New Perspectives on the Control of Salmonella and Related Enteric Pathogens

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Overview of the Presentation

(I) The importance of Salmonella in cattle
(II) Strategies to mitigate Salmonella in cattle
(III) New approaches that use the anti-Salmonella strategies
(IV) Cost effectiveness of these approaches
(V) Conclusions

(I) Importance of Salmonella in Cattle

✓ Three important facets:
  ✓ (A) Attachment to the gut epithelium
  ✓ (B) Invasion of cells lining the gut epithelium
  ✓ (C) Survival within bovine macrophages

✓ (B) Physical invasion of the gut epithelium and entrance into the systemic circulation
✓ Some Salmonella can invade and dislodge cells lining the bovine gut epithelium- causing a hemorrhagic diarrhea.
(I) Importance of *Salmonella* in Cattle

- **(B) Invasion of intestinal cells**

  (i) Attachment
  (ii) Injection of bacterial proteins
  (iii) Rearrangement of actin
  (iv) Formation of a “ruffle”
  (v) Bacterial entry into the cell
  (vi) Cell is damaged and sloughs
  (vii) Underlying vasculature is exposed
  (viii) Hemorrhagic diarrhea

(II) Strategies to mitigate *Salmonella* in cattle

- **(A) Selecting for cattle that are naturally resistant**
- **(B) Altering gene expression in cattle**
- **(C) Altering gene expression in *Salmonella***
- **(D) Directly killing *Salmonella* with antibiotics**

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(I) Importance of *Salmonella* in Cattle

- Less invasive strains will cause a moderate diarrhea in calves.
- Costly because of medical costs and hampered growth and development
- The animal becomes a life-long carrier.

(II) Strategies to mitigate *Salmonella* in cattle

- (A) Selecting for cattle that are naturally resistant
- *Salmonella* exploits proteins within intestinal cells and macrophages during the intestinal invasion and macrophage survival processes.
- Minimizing these proteins will lead to a decreased susceptibility to *Salmonella* infections.
- Certain cattle harbor unique genes that lead to minimized expression of these proteins exploited by *Salmonella*.

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(I) Importance of *Salmonella* in Cattle

- (C) Survival within bovine macrophages (white blood cells)
- Macrophages will engulf *Salmonella* on the basal side of the intestinal lining.
- *Salmonella* survives within the macrophages.
- The macrophages carry the *Salmonella* to organs like the lung (pneumonia) or brain (encephalitis).
- The macrophages also carry the *Salmonella* to lymph nodes, causing a food safety hazard.

(II) Strategies to mitigate *Salmonella* in cattle

- (A) Selecting for cattle that are naturally resistant
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- Minimizing these proteins will lead to a decreased susceptibility to *Salmonella* infections.
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(I) Importance of *Salmonella* in Cattle

- Recent studies indicate that up to 30% of peripheral cattle lymph nodes contain *Salmonella*.
- These lymph nodes are not excised and are unfortunately incorporated into hamburger at slaughter—hard to prevent.
(II) Strategies to mitigate Salmonella in cattle

(B) Altering gene expression in cattle

- It is possible that exogenous compounds (e.g., from supplements) will reduce the expression of the proteins exploited by Salmonella.
- This phenomenon is termed an “epigenetic” change where the genetics of an animal are not altered but the gene expression pattern is transiently altered.

\[
\begin{align*}
\text{Normal expression of the exploited proteins} & \quad \rightarrow \\
\text{supplement} & \quad \rightarrow \text{Reduced expression of the exploited proteins}
\end{align*}
\]

(III) Newly available approaches for mitigating Salmonella in cattle

(A) Selecting for cattle with natural resistance- PSR Genetics LLC
- PSR Genetics LLC identified a cattle genotype conferring natural resistance to Salmonella.
- This genotype leads to reduced expression of the intestinal and macrophage proteins exploited by Salmonella.
- About 35% of non-black cattle possess this genotype, while 5% of black cattle have an analogous resistance-conferring genotype.

(II) Strategies to mitigate Salmonella in cattle

(C) Altering gene expression in Salmonella

- As mentioned, Salmonella injects a series of proteins into the intestinal cells during the invasion process.
- Minimizing the expression of these proteins will lead to a decrease in Salmonella virulence.
- This specific decrease in expression is an outcome of changes in the chemical and/or microbiologic profile of the gut.

(II) Strategies to mitigate Salmonella in cattle

(D) Directly killing Salmonella with antibiotics

- Antibiotics kill Salmonella by perturbing vital processes in the bacteria.
- Unfortunately, Salmonella will form a biofilm in which a subpopulation are protected from exposure to the antibiotic.
- Ultimately, this will lead to antibiotic resistance.

