Probiotics For Pathogen Control

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### Incidence of zoonotic bacterial foodborne pathogens, 1997

<table>
<thead>
<tr>
<th>Zoonoses</th>
<th>Europe</th>
<th>USA</th>
<th>Australia</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>73.0</td>
<td>14.0</td>
<td>38.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>30.0</td>
<td>25.0</td>
<td>100.0</td>
<td>ND</td>
</tr>
<tr>
<td>STEC infections</td>
<td>0.7</td>
<td>2.0</td>
<td>ND</td>
<td>1.7</td>
</tr>
<tr>
<td>Listeria</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Yersinia</td>
<td>2.0</td>
<td>1.0</td>
<td>1.5</td>
<td>ND</td>
</tr>
<tr>
<td>Brucella</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Source:** Rev Sci Tech Int Epiz, 2000 19 (1) 226-239
**Preharvest control of E. coli O157:H7**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Assumption</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modify diet</td>
<td>rehab abnormal GI tract</td>
<td>grass or hay feeding</td>
</tr>
<tr>
<td>biological control</td>
<td>natural predators</td>
<td>phage therapy, colicins</td>
</tr>
<tr>
<td>direct fed microbials</td>
<td>natural competitors</td>
<td>probiotics</td>
</tr>
<tr>
<td>stress reduction</td>
<td>normal homeostasis</td>
<td>shipping mgmt</td>
</tr>
<tr>
<td>genetic resistance</td>
<td>natural resistance</td>
<td>none</td>
</tr>
<tr>
<td><strong>Technological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pharmaceuticals</td>
<td>selective antimicrobials</td>
<td>neomycin, Na chlorate</td>
</tr>
<tr>
<td>vaccination</td>
<td>specific immunity</td>
<td>Finlay, Fort Dodge</td>
</tr>
<tr>
<td>genetic resistance</td>
<td>engineered resistance</td>
<td>none</td>
</tr>
</tbody>
</table>
Effect of oral neomycin (one dose; 22 mg/kg) on STEC O157 & *E coli* fecal shedding in show list steers

Keen *et al.*, unpublished data

* STEC O157 not detected in any day 3 or day 7 feces

Keen *et al.*, unpublished data
“Many studies have found that the use of antibiotics in animals poses significant risks for human health…”

GAO, April, 2004
Probiotics: potential advantages

- inexpensive
- effective single treatment possible
- decreased probability of resistance
- “natural” – regulatory and consumer acceptance
Definitions

- **Probiotics**: live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989)

- **Direct fed microbials (DFM)**: synonymous with probiotics

- **Competitive exclusion (CE) cultures**: a subset of probiotics derived from indigenous intestinal microflora of healthy animals

- **Prebiotics**: nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon

- **Synbiotics**: combined application of pre- and probiotics
Characteristics of an ideal probiotic

- Non-pathogenic
- Remain functional through processing and storage
- Resist gastric acid and bile
- Persist in the gastrointestinal tract, for at least short periods
- Alter microbial populations and/or activity
Probiotics: common genera

- **Bacteria**
  - Lactobacillus
  - Bifidobacterium
  - Enterococcus
  - Bacillus
  - Escherichia
  - Proteus
  - Streptococcus

- **Fungi**
  - Saccharomyces
  - Aspergillus

*Modified from Reid and Friendship, 2002*
Probiotics: mechanism of action

- Competition for receptors
  - on cell surface
  - in mucus layer
  - extracellular matrix
Probiotics: mechanism of action

- Immune enhancement
  - enhanced phagocytosis
  - increased NO production
  - increased antibody production
    - cross reactive antigens
    - bacterial antigen processing
  - antiinflammatory activity
  - alteration of cytokine responses
Probiotics: mechanism of action

- Production of antimicrobial substances
  - organic acids
  - hydrogen peroxide
  - bacteriocins

- Modulation of epithelial cell gene expression and function
  - cytokines
  - prevention of enterocyte apoptosis
  - other
Probiotics: mechanism of action

- Competition for limiting nutrients
  - plausible
  - abundant *in vitro* evidence
  - evidence *in vivo* lacking
Do probiotics work?

