

Lampasas River Watershed Partnership

Bacterial Source Tracking

Elizabeth Casarez, Ph.D.

Post-doctoral Research Associate

George Di Giovanni, Ph.D.

Professor and Faculty Fellow, Environmental Microbiology
Department of Plant Pathology and Microbiology

Texas AgriLife Research Center at El Paso

Texas A&M University System



Elevated Levels of *E. coli* in the Lampasas River

E. coli used as an **INDICATOR** of fecal pollution and health risk
- Higher levels of *E. coli* = Higher probability of health risks



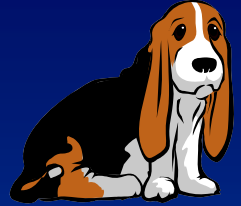
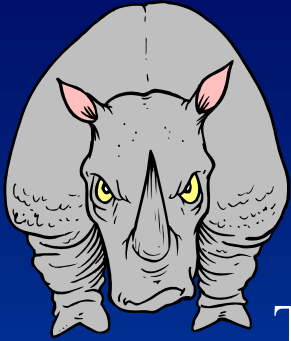
Enteric pathogens are the bad guys

Clean Water Act

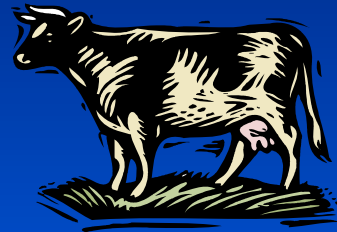
Restore and maintain beneficial uses of water bodies
Standards for Contact Recreation



There's *E. coli* in the Water, But Where Did it Come From?

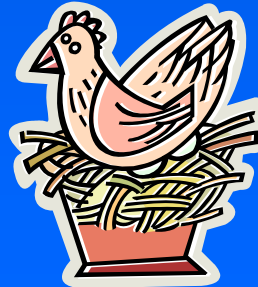
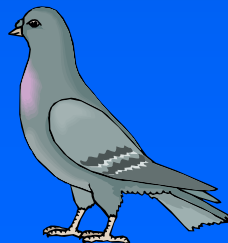
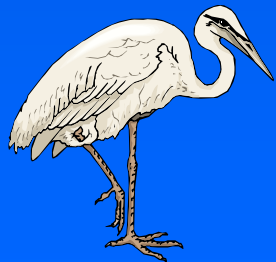
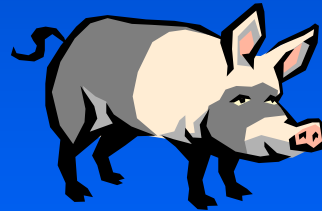


Target Best Management Practices Implementation
Bacterial Source Tracking (BST) as a tool



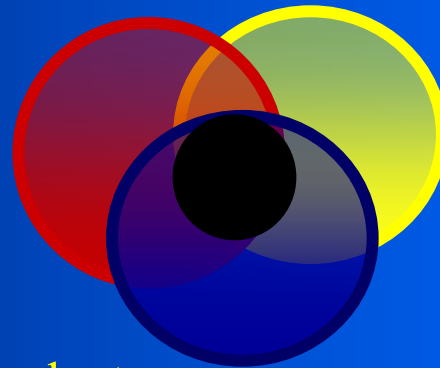
Track fecal pollution sources using *E. coli*
Different animal guts → Different adaptations →
Different *E. coli* strains →

