

Bacterial Source Tracking

Plum Creek

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Where did the Bacteria (*E. coli*) Come From?

- **Potential sources**
 - **Humans**
 - **Domesticated animals**
 - **Wildlife**
- **Methods for determining sources**
 - **Source survey**
 - **Modeling**
 - **Bacterial source tracking**

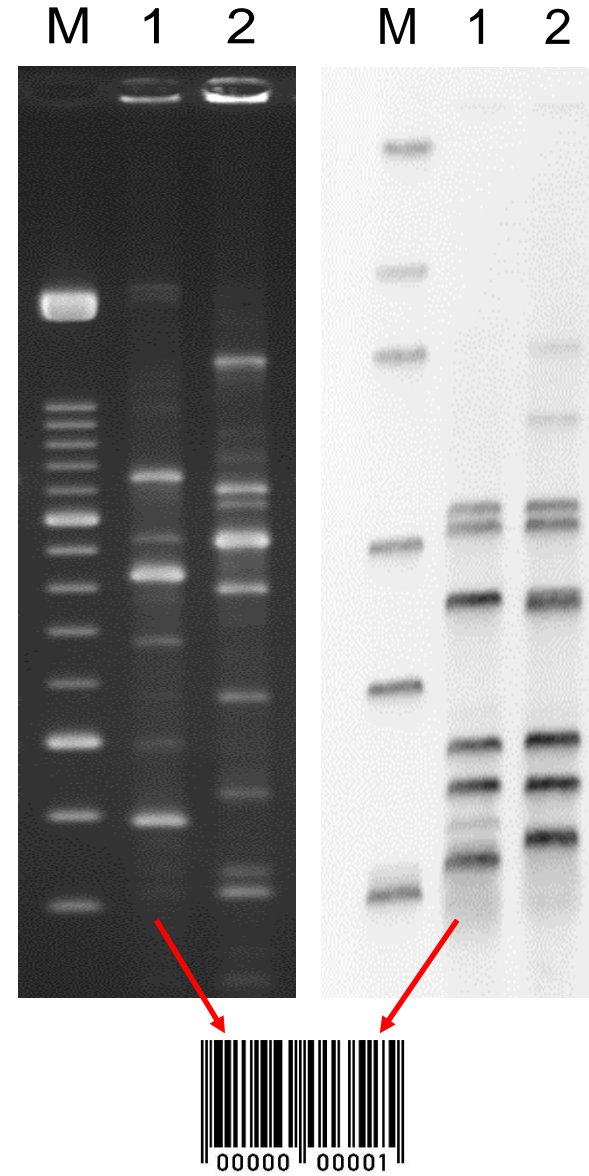
Library-Dependent BST Methods

Methods:

- DNA fingerprinting
 - Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)
 - RiboPrinting® (RP)

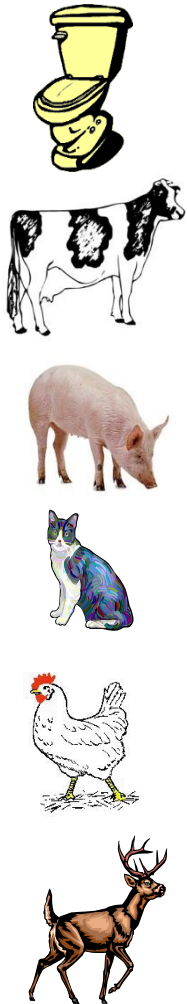
Advantages/Disadvantages:

