Influence of Selected Land Application Strategies on the Fate and Transport of Antimicrobials and Antimicrobial Resistance Genes

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Project Team

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- United States Department of Agriculture
  - Dr. John Gilley
  - Dr. Bryan Woodbury
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Background
Antimicrobials and Antimicrobial Resistance

- Antimicrobials in livestock production
  - Therapeutic level: disease treatment
  - Sub-therapeutic level: prophylaxes and growth promotion
  - A substantial amount not absorbed and released with wastes

- Antimicrobial resistance
  - Emerging in hospitals
  - Emerging in the livestock gut and the environment
  - Commensal and pathogenic bacteria
  - Antimicrobial resistance infections: $20 billion in excess health care costs and 8 million additional hospital days

![Diagram showing antimicrobial pressure and relative abundance of resistant bacteria]
Animal production and crop production in Nebraska
Motivation

- Runoff from fields where manure is land applied can contain antimicrobials and antimicrobial resistance genes (ARG)
- Limited studies investigating the influence of land application strategy and mitigation strategies such as grass buffer strips on antimicrobial and ARG transport
- Limited studies have investigated the fate of both antimicrobial and ARG concurrently
Research Objectives

(1) Evaluate the fate of antimicrobial and ARG after land application of swine manure as a function of land application method

(1) Evaluate removal of antimicrobial and ARG in runoff by a narrow grass hedge
AB and ARGs in runoff – study design

- Manure was collected from US Meat Animal Research Center (USDA) from various animal production barns using different antimicrobials: chlortetracycline, tylosin and bacitracin

<table>
<thead>
<tr>
<th>Manure Slurry</th>
<th>Antimicrobial</th>
<th>ARG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/kg ww)</td>
<td>(mg/kg dw)</td>
</tr>
<tr>
<td>CTC-manure</td>
<td>3.3 ± 1.6</td>
<td>404 ± 138</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYL-manure</td>
<td>0.29 ± 0.12</td>
<td>32.5 ± 7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAC-manure</td>
<td>0.78 ± 0.75</td>
<td>320 ± 31.5</td>
</tr>
<tr>
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</table>
Land Application Methods

Broadcast

Incorporation

Injection

Antimicrobial loading through land application of manure is estimated at the kg/hectare level (Winckler & Grafe, 2001)
Experimental Methods

Manure slurry collected at the USDA Meat Animal Research Center (MARC)

Field testing site at the UNL Roger’s Memorial Farm
Experimental design allowed for statistical analysis of the data

Evaluated in a randomized block design on fields that had never received manure application
Simulated rainfall intensity 70 mm/hr
- 3 sequential events (#1, 2, and 3)
All runoff collected and a composite sample taken
- Stored at -20°C until analysis
Soil cores collected before and after rainfall simulation
## CTC in Runoff

### Graphs

- **Broadcast**
- **Injection**
- **Incorporation**

### Table: Mass Loading (average ± std.error, µg/m²)

<table>
<thead>
<tr>
<th>Run</th>
<th>Mass Loading (average ± std.error, µg/m²)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broadcast</td>
<td>Injection</td>
</tr>
<tr>
<td>1</td>
<td>4.54 ± 1.65</td>
<td>0.30 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>1.11 ± 0.50</td>
<td>0.27 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.15 ± 0.03</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Sum</td>
<td>5.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Fraction in Run 1</td>
<td>0.78</td>
<td>0.45</td>
</tr>
</tbody>
</table>
### Comparison of antimicrobials in runoff

<table>
<thead>
<tr>
<th>Rainfall Event Number</th>
<th>Broadcast (µg/m²)</th>
<th>Injection (µg/m²)</th>
<th>Incorporation (µg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280.41 ± 213.66</td>
<td>5.23 ± 2.28</td>
<td>33.56 ± 22.45</td>
</tr>
<tr>
<td>2</td>
<td>89.02 ± 70.28</td>
<td>4.59 ± 1.84</td>
<td>11.55 ± 1.64</td>
</tr>
<tr>
<td>3</td>
<td>56.37 ± 34.12</td>
<td>1.73 ± 1.25</td>
<td>4.50 ± 0.56</td>
</tr>
<tr>
<td>Sum</td>
<td>425.90</td>
<td>11.55</td>
<td>49.61</td>
</tr>
</tbody>
</table>

#### Tylosin mass transport in runoff

<table>
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Fraction in Run 1: 0.66, 0.45, 0.68
ARGs in Runoff

- Highest loads of antimicrobials and ARGs occurred in the runoff during the 1\textsuperscript{st} rain event.
- Broadcast application leads to more antimicrobial and ARGs in runoff than injection and incorporation.
Narrow Grass Hedges – Study Design
Antibiotic removal by grass hedge

Tylosin in Runoff (µg L⁻¹)

Rainfall Event Number

- w/o NGH
- w/ NGH
ARG removal by grass hedge

- **erm(B)** (copies mL$^{-1}$) vs Rainfall Event Number:
  - Without grass hedge
  - With grass hedge

- **16S rRNA gene** (copies mL$^{-1}$) vs Rainfall Event Number:
  - Without grass hedge
  - With grass hedge

Legend:
- Red: without grass hedge
- Pink with crosshatch: with grass hedge
ARG occurrence in check plots

- **erm(B)** (copies mL\(^{-1}\))
  - Rainfall Event Number
  - Without grass hedge: 1.0 x 10^2
  - With grass hedge: <MDL, <MDL, <MDL>

- **16S rRNA gene** (copies mL\(^{-1}\))
  - Rainfall Event Number
  - Without grass hedge: 1.0 x 10^6
  - With grass hedge: 1.0 x 10^9
AB and ARG occurrence in soils

Tylosin in Soil (ng g\(^{-1}\) dw)

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<td>&lt;MDL &lt;MDL &lt;MDL</td>
</tr>
<tr>
<td>After Manure Application</td>
<td>&lt;MDL</td>
</tr>
<tr>
<td>After Rainfall Events</td>
<td>&lt; MDL</td>
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erm(B) in Soil (copies g\(^{-1}\) dw)

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Control plot
Amended plot
Conclusions

- Narrow grass hedges reduced tylosin in runoff by over an order of magnitude, likely due to enhanced infiltration or sorption.
- \textit{erm}(B) and 16S RNA were also removed by grass hedges.
  - Consistent with prior work on removal of suspended sediment and fecal coliforms.
- Tylosin and \textit{erm}(B) occurrence in soil can be attributable to manure application.
Publications Resulting from this Work


Questions? sbartelt2@unl.edu