Phenotypic Differences
Genetic Differences

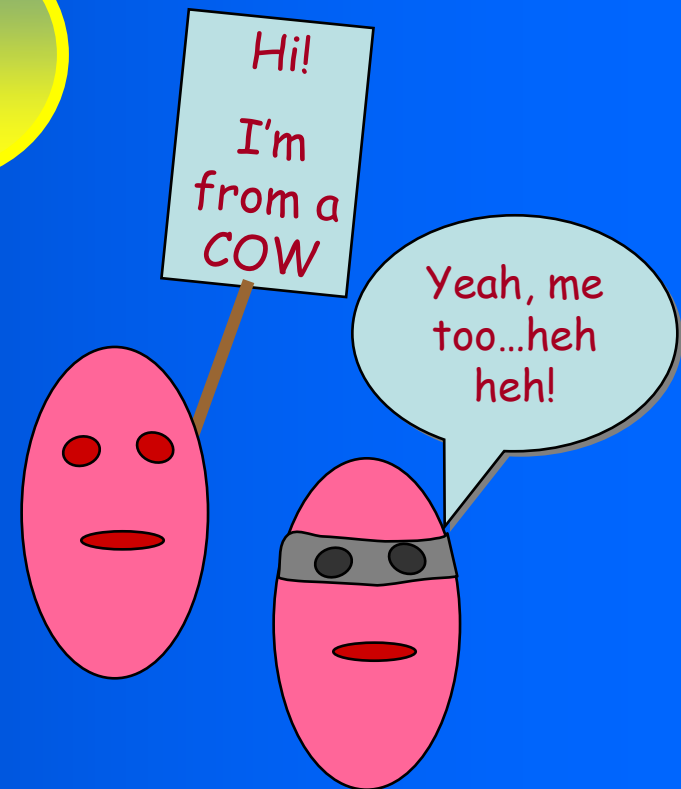


Nature of the Beast

- Different strains of *E. coli* in a single individual
- Different strains in different individuals
- Similar strains in similar environments
- Strains of *E. coli* present dependent on:
 - Animal species
 - Gut type
 - Diet
 - Environment
 - Interactions
 - Geography?
 - Time?



Cosmopolitan strains



Bacterial Source Tracking

- BST - laboratory tests to find differences in DNA sequence (strains)
 - Correlate differences with specific source host
 - Determine if *E. coli* in water samples came from animal or human feces
- Most BST methods are **Library Dependent**
 - Need database of reference bacteria from known animal and human sources
- “Local” watershed libraries currently considered most useful
 - Cost and time considerations



TSSWCB Assessment of Lake Waco and Belton Lake (N. Bosque)

- One of the first BST studies in the state (~2003)
- >3700 *E. coli* from 765 fecal and 415 water samples
- Compared 4 different BST methods
 - ERIC-PCR, RiboPrinting, PFGE, KB-ARA
- **Results: Wildlife was major source, followed by cattle and humans**
- **Lessons learned**
 - Identified pollution sources may differ from assumed sources—assumed was dairy cattle
 - 2-method composite for good results
 - Cross identification between livestock
 - Cosmopolitan isolates can confound results
 - Big libraries are expensive
- **Will results for Lampasas be similar?**
 - Cannot predict
 - Geographically similar, but temporally different

Texas *E. coli* BST Library

Self –validated isolates from 7 Texas watersheds

1309 isolates from 1185 source samples

Thousands of *E. coli* isolates screened from Lake Waco; Belton Lake; San Antonio River; Lake Granbury; Buck Creek; Upper Trinity River; Upper Oyster Creek

Table 1. Texas *E. coli* BST library (ver. 8-10) composition and rates of correct classification

Source Class (number of isolates/samples)	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification	Left Unidentified (unique patterns)
Human (374/327)	29%	89%	19%
Livestock and Pets (462/424)	35%	83%	20%
Wildlife (473/434)	36%	86%	18%

