Bacterial Source Tracking
Identification of Fecal Pollution Sources Impacting Buck Creek

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There Are *E. coli* in the Water, But Where Did They Come From?

Develop Watershed Protection Plans

Can Bacterial Source Tracking (BST) be a tool?

Track fecal pollution sources using *E. coli*

Different guts → Different adaptations → Different *E. coli* strains →

Genetic Differences

Phenotypic Differences
There Are *E. coli* in the Water, But Where Did They Come From?

- BST - laboratory tests to determine if *E. coli* in water samples came from animal or human feces

- Most *E. coli* 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
Approach
Isolation of *E. coli* From Source and Water Samples

- *E. coli* isolation from samples using same media for compliance water monitoring
  - USEPA Method 1603 – modified mTEC medium
  - Confirmation of β-D-glucuronidase activity of isolates using NA-MUG (same as Colilert and Quanti-Tray)
  - No broth enrichment or clinical media - avoid selecting different populations of *E. coli*
Isolation of *E. coli* From Feces and Water

Fecal Specimens

Water Sample Filtered and Filter Placed on Modified mTEC Medium (EPA Method 1603)

Modified mTEC Medium

*E. coli* Colonies

Each *E. coli* colony is an “isolate”
E. coli 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
E. coli BST Technique 2

HindIII Automated RiboPrinting

- Another DNA fingerprinting test
- Also confirms isolates as E. coli
Data Analysis

- Applied Maths BioNumerics software
- Library accuracy - jackknife rates of correct classification (RCC) or average RCC (ARCC)
- Comparison between different BST techniques
  - Data from different BST techniques analyzed within defined parameters
  - Composite data sets
Data Analysis

Best Match Approach

- DNA fingerprints – Pearson correlation curve-based analyses
- “Best Match” approach with minimum similarity cutoff based on laboratory QC data
  - Water isolate must match library isolate ≥ minimum similarity or unidentified
  - Identification to single library isolate with highest similarity – max similarity approach
Best ERIC-PCR Match (96.9% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library

Best RiboPrint Match (95.8% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library
Data Analysis
Best Match Approach

No Match (Unidentified) Water Isolate, Best ERIC-PCR Match of only 82.4% Sim Library Isolate

No Match (Unidentified) Water Isolate, Best RiboPrint Match of only 65.9% Sim to Library Isolate
ERIC-RP Composite Data Sets

Minimum similarity for match

≥ 80% identical
Texas *E. coli* BST Library (ver. 10-09)

**Library Composition**

- **Wildlife** (374 samples) n=413
- **Human** (327 samples) n=377
- **Domestic animals** (344 samples) n=383

**Library Identification Accuracy**

- 1172 isolates from 1045 different human and animal fecal samples
- 87% ARCC

![Pie chart and bar graph showing the composition and identification accuracy of the library.](chart.png)
What are Bacteroidales?

- Human and animal fecal bacteria similar to *E. coli*
- Order *Bacteroidales* (or class *Bacteroidetes*) include several different genera and species of bacteria, including *Bacteroides* and *Prevotella* spp.
- Obligate anaerobes – difficult to grow and less likely to multiply in the environment
- More abundant in feces than *E. coli*

Many different *Bacteroidales* spp./strains shared between different animals and humans

Markers (PCR primers) developed to subgroups of *Bacteroidales* that appear host specific
Library Independent Screening of Pollution Sources Using *Bacteroidales* PCR

- Markers available for:
  - General marker (humans and animals)
  - Ruminants (cattle, deer, elk, sheep, llama)
  - Humans
  - Hogs (including feral hogs)
  - Horses

- Rapid and less expensive than library methods
- Multiple studies indicate approx. 90% specificity
- Only qualitative or semi-quantitative detection
- Limited markers for wildlife and birds
Sample Processing For *Bacteroidales* PCR

- Water samples for *E. coli* counts and *Bacteroidales* PCR collected at same time
- Water samples filtered similar to process for *E. coli* analysis
- DNA extracted from filtered water concentrate
- PCR testing for *Bacteroidales* PCR markers
  - Presence/absence detection
- Both viable and dead *Bacteroidales* bacteria are detected, and therefore both recent and older contamination detected
Bacteroidales PCR

Human Marker

Ruminant Marker
Hog/Feral Hog *Bacteroidales* PCR
BST for Buck Creek

- Water samples collected 2007-2009, mostly routine low-flow conditions
- 350 *E. coli* isolates from water
  - Identification using Texas Library, including some *E. coli* isolates from Buck Creek
  - 53 *E. coli* isolates from 28 Buck Creek known source samples, 31 isolates from the 28 source samples selected for library
- 79 water samples (10 to 20 per station) for *Bacteroidales* analyses
  - General marker – general indication of human and/or animal fecal pollution
  - Human marker
  - Hog marker – including feral hogs
  - Ruminant marker – cattle, deer, llamas, sheep
Results reported by station