- More discriminating
- Allows ranking of sources
- More expensive

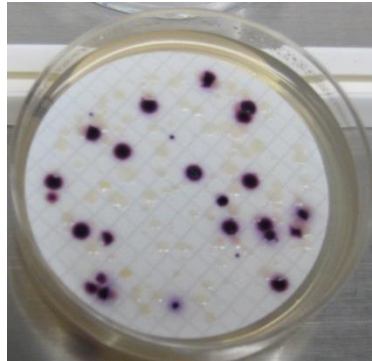


Development of Texas *E. coli* BST Library

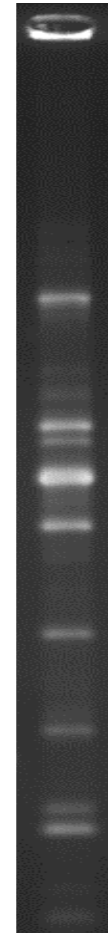
Sources



Isolate
→
E. coli



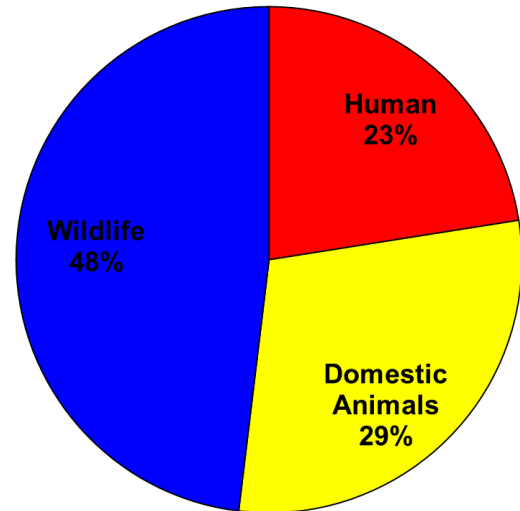
DNA
→
Fingerprint



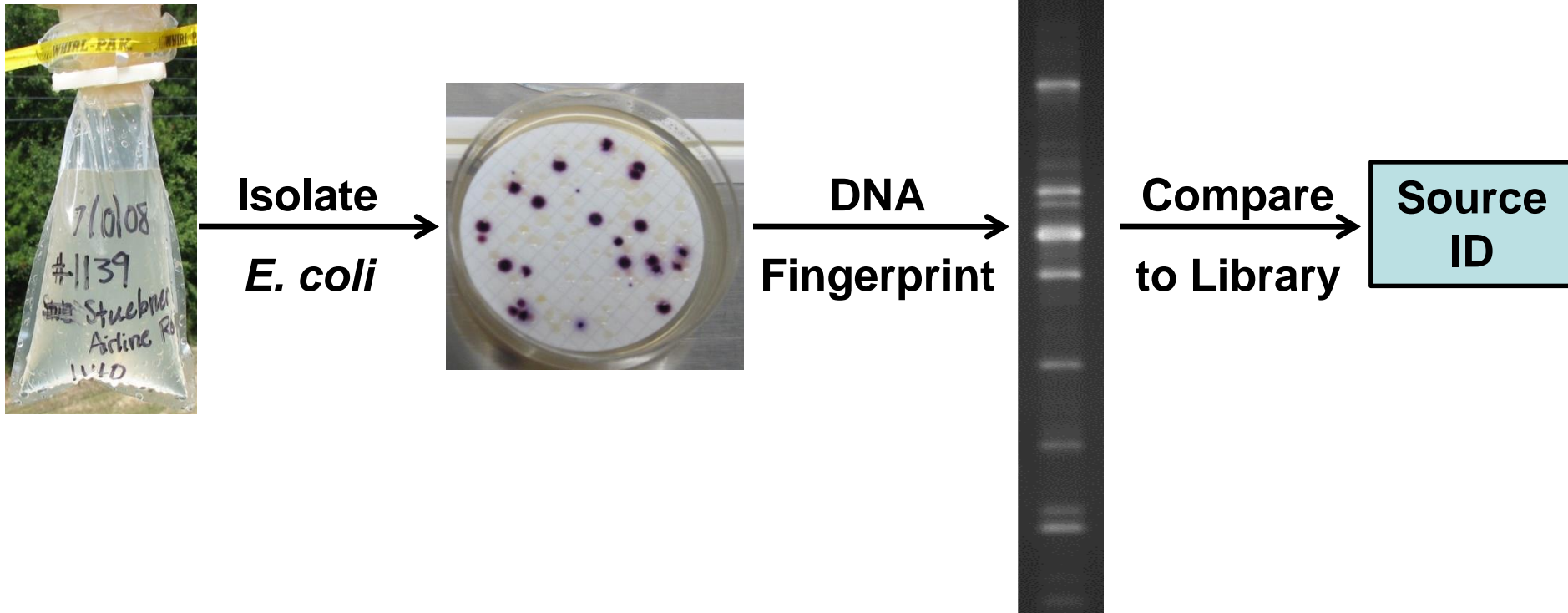
Add to
→
Library

Texas *E. coli* BST Library (v. 12-17)

- Contains 1,853 *E. coli* isolates from 1,612 different human and animal samples
- Developed by collecting over 4,000 domestic sewage, wildlife, livestock, and pet fecal samples and screening over 7,000 isolates for clones and host specificity
- Samples from 20 watersheds across Texas for BST including:
 - Plum Creek
 - San Antonio
 - Lake Granbury
 - Oyster Creek / Trinity River
 - Waco / Belton Lake
 - Little Brazos River Tributaries
 - Attoyac Bayou
- Additional isolates being added from ongoing and future BST projects in other areas of Texas



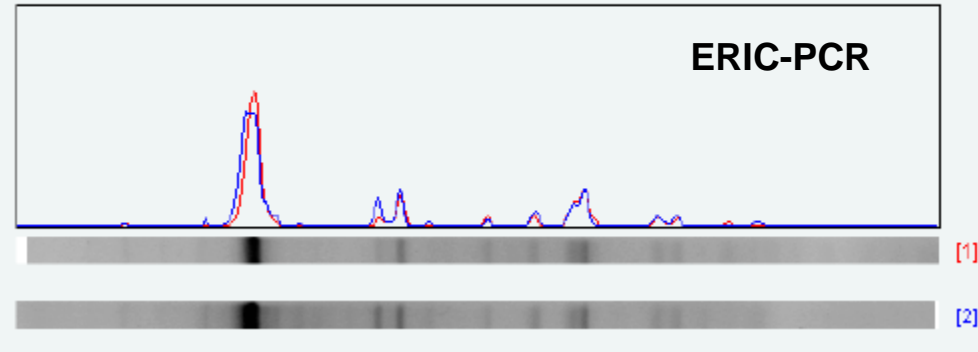
Use of Texas *E. coli* BST Library for Identifying Water Isolates



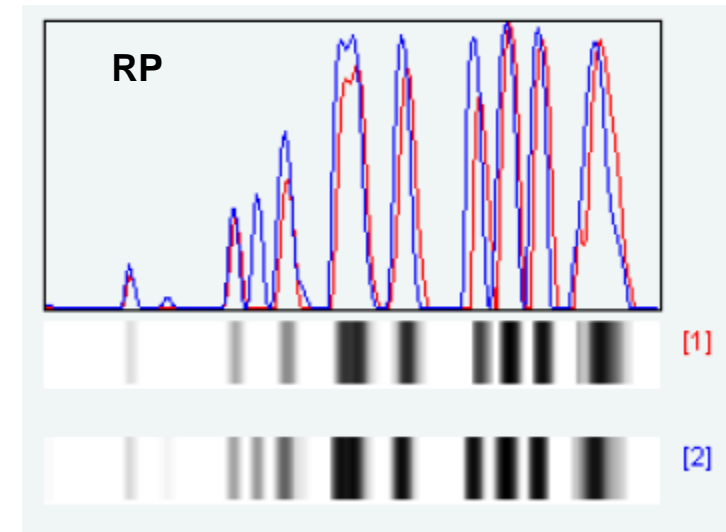
Comparison to Texas *E. coli* BST Library

- Best match approach with 80% minimum similarity cutoff based on laboratory QC data
 - Water isolate must match library isolate $\geq 80\%$ similarity or it is considered unidentified
 - Identification to single library isolate with highest similarity – max similarity approach

