

Overview of Bacterial Source Tracking Methods

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BST Target Organisms

- **Bacterial v. Microbial Source Tracking**
- **Different targets:**
 - *E. coli*
 - *Bacteroidales*
 - **Bacteriophage**
 - **Human viruses**
 - **Chemicals**
- **Library-dependent v. library-independent**

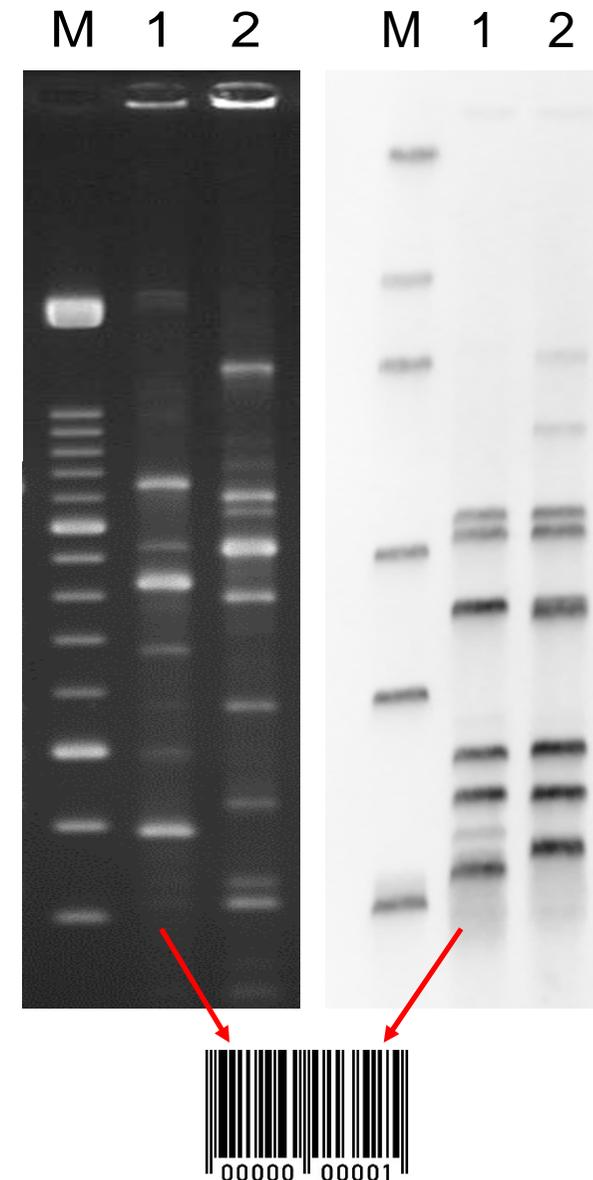
Library-Dependent BST Methods

Methods:

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

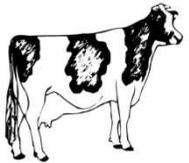
Advantages/Disadvantages:

- More discriminating
- Allows ranking of sources
- More expensive

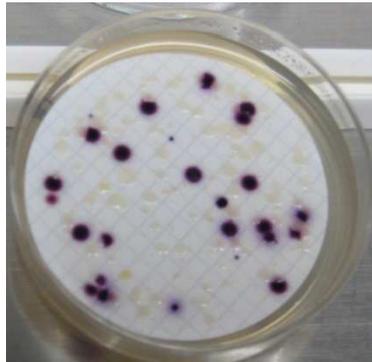


Development of Texas *E. coli* BST Library

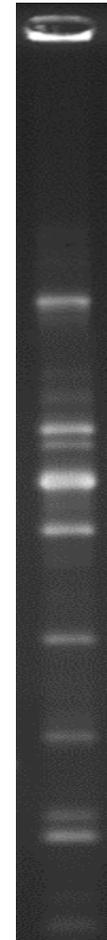
Sources



Isolate
→
E. coli



DNA
→
Fingerprint

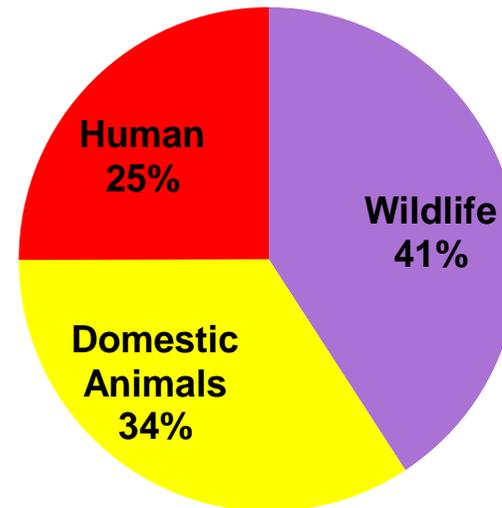


→
Add to
Library

Texas *E. coli* BST Library (v. 5-15)

