

Annual Variation of the Sources of *Escherichia coli* in the Lamoille River Basin, Vermont

Helen Birk
Vermont EPSCoR Streams Project
Rubenstein School for the Environment
and Natural Resources '12



What is the problem?

- Among the 19% of total miles of assessed rivers and streams in the United States, 39% have been found to be impaired according to the EPA.
- Of the impaired, 35% are polluted by fecal coliforms, which are listed as the first leading factor of water quality impairment in the rivers and streams and the second leading factor in estuaries. (USEPA 2000)

Why Should You Care?

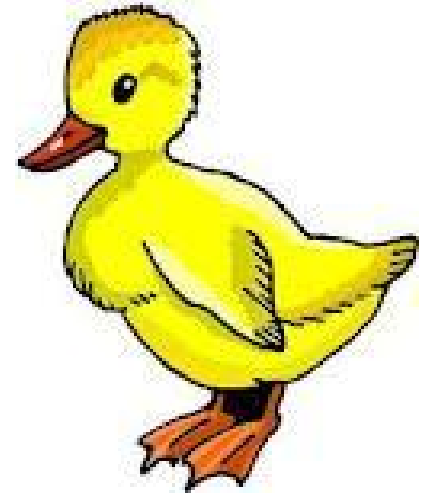
- Impaired waters are no longer swimmable or fishable until cleaned
- Such restrictions can hurt the economy and your own recreational use
- Can cause algae blooms and lead to eutrofication of water bodies
- Can even cause sickness in humans



How Can This Be Monitored?

- *Escherichia coli* is a bacterium and type of fecal coliform bacteria that is commonly found in the lower intestine of warm-blooded organisms (Feng *et. al* 2002).
- The bacterium can be transported outside of the body through feces where it can sustain in the environment for up to two weeks (USEPA 2005).
- Due to its ability to exist outside the body for a short time, *E. coli* can be used as an indicator for fecal contamination in the environment (CDC 2011).