Similarity: 96.94%

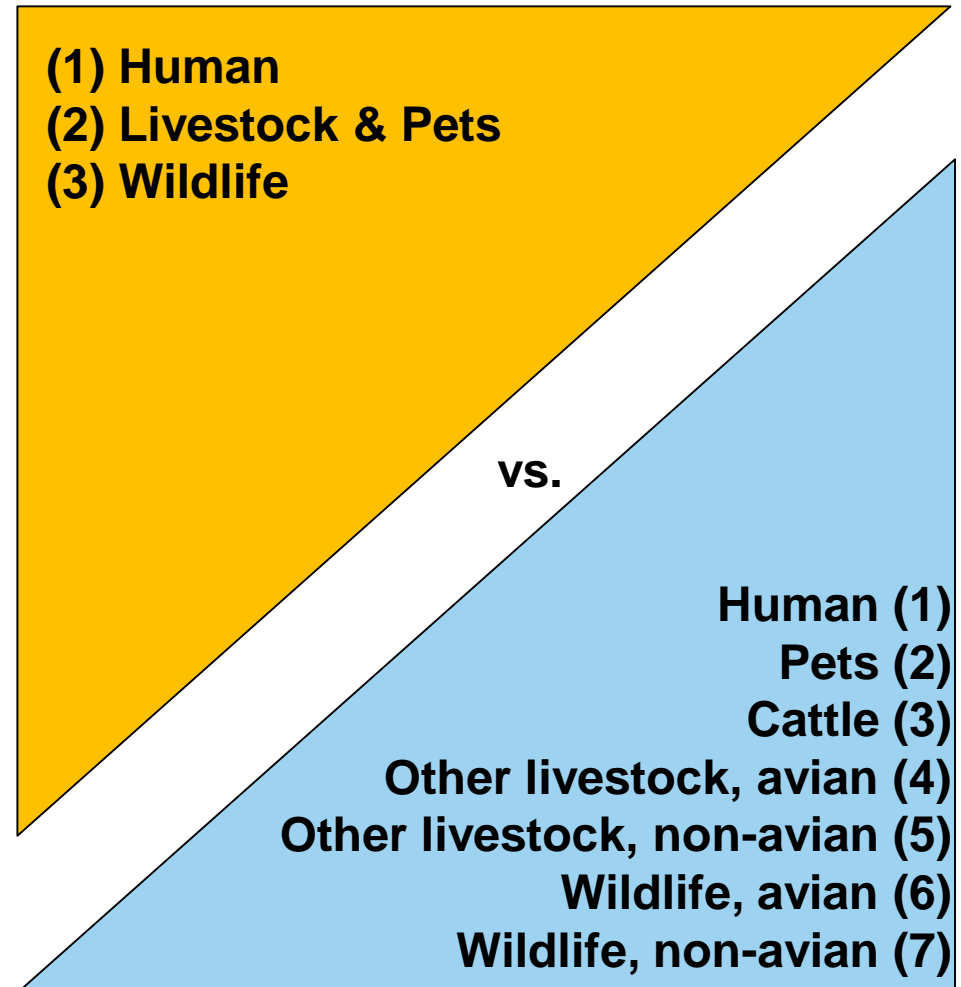


Similarity: 95.82%



Three-way v. Seven-way Split of Results

- **Using the results**
 - Is it from human sources?
 - Is it from livestock?
 - Is it from wildlife?
- **Biology**
 - Large variety of wildlife
 - Cosmopolitan strains
 - Geographical and temporal differences
- **Statistics**
 - Number of isolates collected
 - May only use three-way split for limited studies



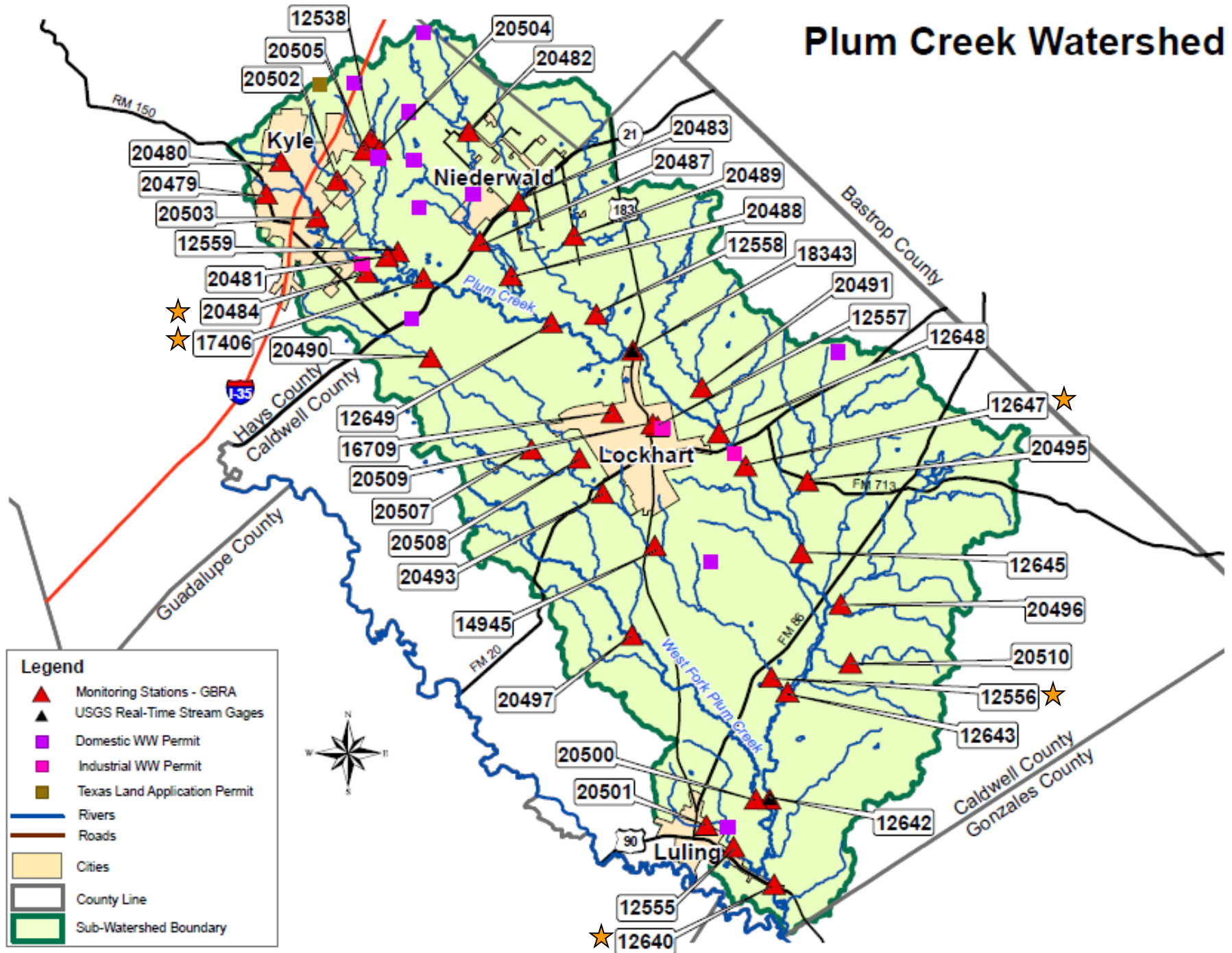
Texas *E. coli* BST Library Composition & Rates of Correct Classification (RCC)

Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio***	Left Unidentified (unique patterns)
HUMAN	417	351	23%	100	4.3	22%
DOMESTIC ANIMALS	545	500	29%	100	3.4	19%
Pets	83	74	4%	84	21.0	41%
Cattle	244	225	13%	94	7.2	11%
Avian Livestock	96	84	5%	89	17.8	27%
Other Non-Avian Livestock	122	117	7%	90	12.8	15%
WILDLIFE	891	761	48%	100	2.1	16%
Avian Wildlife	272	250	15%	79	5.3	18%
Non-Avian Wildlife	619	511	33%	91	2.8	15%
Overall	1853	1612		ARCC** = 100% (3-way) 91% (7-way)		18%

BST for Plum Creek

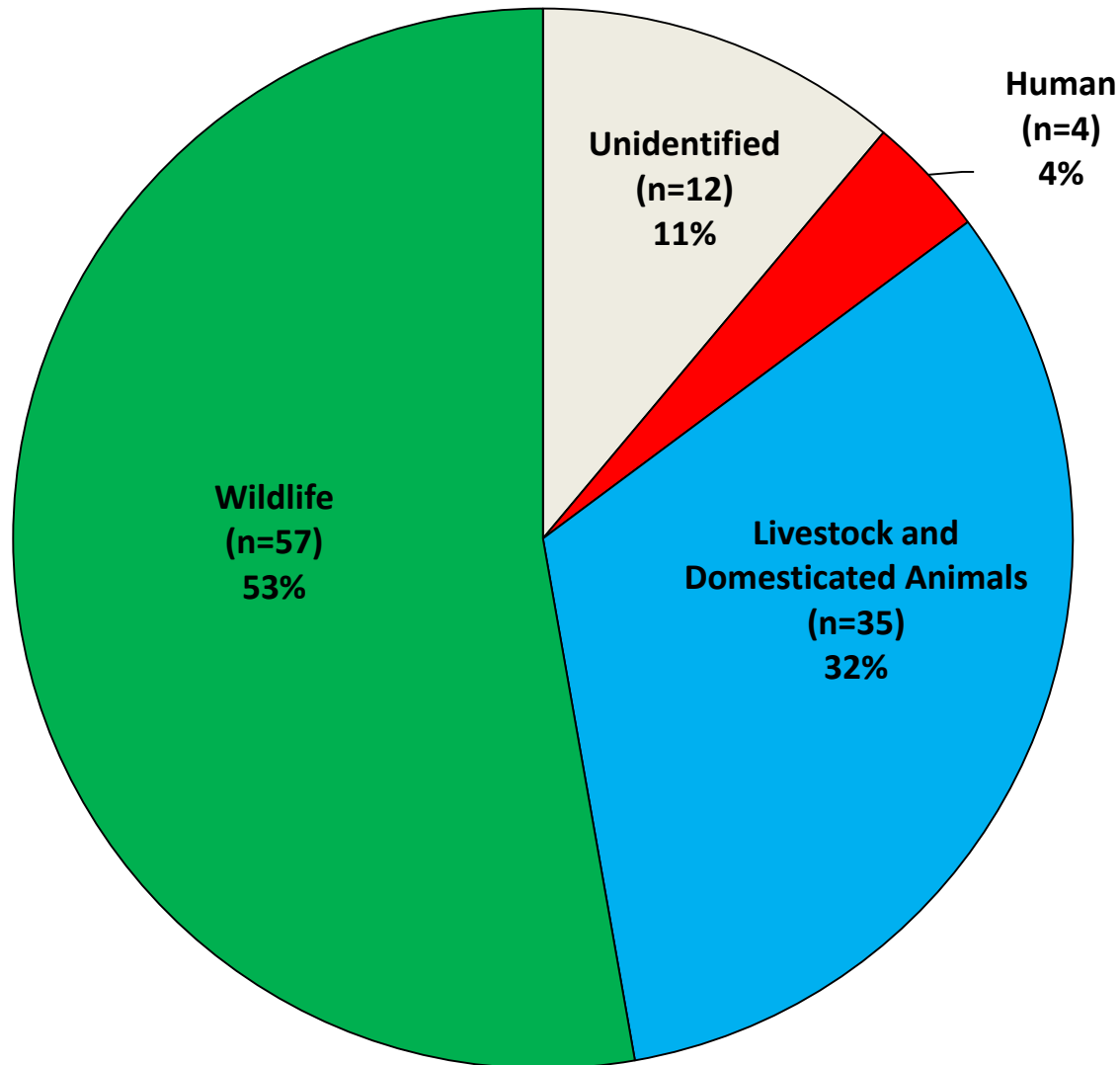
- **Addition of Known-Source *E. coli* Isolates**
 - **Isolated and DNA fingerprinted 76 *E. coli* from Plum Creek fecal/wastewater samples for addition to the Texas *E. coli* BST Library**
 - **Wastewater, poultry, cattle, wildlife, feral hogs, etc.**
- **Characterization of Water *E. coli* Isolates**
 - **Isolated *E. coli* from water samples collected monthly at five sites over one year (60 samples)**
 - **DNA fingerprinted 108 *E. coli* isolates and compared to Texas *E. coli* BST Library for source identification**

