

Bacterial Source Tracking in the Double Bayou Watershed

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BST Target Organisms

- **Bacterial v. Microbial Source Tracking**
- **Different targets:**
 - *E. coli*
 - *Bacteroidales*
 - **Bacteriophage**
 - **Human viruses**
 - **Animal cells**
 - **Chemicals**

BST Approaches

- **Culture-based (library-dependent)**
 - Isolate bacteria
 - Phenotypic/genotypic characterization
 - Compare to isolates from known-source samples
- **Marker-based (library-independent)**
 - Extract DNA from samples
 - Use PCR-based methods to detect/quantify source-specific markers
- **Sequencing-based**
 - 16S rRNA gene, metagenomic

History of BST Use in Texas

- **Lake Waco/Belton Project Findings**
 - Initiated Sep. 2002 with funding from TSSWCB
 - 4-method composite performed better than individual methods
 - 2-method composites appeared promising
 - ERIC-ARA = lower cost but more sample & data processing
 - ERIC-RP = higher cost but automated
- **TMDL Task Force Report – 2007**
 - Confirmed ERIC-RP as recommended method

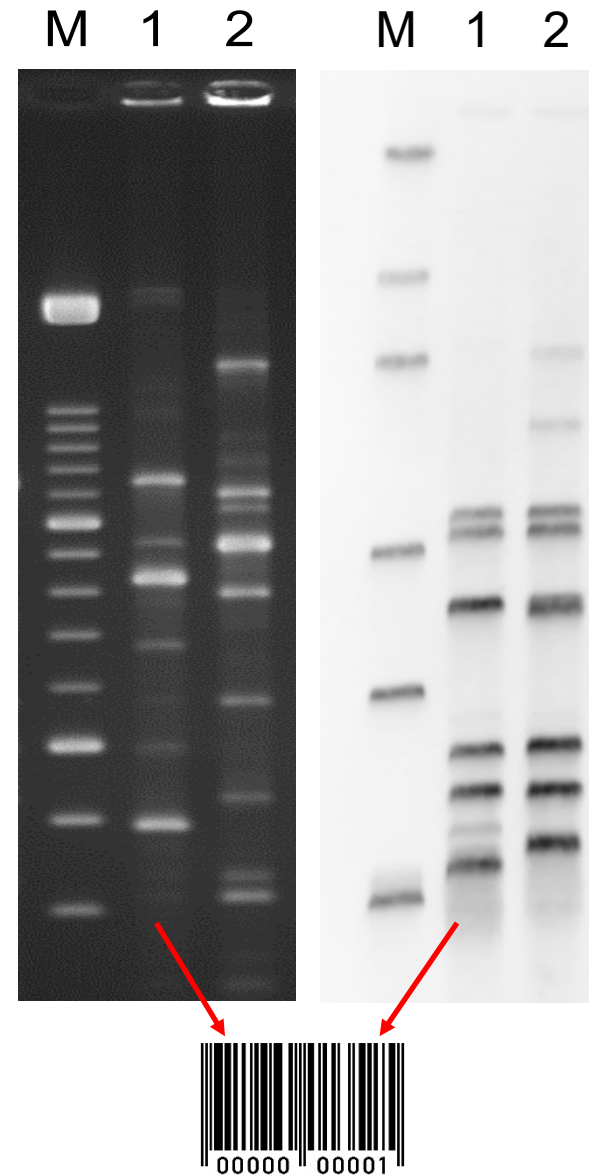
ERIC-RP DNA Fingerprinting

DNA fingerprinting:

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

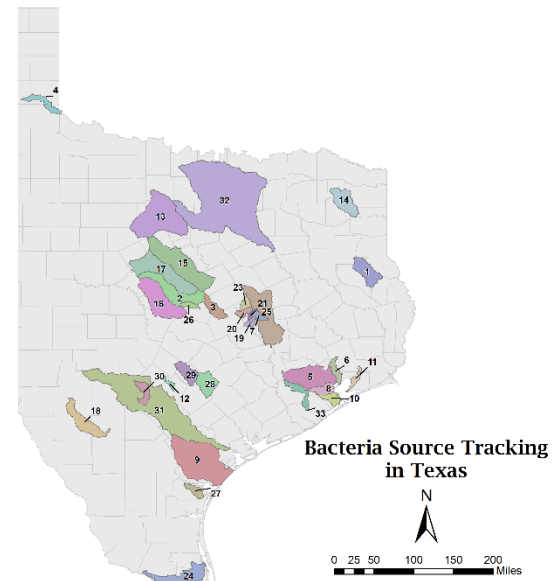
Advantages/Disadvantages:

- More discriminating
- Allows ranking of sources
- Relatively expensive

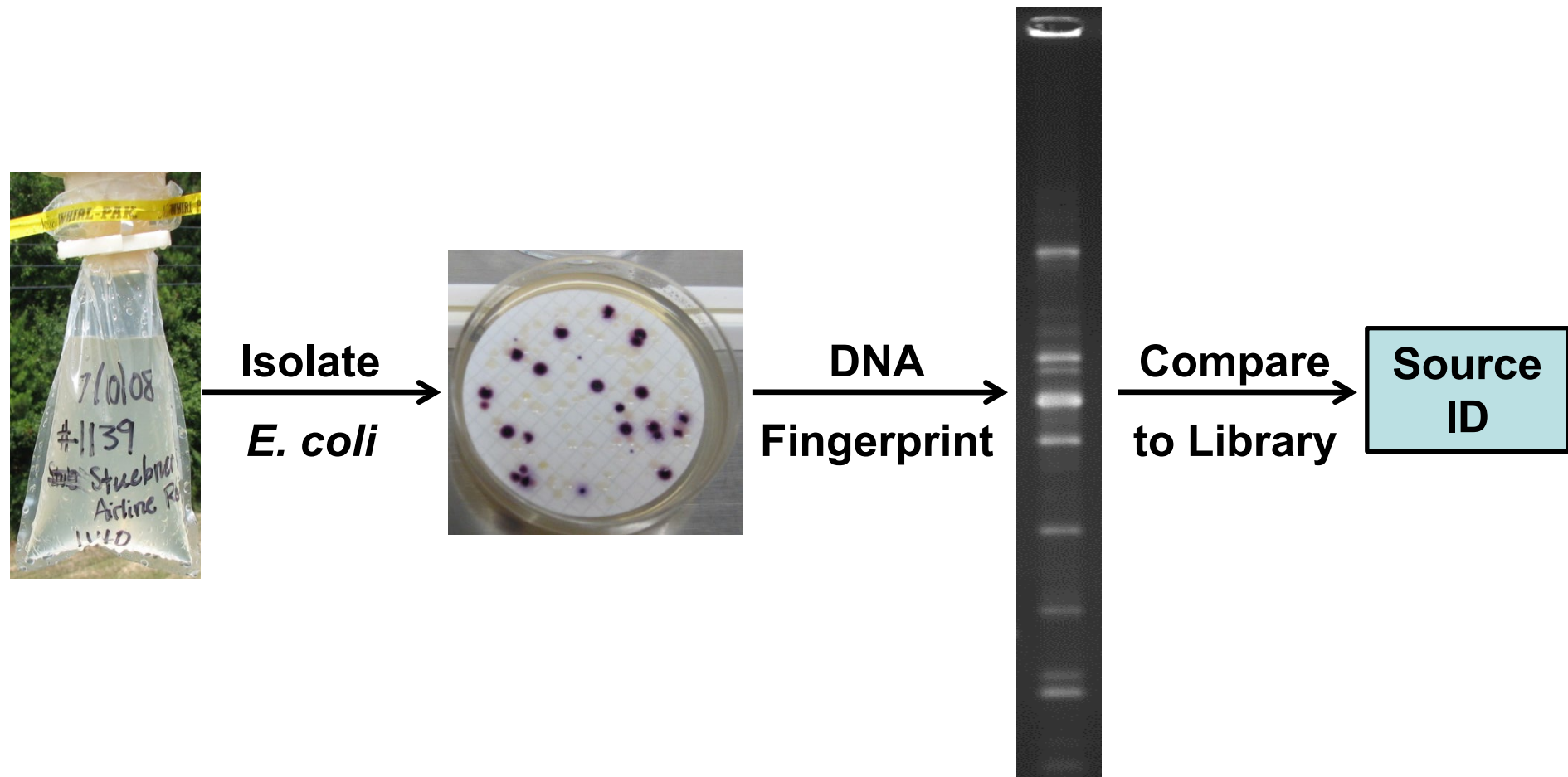


Texas *E. coli* BST Library (v. 04-22)

- Contains 1,942 *E. coli* isolates from 1,775 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