Microbial Source Tracking

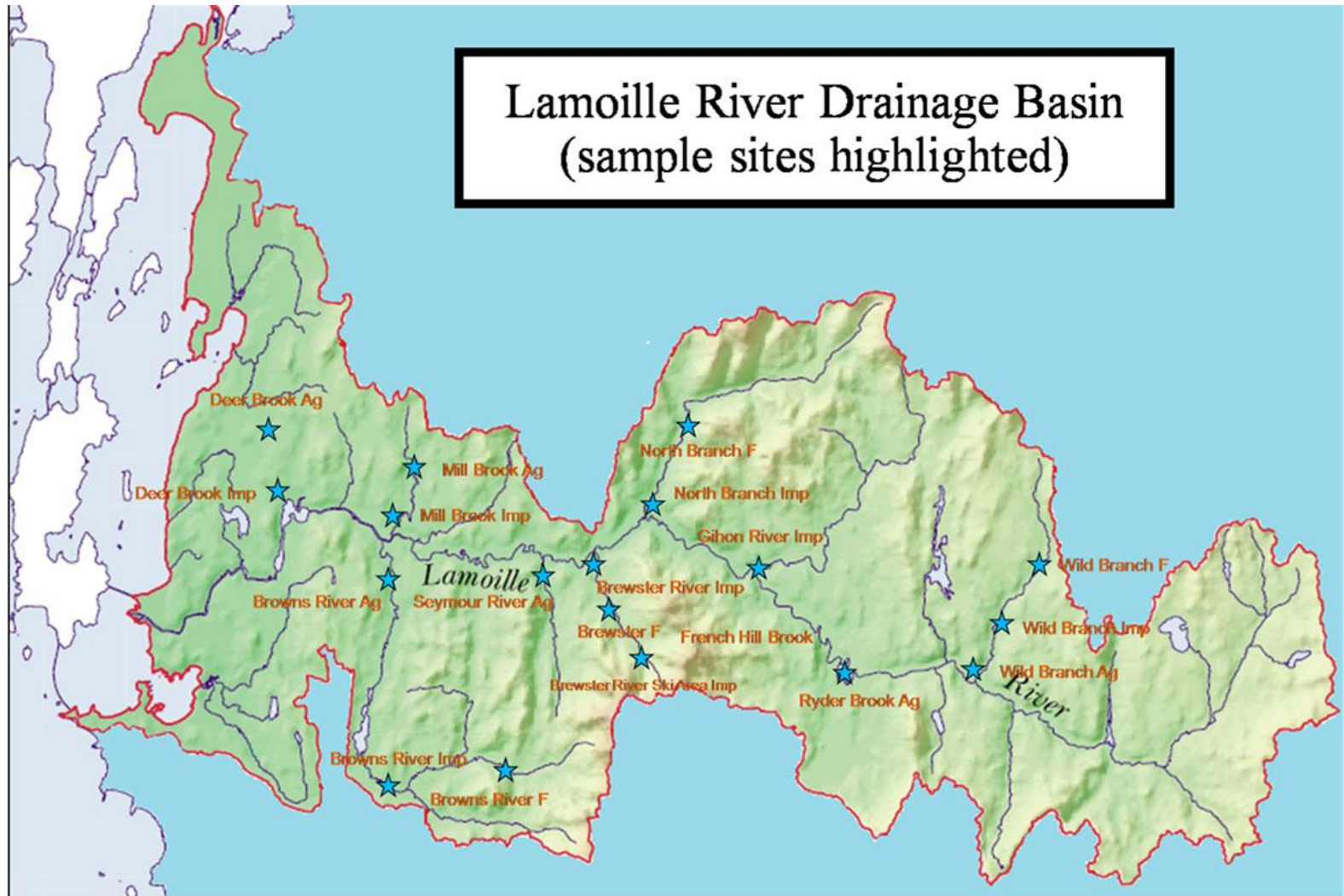


Microbial source tracking (MST) of *E. coli* can be conducted to help identify non-point sources of fecal indicator bacteria (USEPA 2005).

Question

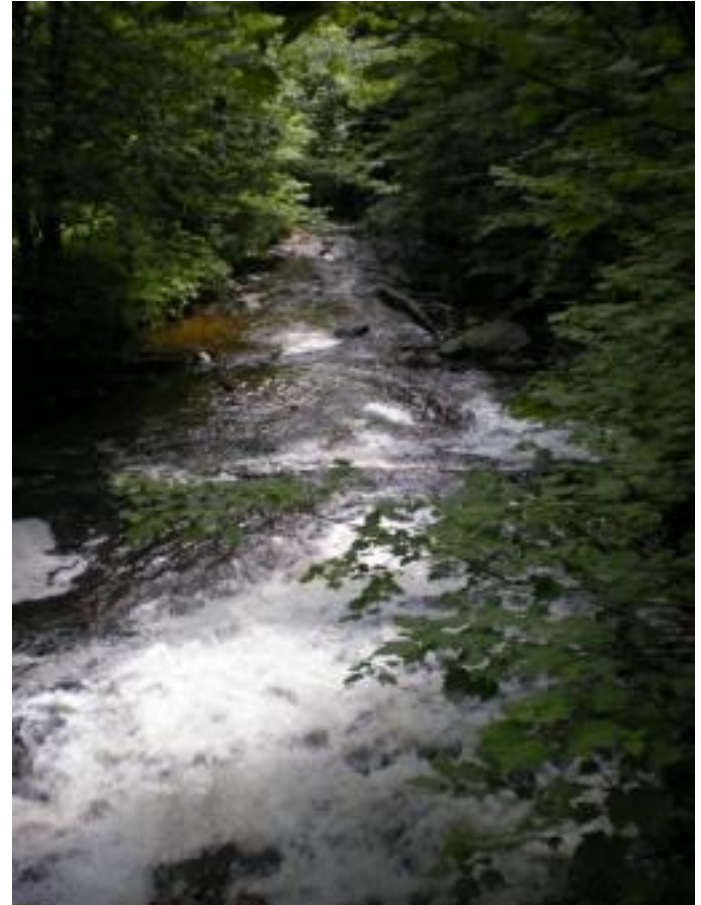
- The purpose of this study is to determine whether sources of *E. coli* in the Lamoille River Basin will change over 4 years. Water discharge will also be monitored to determine whether discharge has a significant effect on the sources of *E. coli* in the river basin.
- If sources are stable, then further investigation can be made to determine what type of *E. coli* will be present in waterways due to nearby land use and whether or not local waterways are at risk of being impaired.