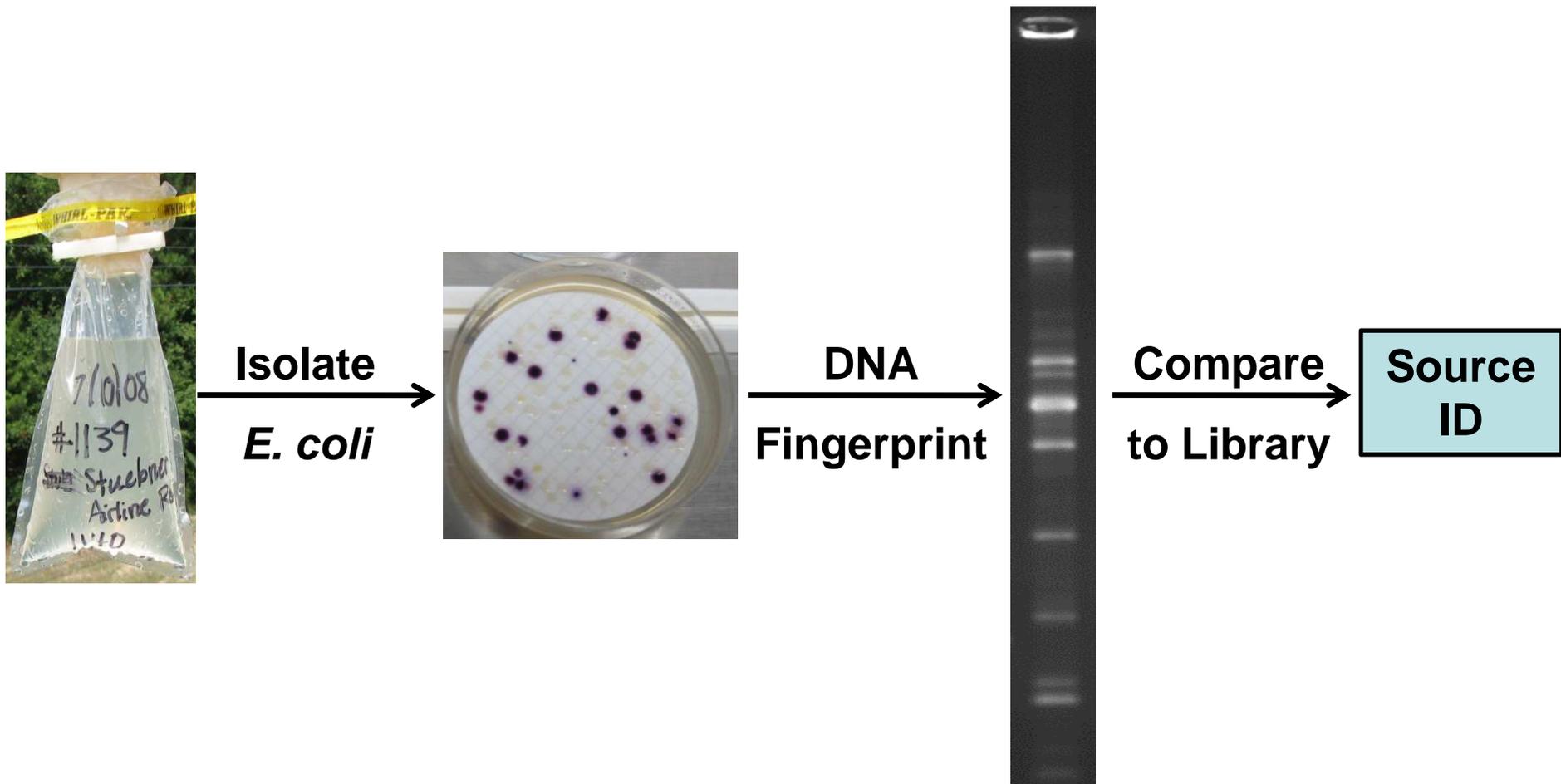
- Contains 1,765 *E. coli* isolates from 1,554 different human and animal samples
- Developed by collecting over 3,500 domestic sewage, wildlife, livestock, and pet fecal samples and screening over 6,000 isolates for clones and host specificity
- Samples from 13 watersheds across Texas for BST including:

- Waco / Belton Lake
- San Antonio
- Lake Granbury
- Oyster Creek / Trinity River
- Buck Creek
- Little Brazos River Tributaries
- Attoyac Bayou



- Additional isolates being added from ongoing and future BST projects in other areas of Texas