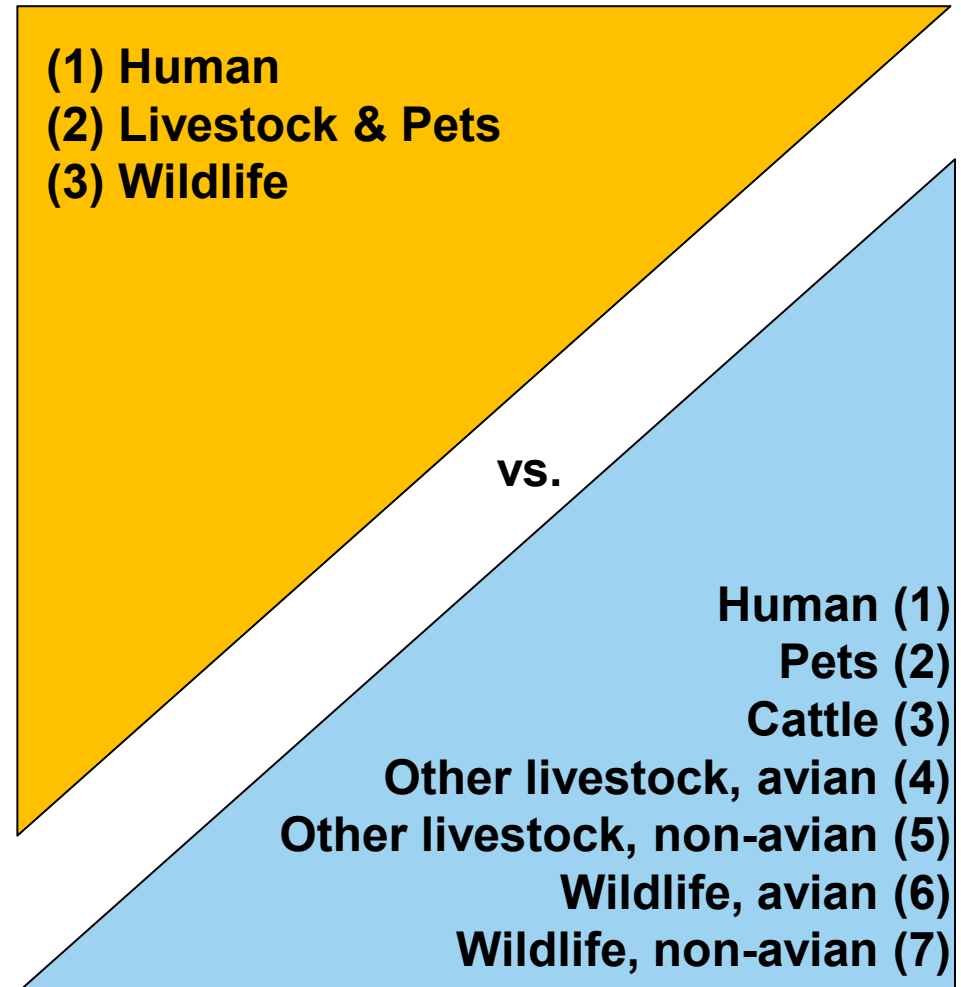


Use of Texas *E. coli* BST Library for Identifying Water Isolates



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
 - Geographical and temporal differences
 - Cosmopolitan strains
- **Statistics**
 - Number of isolates collected
 - May only use three-way split for limited studies