Study Area



Field Methods

- 19 stream sites were surveyed throughout the Lamoille River Basin in Vermont in June from 2008 to 2011.
- Each year, stream water samples were collected in sterile Whirl-Pak® bags and brought back to the laboratory on ice to process within 6 hours.
- Discharge was measured using an acoustic doppler SonTek®/YSI (Sand Diego, CA) FlowTracker using the Vermont EPSCoR mid stream method.

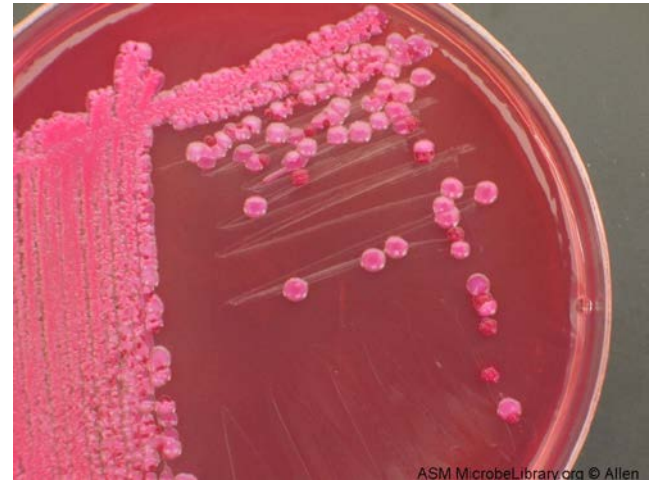


French Hill Study Site

Lab Methods

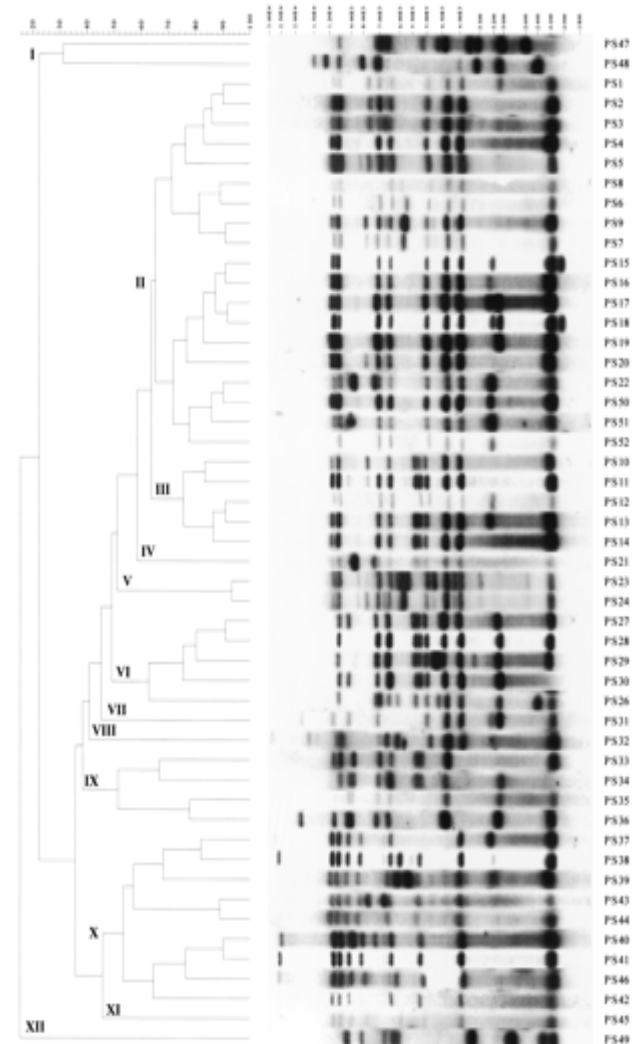
Microbial Source Tracking (MST) method (USEPA 2005):