Plum Creek Watershed



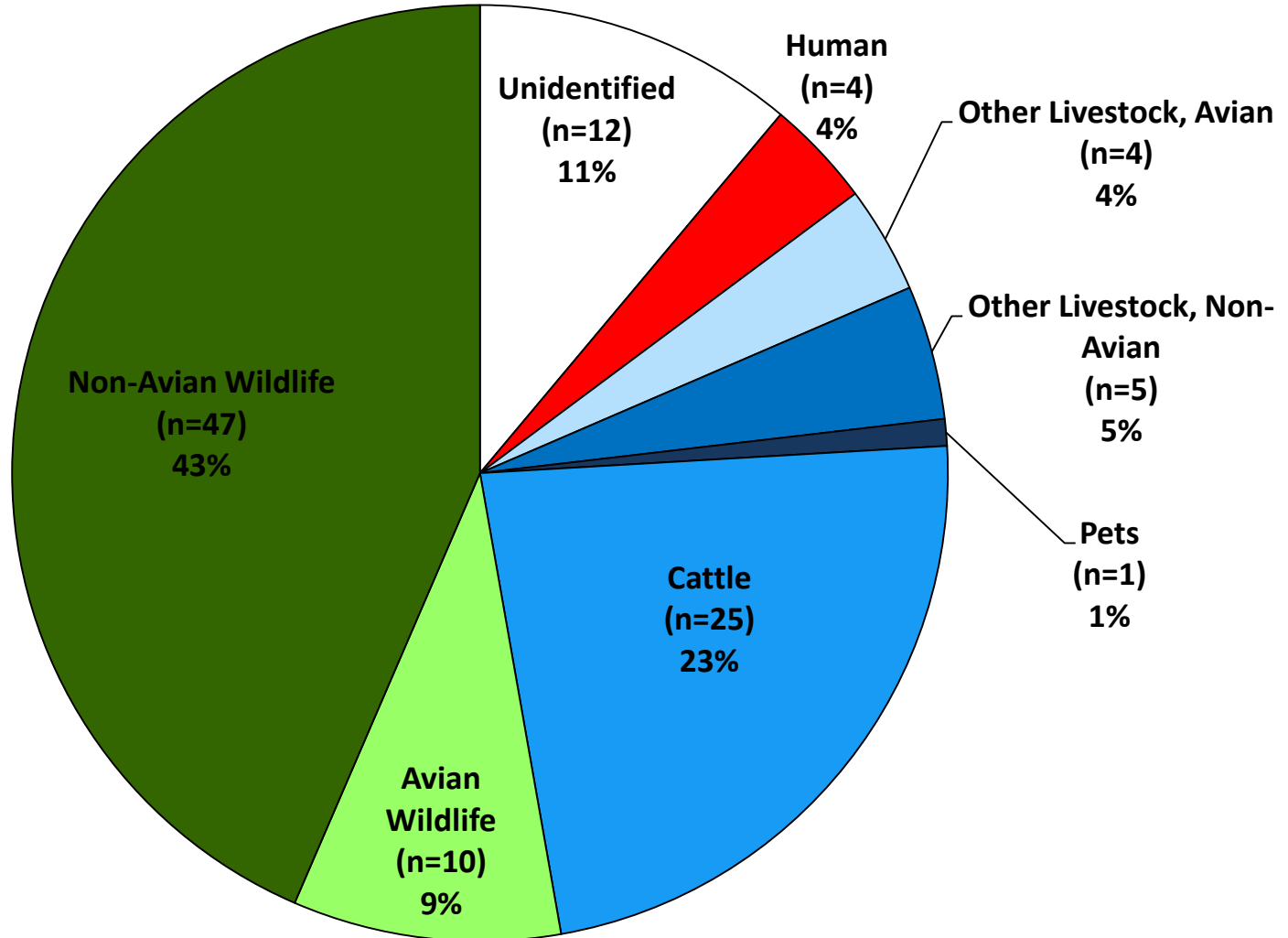
E. coli BST Results

3-Way Split



E. coli BST Results

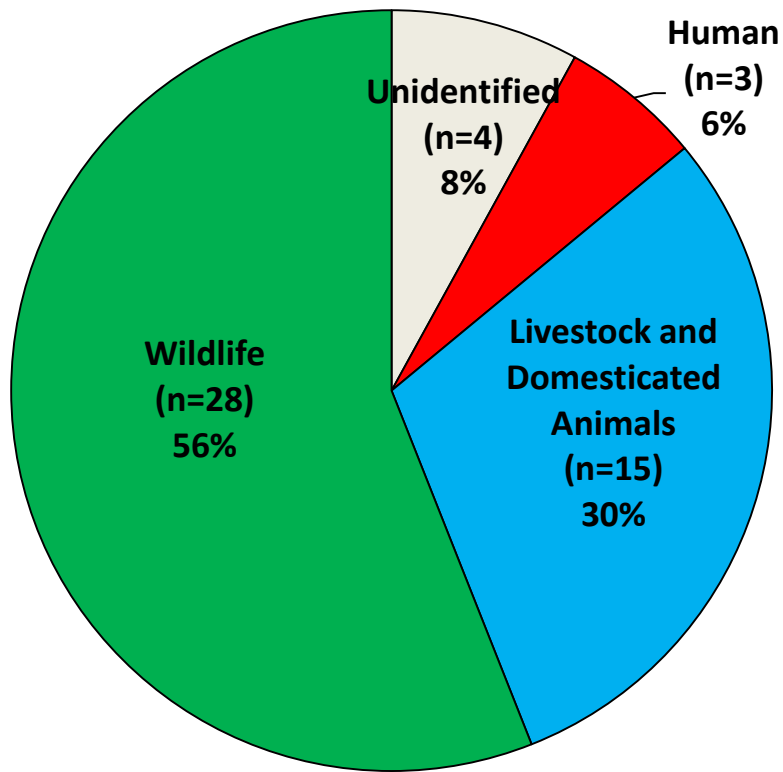