BST for Double Bayou

- BST on Tributaries of Trinity and Galveston Bays

- Funded by TCEQ

- One Double Bayou site

- Twelve water samples

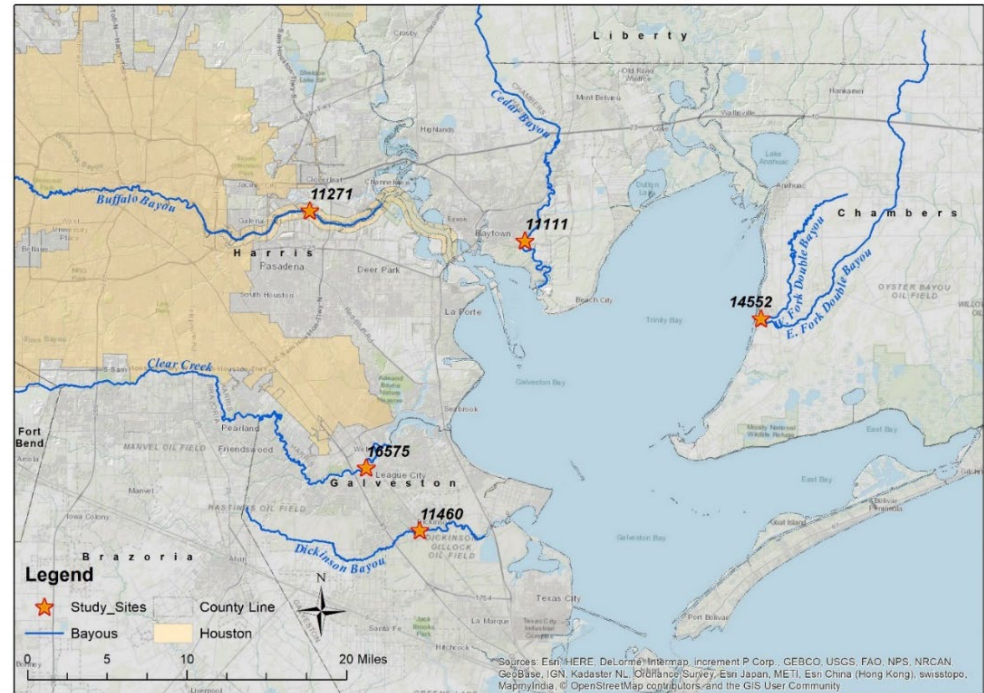
- ~Monthly

- April 2018 – April 2019

- Water *E. coli* Isolates

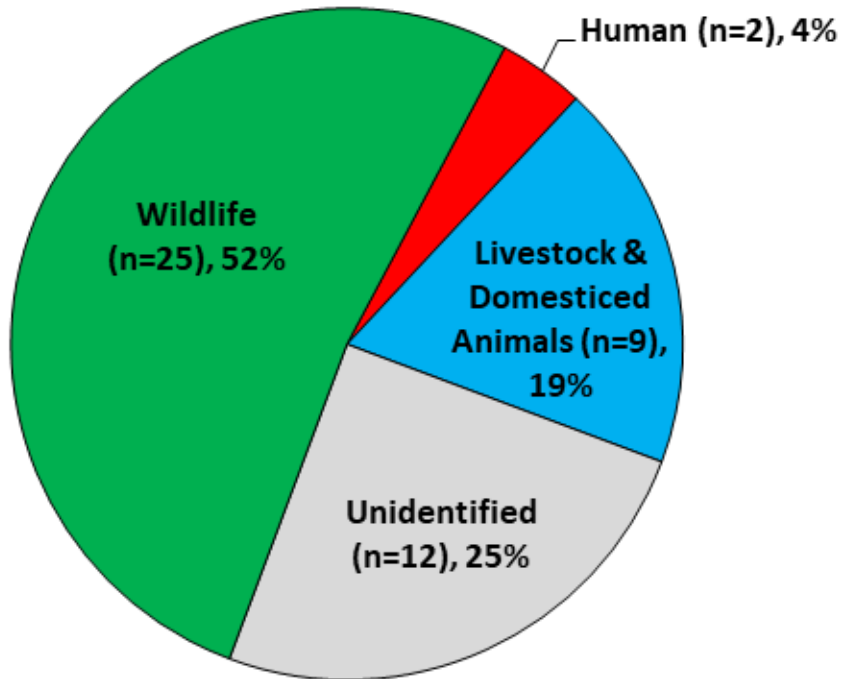
- 4 per water sample

- 48 total isolates DNA fingerprinted and compared to Texas *E. coli* BST Library for source identification