- Filter samples onto a 0.45 μ m membrane filter
- Grow bacterium on MI media
- Confirm *E. coli* identifications on MacConkey agar and with Enterotube II



Ribotyping and Identification

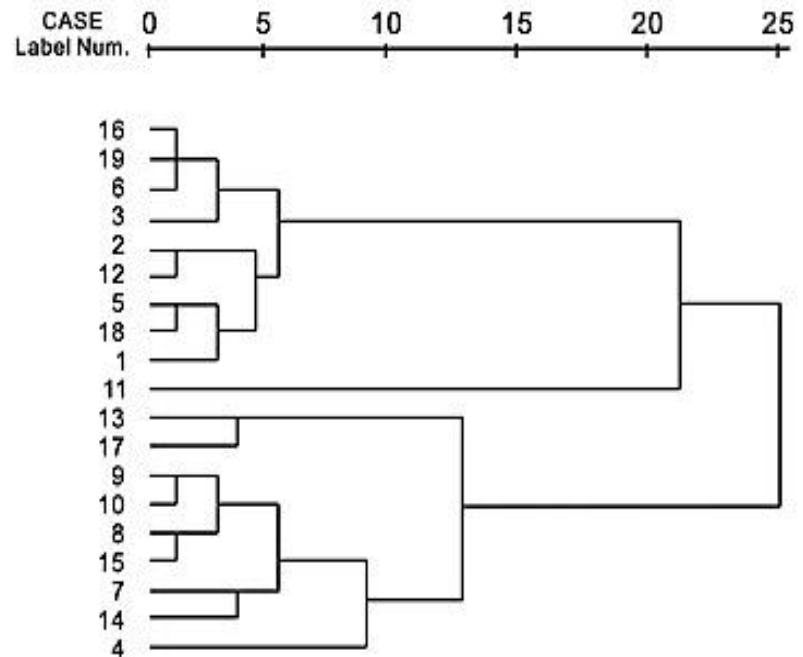
- After confirming *E. coli* identifications, a RiboPrinter was used to generate genetic bar codes based on RFLP analysis of the 16S section of mitochondrial DNA
- Genetic bar codes were then matched with existing library to find sources of *E. coli*



Evaluating Similarity

A Cluster Analysis was used to determine source species for isolates:

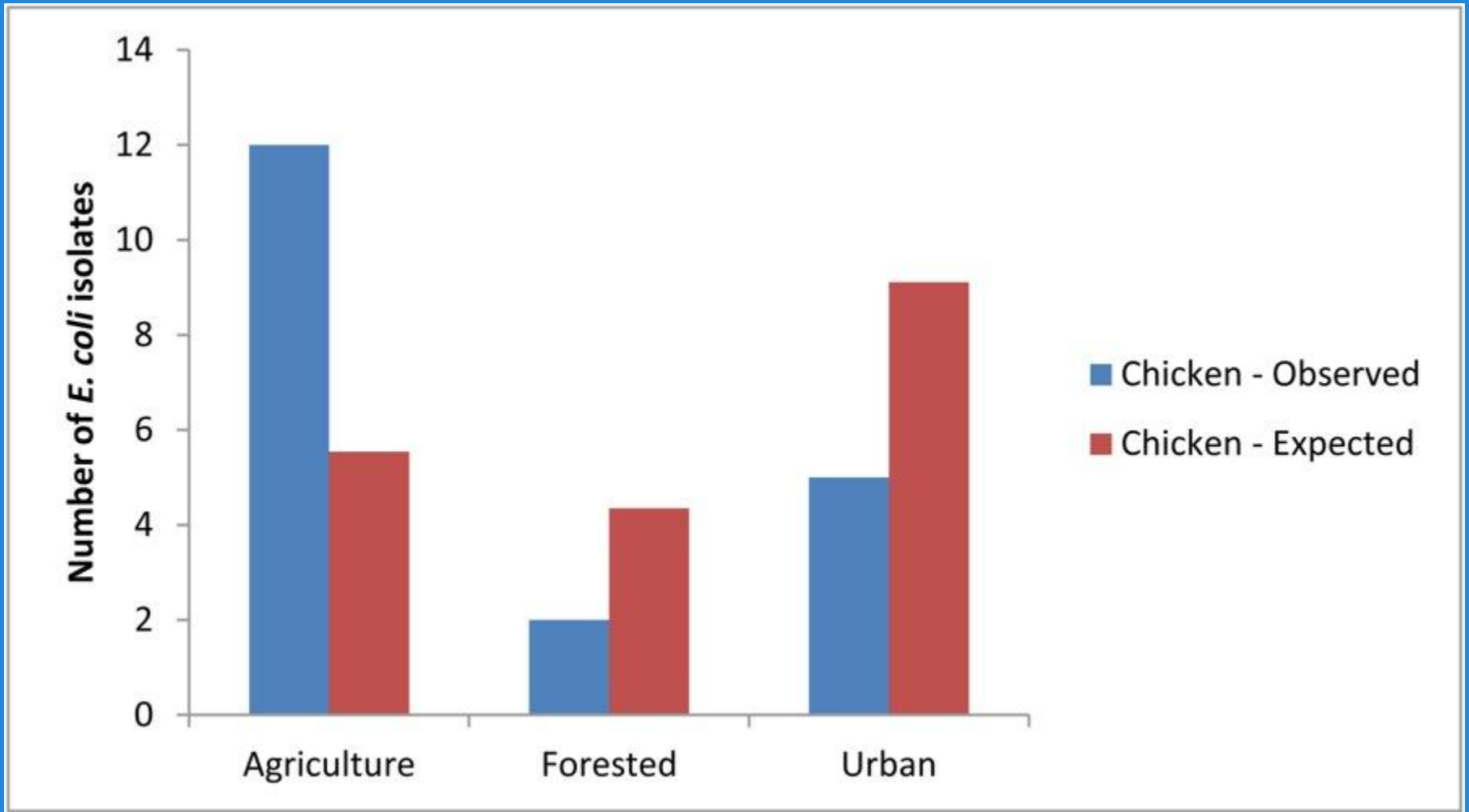
- 80% cut off was used for 80% similarity
- 90% cut off was used for 90% similarity



