Bacterial Source Tracking Identification of Fecal Pollution Sources Impacting Buck Creek

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Acknowledgments

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    Joy Truesdale
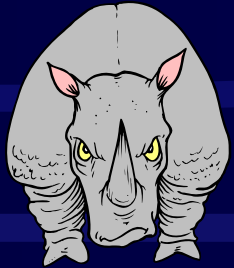
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    Phyllis Dyer

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

Develop Watershed Protection Plans

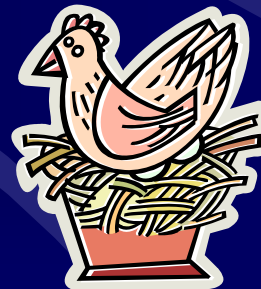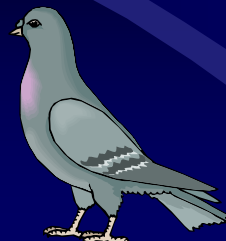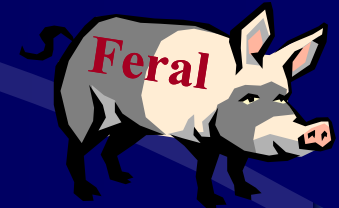**Can Bacterial Source Tracking (BST) be a tool?**

Track fecal pollution sources using *E. coli*

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

Genetic Differences

Phenotypic Differences

Feral

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**

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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***
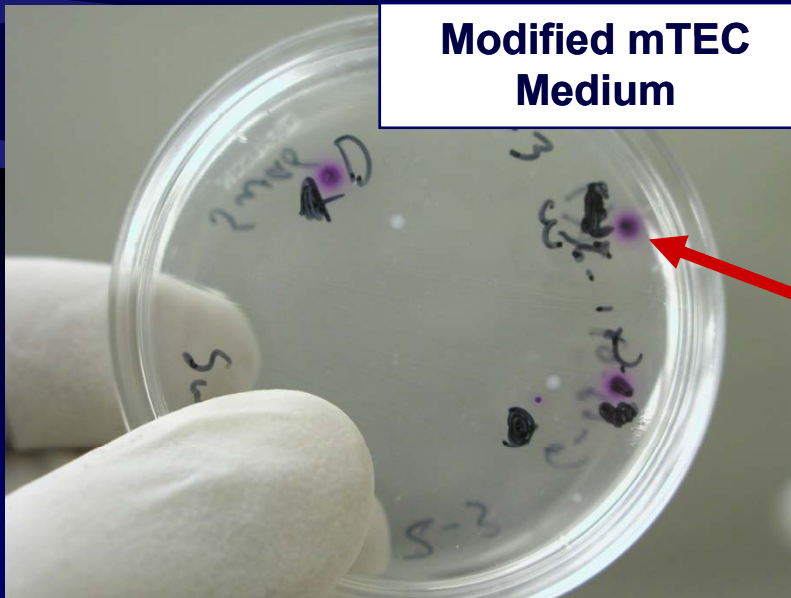
Isolation of *E. coli* From Feces and Water

Fecal Specimens

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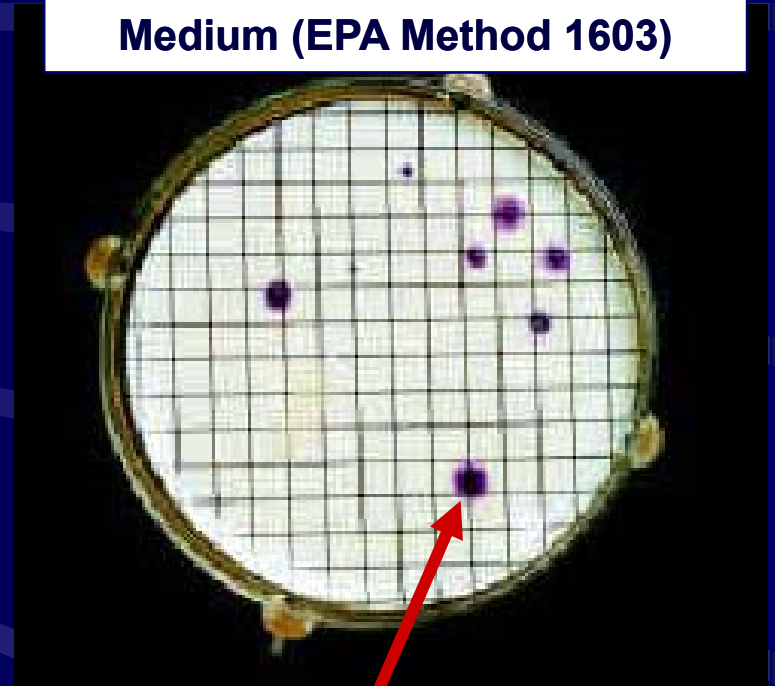
**Modified mTEC Medium**

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
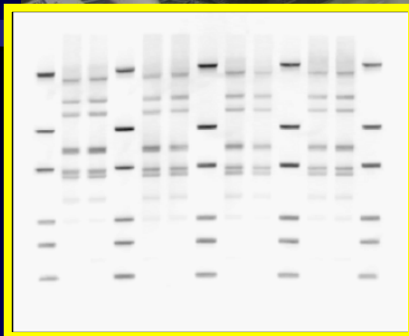- Different strains of *E. coli* have different fingerprints

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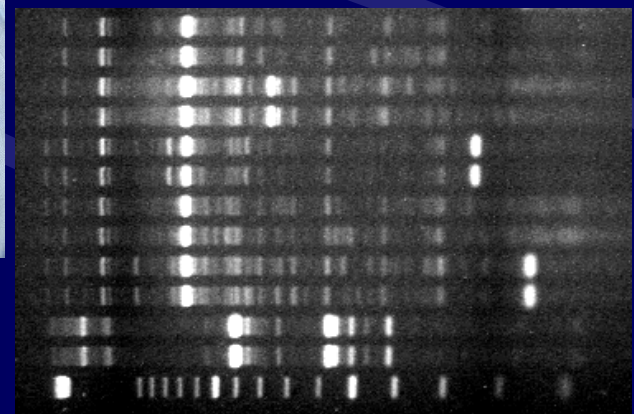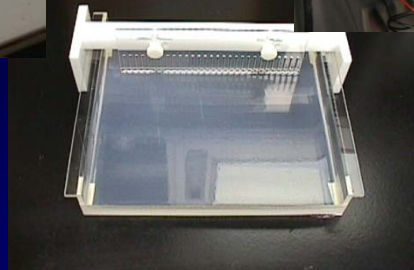
E. coli BST Technique 2
*Hin*dIII Automated RiboPrinting