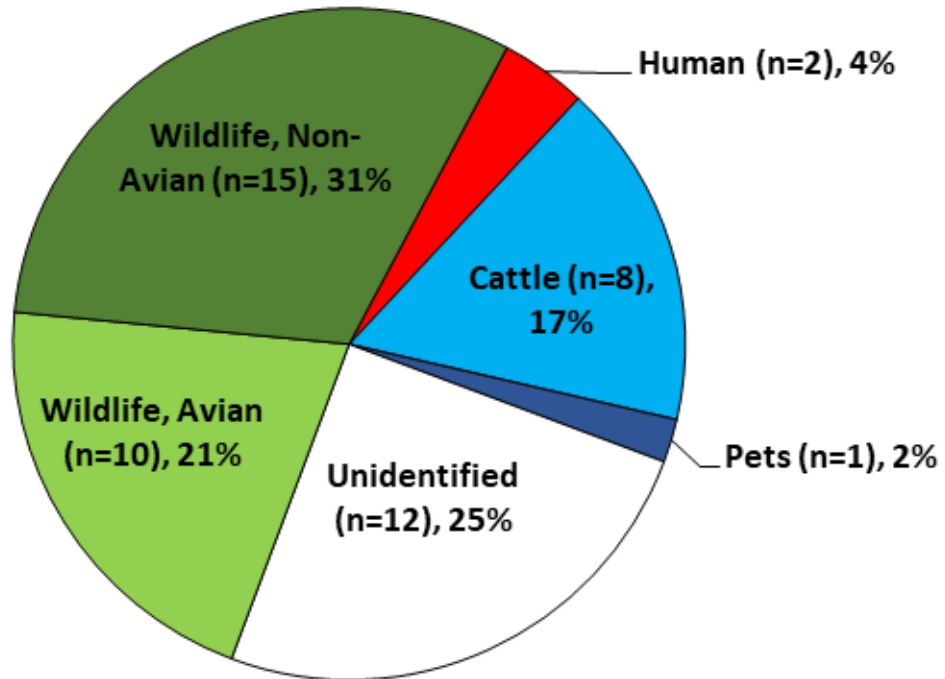


Double Bayou BST Results

3-way Split

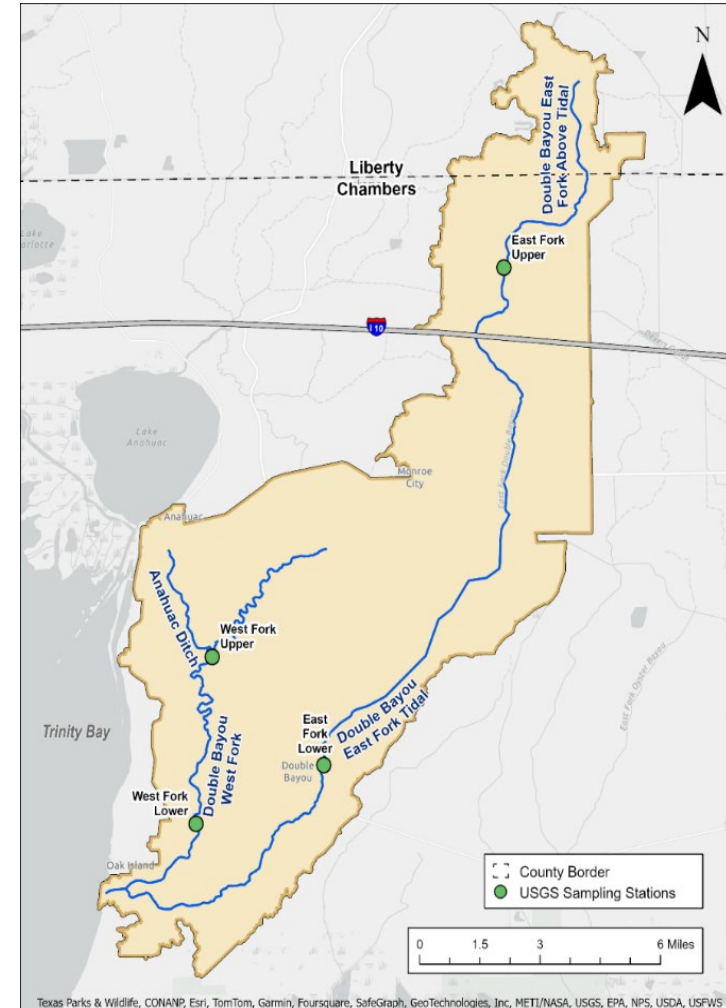


7-way Split



BST for Double Bayou

- **Funded by Texas State Soil and Water Conservation Board**
- **Four sites**
- **Twenty water samples**
 - **July 2023 – February 2024**
 - **Routine (ambient) = 4 rounds**
 - **Stormwater = 1 round**
- **Water *E. coli* Isolates**
 - **7-8 per water sample**
 - **150 total DNA fingerprinted and compared to Texas *E. coli* BST Library for source identification**