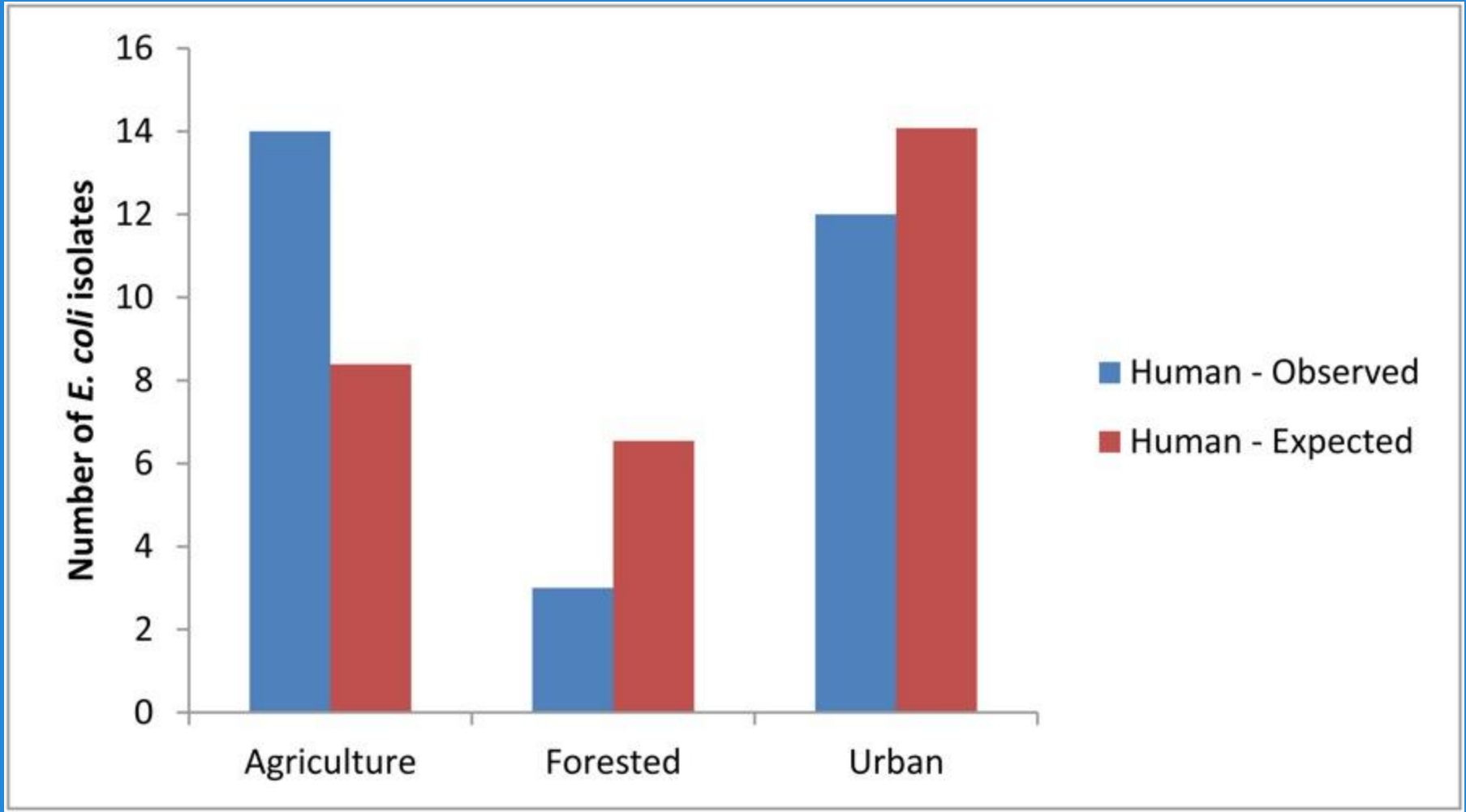
Source of E. coli	Significant Difference in Annual Variation*	Percent Gene Similarity	Suspect Years
Chicken	No Significant Difference	90%	
Cow, Deer, Human	No Significant Difference	90%	
Dog	No Significant Difference	90%	
Human	Significant Difference	90%	2010
Bear, Cow, Deer, Goat, Goose, Horse, Human	Significant Difference	80%	2008 and 2009
Chicken, Cow, Dog, Goat, Horse, Human, Pig	No Significant Difference	80%	
Cow, Deer, Dog, Goat, Human, Llama	Significant Difference	80%	2009 and 2011

Table 1: Annual Variation of Source Species of *E. coli* in the Lamollie River Basin, VT