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

Sample Number	Label	RiboGroup	Similarity to Selected 295-21-S-1	RiboPrint ® Pattern
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	

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**

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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
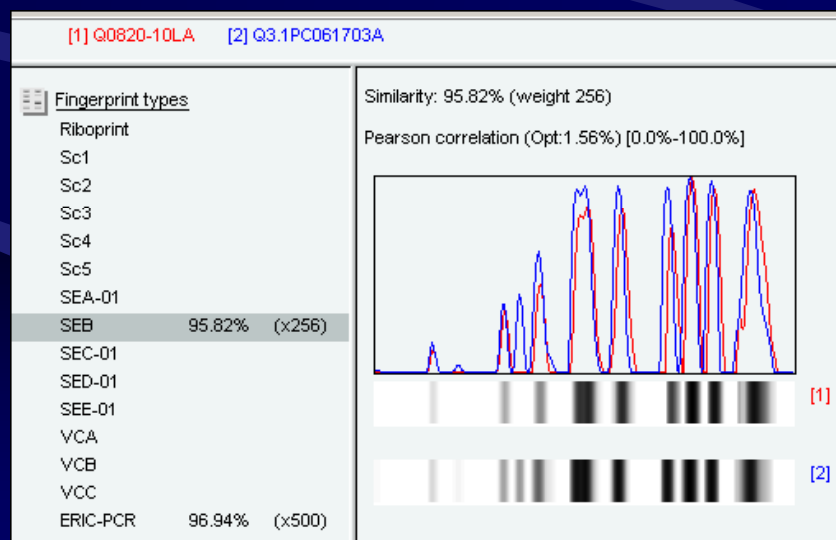
Data Analysis
Best Match Approach

Best ERIC-PCR Match (96.9% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library
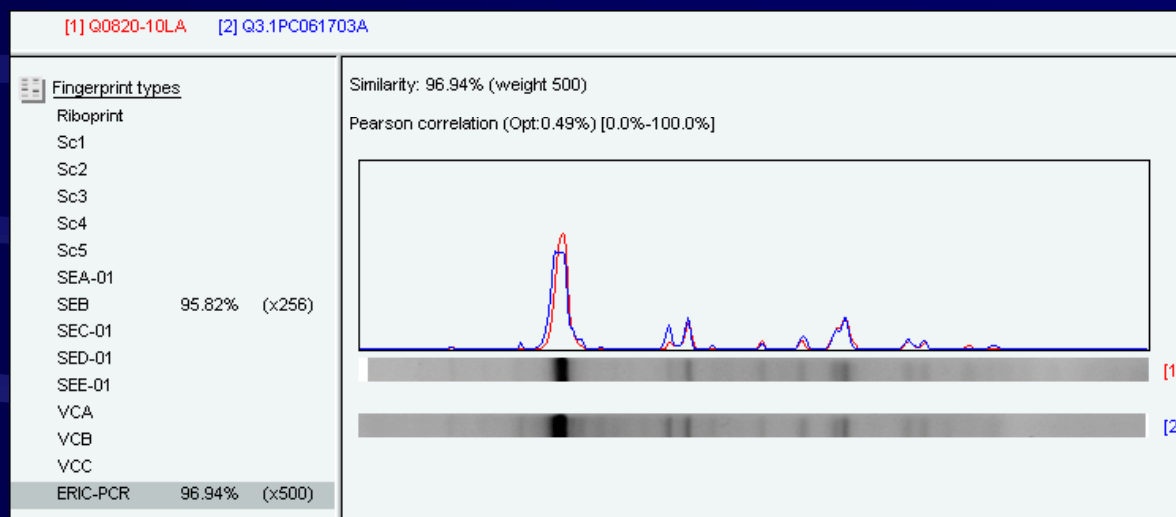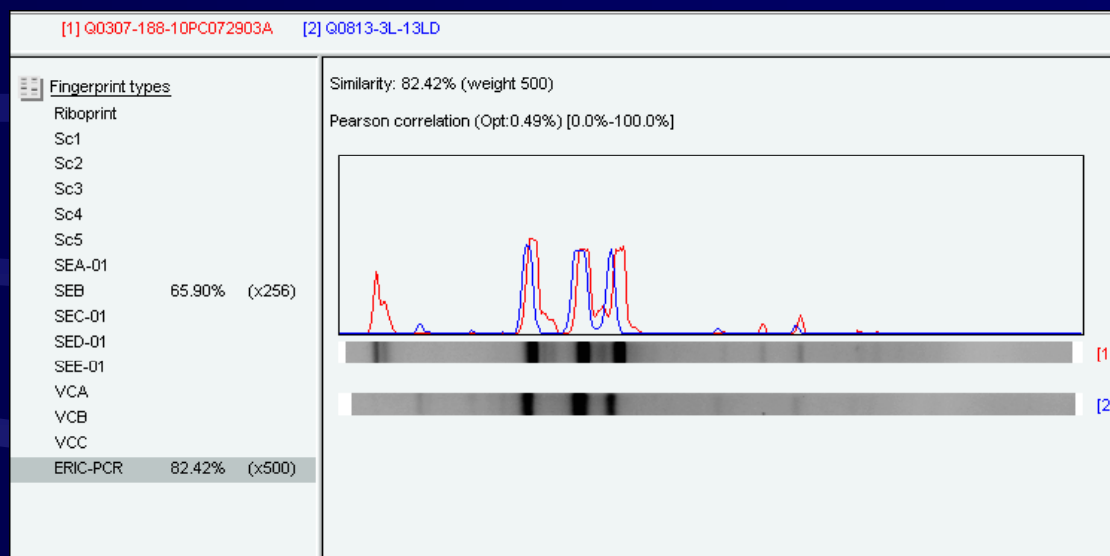

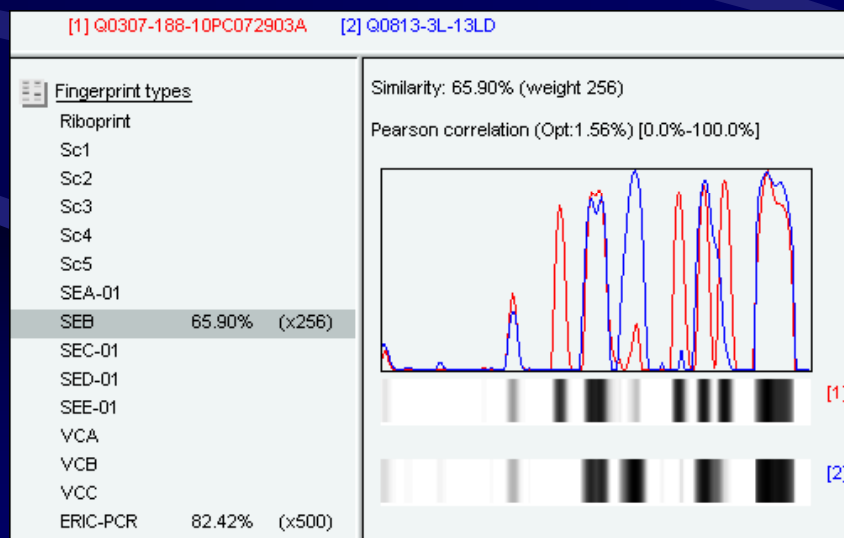**Best RiboPrint Match (95.8% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library**