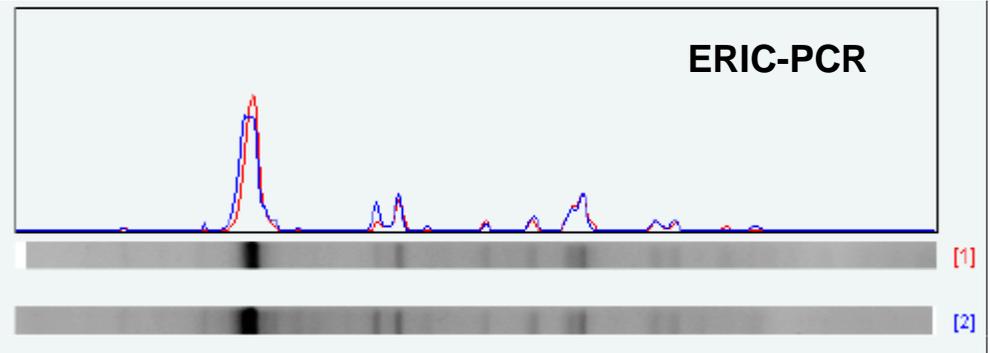
Use of Texas *E. coli* BST Library for Identifying Water Isolates



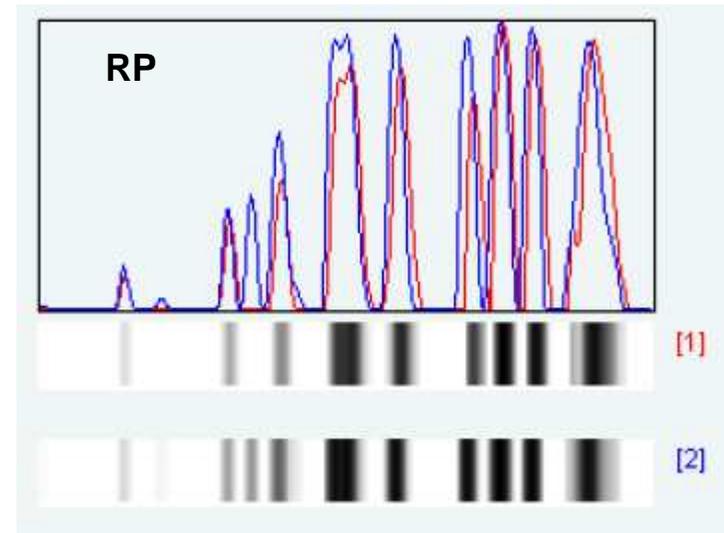
Comparison to Texas *E. coli* BST Library

