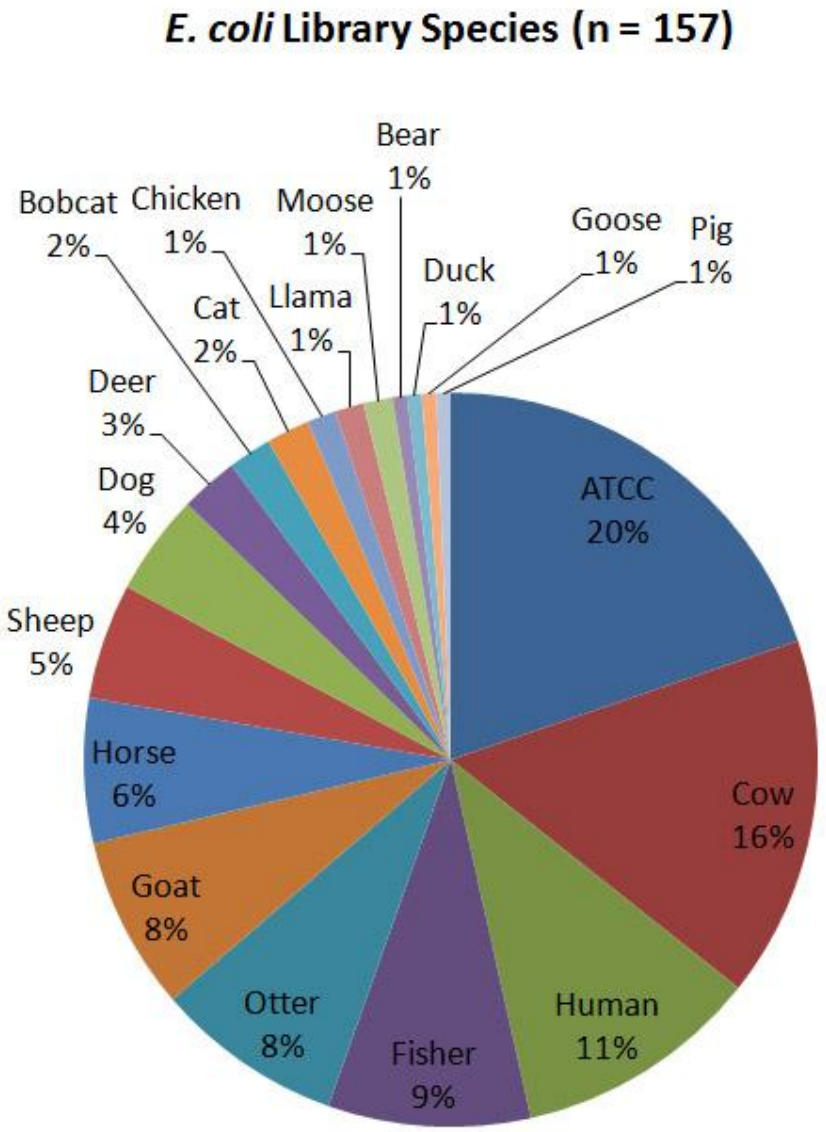
Dice's Similarity

- Library *E. coli*
- Degrees of Similarity
  - 90%
  - 80%



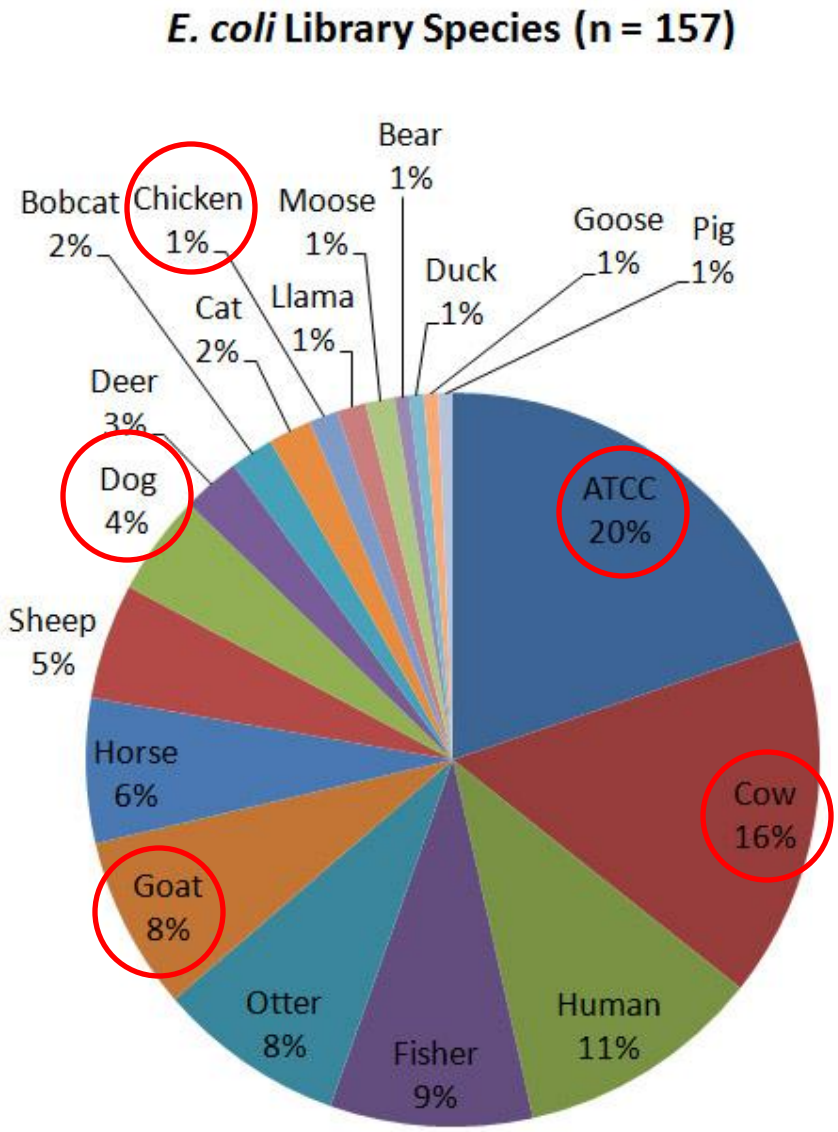
Library

- 126 isolates of *E. coli* from local warm-blooded animals
- 18 Library Species
  - 8 Wildlife
    - 6 mammals
    - 2 birds
  - 7 Agricultural
    - 6 mammals
    - 1 bird
  - 2 Domestic
  - Human
  - ATCC Reference Standard