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Data Analysis
Best Match Approach

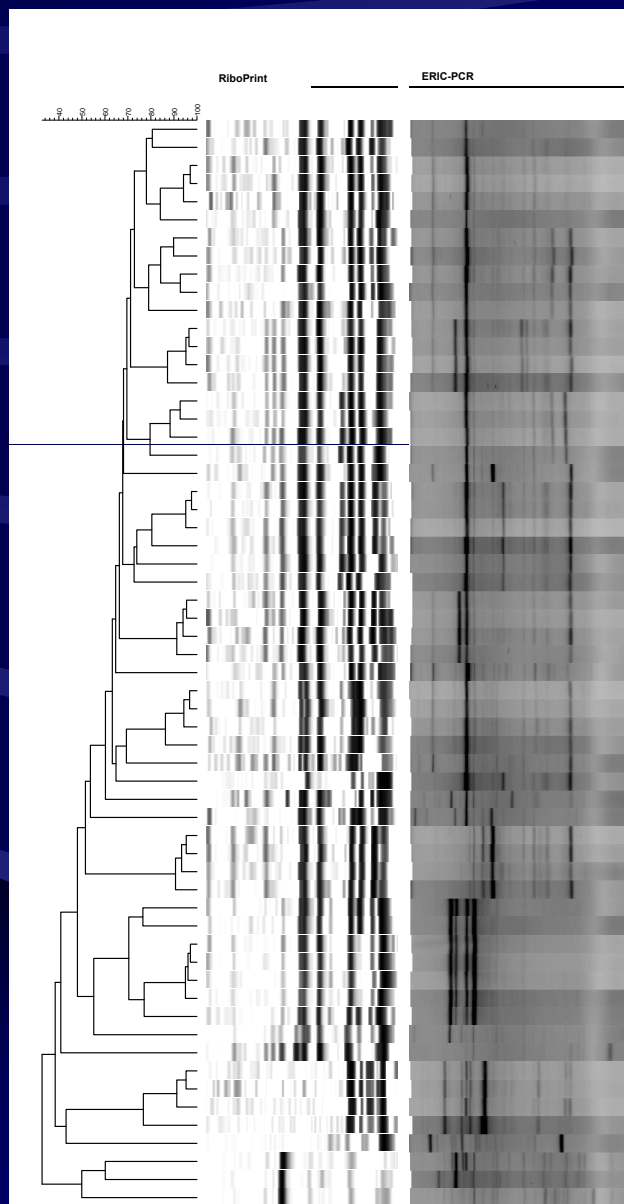**No Match (Unidentified) Water Isolate, Best ERIC-PCR Match of only 82.4% Sim Library Isolate**

[1] Q0307-188-10PC072903A [2] Q0813-3L-13LD

Fingerprint types
Riboprint
Sc1
Sc2
Sc3
Sc4
Sc5
SEA-01
SEB   65.90%   (x256)
SEC-01
SED-01
SEE-01
VCA
VCB
VCC
ERIC-PCR   82.42%   (x500)

Similarity: 82.42% (weight 500)

Pearson correlation (Opt:0.49%) [0.0%-100.0%]

[1]
[2]

No Match (Unidentified) Water Isolate, Best RiboPrint Match of only 65.9% Sim to Library Isolate

[1] Q0307-188-10PC072903A [2] Q0813-3L-13LD

Fingerprint types
Riboprint
Sc1
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SEE-01
VCA
VCB
VCC
ERIC-PCR   82.42%   (x500)

Similarity: 65.90% (weight 256)

Pearson correlation (Opt:1.56%) [0.0%-100.0%]
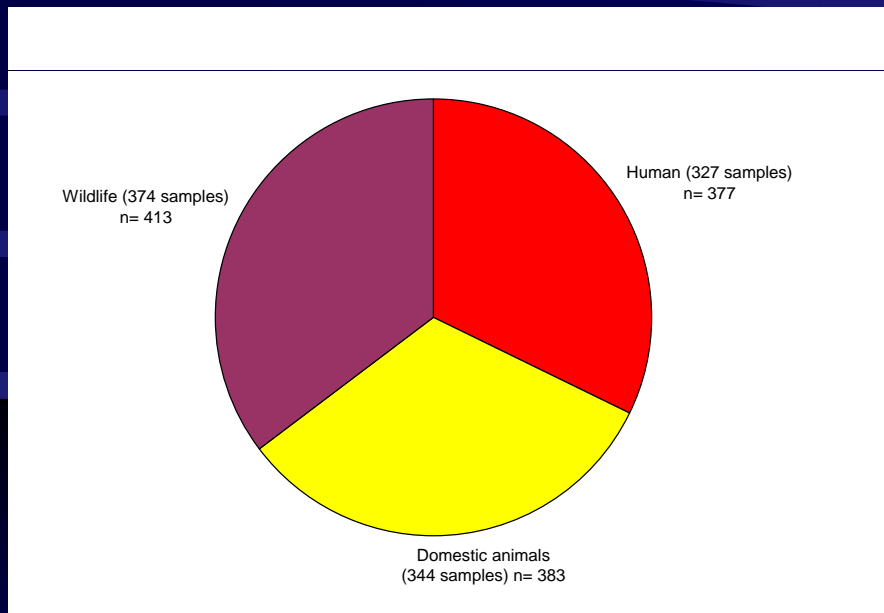
[1]
[2]