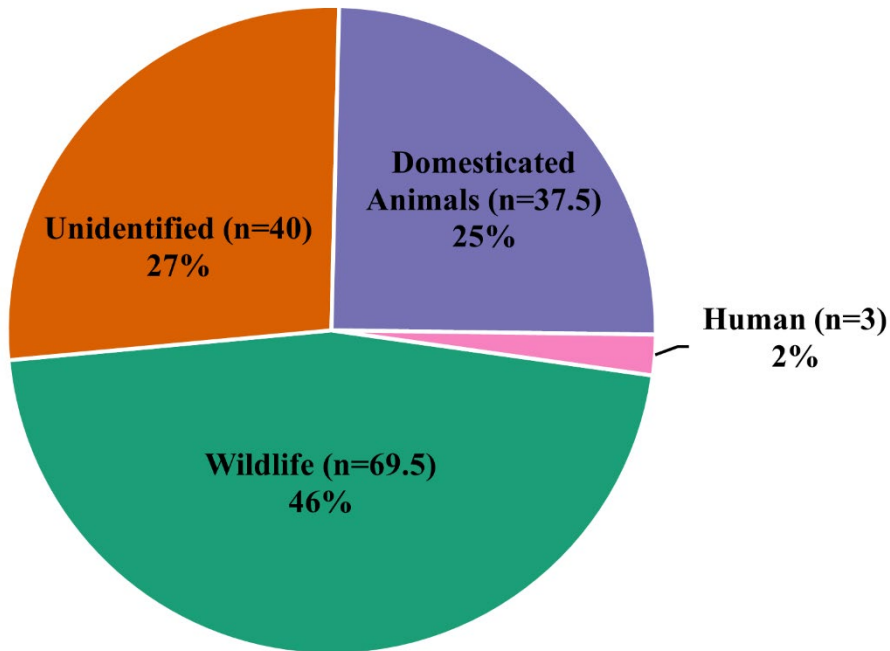


Four USGS sampling locations in the Double Bayou watershed

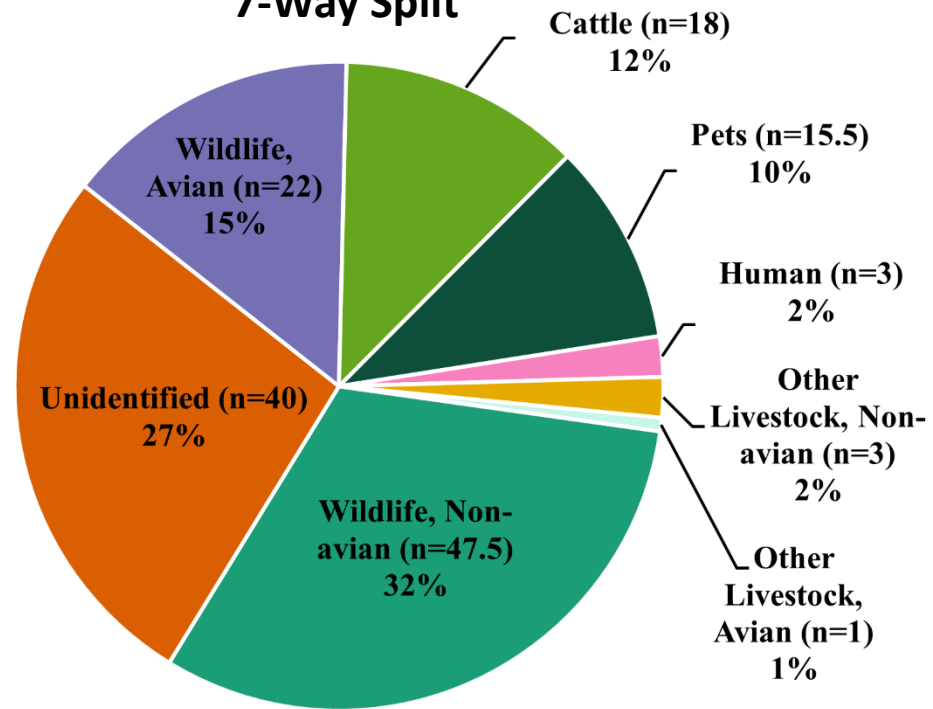
Double Bayou BST Results

Overall Results

3-Way Split

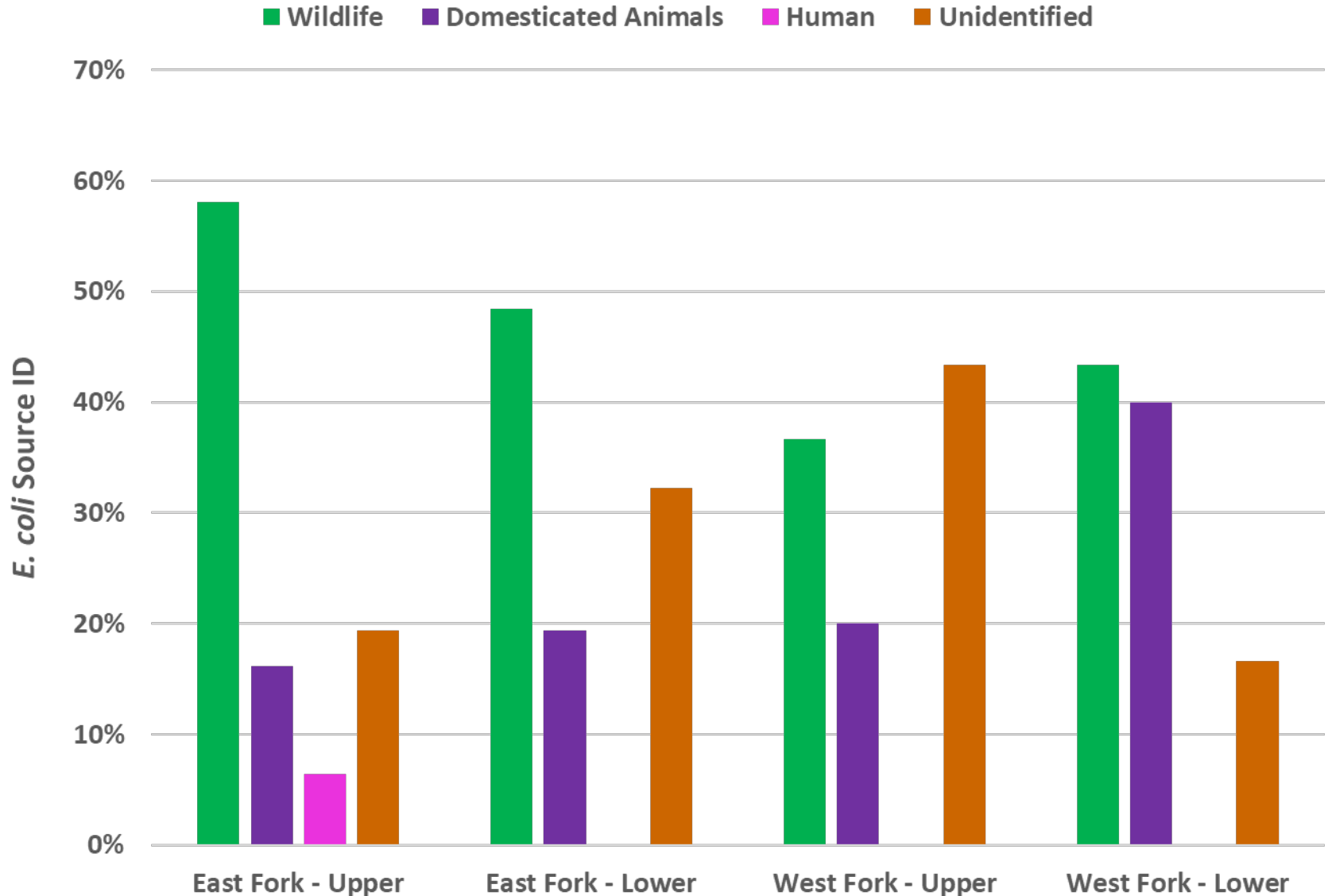


7-Way Split



Double Bayou BST Results

Routine Samples (3-Way Split)



BST Summary

- **Major *E. coli* sources at most sites appear to include wildlife (non-avian and avian)**
- **Domesticated animal sources detected at all sites and highest at West Fork Lower site**
- **Human source contributions detected at some sites but represented a limited portion of *E. coli***
- **Relatively high proportion of unidentified isolates at some sites indicating ‘unique’ organisms not represented in library**

Use of BST Results

- **Reconcile with:**
 - **Indicator bacteria levels**
 - **Land use**
 - **Watershed source survey**
 - **Modeling**
 - **Stakeholder input**
 - **Common sense**

Questions?

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