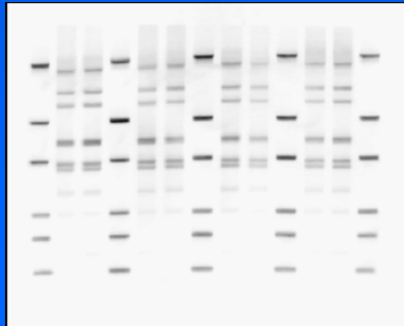
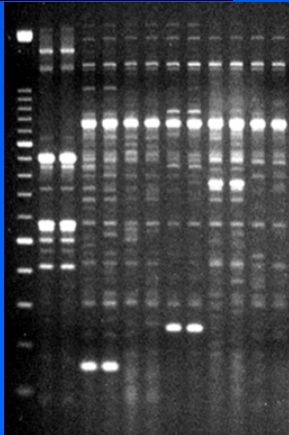
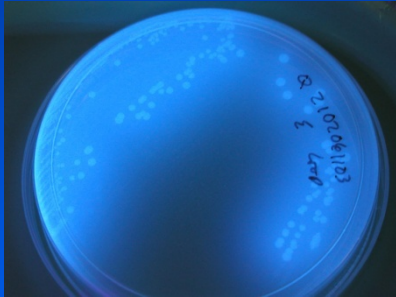
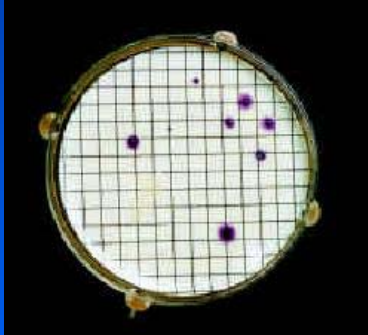
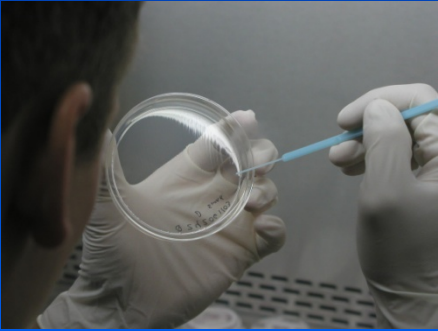
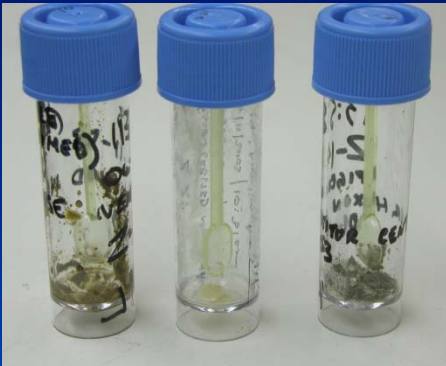
Statewide library works about as well as a small local library but still room for improvement
—supplement with local known sources

50 local known source samples needed

Different individual animals*; swab or fresh excretion
5 isolates/sample archived; 3 isolates/sample screened
self-validated isolates will be incorporated into State library

- Identify potential sources
- What are sources of special concern?
- Can we access these sources?
 - Landowner permission
 - Collection permission
 - Wildlife collections
- Challenge and expand current state library

Bacterial Source Tracking (BST) Studies



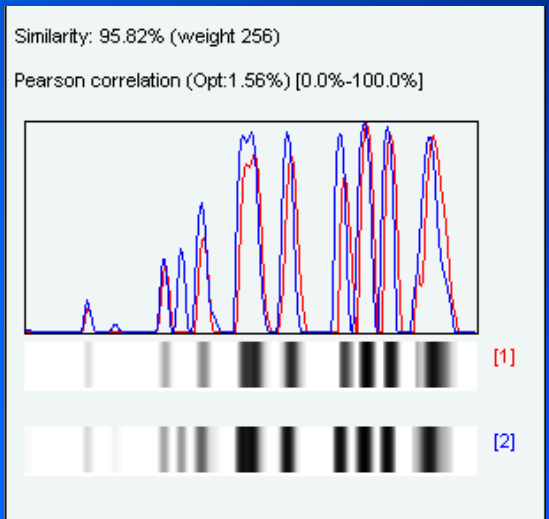
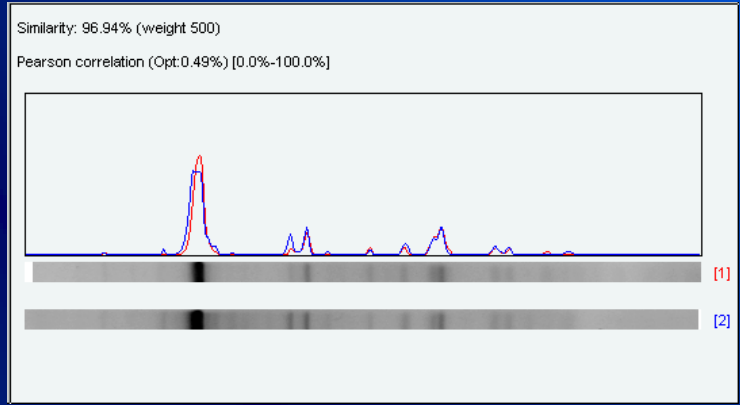
Data Analysis