ERIC-RP Composite Data Sets

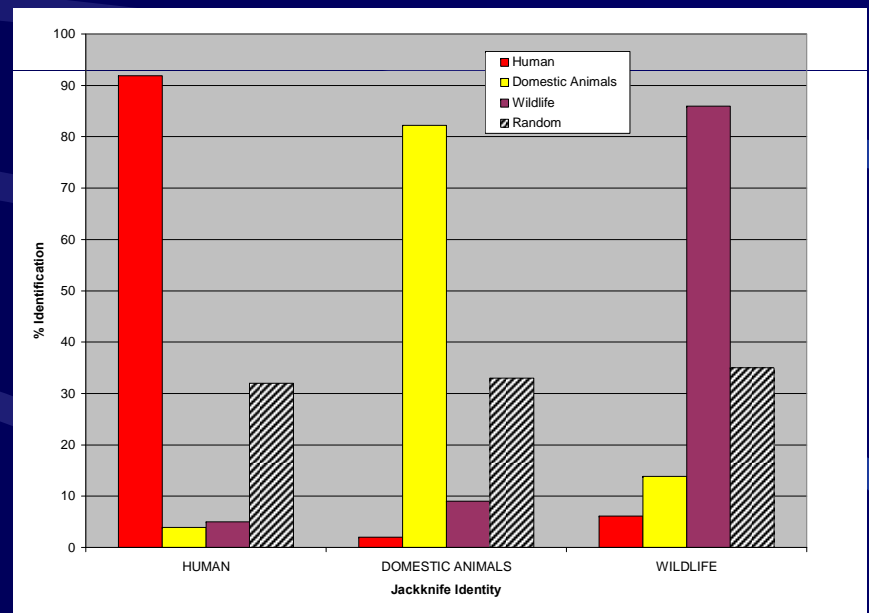**Minimum similarity for match**

≥ 80% identical

Texas *E. coli* BST Library
(ver. 10-09)

Library Composition

1172 isolates from 1045 different human and animal fecal samples

Library Identification Accuracy

87% ARCC

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Library Independent Screening of Pollution Sources Using *Bacteroidales* PCR

U **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***

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

U **Markers (PCR primers) developed to subgroups of *Bacteroidales* that appear host specific**

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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**

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# Sample Processing For *Bacteroidales* PCR

- Ʊ **Water samples for *E. coli* counts and *Bacteroidales* PCR collected at same time**

- Ʊ **Water samples filtered similar to process for *E. coli* analysis**

- Ʊ **DNA extracted from filtered water concentrate**

- Ʊ **PCR testing for *Bacteroidales* PCR markers**
  - ◊ **Presence/absence detection**

- Ʊ **Both viable and dead *Bacteroidales* bacteria are detected, and therefore both recent and older contamination detected**
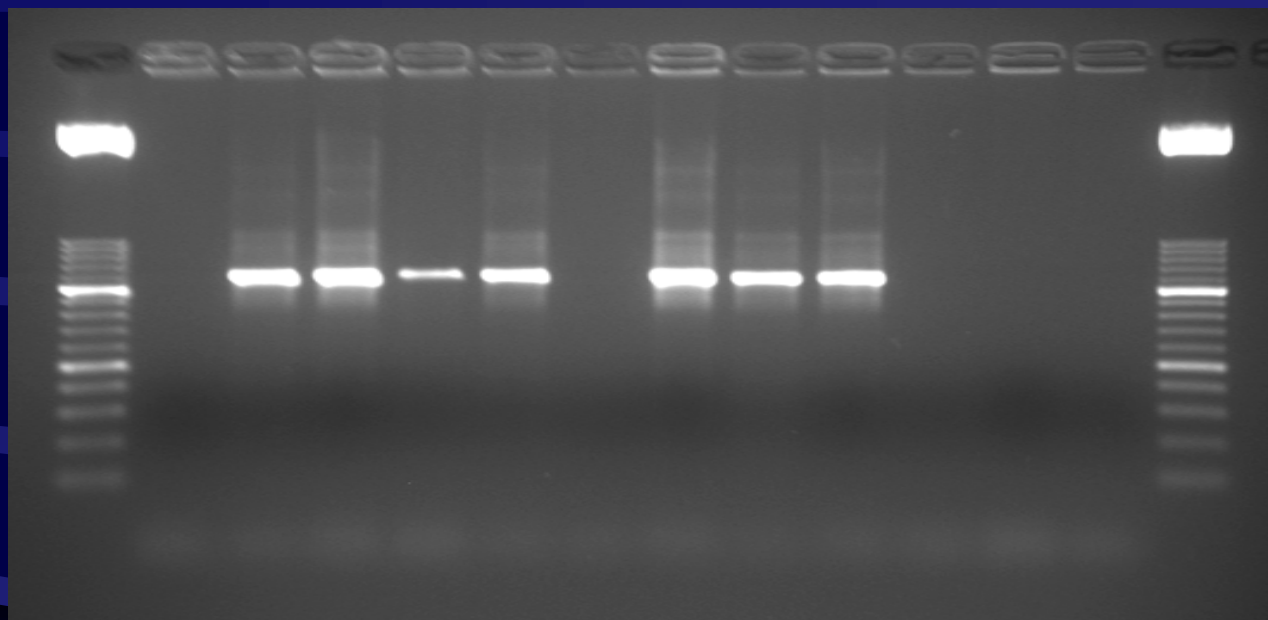
Bacteroidales PCR

Human Marker

Ruminant Marker

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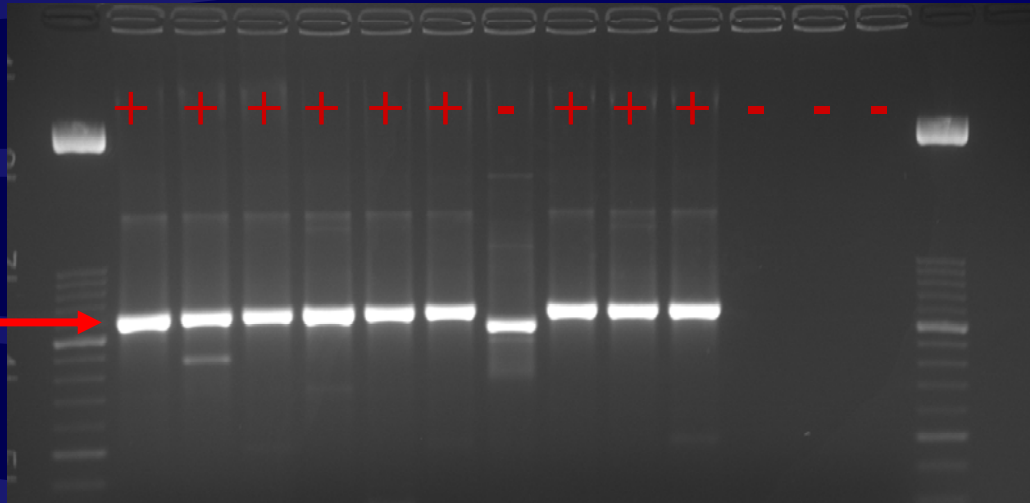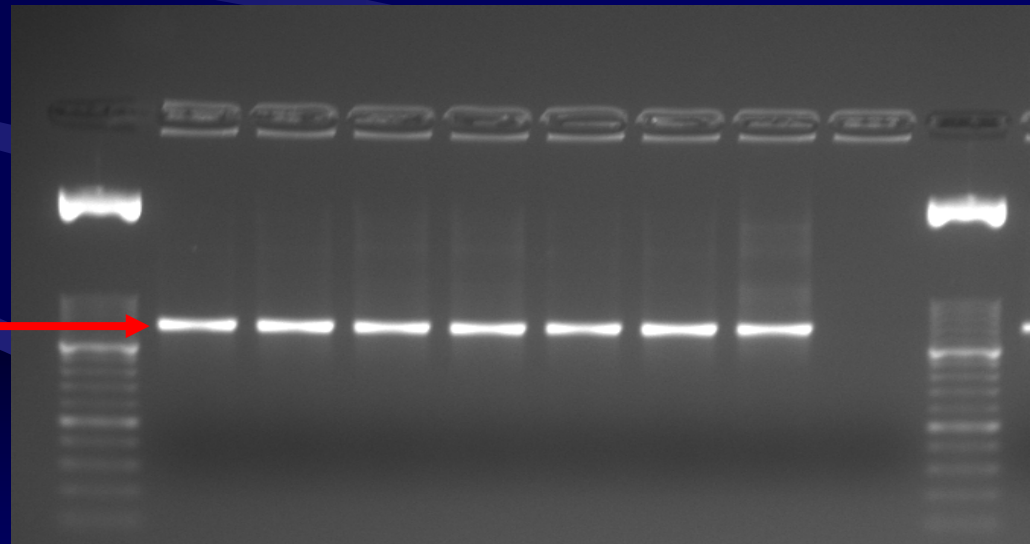
Hog/Feral Hog *Bacteroidales* PCR