7-Way Split



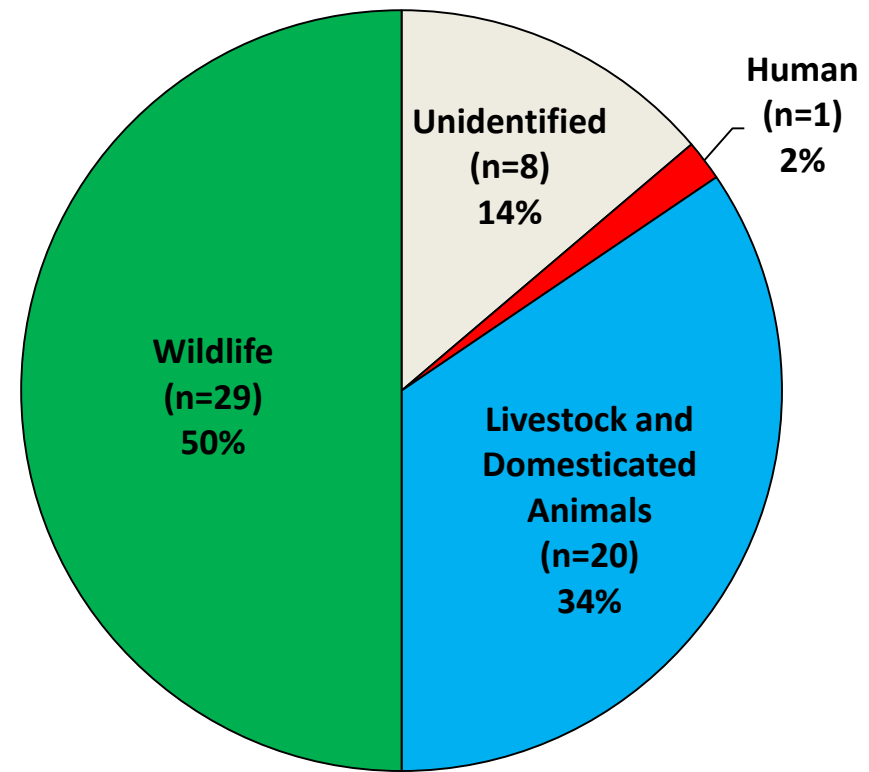
E. coli BST Results

Dry v. Wet Conditions (3-Way Split)

Dry (5)



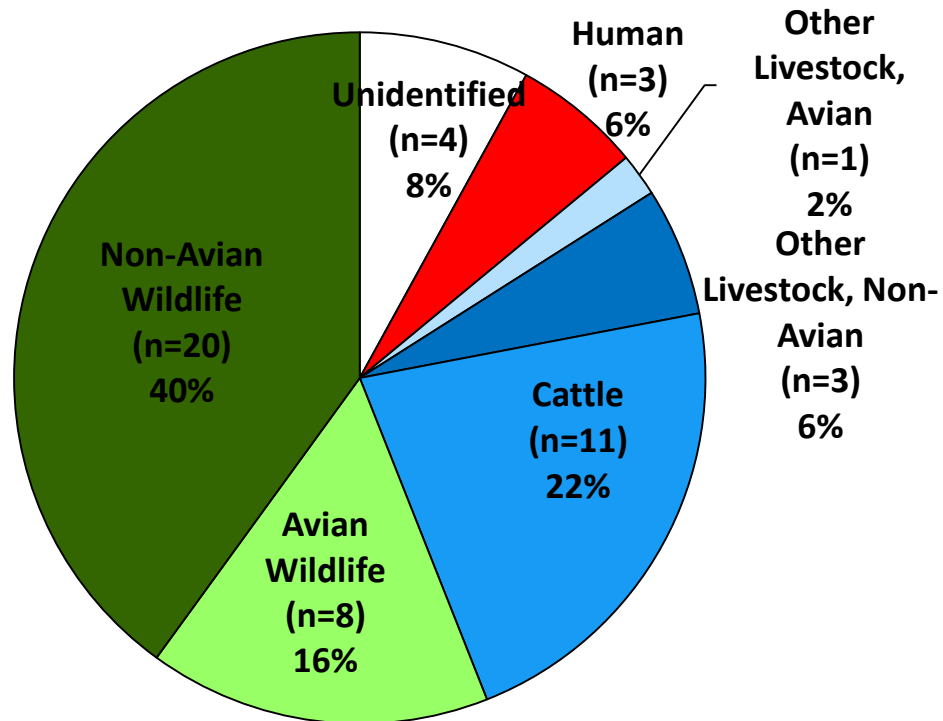
Wet (7)