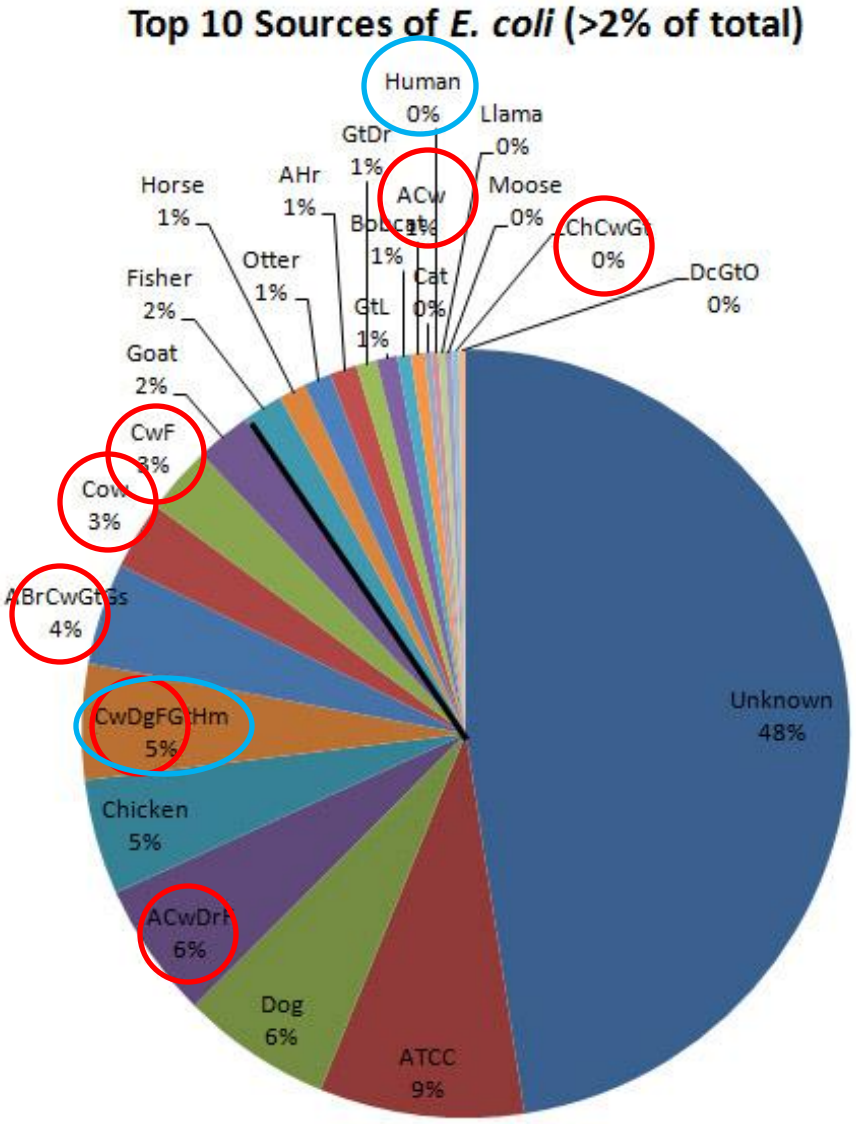
Streams

- The single-source species that show up most often (>2%) in streams
  - At the 90% similarity threshold

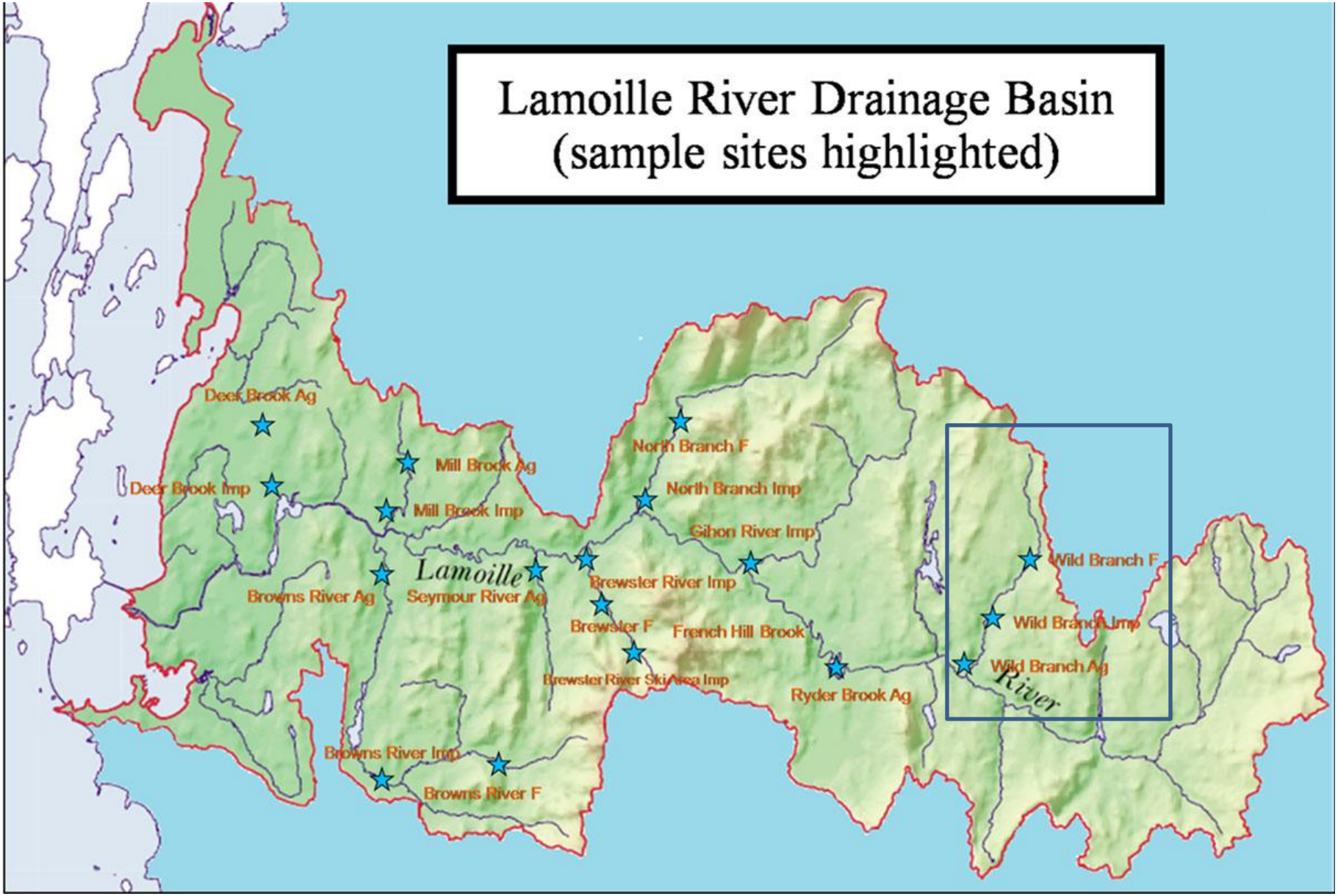


E. coli in the Lamoille Basin

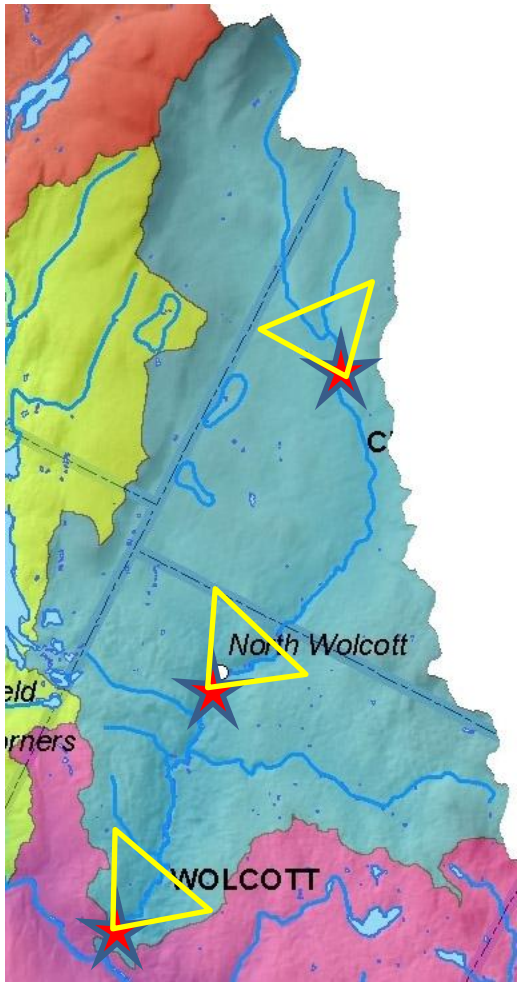
- 10 source species > 2%
- Dog, Chicken, Cow, and Goat are the major single-source species
- Cow – 3%
  - Potentially cow = 21.5%
- Human insignificant (0.3%)
  - Potentially human = 5.15%
- Many sources are unidentified