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

BST for Buck Creek

Ʊ **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

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Buck Creek Sampling Sites
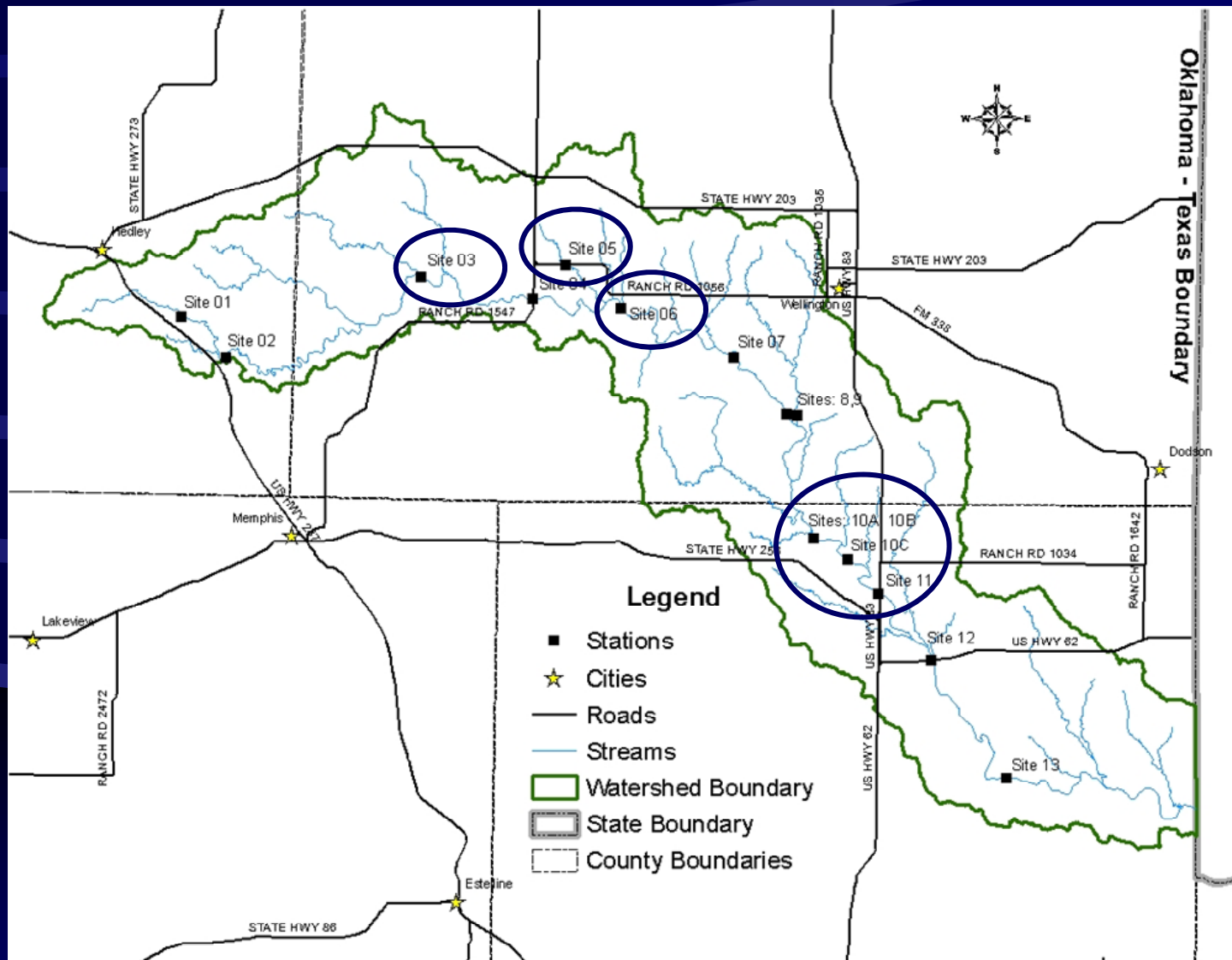
Considerations For Interpreting BST Results

U **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)**

U **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**

U ***Bacteroidales* results reported as frequency of detection (presence/absence), but not abundance or level of marker present**

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Considerations For Interpreting BST Results