- **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%

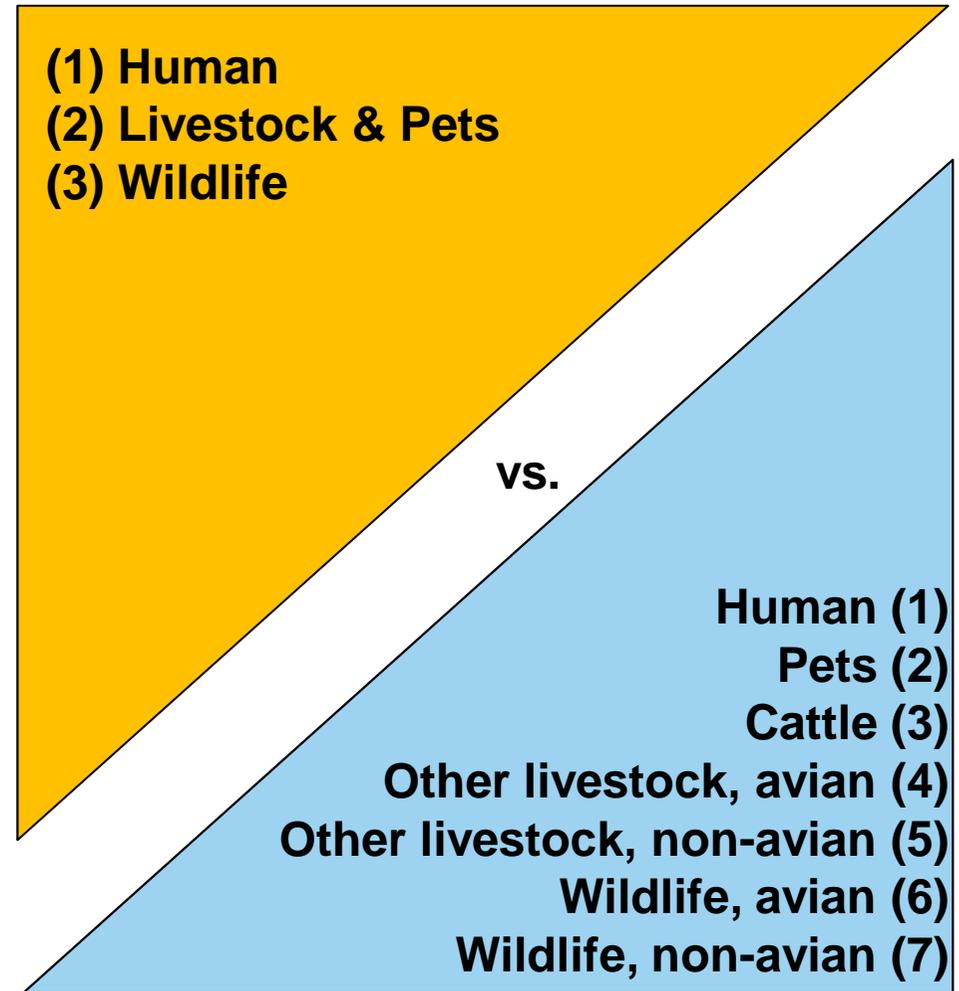


Similarity: 95.82%



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



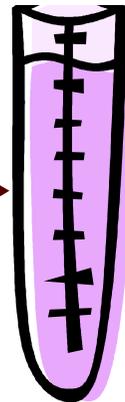
Texas *E. coli* BST Library Composition & Rates of Correct Classification (RCC)

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	364	315	24%	100	4.2	22
DOMESTIC ANIMALS	531	474	35%	100	2.9	19
Pets	86	76	6%	83	13.8	40
Cattle	237	207	16%	93	5.8	11
Avian Livestock	96	83	6%	89	14.8	25
Other Non-Avian Livestock	112	108	7%	90	12.9	14
WILDLIFE	629	569	41%	100	2.4	19
Avian Wildlife	239	221	16%	85	5.3	21
Non-Avian Wildlife	390	348	26%	92	3.5	17
Overall	1524	1358		ARCC** = 100% (3-way) 92% (7-way)		20%

Library Independent BST



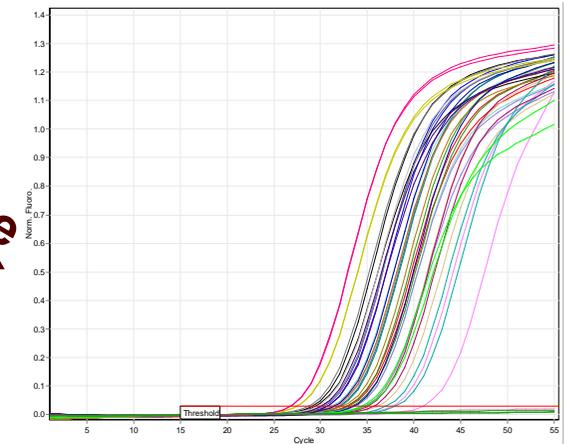
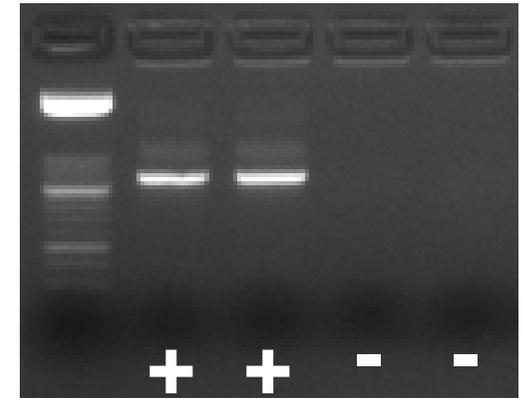
Extract
DNA



PCR amplify
target sequence

Presence/
Absence

Quantitative



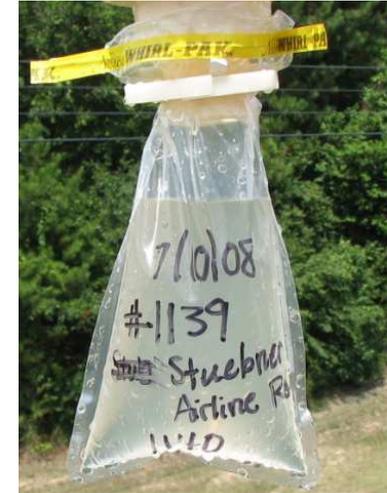
Advantages:

- Cost
- Time

Library-Independent BST

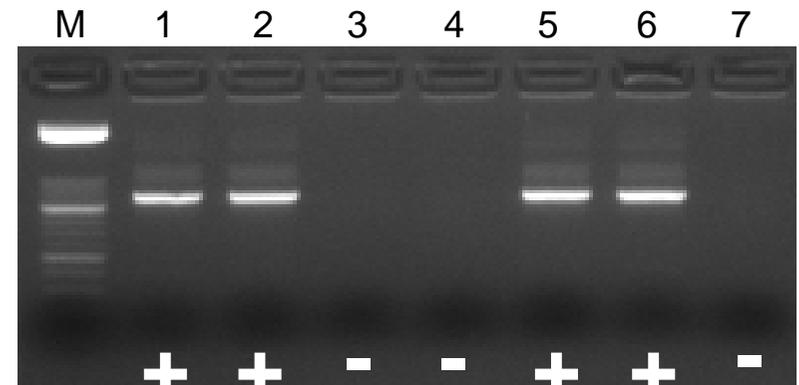
Approach:

- Genotypic detection of microorganisms based on marker genes (DNA)
- Does not require known-source library
- Most common approach targets



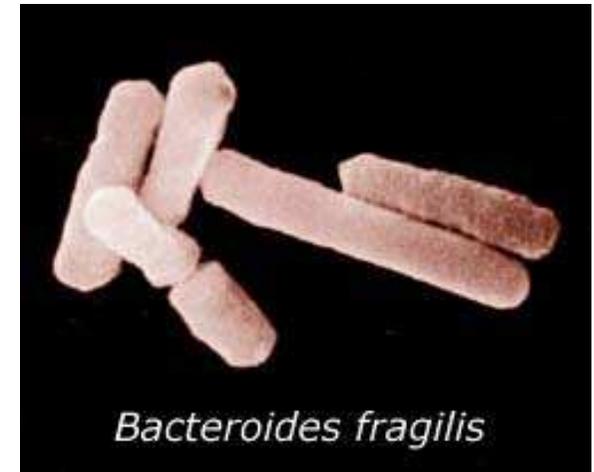
Bacteroidales

- Presence/absence
- Relative abundance



What are *Bacteroidales*?

- More abundant in feces than *E. coli*
- Not pathogens
- Obligate anaerobes – less likely to multiply in environment
- Subgroups appear to be host specific
- Markers available for humans, ruminants, horse, hog
 - Others being tested (e.g., poultry)
 - Limited wildlife markers



http://www.sourcemolecular.com/newsite/_images/bacteroidetes.jpg

Use of BST Results

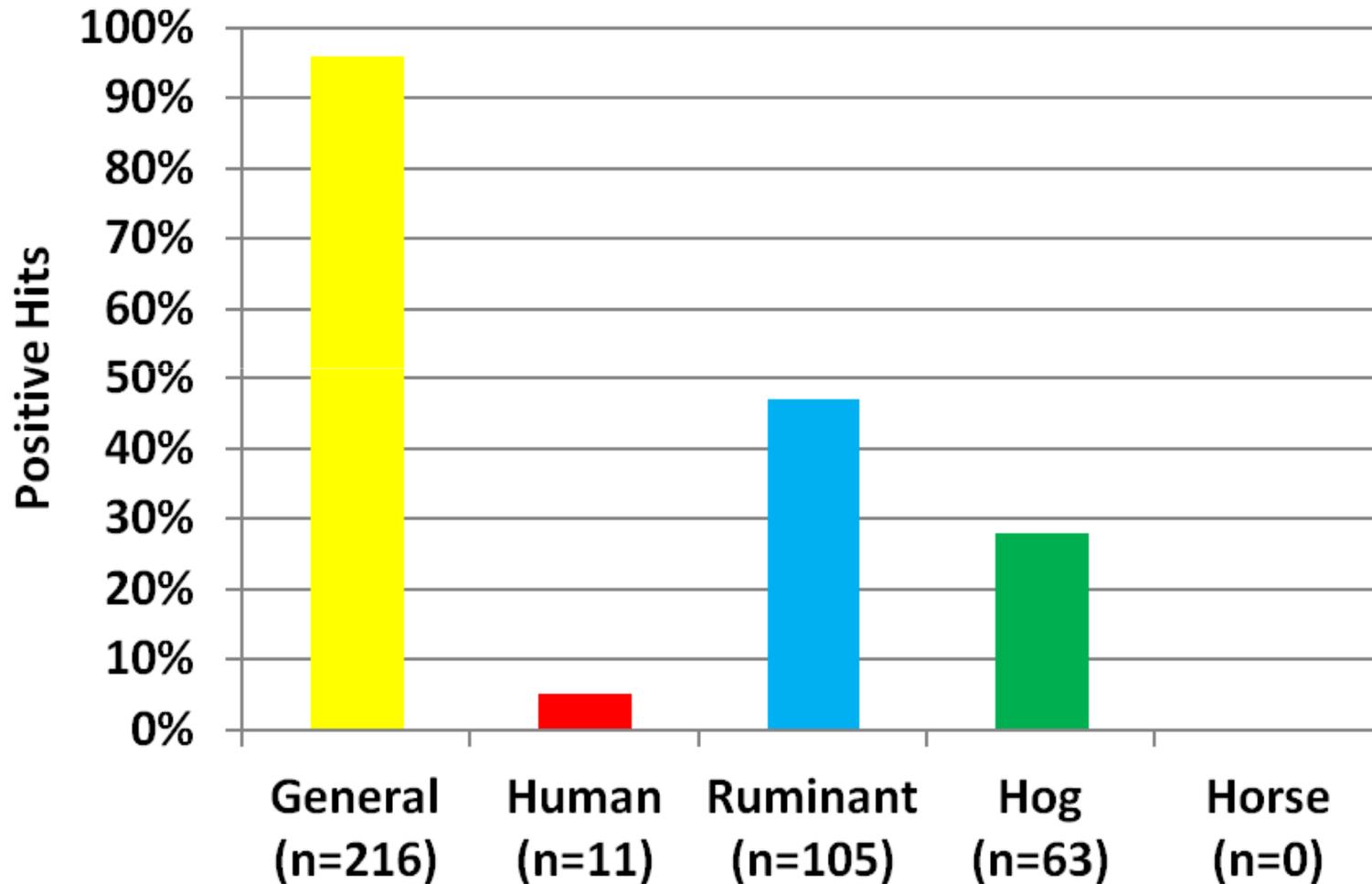
- **Reconcile with:**
 - **Land use**
 - **Watershed source survey**
 - **Modeling**
 - **Stakeholder input**
 - **Common sense**