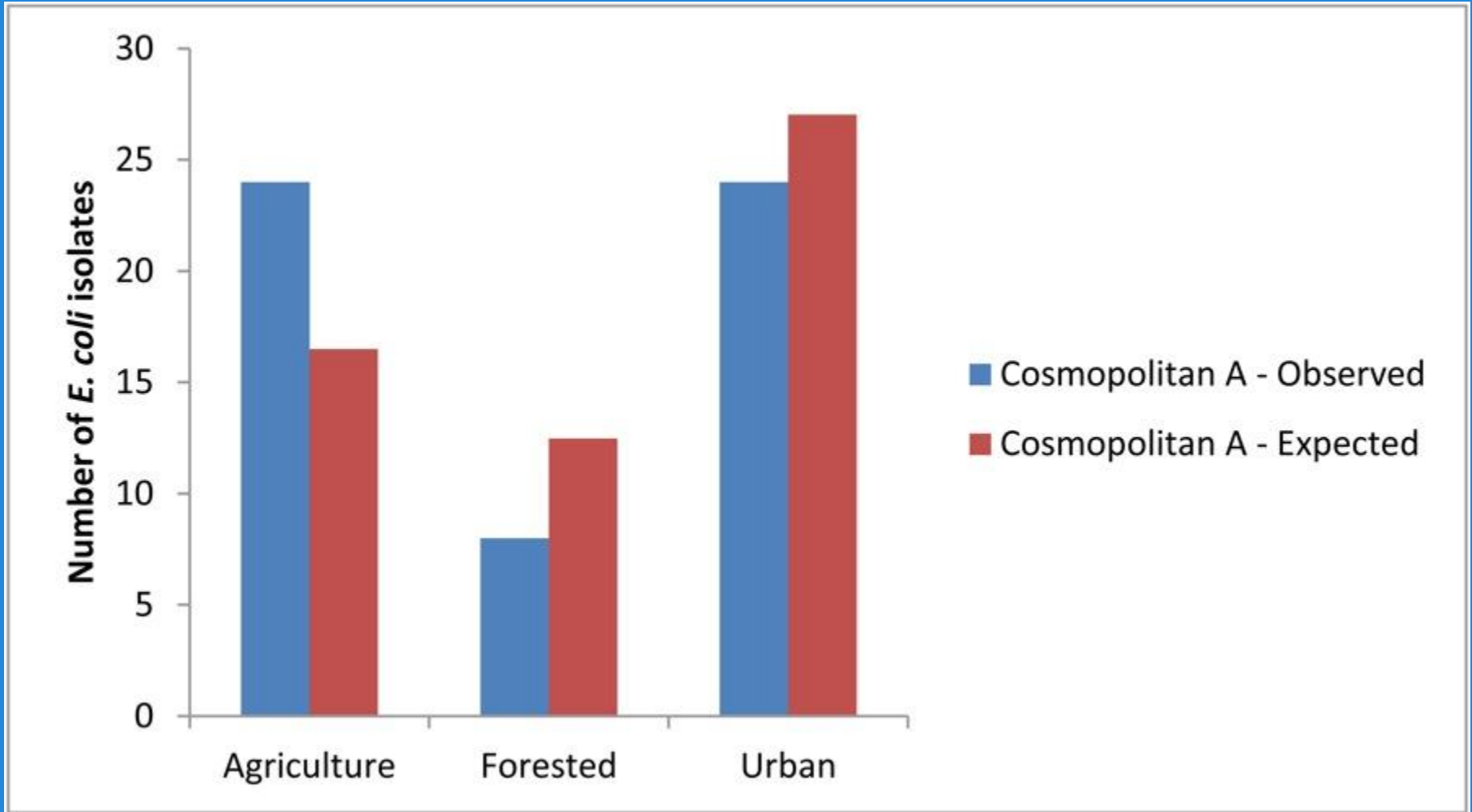
* Significance was determined using a .05 alpha level and a Chi-Squared Test of Independence.