U *Bacteroidales* **PCR specificity typically 90%**

U *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**

BST Results For Station BC03
CR 40; Collingsworth County
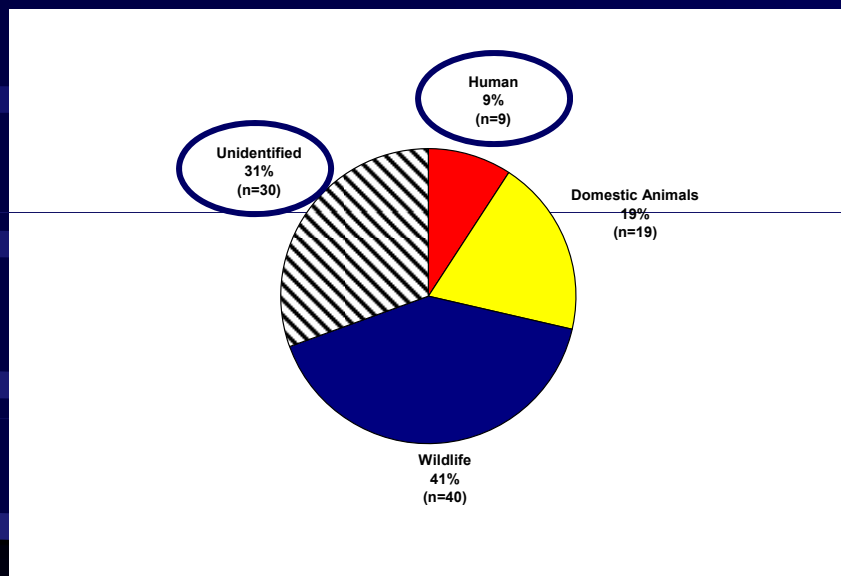
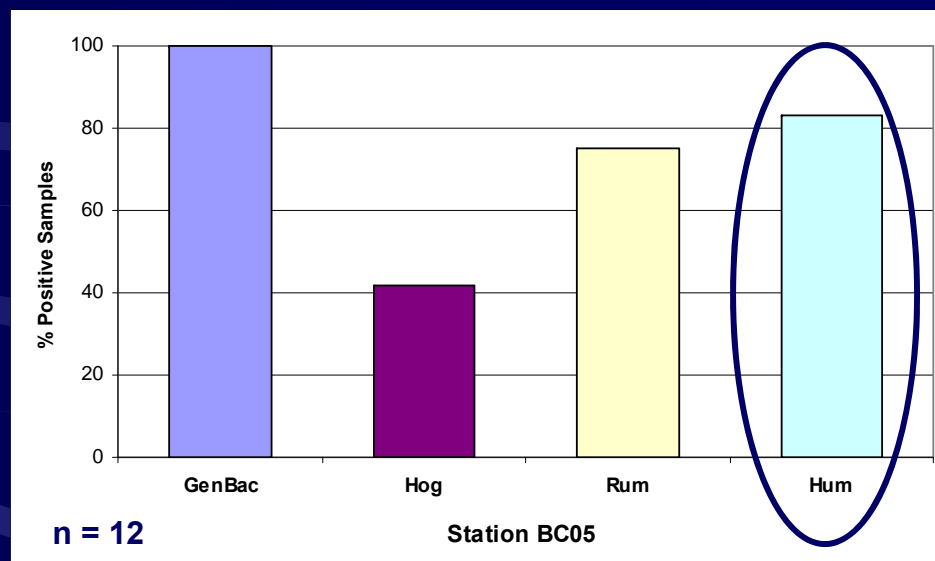**E. coli Source Identification**
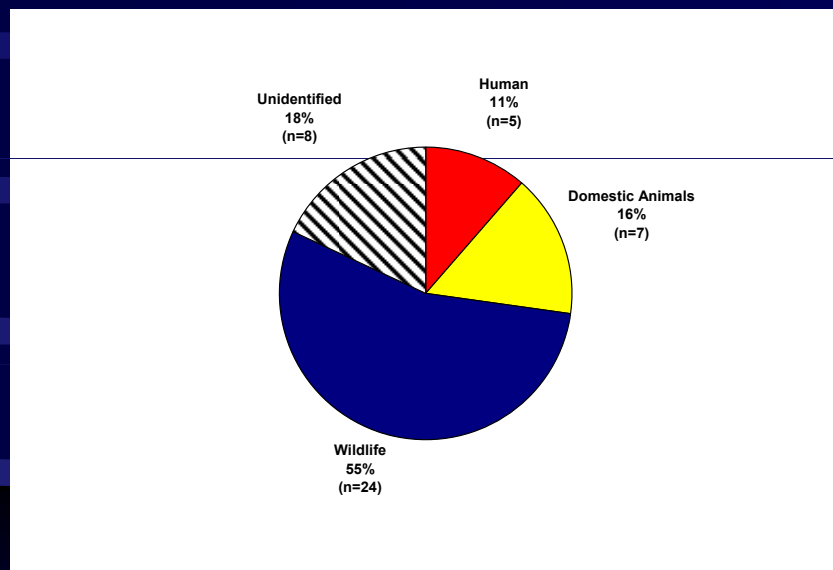
Bacteroidales Marker Occurrence
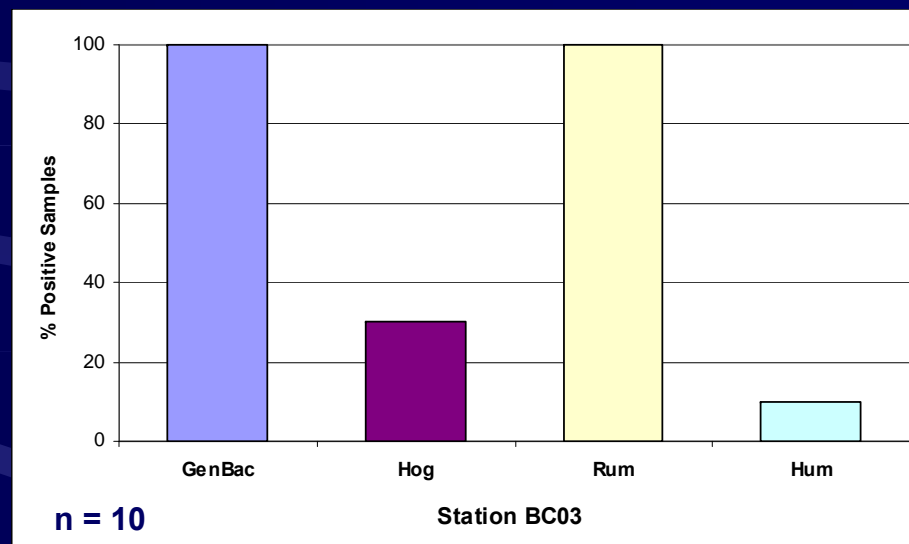
E. coli geo. mean during BST sample collection = 8.4 CFU/100 ml

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BST Results For Station BC05
FM 1056; Collingsworth County