(III) Newly available approaches for mitigating Salmonella in cattle

(A) Selecting for cattle that are naturally resistant

There is a direct correlation between the presence of the PSR gene and susceptibility of live cattle to Salmonella infection. Even 10X the challenge dose could not produce illness or any shedding of Salmonella in the feces in these cattle.
(III) Newly available approaches for mitigating *Salmonella* in cattle

(A) Selecting for cattle that are naturally resistant

![Graph showing % of lymph nodes contaminated](image)

The peripheral lymph nodes of PSR/PSR cattle are not susceptible to contamination by two of the *Salmonella* strains implicated in this emerging problem.

How does the PSR/PSR genotype inhibit *Salmonella*?

- For non-black cattle, these animals lack one protein exploited by *Salmonella* during the invasion process and they lack one protein exploited by *Salmonella* during the macrophage survival process.
- For black cattle, these animals have a diminished expression of 3-6 proteins exploited by *Salmonella*.
- A genetic test is available for identifying and propagating cattle containing the genotype.

(B) Altering expression in cattle- Diamond V products

(C) Altering genes in *Salmonella*- Diamond V products

- (i) Calf diarrhea (*Salmonella* and *E. coli* K99)- Diamond V SmartCare® & Original XPC™
- (ii) Adult salmonellosis- Prototype NaturSafe
- (iii) Lymph node persistence- Prototype NaturSafe

(III) Newly available approaches for mitigating *Salmonella* in cattle

- Proprietary active compounds- DV Bioactives™ that likely support immune function
- Available for incorporation into milk replacer (SmartCare @ 0.15%) and starter feed (Original XPC @ 3.5gm/head/day)
- Recent blinded studies demonstrate that these products prevent the untoward effects of *S. Typhimurium* in dairy calves on milk.

Diamond V’s SmartCare & Original XPC

- Calories were fed these products for two weeks then challenged with *S. Typhimurium*.
- Calories were fed these products for another three weeks during which various clinical parameters were measured.
- The DV Bioactives (SCFP) significantly diminished the incidence of fever.

Diamond V’s SmartCare & Original XPC

- DV Bioactives significantly diminished the severity and incidence of diarrhea throughout the study.
**Diamond V’s SmartCare & Original XPC**

- **DV Bioactives** significantly diminished the prevalence of calves shedding *Salmonella* in their feces.

- **Mitigating Adult Bovine Salmonellosis**
  - Diamond V is launching a "next generation" product (NutriTek®) for use in dairy cattle.
  - Diamond V is working on a similar technology to be used in beef cattle (NaturSafe).
  - Our investigator-blinded studies with NaturSafe reveals a protective effect against salmonellosis.
  - N=200 animals per group; naturally infected with various *Salmonella*; Control includes an antibiotic

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**Materials and Methods**

- **Treatments**
  - **Positive Control**
    - Monensin at 300 mg/head/day,
    - Tylosin at 90 mg/head/day, and
    - Direct fed microbial at 50 mg/head/d
  - **NaturSafe**
    - Supplemented at rate 18 g/head/d
    - No monensin, tylosin, or a direct fed microbial
Materials and Methods

- **Liver abscess**
  - **Classifications**
    - With O
      - No abscesses
    - With A\(^{-}\) or A
      - 1 to 2 small abscesses
      - Up to 2 to 4 well organized abscesses
    - With A\(^{+}\)
      - Multiple large abscesses
      - Tissue inflammation around abscess and adhesions

Liver Abscesses

<table>
<thead>
<tr>
<th>Positive Control(^{1})</th>
<th>NaturSafe</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcasses, n</td>
<td>740</td>
<td>735</td>
<td>--</td>
</tr>
<tr>
<td>Liver abscesses, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(^{-})</td>
<td>6.9</td>
<td>3.3</td>
<td>0.93</td>
</tr>
<tr>
<td>A</td>
<td>5.0</td>
<td>3.5</td>
<td>1.06</td>
</tr>
<tr>
<td>A(^{+})</td>
<td>7.4</td>
<td>7.7</td>
<td>1.19</td>
</tr>
<tr>
<td>Total Condemned</td>
<td>19.3</td>
<td>14.5</td>
<td>1.92</td>
</tr>
</tbody>
</table>

\(^{1}\)Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype.

Overview

<table>
<thead>
<tr>
<th>Positive Control(^{1})</th>
<th>NaturSafe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifers, n</td>
<td>748</td>
</tr>
<tr>
<td>Pens, n</td>
<td>10</td>
</tr>
<tr>
<td>Days on feed</td>
<td>136</td>
</tr>
<tr>
<td>Bunk space, in/ head</td>
<td>14.4</td>
</tr>
<tr>
<td>Pen space, ft(^{2})/head</td>
<td>231</td>
</tr>
</tbody>
</table>

\(^{1}\)Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype.