BST for Attoyac Bayou

- **Limited, library-dependent**
 - Analyze *E. coli* from ~100 water samples from across the study area using both ERIC-PCR and RP fingerprinting
 - Add ~100 known-source *E. coli* isolates from the area to the Texas *E. coli* BST Library
 - Wastewater, poultry, cattle, wildlife, etc.
- **Library-independent**
 - Analyze ~250 water samples from across the study area using *Bacteroidales* PCR for human, ruminant, hog, and horse markers

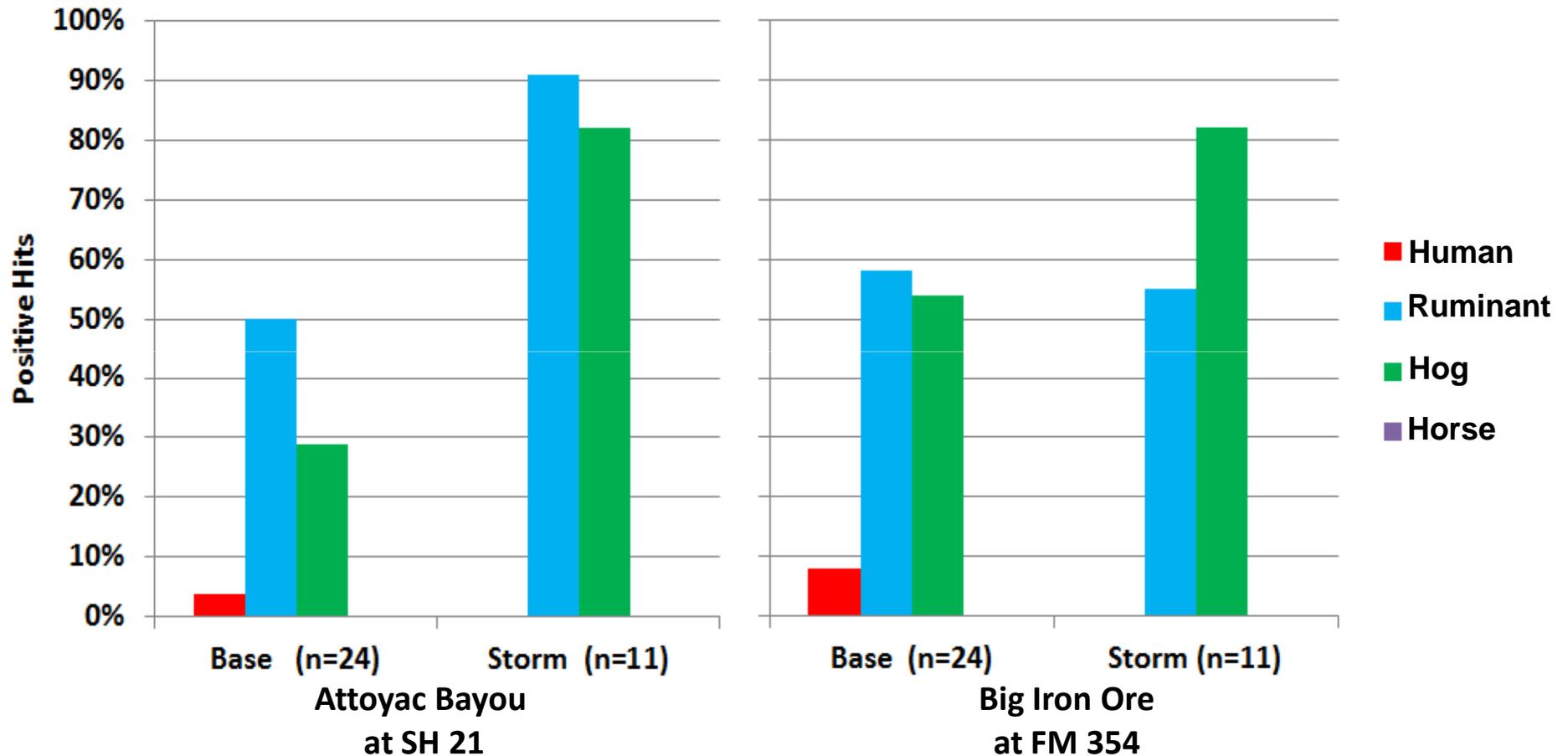
Bacteroidales BST Results

Base Flow Samples (n=225)