E. coli Source Identification

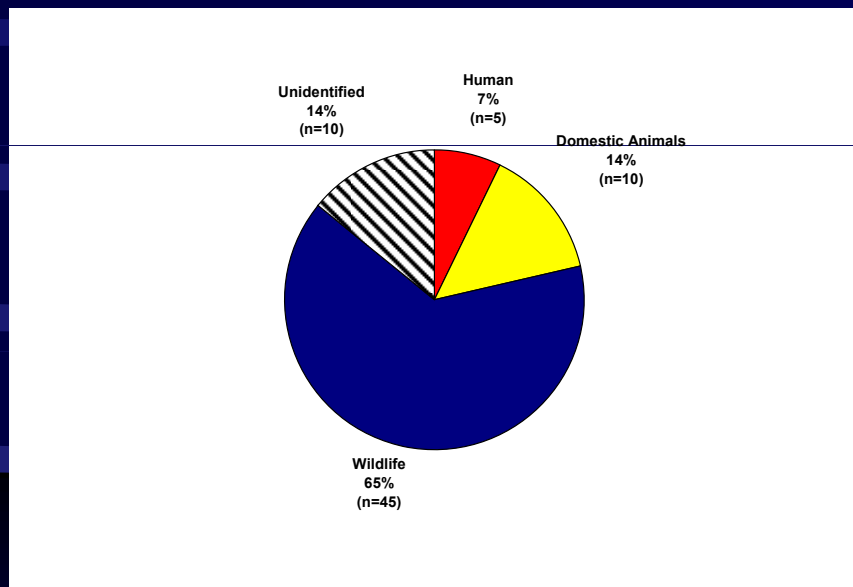## *Bacteroidales* Marker Occurrence

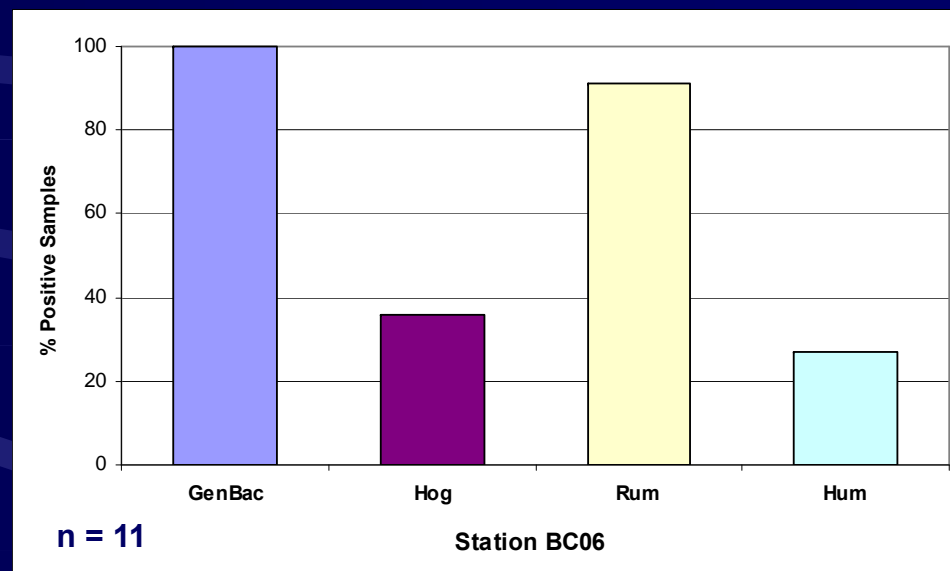*E. coli* geo. mean during BST sample collection = 48.0 CFU/100 ml

- 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

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BST Results For Station BC06
CR 110; Collingsworth County

E. coli Source Identification

Unidentified
14%
(n=10)

Human
7%
(n=5)

Domestic Animals
14%
(n=10)

Wildlife
65%
(n=45)

Bacteroidales Marker Occurrence

% Positive Samples

GenBac   Hog   Rum   Hum

n = 11

Station BC06

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

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BST Results For Station BC10A
SH 256; Childress County

E. coli Source Identification

Unidentified 17% (n=13)

Human 11% (n=8)

Domestic Animals 17% (n=13)

Wildlife 55% (n=42)

Bacteroidales Marker Occurrence

n = 14
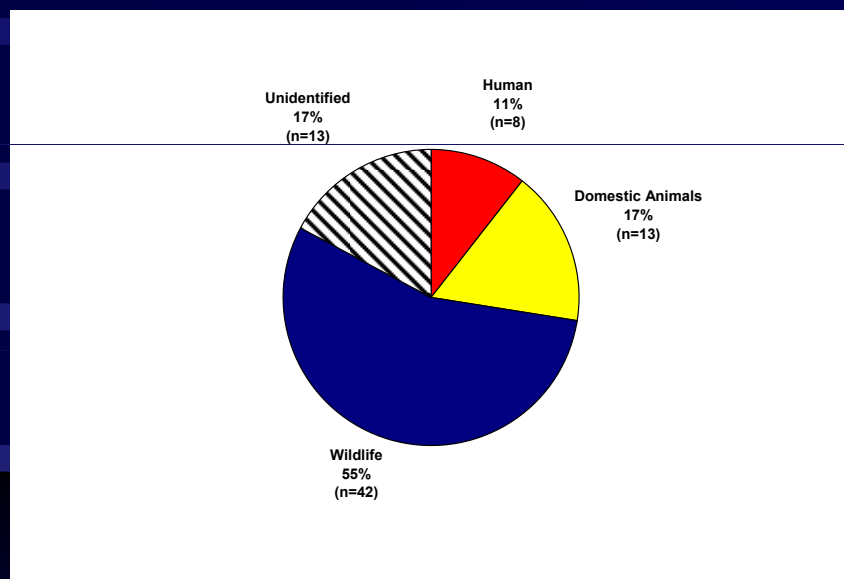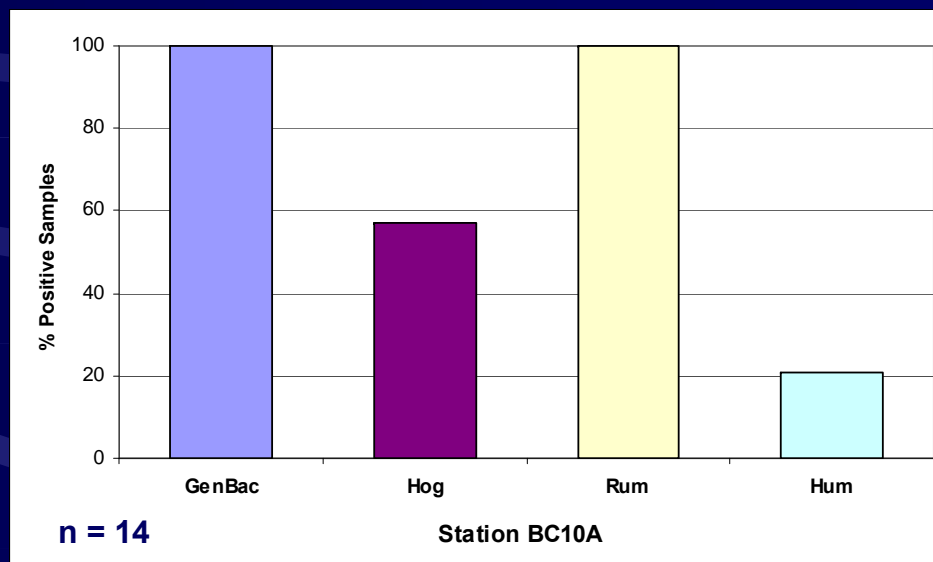
Station BC10A