For 90 % cluster similarity, n = 349 Ribotyped

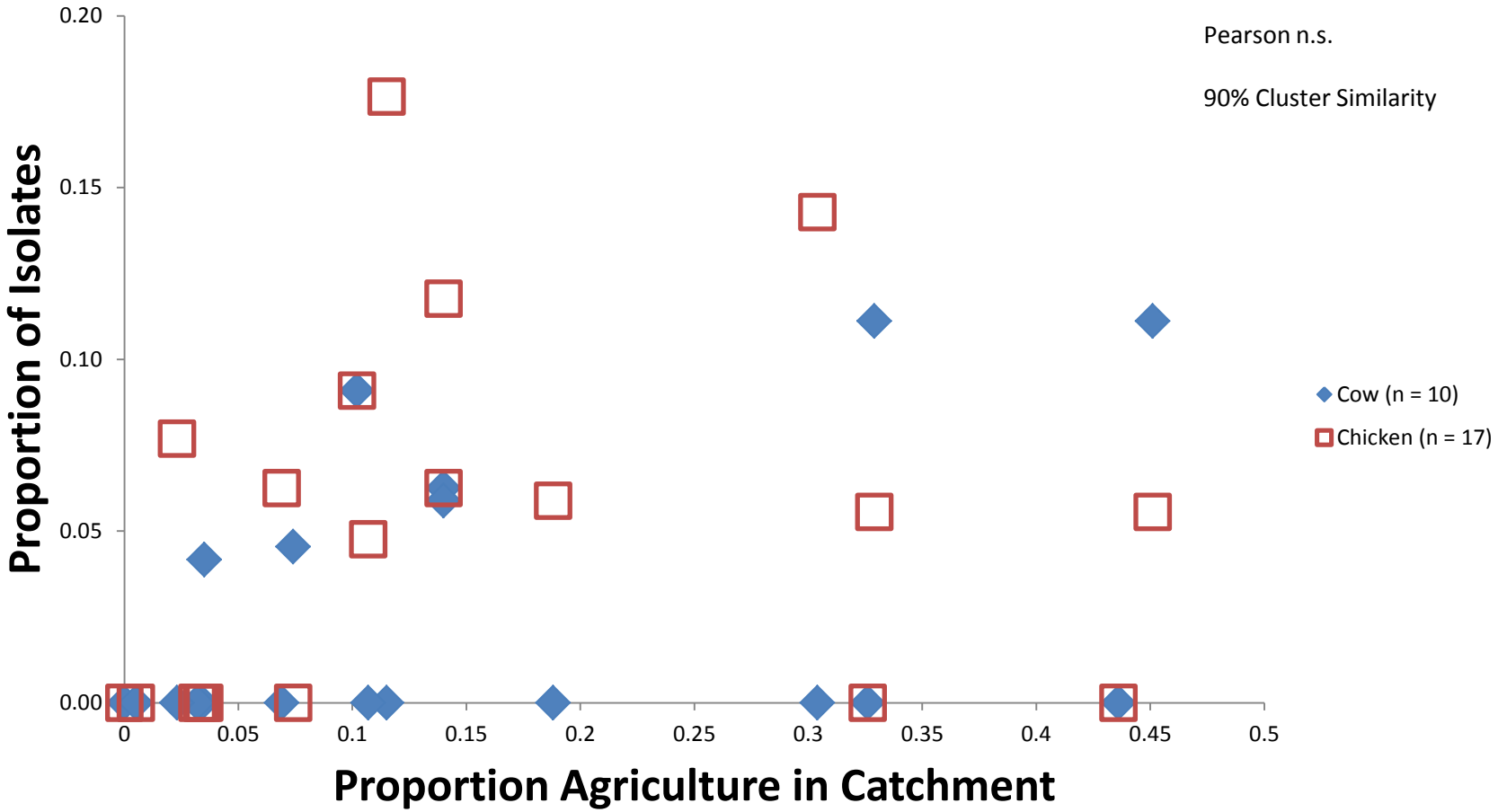

Lamoille River Drainage Basin
(sample sites highlighted)
Deer Brook Ag
Deer Brook Imp
Mill Brook Ag
Mill Brook Imp
North Branch F
North Branch Imp
Gibon River Imp
Lamoille
Browns River Ag
Seymour River Ag
Brewster River Imp
Brewster F
French Hill Brook
Brewster River Ski Area Imp
Ryder Brook Ag
Wild Branch F
Wild Branch Imp
Wild Branch Ag
River
Browns River Imp
Browns River F

Land Use Measurements



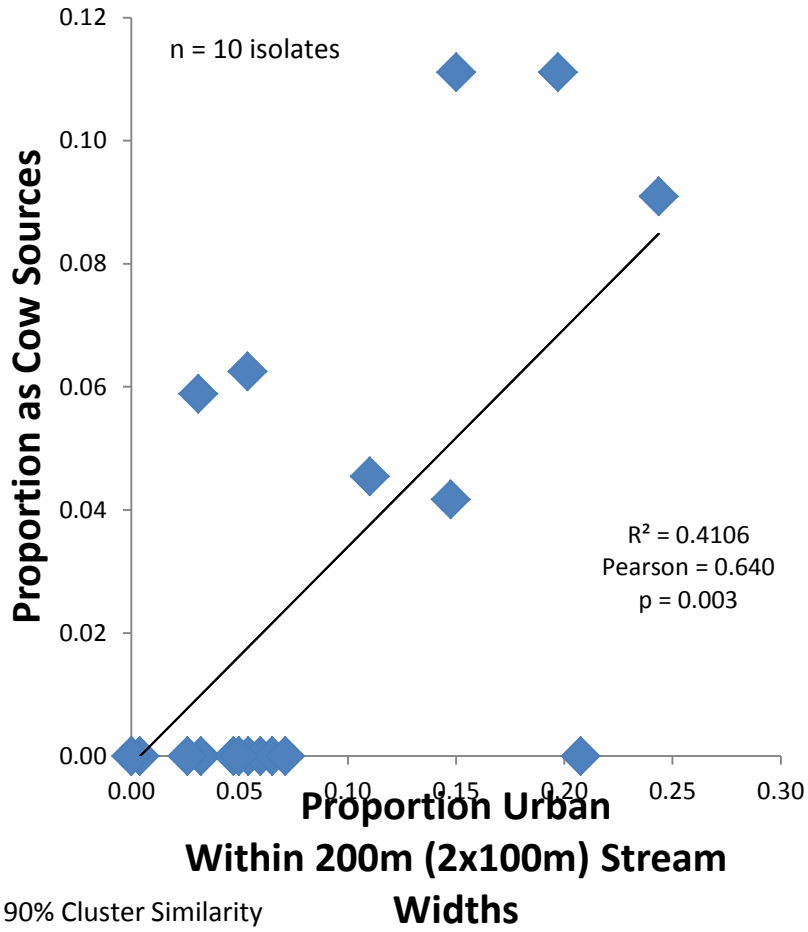
- Entire catchment upstream of the site
- Catchment 1-mile upstream
- Corridor 100 m on each side of stream (200 m belt transect) going 1-mile upstream
- Corridor 3 stream widths on each side going 1-mile upstream