Best Match Approach

ERIC-PCR

Enterobacterial Repetitive Intergenic Consensus sequence--Polymerase Chain Reaction

Best ERIC-PCR Match



HindIII Automated Ribotyping

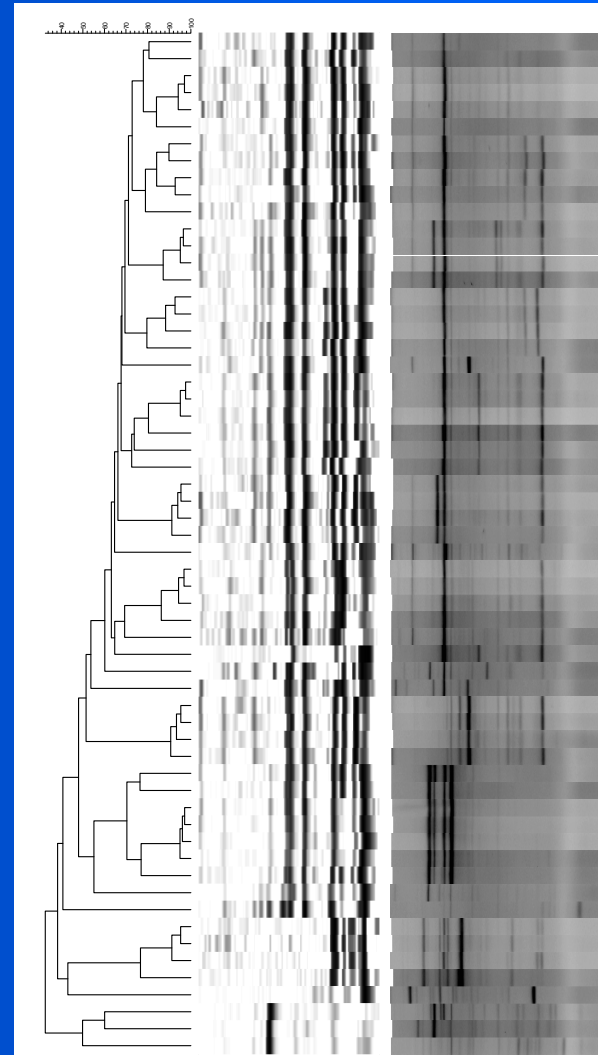
Best RiboPrint Match

Data Analysis: Best Match Approach

Composite Data Sets

- Applied Maths Bionumerics Software
 - Generate a family tree based on similarity
- “Best Match” approach with minimum similarity cutoff based on laboratory QC data
 - Water isolate must match library isolate \geq minimum similarity or unidentified
 - Identification to *single library isolate* with highest similarity – max similarity approach

80% similarity or BUST!