**efficacy vs. effectiveness**

- **efficacy**: statistically significant effect of a treatment in a controlled setting
- **effectiveness**: significant effect of a treatment under field conditions and outcomes relevant to the problem

How do we define effectiveness for a given pathogen?
Correlation between preharvest and postharvest prevalence of EHEC O157 by day of sampling

\[ r = 0.79 \]
\[ p = 0.02 \]

Elder et al., PNAS 2000
Elements of intervention study evaluation

- **Design**
  - study question
  - type of study
  - study population/groups

- **Methodology**
  - what outcome is being measured
  - how is it being measured
  - magnitude of effect

- **Analysis**
  - statistical analysis
  - interpretation
Design effects on evaluation of intervention literature

**Effectiveness**
- Systematic reviews and meta-analysis
- Randomized, controlled, blinded (RCT) trials
- Cohort studies
- Case control studies
- Case series
- Case reports
- Animal research
- In vitro “test tube” research

**Efficacy**
- Clinical & laboratory studies
- Epidemiologic studies
- RCT’s and multi-study analyses

**Evidence**
- Strong
- Weak

**EBM Class**
- I
- II
- III
- IV
- V

**Results**
- Few positive
- Positive bias
Many instances of inappropriate use of statistics

Several instances where this leads to erroneous conclusions about the efficacy of an intervention
### Efficacy?

#### Study 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>n positive</th>
<th>% positive (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td>40</td>
<td>10</td>
<td>25.0% (13.24 - 41.52)</td>
</tr>
<tr>
<td>Treatment:</td>
<td>68</td>
<td>7</td>
<td>10.3% (4.59 - 20.65)</td>
</tr>
</tbody>
</table>

**p = 0.08**

#### Study 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>n positive</th>
<th>% positive (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td>203</td>
<td>10</td>
<td>4.9% (2.52 - 9.14)</td>
</tr>
<tr>
<td>Treatment:</td>
<td>201</td>
<td>3</td>
<td>1.5% (0.39 - 4.65)</td>
</tr>
</tbody>
</table>

**p = 0.09**
**Feedlot pen-to-pen variation in fecal STEC O157 shedding**

- 455 show list cross bred steers in 12 adjacent feedlot pens
- Fecal census June 28-30, 1999

### Fecal STEC O157 prevalence by pen

<table>
<thead>
<tr>
<th>Pen</th>
<th>123</th>
<th>124</th>
<th>125</th>
<th>126</th>
<th>127</th>
<th>128</th>
<th>129</th>
<th>130</th>
<th>131</th>
<th>132</th>
<th>133</th>
<th>134</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/68</td>
<td>0/68</td>
<td>1/32</td>
<td>1/32</td>
<td>10/41</td>
<td>23/28</td>
<td>4/28</td>
<td>1/28</td>
<td>18/28</td>
<td>3/42</td>
<td>2/30</td>
<td>0/30</td>
<td></td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0%</td>
<td>3.1%</td>
<td>3.1%</td>
<td>24.4%</td>
<td>82.1%</td>
<td>14.3%</td>
<td>3.6%</td>
<td>64.3%</td>
<td>7.1%</td>
<td>6.7%</td>
<td>0.0%</td>
<td></td>
</tr>
</tbody>
</table>

- = water source  
- = 2 lowest prev pens  
- = 2 highest prev pens

**Range:** 0.0% to 82.1%; **Mean:** 17.5%;  
**Median:** 5.1%; **Std dev:** 27.2%

### Overall prevalence

64 /455 = 14.1% (11.1-17.7)

### Pen prevalence

10/12 = 83.3% (50.9-97.1)
Effect of clustering by pen on analysis of treatment effects

If we randomly assign *pens* to treatment or control groups, ~80% of the time there will be a statistically significant (p<0.05) difference in pooled prevalence attributable to treatment.

Only 5% of the time will there be an apparent effect if *individuals* are randomly assigned to treatment or control groups.