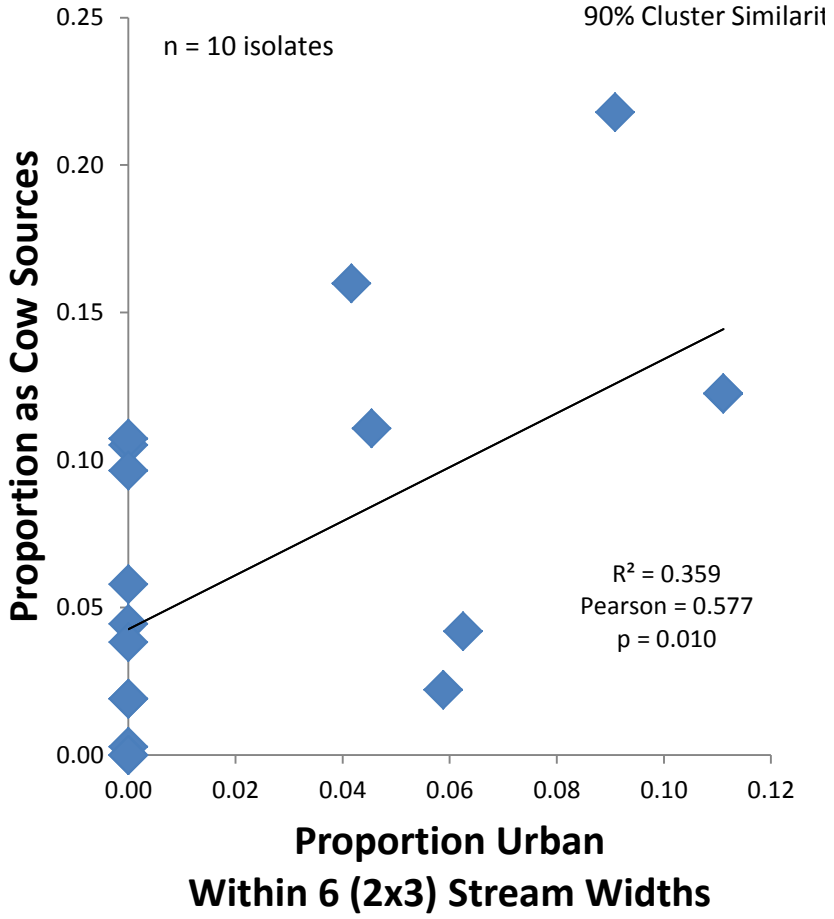
Cow and Chicken: everywhere



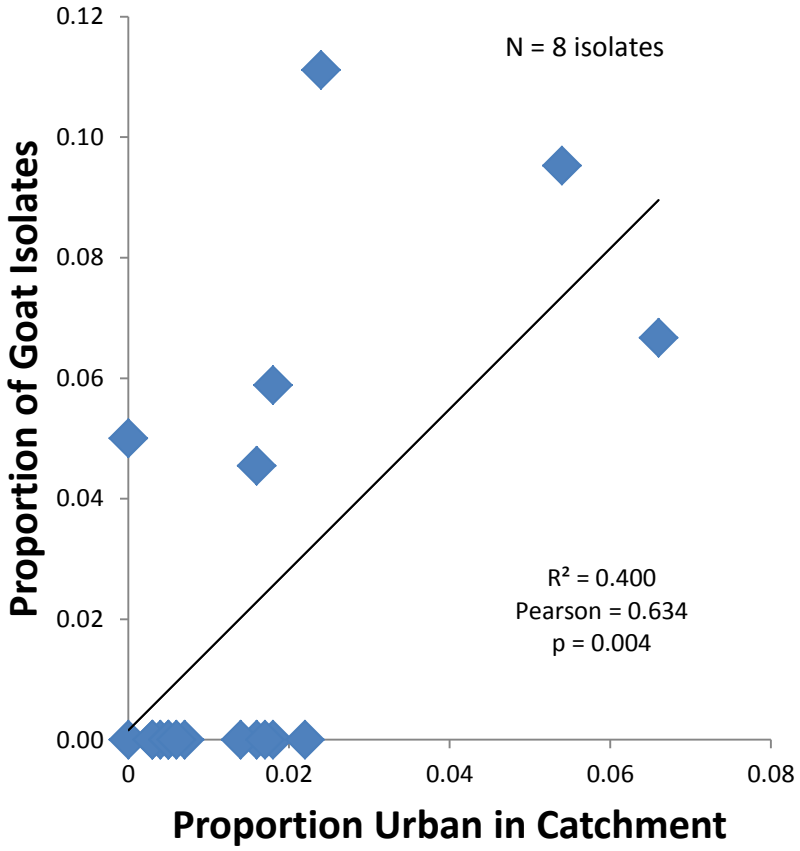
At 90% similarity threshold

Cow: more likely if riparian is urban

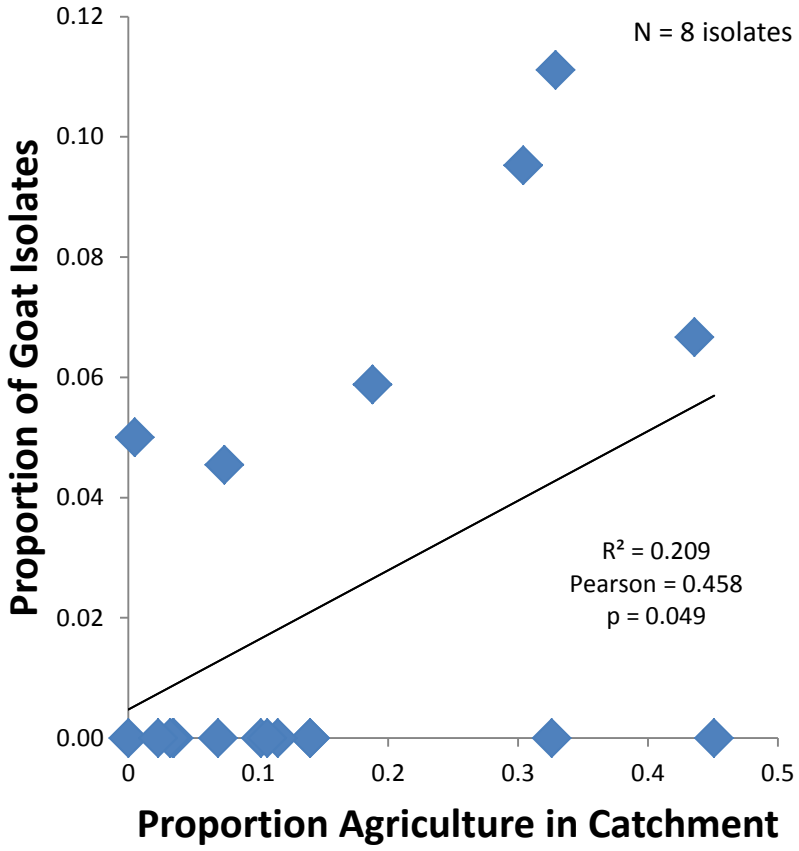




Goat: likely in urban and agricultural

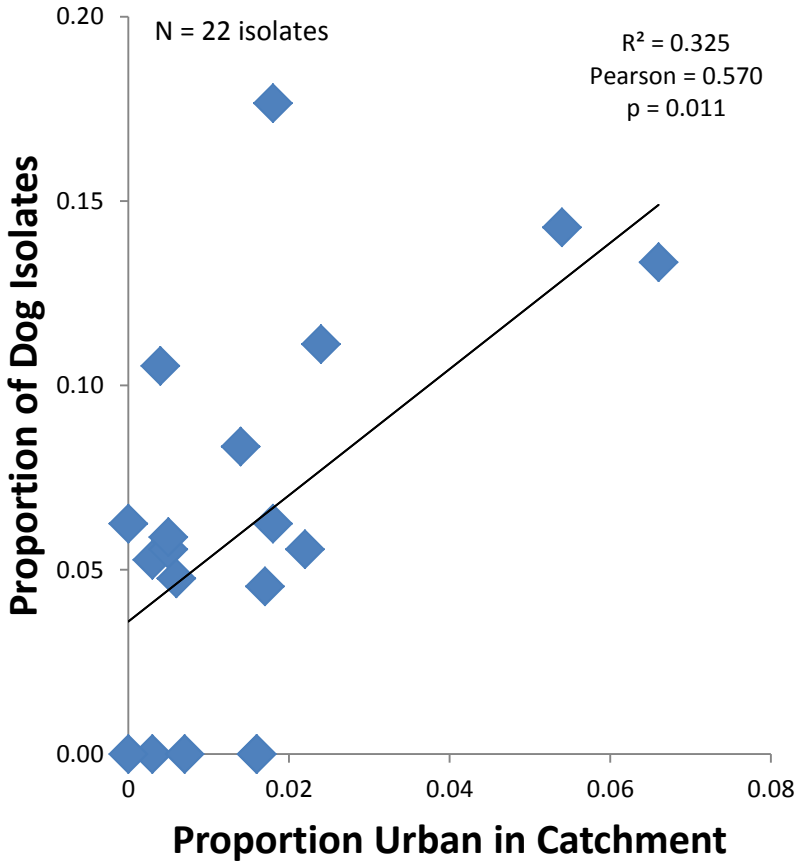


90% Cluster Similarity

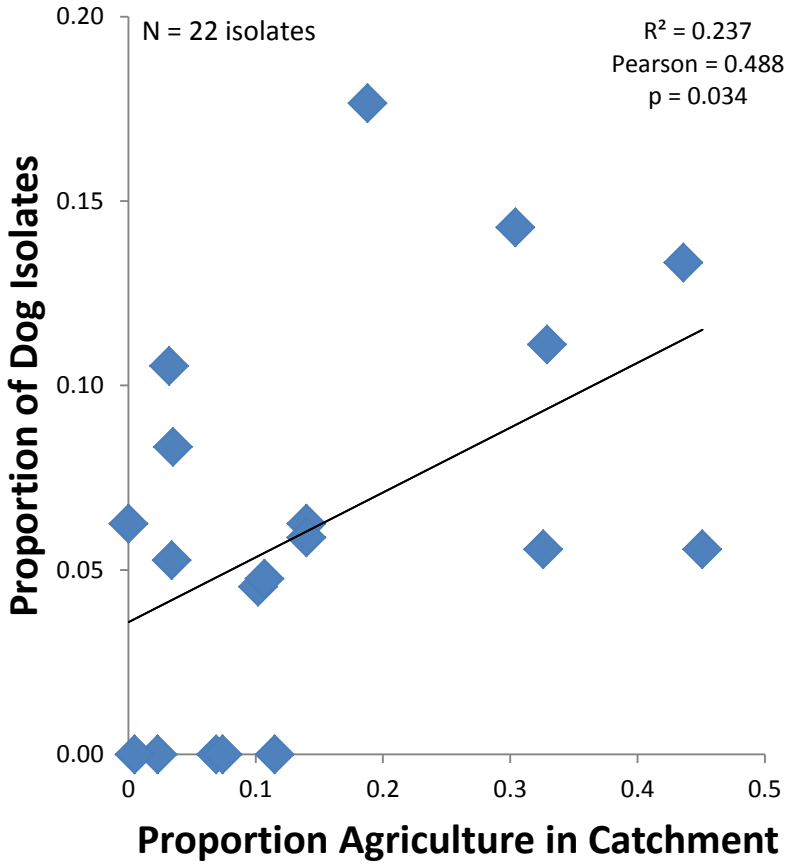


90% Cluster Similarity

Dog: likely in urban and agricultural

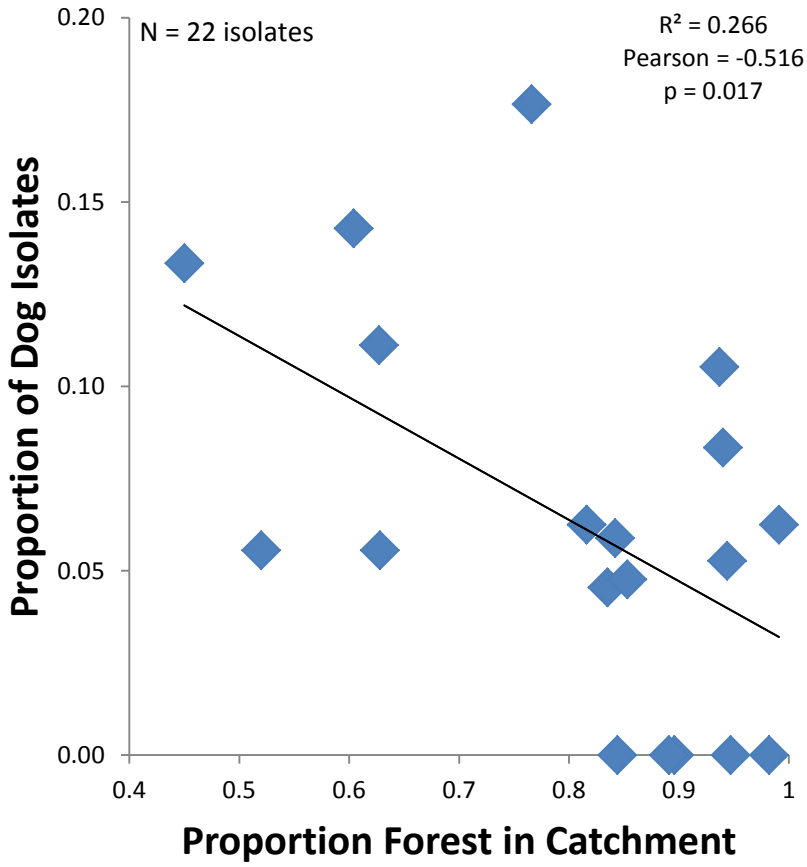


90% Cluster Similarity

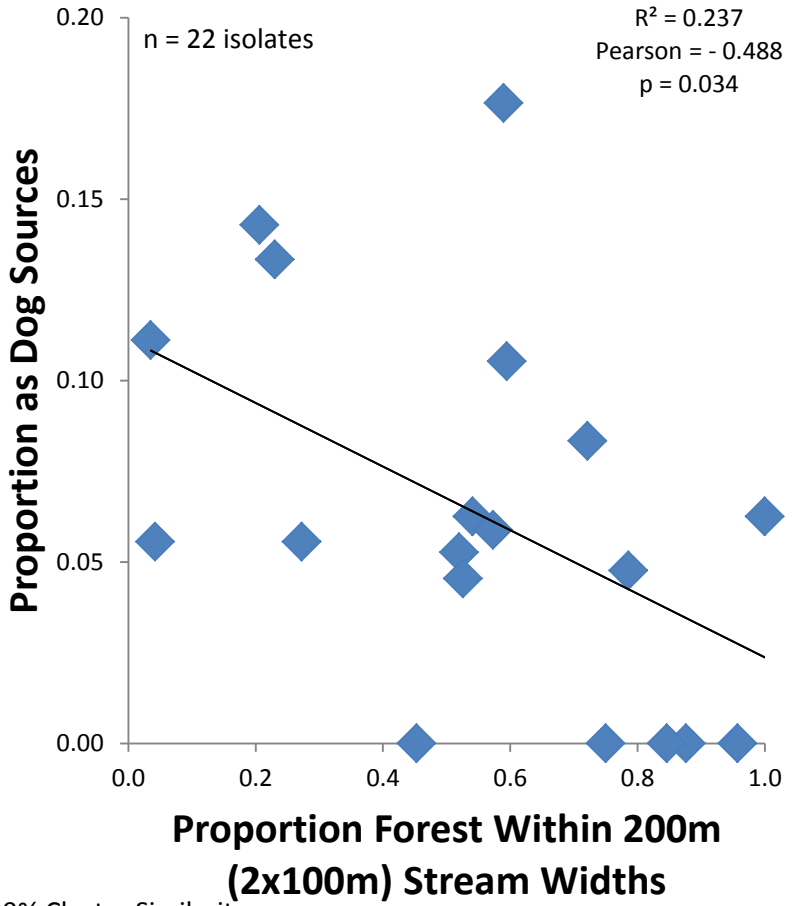