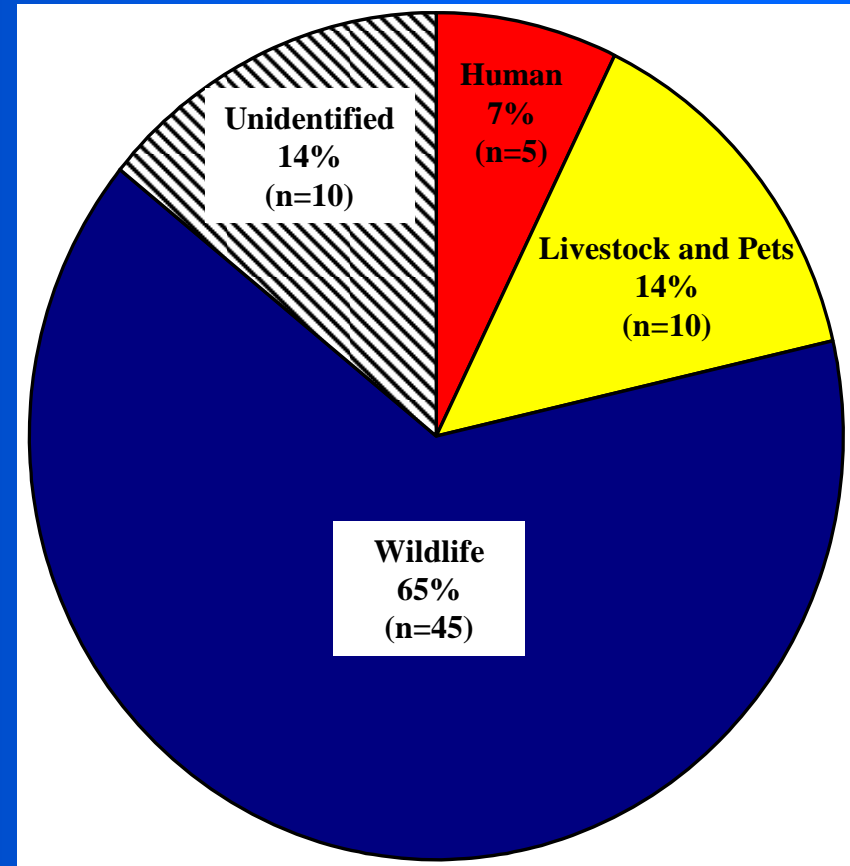
Three-way vs. Six-way split of Results

- **Using the results**
 - Is it from human sources?
 - Is it from livestock?
 - Is it from wildlife?
- **Biology**
 - Cross identification between livestock
 - Large variety of wildlife
 - Cosmopolitan strains
 - Geographical and temporal differences
- **Statistics**
 - Number of isolates collected



Example of *E. coli* BST Results

- **Matches or “hits” for *E. coli* split into 3 categories**
 - ie., Human, Livestock and Pets, Wildlife
- **Some *E. coli* cannot be identified**
 - If < 80% similarity, classified as “Unidentified”
 - Particular strain fingerprint not in library
- **Estimate of pollution source contribution**
- **Provides rank order**
 - Not absolute percentages: ~30% = ~40%



Interpretation of Results

- Local known source isolates will be compared to Texas *E. coli* BST Library
 - self-validated isolates will be incorporated
- 5 *E. coli* isolates from each water sample monthly over 12 months for 15 sites
 - 5 * 12 = 60 isolates per site
- 3-way split of source classes per site
 - May be able to use 6-way split for entire watershed as a whole
- Along with BST data, incorporate:
 - *E. coli* counts data
 - Comparisons between water stations and over time
 - Knowledge of local area
 - Modeling
 - **Common Sense**



to interpret results for recommendations for Best Management Practices

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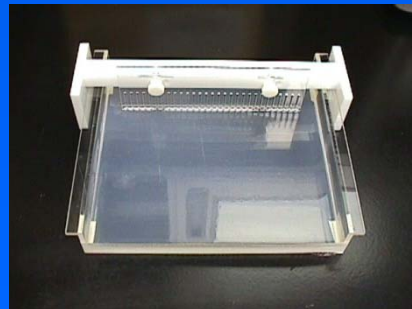
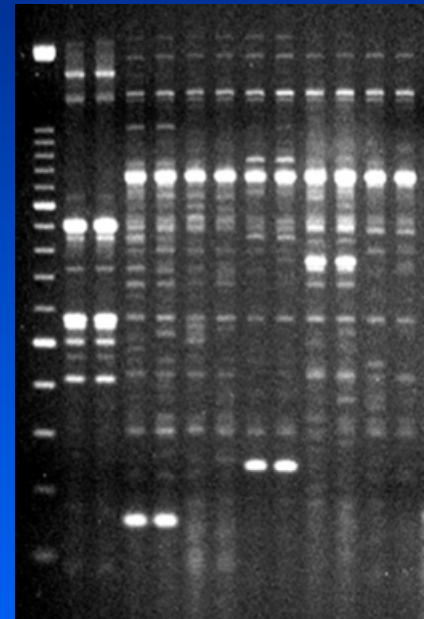
Texas State Soil and Water Conservation Board (TSSWCB)