- BC03 - CR 40; Collingsworth County
- BC05 - FM 1056; Collingsworth County
- BC06 - CR 110; Collingsworth County
- BC10A - SH 256; Childress County
- BC10C - SH 256; Childress County
- BC11 - US 83; Childress County
Considerations For Interpreting BST Results

- Identification of *E. coli* water isolates at each station presented as pie charts
  - Provides *an estimate* of pollution source contribution
  - 3-way split – human, wildlife (including feral hogs), domestic animals (includes livestock and pets)

- OK to compare *E. coli* results to *Bacteroidales* results, but remember not exactly same pollution source classifications
  - E.g. Domestic animals vs. ruminants, wildlife vs. ruminants/hogs

- *Bacteroidales* results reported as frequency of detection (presence/absence), but not abundance or level of marker present
Considerations For Interpreting BST Results

**Bacteroidales** PCR specificity typically 90%

**However**

- Human marker – occasional cross-reactivity with coyote and raccoon feces
- Ruminant marker – cross-reactivity with almost all hog/feral hog feces and occasionally with some other animals but not humans
**E. coli Source Identification**

- **Human**: 11% (n=5)
- **Domestic Animals**: 16% (n=7)
- **Wildlife**: 35% (n=24)
- **Unidentified**: 18% (n=8)

**Bacteroidales Marker Occurrence**

- **GenBac**: 100%
- **Hog**: 30%
- **Rum**: 90%
- **Hum**: 0%

**E. coli** geo. mean during BST sample collection = 8.4 CFU/100 ml
BST Results For Station BC05
FM 1056; Collingsworth County

**E. coli Source Identification**

- **Human**: 9% (n=6)
- **Domestic Animals**: 19% (n=19)
- **Wildlife**: 41% (n=48)
- **Unidentified**: 31% (n=30)

**Bacteroidales Marker Occurrence**

- **GenBac**: 100%
- **Hog**: 80%
- **Rum**: 60%
- **Hum**: 40%

n = 12

**E. coli** geo. mean during BST sample collection = 48.0 CFU/100 ml

- Highest occurrence of unidentified **E. coli**
- Frequent human **Bacteroidales** marker detection, but average human **E. coli** occurrence suggests pollution from distant source or significant but infrequent pollution
**BST Results For Station BC06**

**CR 110; Collingsworth County**

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**E. coli Source Identification**

- **Wildlife**: 65% (n=45)
- **Unidentified**: 14% (n=10)
- **Domestic Animals**: 14% (n=10)
- **Human**: 7% (n=5)

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**Bacteroidales Marker Occurrence**

- **GenBac**: 100%
- **Hog**: 40%
- **Rum**: 80%
- **Hum**: 10%

- **E. coli** geo. mean during BST sample collection = 24.8 CFU/100 ml

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**n = 11**
E. coli Source Identification

- Wildlife: 55% (n=42)
- Unidentified: 17% (n=13)
- Domestic Animals: 17% (n=13)
- Human: 11% (n=8)

Bacteroidales Marker Occurrence

- GenBac: 100%
- Hog: 80%
- Rum: 40%
- Hum: 0%

n = 14

E. coli geo. mean during BST sample collection = 40.8 CFU/100 ml
**BST Results For Station BC10C**  
**SH 256; Childress County**

**E. coli Source Identification**

- Unidentified: 18% (n=12)
- Human: 18% (n=12)
- Domestic Animals: 12% (n=8)
- Wildlife: 52% (n=36)

**E. coli geo. mean during BST sample collection = 18.9 CFU/100 ml**

- High occurrence of human *E. coli* and frequent human *Bacteroidales* marker detection suggest frequent pollution

- **However**, low geo. mean levels of *E. coli*, so not likely a significant pollution load
BST Results For Station BC11
US 83; Childress County

**E. coli Source Identification**

- **Wildlife**: 62% (n=44)
- **Unidentified**: 9% (n=6)
- **Human**: 19% (n=7)
- **Domestic Animals**: 19% (n=13)

**Bacteroidales Marker Occurrence**

- GenBac: 100%
- Hog: 40%
- Rum: 20%
- Hum: 0%

\[ n = 12 \]

**E. coli geo. mean during BST sample collection = 14.1 CFU/100 ml**
Summary of BST Results

- Approximately 50% of fecal pollution is derived from wildlife, including feral hogs and deer.

- Domestic animals/livestock pollution contributing approximately 20% of fecal pollution, and high frequency of ruminant marker observed across all stations.
  - Continue efforts to minimize livestock impacts, since this pollution source may be more feasibly controlled than wildlife.

- Stations BC05 and BC10C need further investigation to identify sources of human pollution.
  - Need to investigate possible sources near Station BC05 – reunion center, illegal dumping?
  - Strong evidence for human fecal pollution at Station 10C – role of coyotes and raccoons?

- Knowledge and input of stakeholders valued!