90% Cluster Similarity

Dog: less likely in forest

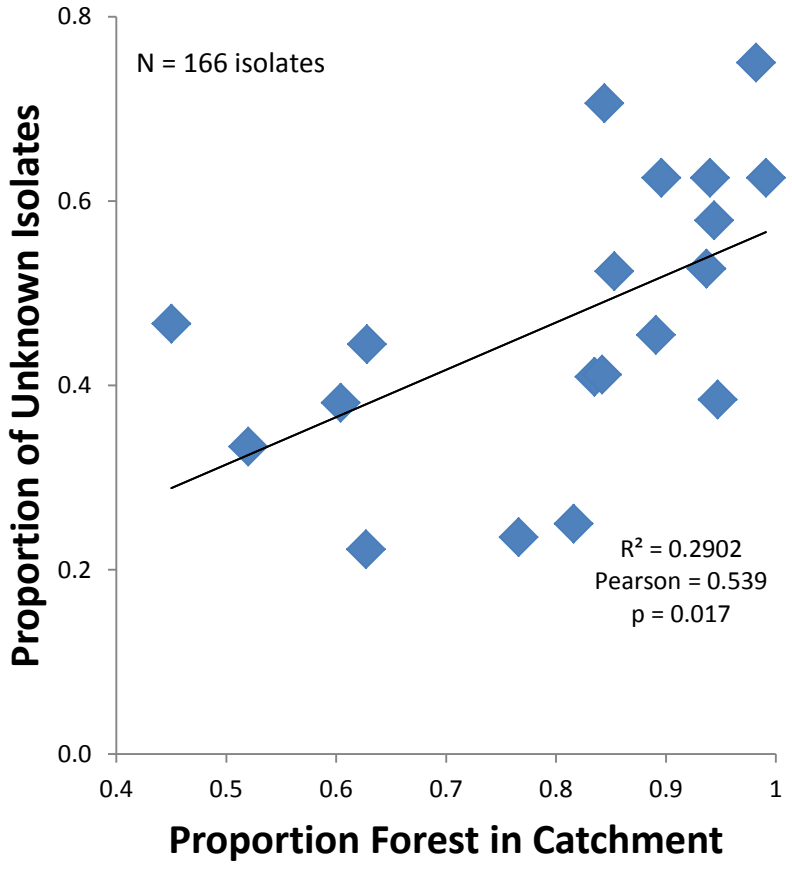


90% Cluster Similarity

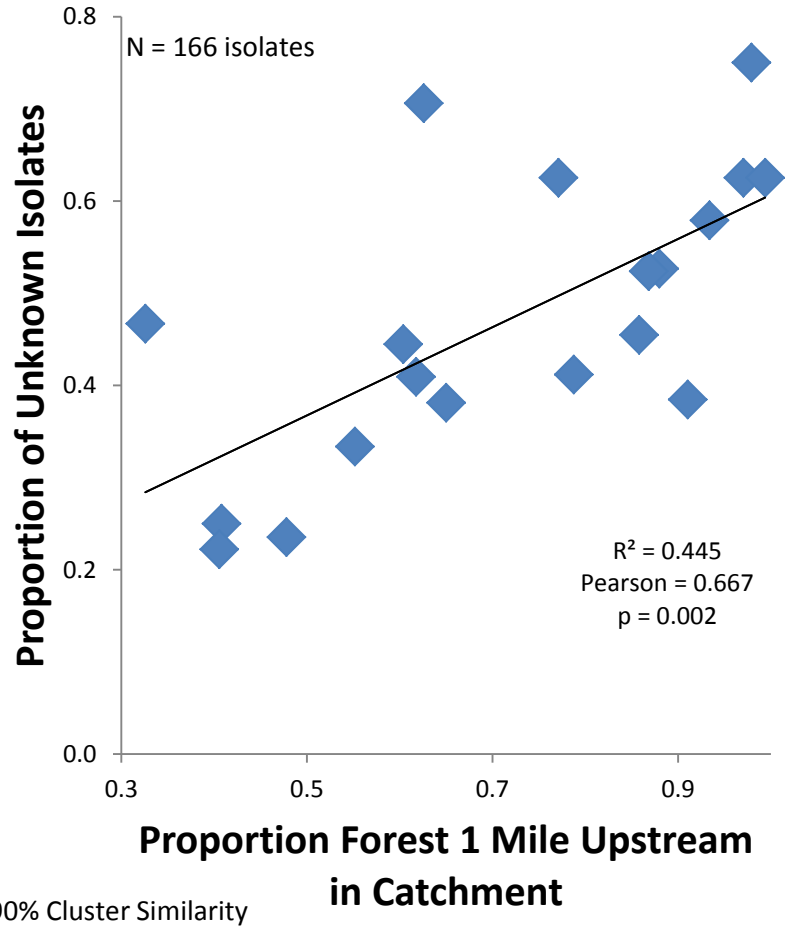


90% Cluster Similarity

Unidentified *E. coli*: forests

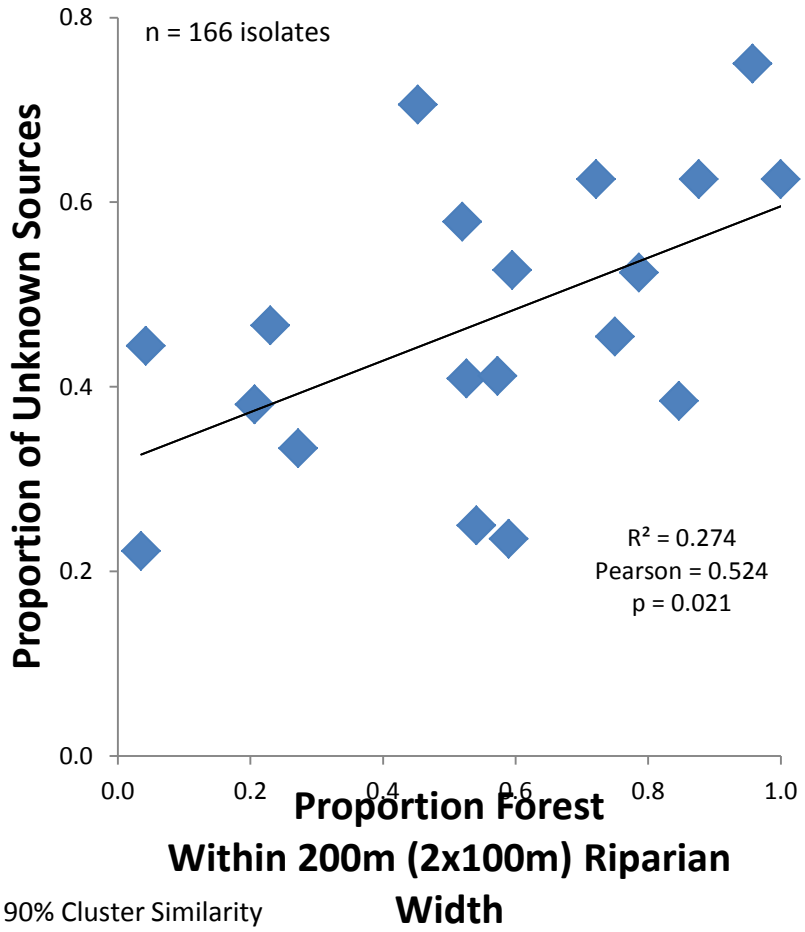


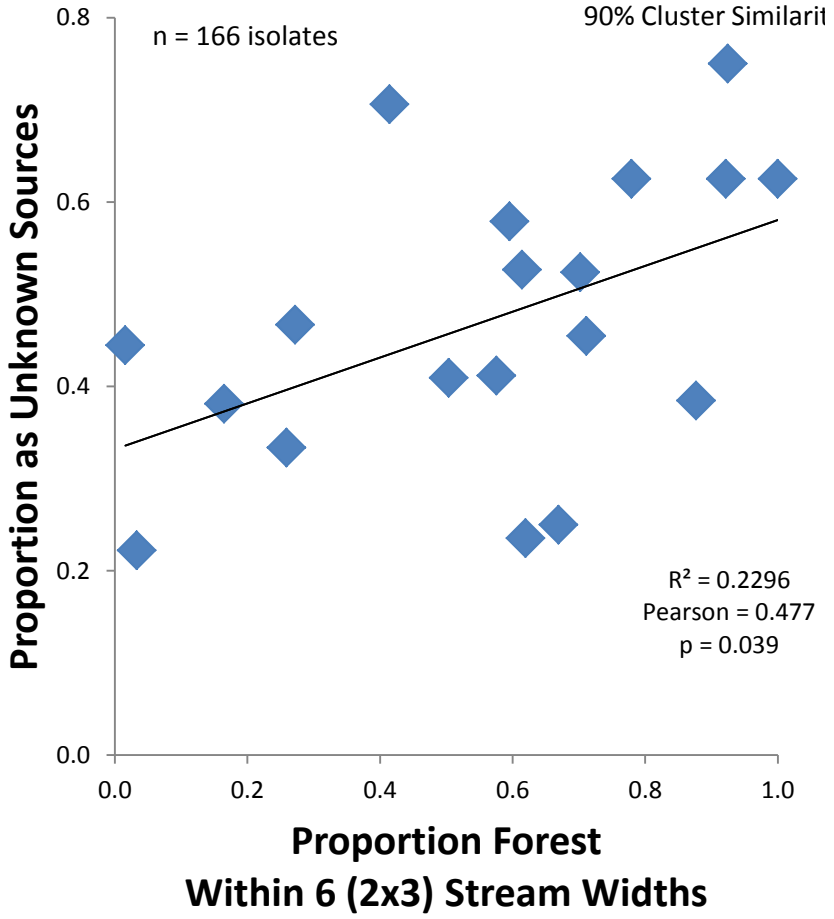
90% Cluster Similarity



90% Cluster Similarity

Unidentified *E. coli*: forests





Summary of Land-Use Classifications

- Entire Catchment
  - Dog: urban and agricultural, less in forest
  - Goat: urban and agricultural, less in forest