BST Technique 1

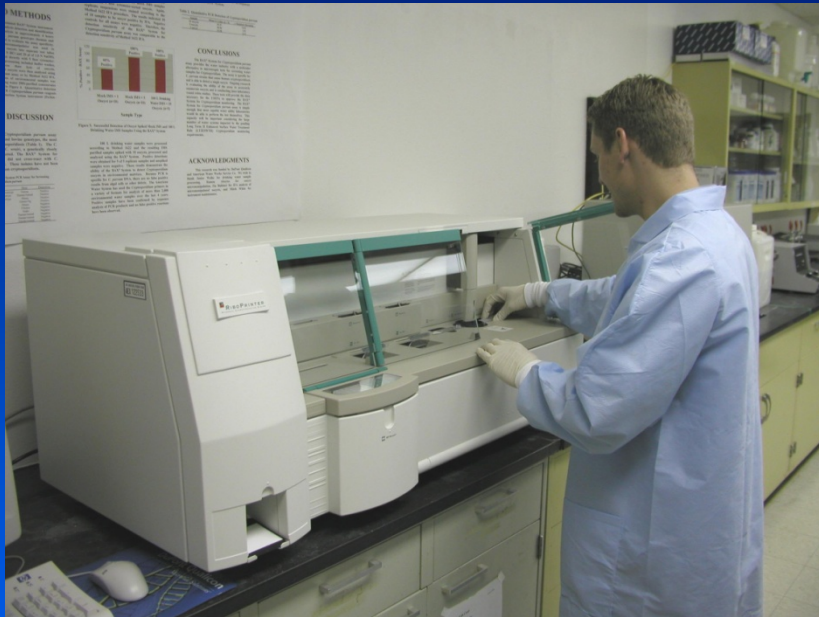
ERIC-PCR Fingerprinting

- Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR)
- Method of generating a “DNA Fingerprint” for each *E. coli* isolate
- Different strains of *E. coli* have different fingerprints

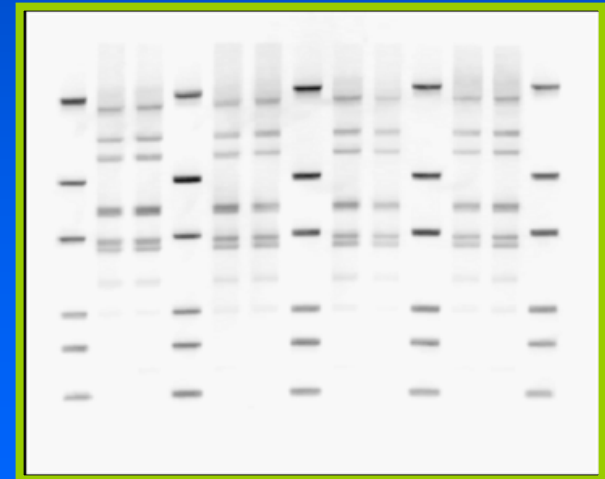


BST Technique 2

HindIII Automated Ribotyping



- Another DNA Fingerprinting Test
- Also Confirms Isolates as *E. coli*



Sample Number	Label	RiboGroup	Similarity to Selected 295-21-S-1	RiboPrint® Pattern				
				1 kbp	5	10	15	50
295-21-S-1	QC 101	HindIII 295-21-S-1	1.00					
295-21-S-2	QC 101	HindIII 295-21-S-1	0.98					
295-21-S-3	QC 101	HindIII 295-19-S-1	0.95					
295-21-S-4	QC 101	HindIII 295-21-S-1	0.97					
295-21-S-5	QC 101	HindIII 295-21-S-1	0.97					
295-21-S-6	QC 101	HindIII 295-21-S-1	0.96					
295-21-S-7	QC 101	HindIII 295-21-S-1	0.93					
295-21-S-8	QC 101	HindIII 295-21-S-1	0.94					

Texas *E. coli* BST Library (8-10)

