Fate and transport of *E. coli* in rural Texas streams & landscapes

Texas Watershed Coordinator Roundtable
Bacteria Dynamics, Assessment Methods, and BMPs

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Austin, Texas - July 27, 2011
Cedar Creek watershed
Area ~ 131 Sq. miles
Objectives

- Identify and characterize fecal contamination sources in Cedar Creek watershed and quantify the *E. coli* concentration from the sources
- Monitor survival, growth, and re-growth of *E. coli* under different environmental (temperature and moisture) conditions
Total number of fecal samples analyzed:

- Raccoons: 221
- White-tailed deer: 10
- Opossums: 140
- Feral Hogs: 41
- Cows: 34

By trapping animals or collecting fresh feces from two sub-watersheds.
Average *E. coli* concentrations (log CFU/g) in different feces

- **White-tailed deer** (n=10):
  - R: $1.40 \times 10^4 - 5.60 \times 10^7$
  - M: $3.75 \times 10^5$

- **Feral hog** (n=41):
  - R: $2.40 \times 10^5 - 4.10 \times 10^9$
  - M: $1.18 \times 10^7$

- **Raccoon** (n=221):
  - R: $5.00 \times 10^2 - 4.30 \times 10^{11}$
  - M: $4.50 \times 10^7$

- **Virginia opossum** (n=140):
  - R: $1.00 \times 10^2 - 1.21 \times 10^{11}$
  - M: $6.55 \times 10^7$

- **Cow** (n=34):
  - R: $1.00 \times 10^2 - 1.20 \times 10^7$
  - M: $2.43 \times 10^4$
Summer:
Average *E. coli* concentrations (Log CFU/g)

*Note: summer months from March through September with an average mean temperature ranging from 60 – 80 degrees F*

**White-tailed deer (n=6)**

R = $1.40 \times 10^4 - 5.60 \times 10^7$

M = $3.75 \times 10^5$

**Feral hog (n=21)**

R = $2.40 \times 10^5 - 4.30 \times 10^7$

M = $6.65 \times 10^7$

**Raccoon (n=104)**

R = $5.03 \times 10^5$

M = $8.10 \times 10^7$

**Virginia opossum (n=64)**

R = $1.00 \times 10^5$

M = $1.21 \times 10^7$

**Cow (n=25)**

R = $1.00 \times 10^2$

M = $2.93 \times 10^5$

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Winter:
Average *E. coli* concentrations (Log CFU/g)

*Note: winter months from October through February with an average mean temperature ranging from 53 – 70 degrees F*
# Statistics

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raccoon vs. Opossum</td>
<td>Not significant</td>
</tr>
<tr>
<td>Raccoon vs. Hog</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Raccoon vs. Deer</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Raccoon vs. Cow</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Opossum vs. Hog</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Opossum vs. Deer</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Opossum vs. Cow</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Hog vs. Deer</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Hog vs. Cow</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Deer vs. Cow</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Species vs. Season</td>
<td>Significant at 95% CI</td>
</tr>
<tr>
<td>Interaction between Species and Season</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

* Data was normalized through log transformation
*E. coli* (in feces) survival in Creek water: Results

Study 1 ("Long term" – 1 week)
E. coli (in feces) survival in water – Study 1

- Three random fecal samples from each species
  - 10 g fecal material added to 1000 mL autoclaved creek water
  - pH ~ 7
  - Divided into 4 parts (~ 250 mL)
  - Kept at four different temperatures (0°C, 10°C, 20°C, & 50°C)
  - E. coli concentration enumerated after 60 min (Day 0), 24 h (Day 1), 72 h (Day 3), 120 h (Day 5) & 168 h (Day 7)
= 180 plate counts
~ 200 plates
X
5 species
Psychrophile  Mesophile  Thermophile

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Psychrophile</th>
<th>Mesophile</th>
<th>Thermophile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 °C</td>
<td>1.39E+05</td>
<td>1.20E+05</td>
<td>1.47E+05</td>
</tr>
<tr>
<td>10 °C</td>
<td>1.34E+05</td>
<td>2.72E+08</td>
<td>0</td>
</tr>
<tr>
<td>20 °C</td>
<td>1.29E+05</td>
<td>1.70E+05</td>
<td>0</td>
</tr>
<tr>
<td>50 °C</td>
<td>1.34E+05</td>
<td>2.72E+08</td>
<td>0</td>
</tr>
</tbody>
</table>