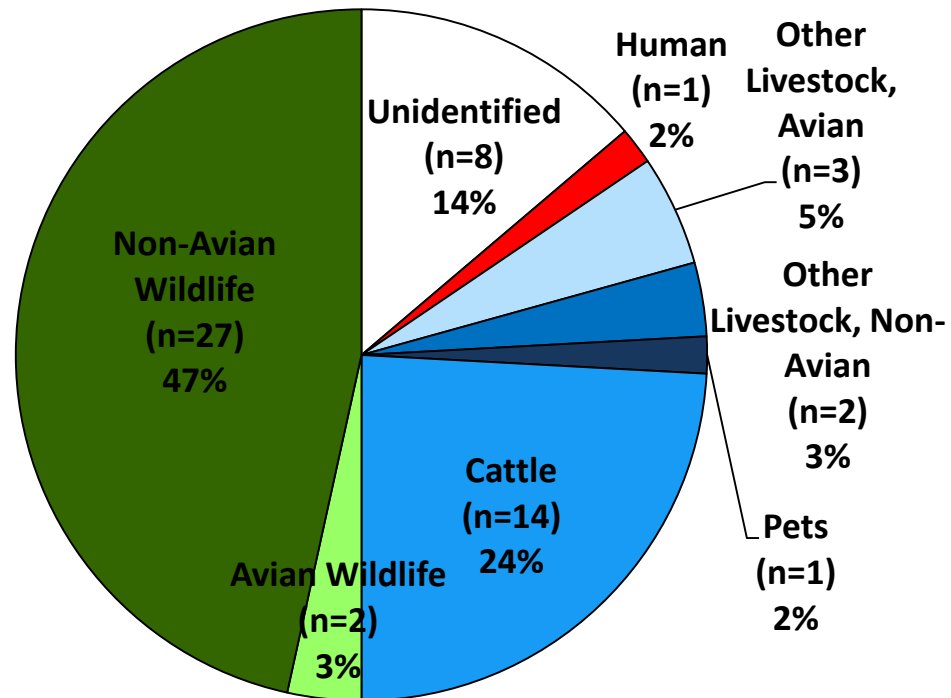
E. coli BST Results

Dry v. Wet Conditions (7-Way Split)

Dry (5)

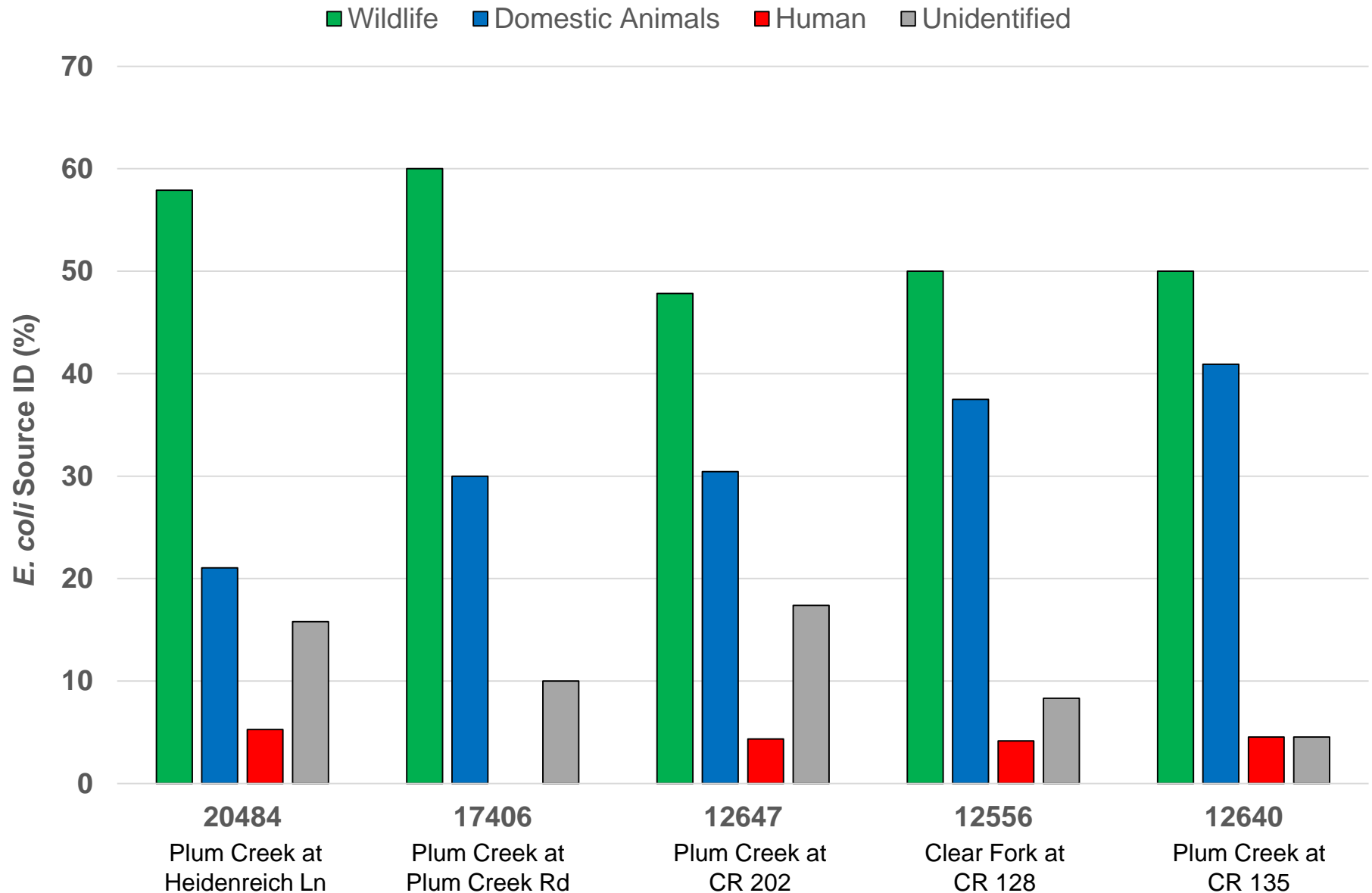


Wet (7)