  - ATCC: catchment area
- Catchment 1-mile upstream
  - Unknown: forests
- Buffer 100 m each side
  - Cow: urban
  - Dog: less in forest
  - Others strongly influenced by outliers
- Buffer 3-stream widths on each side
  - Cow: urban

At 90% similarity threshold

Summary of Microbial Sources

- Chicken is common, but not related to land use
- Cow
  - Significant correlation between urban and agricultural land use
- Dog – where there are more people (urban & agriculture, less forest)
- Goat – agriculture & urban (less forest)
- Mixed sources unrelated to land use
- Human very rare
- Source species abundance rarely related to area (acres)

At 90% similarity threshold

Goals

- Increase sample size for library
  - Especially less-represented domestic species
  - Wildlife too
- Increase field isolates
  - Decrease the number of zeros in the data set
  - Increase the number of isolates at each site (> 400)

Acknowledgements

- Vermont EPSCoR
  - Judy Van Houten, Kelvin Chu
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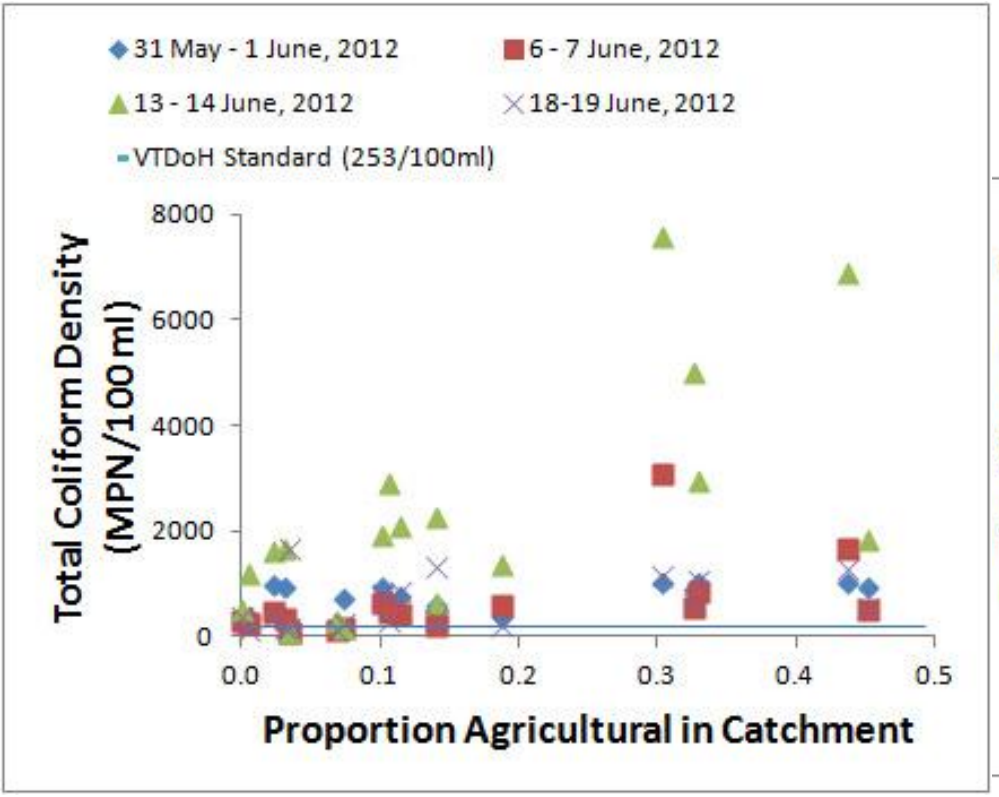
- Rebecca Richert
- John Dawkins