<table>
<thead>
<tr>
<th>Pen</th>
<th>1/68</th>
<th>0/68</th>
<th>1/32</th>
<th>1/32</th>
<th>10/41</th>
<th>23/28</th>
<th>4/28</th>
<th>1/28</th>
<th>18/28</th>
<th>3/42</th>
<th>2/30</th>
<th>0/30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5%</td>
<td>0.0%</td>
<td>3.1%</td>
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<td>82.1%</td>
<td>14.3%</td>
<td>3.6%</td>
<td>64.3%</td>
<td>7.1%</td>
<td>6.7%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

^ = control

<table>
<thead>
<tr>
<th>Iteration</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>18.5</td>
<td>19.9</td>
<td>16.2</td>
<td>28.9</td>
<td>15.4</td>
</tr>
<tr>
<td>treatment</td>
<td>10.9</td>
<td>9.4</td>
<td>11.9</td>
<td>2.7</td>
<td>13.1</td>
</tr>
</tbody>
</table>

p<

0.03* 0.01* 0.24 0.01* 0.89
### Effect of select probiotics on *Salmonella enterica* Enteritidis prevalence in chickens

<table>
<thead>
<tr>
<th>Study type</th>
<th>Probiotic genera</th>
<th>Control</th>
<th>Probiotic</th>
<th>Max. effect (%)</th>
<th>p-value</th>
<th>n</th>
<th>n x effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>Avigard</td>
<td>87</td>
<td>0</td>
<td>100</td>
<td>&lt;0.05</td>
<td>*</td>
<td>*</td>
<td>Davies, 2003</td>
</tr>
<tr>
<td>V</td>
<td><em>Lactobacillus</em></td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>&gt;0.05</td>
<td>5</td>
<td>0</td>
<td>LaRagione, 2004</td>
</tr>
<tr>
<td>V</td>
<td>Undefined CE</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>&lt;0.05</td>
<td>50</td>
<td>50</td>
<td>Andreatti-Filho, 2003</td>
</tr>
<tr>
<td>V</td>
<td>Avigard</td>
<td>75</td>
<td>10</td>
<td>87</td>
<td>&lt;0.05</td>
<td>10</td>
<td>7</td>
<td>Seo, 2000</td>
</tr>
<tr>
<td>V</td>
<td>Broilact</td>
<td>40</td>
<td>10</td>
<td>75</td>
<td>&gt;0.05</td>
<td>10</td>
<td>3</td>
<td>Fukata, 1999</td>
</tr>
</tbody>
</table>

* *not calculable from the data available*
Effect of select probiotics on fecal prevalence of *E. coli* O157:H7 in cattle

<table>
<thead>
<tr>
<th>Study type</th>
<th>Probiotic genera</th>
<th>Control</th>
<th>Probiotic</th>
<th>Max. effect</th>
<th>p-value</th>
<th>n</th>
<th>n x effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td><em>Lactobacillus, Propionibacterium</em></td>
<td>28</td>
<td>14</td>
<td>50.9</td>
<td>&gt;0.09</td>
<td>60</td>
<td>8</td>
<td>Elam, 2003</td>
</tr>
<tr>
<td>III</td>
<td><em>Lactobacillus</em></td>
<td>65</td>
<td>45</td>
<td>30.8</td>
<td>&lt;0.04</td>
<td>60</td>
<td>12</td>
<td>Brashears, 2003</td>
</tr>
<tr>
<td>V</td>
<td><em>Escherichia</em></td>
<td>67</td>
<td>0</td>
<td>100</td>
<td>&lt;0.05</td>
<td>6</td>
<td>6</td>
<td>Tkalcic, 2003</td>
</tr>
<tr>
<td>V</td>
<td><em>Escherichia</em></td>
<td>57</td>
<td>29</td>
<td>49.1</td>
<td>&gt;0.05</td>
<td>7</td>
<td>2</td>
<td>Zhao, 2003</td>
</tr>
</tbody>
</table>
Do probiotics work? … Possibly

There is a general basis for efficacy of probiotics for reduction of carriage of some food-borne pathogens.

Well-designed, peer-reviewed clinical/field trials are required before probiotics should be considered effective components of an overall pathogen reduction program.