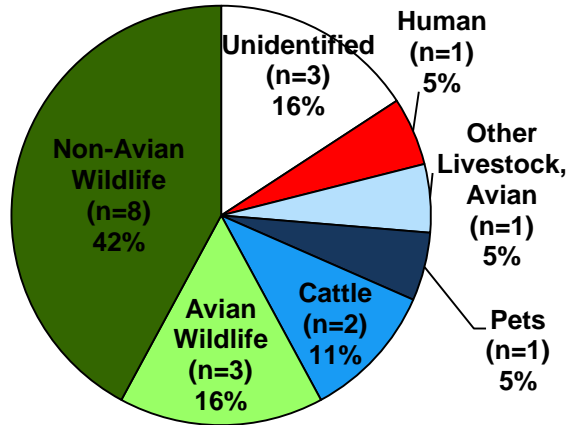


E. coli BST Results

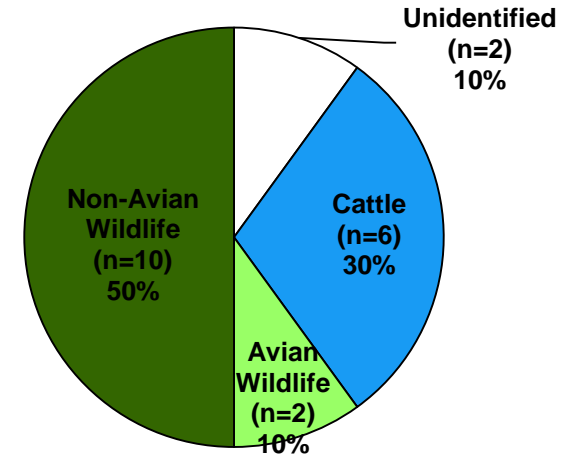
5 Sampling Sites (3-Way Split)



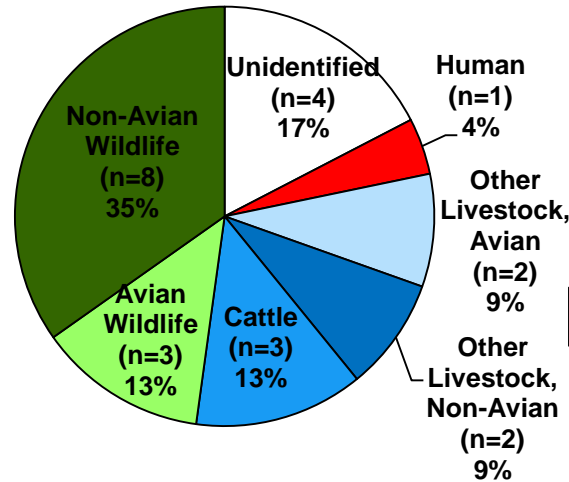
20484 – Plum Creek at Heidenreich Ln



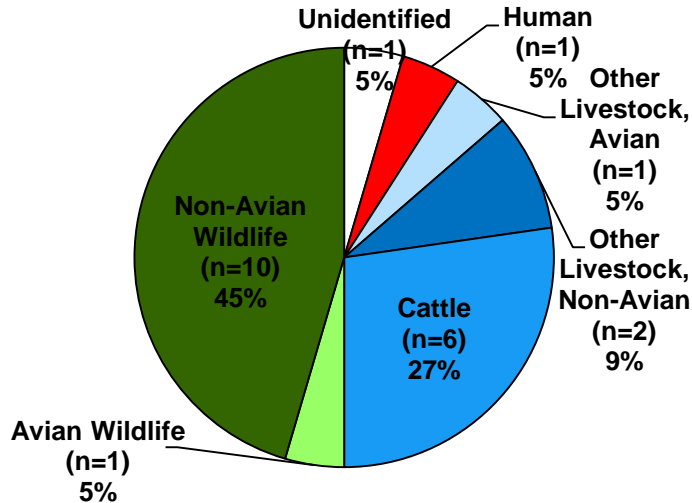
17406 – Plum Creek at Plum Creek Rd



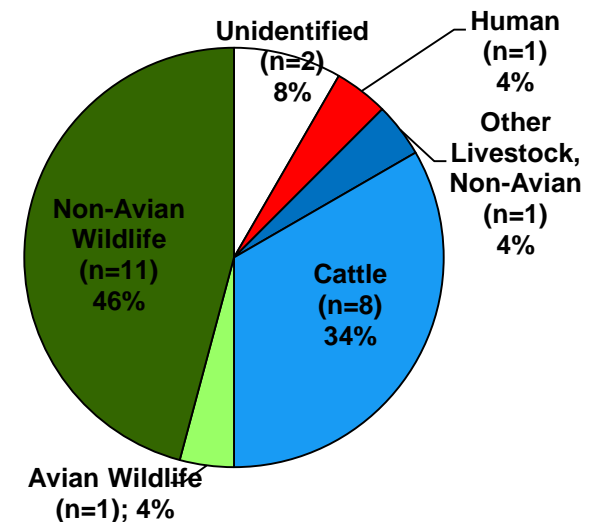
12647 – Plum Creek at CR 202



12640 – Plum Creek at CR 135



12556 – Clear Fork at CR 128



BST Summary

- Major *E. coli* sources in tested samples appear to be wildlife (feral hogs, small mammals, deer, birds) as well as domesticated animals (cattle)
- Domesticated animal contributions trended higher in samples from lower in the watershed
- Limited proportion of human *E. coli* isolates detected; primarily seen in samples collected below WWTF outfalls

Use of BST Results

- Reconcile with:
 - *E. coli* enumeration data
 - Land use
 - Watershed source survey
 - Modeling
 - Stakeholder input
 - Common sense

Questions?

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