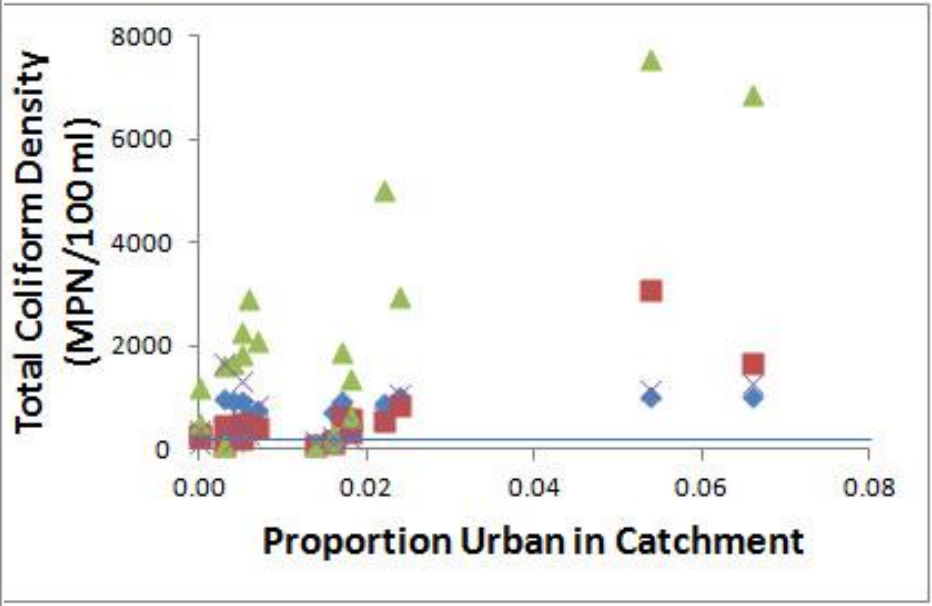
Literature Cited

- Jones, S.H. 2007. Microbial Pollution Source Tracking at New Castle Beach – A final report to the New Hampshire Department of Environmental Services, Durham, New Hampshire.
- Santo-Domingo, J, J Hansel, M Molina, R Oshiro, O C Shanks, G N Stelma, T Edge, J Griffith, V Harwood, M Jenkins, A Layton, C Nakatsu, M Sadoswky, J Stewart, D Stoeckel, B Wiggins, and J Wilbur. 2005. Microbial Source Tracking Guide Document. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/064

Total Coliform Bacteria

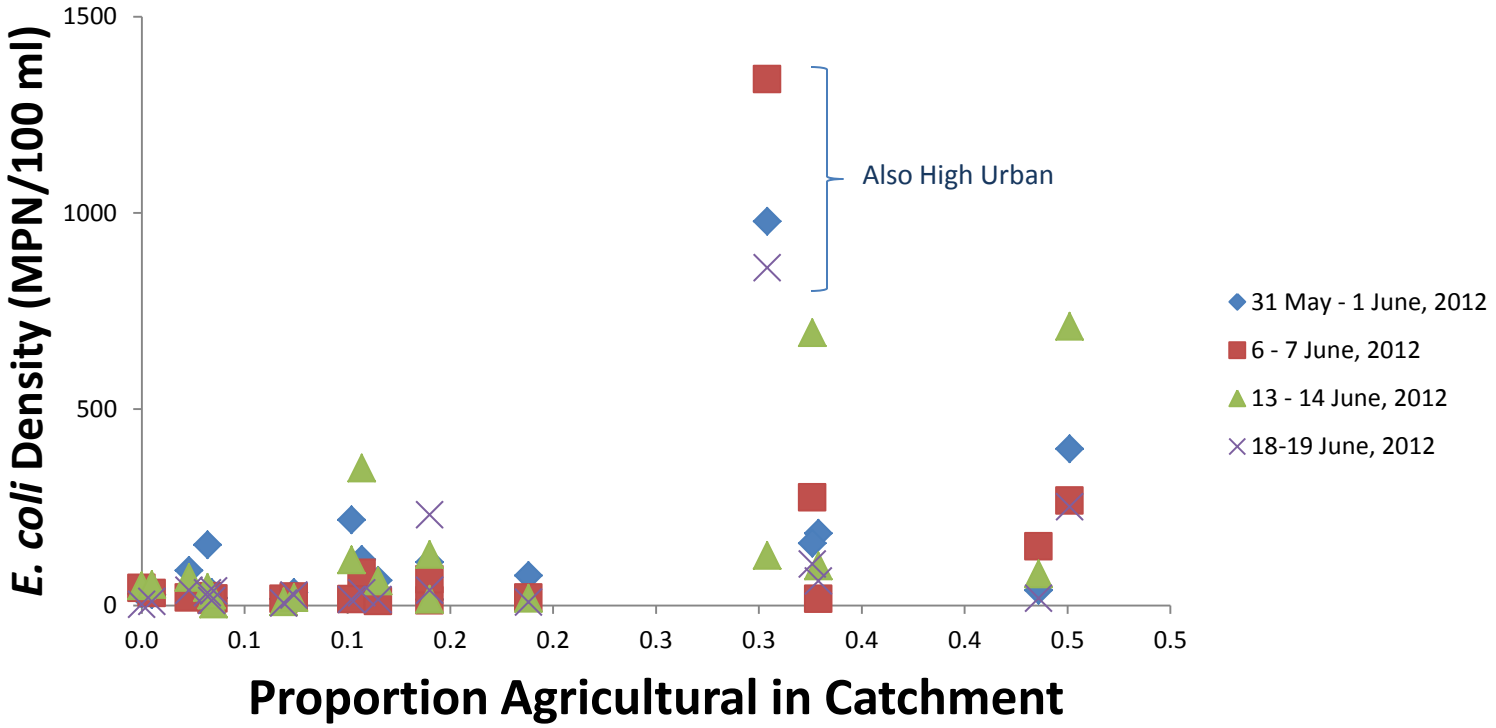
- 79 % with Total Coliform > 253 *E. coli*/100 ml
- More likely in agricultural and urban areas





E. coli

- More likely in some agricultural areas.



Mixed Source Species

Mixed Source Species	ATCC	Bear	Cow	Deer	Dog	Fisher	Goat	Goose	Human	Number of Stream *E. coli*
1	X	X	X				X	X		15
2	X		X	X		X				20
3			X			X				10
4			X		X	X	X		X	17

- Adapted to multiple environments or source species?
- Transient in overlapping habitats: ingestion & digestion of prey, feces, or other food sources?

(Jones 2007)

For 90 % cluster similarity, n > 7 (>2%) occurrences