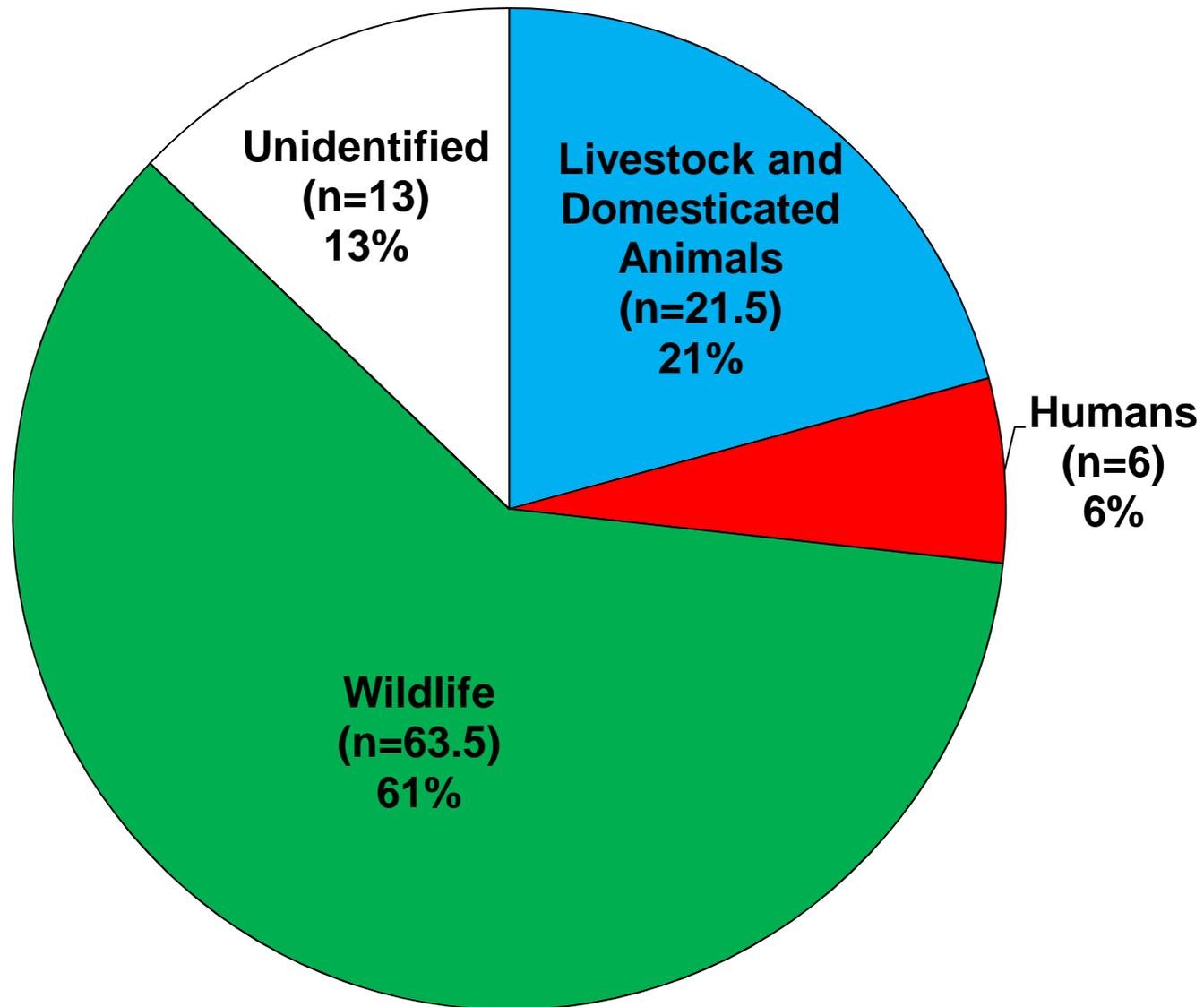
Bacteroidales BST Results

Base Flow vs Storm Flow



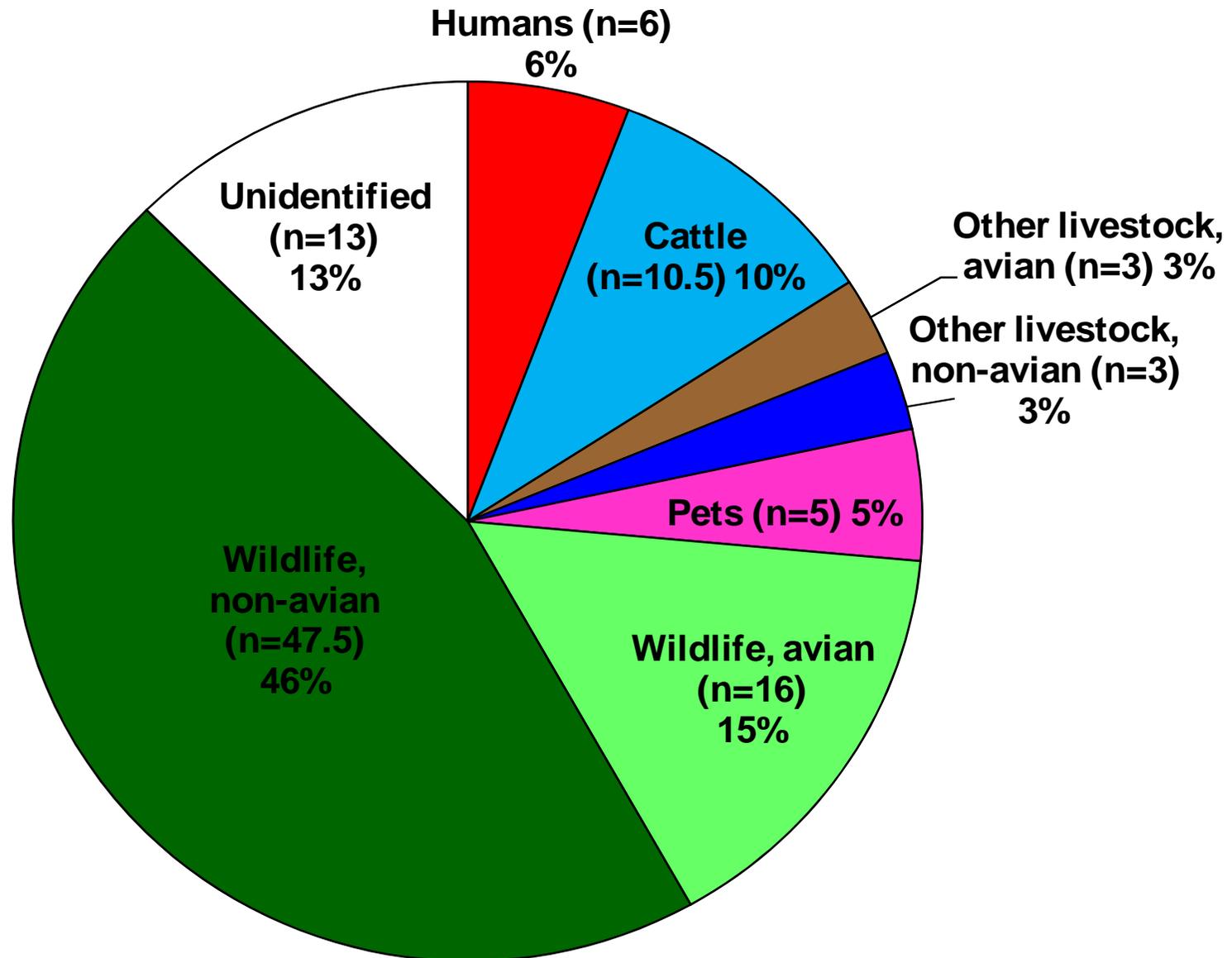
E. coli BST Results

Base + Storm Samples – 3-Way Split



E. coli BST Results

Base + Storm Samples (7-Way Split)



BST Summary

- **Library-Independent Analysis**
 - Ruminant and hog (feral) markers most common
 - Spike in ruminant and hog hits during storm events
- **Limited Library-Dependent Analysis**
 - Major *E. coli* sources in watershed appear to be wildlife (feral hogs, small mammals, avian wildlife) as well as domesticated animals (cattle)
 - Texas *E. coli* BST Library additions from Attoyac Bayou
 - Significant effort to include isolates from poultry litter

Future Methods & Approaches

1. Identify the “Unidentified”

- Continue expansion of BST library
- Evaluating “naturalized” *E. coli*

2. Improve Library-Independent BST

- Limited markers, but new markers being developed
- Geographic stability of markers
- Quantification?

BST for Your Watershed?

- **What is the Goal of BST?**
 - **Characterize watershed or monitor specific sources?**
 - **How many potential sources?**
 - **All, most numerous...**
 - **One or a few (e.g., human)**
 - **What level of resolution is needed?**
 - **Individual species**
 - **Groups (e.g., humans, domesticated animals, and wildlife)**
 - **Presence/absence, relative ranking, or absolute number for various sources**

Questions?

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