E. coli (Log CFU/100ml)

Day 0  Day 1  Day 3  Day 5  Day 7

No Growth
$E. coli$ (Log CFU/100ml) vs. Time (h) for different species at 0°C.
The diagram shows the change in E. coli (Log CFU/100ml) over time (h) for different species at 10°C. The species include Cow, Raccoon, Opossum, Feral Hog, and Deer. The y-axis represents the log of CFU per 100ml, ranging from 1.00E+00 to 1.00E+10, while the x-axis represents time (h) ranging from 0 to 180.
Graph showing the survival of E. coli (Log CFU/100ml) at 50°C for different animals: Cow, Raccoon, Opossum, Feral Hog, and Deer.
E. coli growth/decay in Creek water: Results

Study 2 ("short term" – 1 day)
E. coli growth/decay in water – Study 2

- E. coli isolates from three random fecal samples of each species
  - E. coli isolates were spiked to autoclaved creek water
  - 100 mL vials were kept at four different temperatures (0°C, 10°C, 25°C, & 35°C) in triplicates.
  - E. coli concentration enumerated after 0, 60, 120, 300, 600, & 1440 min.
<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Count</td>
<td>216</td>
<td>220</td>
<td>215</td>
<td>220</td>
<td>225</td>
<td>220</td>
</tr>
<tr>
<td>Species</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

= 216 plate counts
~ 240 plates

3 replicates

2 species
E. coli (Log CFU/100ml)

Time (h)

35°C
E. coli (Log CFU/100ml)

Time (h)

25°C
E. coli (Log CFU/100ml)

Time (h)

35°C
E. coli growth/decay in water

- What do the results mean? at 0°C

4 2.6 hours
at 10°C

2.4 hours

3.6 hours

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at 25°C

2.0  1.8 hours
at 35°C

1.0 1.0 hours
E. coli survival in soil: Results

Study 1 (“Long term” – 1 week)
**E. coli** survival in Soil – Study 1

- **E. coli** strains isolated from the same samples
  - Isolates cultured in LB Broth for 24 h
  - One mL added to 30 g over dried and autoclaved Cedar Creek soil (sandy loam soil)
  - Sterile de-ionized water added to each 30 g soil + 1 ml broth mixture create four moisture conditions (4%, 25%, 57%, and 83%)
- Stored at room temperature
- **E. coli** concentration enumerated at after 60 min (Day 0), 24 h (Day 1), 72 h (Day 3), 120 h (Day 5) & 168 h (Day 7)
<table>
<thead>
<tr>
<th>day</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
</tr>
<tr>
<td>20%</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
</tr>
<tr>
<td>50%</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
</tr>
<tr>
<td>75%</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
<td>◦</td>
</tr>
</tbody>
</table>

= 180 plate counts
~ 200 plates

X

3 replicates

X

2 species
Aerobic Facultative Anaerobic

E. coli (Log CFU/100ml)

Day 0 · Day 1 · Day 3 · Day 5 · Day 7
Aerobic Facultative Anaerobic

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Aerobic</th>
<th>Facultative</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>1.29E+05</td>
<td>2.18E+07</td>
<td>5.00E+06</td>
</tr>
<tr>
<td>20%</td>
<td>5.70E+08</td>
<td>5.08E+09</td>
<td>2.00E+04</td>
</tr>
<tr>
<td>50%</td>
<td>1.55E+09</td>
<td>1.56E+09</td>
<td>5.70E+08</td>
</tr>
<tr>
<td>75%</td>
<td>3.08E+09</td>
<td>3.04E+09</td>
<td>1.55E+09</td>
</tr>
</tbody>
</table>

E. coli (Log CFU/100ml)

Day 0  Day 1  Day 3  Day 5  Day 7
E. coli survival in soil

- What do the results mean? at 4% moisture

0.8 days 1.2 days
at 25% moisture

1.4 1 days
at 57% moisture

1.8 days

1.1 days
at 83% moisture

1.8 days

1.6 days
Acknowledgment

- Graduate students:
  - Reema Padia (MS, completed)
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  - Bailey Sullivan (Ph.D.,)

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