E. coli geo. mean during BST sample collection = 40.8 CFU/100 ml

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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)

Bacteroidales Marker Occurrence

% Positive Samples

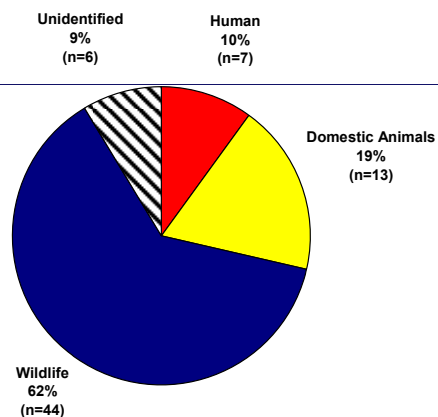GenBac   Hog   Rum   Hum

Station BC10C

n = 20
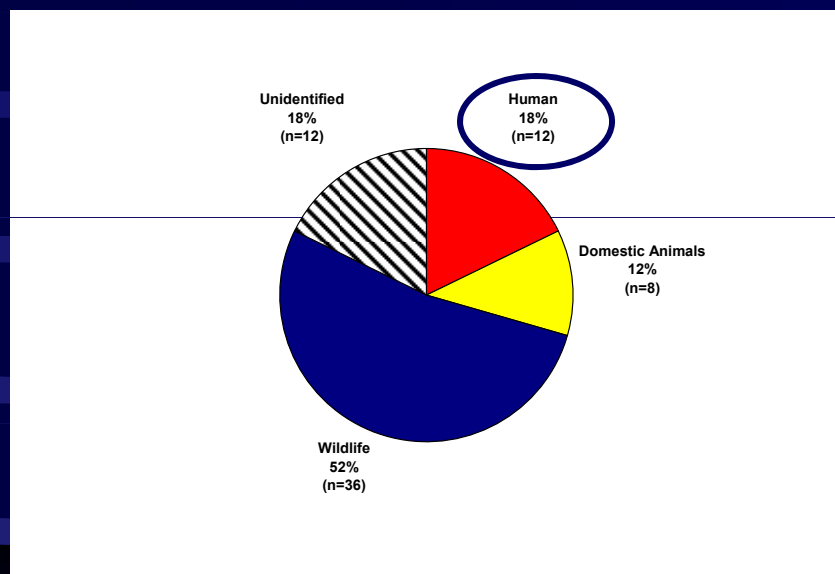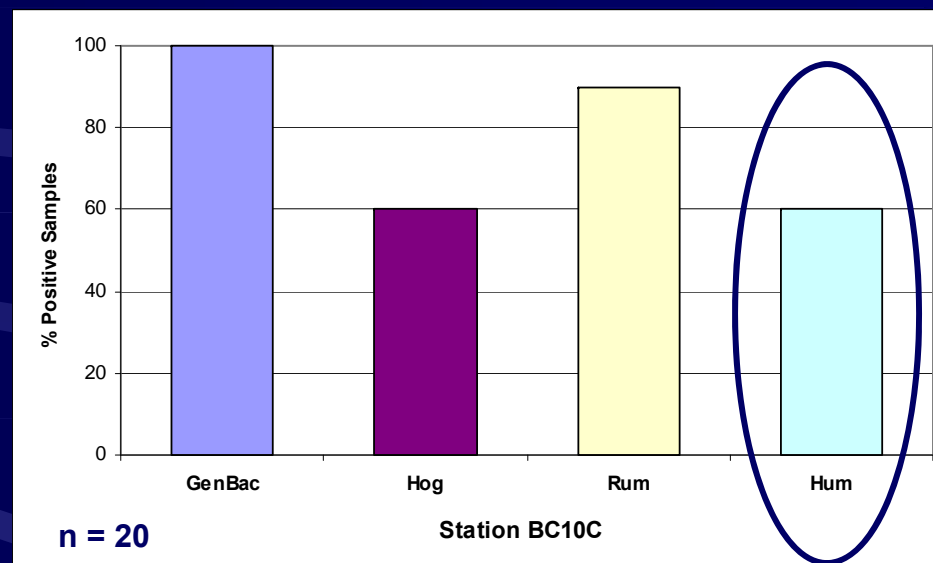
E. coli geo. mean during BST sample collection = 18.9 CFU/100 ml

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

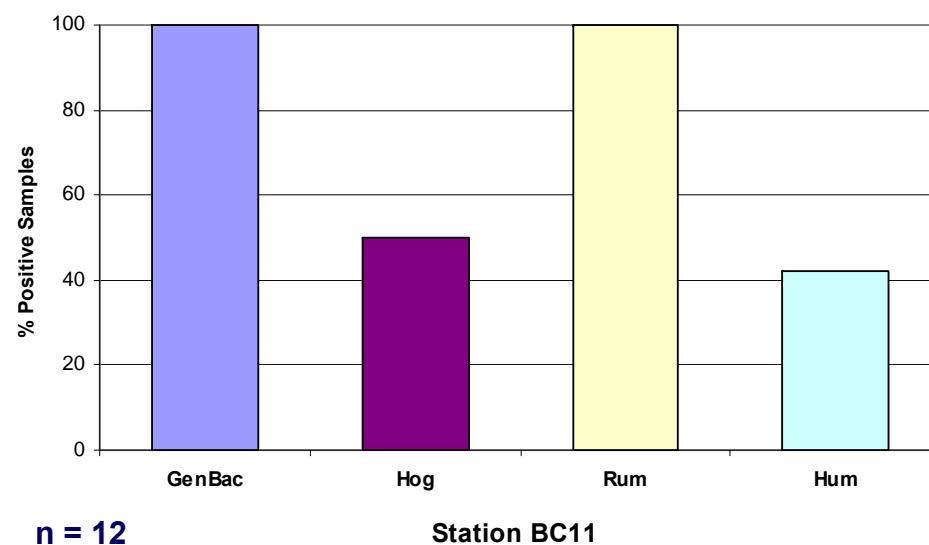- *However*, low geo. mean levels of *E. coli*, so not likely a significant pollution load

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BST Results For Station BC11
US 83; Childress County

E. coli Source Identification

Unidentified
9%
(n=6)

Human
10%
(n=7)

Domestic Animals
19%
(n=13)

Wildlife
62%
(n=44)

Bacteroidales Marker Occurrence

% Positive Samples

GenBac   Hog   Rum   Hum

n = 12   Station BC11

E. coli geo. mean during BST sample collection = 14.1 CFU/100 ml

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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!*

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