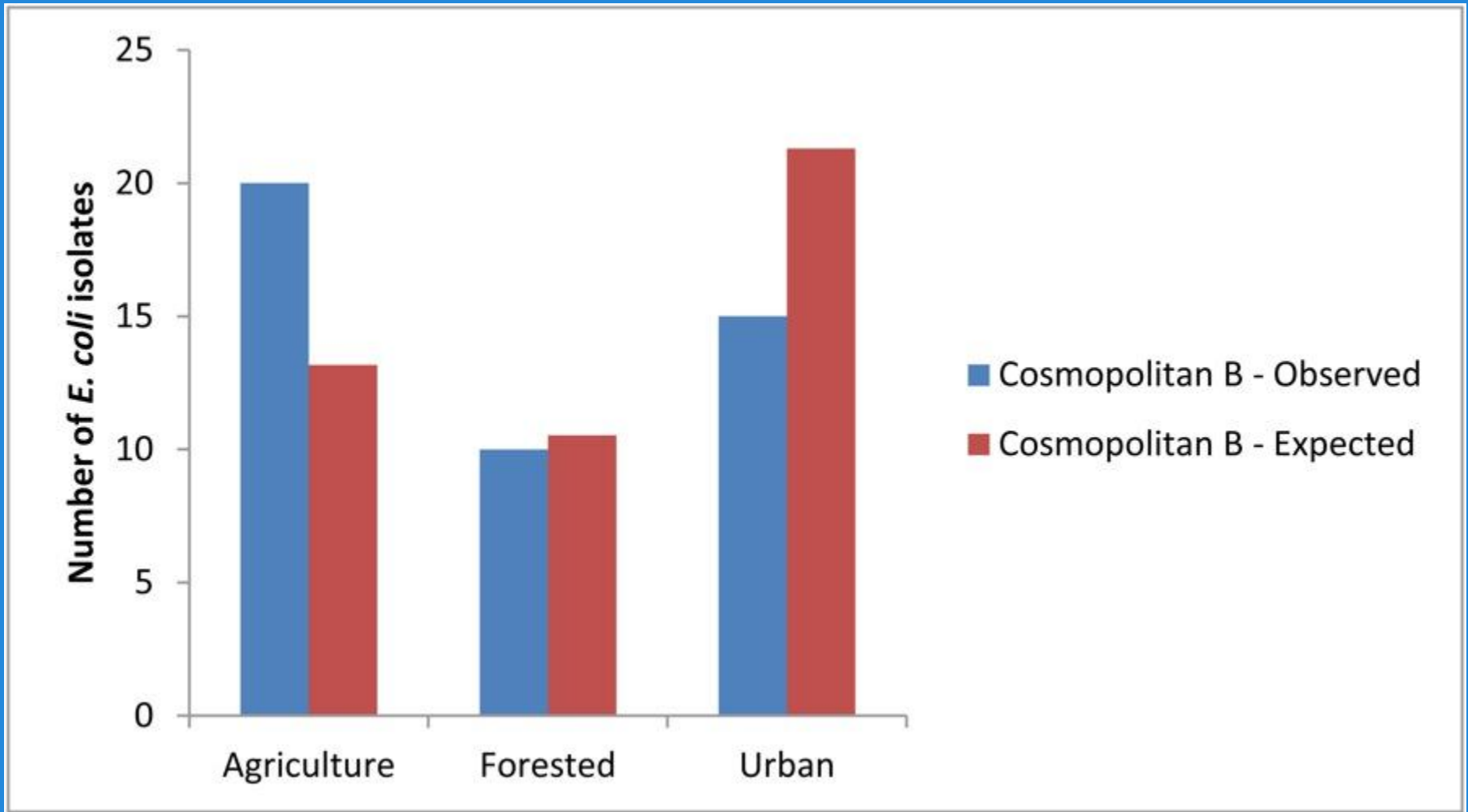
Chicken *E. coli* was found to be significantly higher than expected in agricultural areas



Human *E. coli* was found to be significantly higher than expected in agricultural areas



Cosmopolitan A (Chicken, Cow, Dog, Goat, Horse, Human, Pig) was found to be significantly higher than expected in agricultural areas



Cosmopolitan B (Cow, Deer, Dog, Goat, Human, Llama) was found to be significantly higher than expected in agricultural areas and the same in forested

Acknowledgements

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Works Cited

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