Performance

<table>
<thead>
<tr>
<th>Body weight, lb</th>
<th>Positive Control(^{1})</th>
<th>DV Prototype</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>790</td>
<td>793</td>
<td>7.50</td>
<td>0.33</td>
</tr>
<tr>
<td>Final(^{2})</td>
<td>1,278</td>
<td>1,280</td>
<td>7.10</td>
<td>0.80</td>
</tr>
<tr>
<td>ADG, lb/day</td>
<td>3.61</td>
<td>3.59</td>
<td>0.043</td>
<td>0.73</td>
</tr>
<tr>
<td>DMI, lb/day</td>
<td>22.7</td>
<td>23.0</td>
<td>0.33</td>
<td>0.09</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>6.29</td>
<td>6.40</td>
<td>0.074</td>
<td>0.16</td>
</tr>
<tr>
<td>Yield adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final BW, lb(^{1})</td>
<td>1,266</td>
<td>1,269</td>
<td>9.30</td>
<td>0.64</td>
</tr>
<tr>
<td>ADG, lb/day</td>
<td>3.51</td>
<td>3.51</td>
<td>0.041</td>
<td>0.94</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>6.46</td>
<td>6.57</td>
<td>0.122</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\(^{1}\)Positive Control contains monensin, tylosin, and a direct fed microbial. These are not included in diet of DV Prototype.

\(^{2}\)Final BW shrunk 4%.

\(^{1}\)Yield adjusted BW calculated by dividing HCW by a common dressing yield of 63.75.

Mitigating Adult Bovine Salmonellosis

✔ NaturSafe led to a decrease in the presence of *Salmonella* in the feces.

Salmonella invasion assays

- **Salmonella Isolates**
- **Tissue culture cells**

- Recover and count

- Invaded cell

Mitigating Adult Bovine Salmonellosis
✓ NaturSafe led to a decrease in the invasiveness of *Salmonella* recovered from feces.

How are the Diamond V products reducing the presence of *Salmonella*?
✓ Studies from other species reveal that the Diamond V products rebalance the immune system, by epigenetically activating gene expression events.
✓ This rebalancing allows the immune system to appropriately and efficiently respond to pathogens.
✓ This likely facilitates an enhanced killing of *Salmonella* within macrophages.
✓ Since *Salmonella* cannot form biofilms within macrophages, the chance for resistance is minimal.

Mitigating Lymph Node Infiltration
✓ NaturSafe led to a decreased load of *Salmonella* in subiliac lymph nodes.

Diamond V’s SmartCare & Original XPC
White blood cells from XPC-fed calves were more efficient at killing *S. Dublin* and *S. Typhimurium*.

Mitigating Lymph Node Infiltration
✓ NaturSafe led to a decreased prevalence of *Salmonella* in subiliac lymph nodes.

Diamond V’s SmartCare & Original XPC
White blood cells from XPC-fed calves were more efficient at killing *E. coli* K88 and K99.
How are the Diamond V products inhibiting the invasiveness (and thus virulence) of *Salmonella*?

- Diamond V Bioactives alter the ruminal and intestinal microbiomes.
- The microbial alterations lead to epigenetic changes in *Salmonella*, altering its invasiveness and virulence.
- Our studies revealed that multiple Diamond V products are capable of inhibiting the expression of the major *Salmonella* invasion-regulating gene designated as *hila*.

### (IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

- The PSR gene appears to be correlated with enhanced production benefits.
- Cattle with the PSR gene are healthier than other cattle.
- PSR/PSR cattle ranked the best in regards to marbling, average daily gain, longevity, and fertility.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Marbling</th>
<th>Daily Gain</th>
<th>Longevity</th>
<th>Fertility</th>
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<tbody>
<tr>
<td>PSR/PSR</td>
<td>1st</td>
<td>1st</td>
<td>1st</td>
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</tr>
<tr>
<td>PSR/psr</td>
<td>2nd</td>
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### (IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

- SmartCare and Original XPC are useful antibiotic-free feed and milk replacer additives for combating salmonellosis in pre-weaned dairy calves, while also enhancing growth.

### Anti-Resistance Properties of the New Approaches for *Salmonella* in Cattle

- Cattle lacking a protein exploited by *Salmonella*- the bacteria will simply go elsewhere.
- Killing *Salmonella* in macrophages where biofilms cannot exist- no chance for resistance.
- Inhibiting virulence- not adverse for *Salmonella* since it has the option to adopt or not adopt the pathogenic lifestyle; no resistance response.

### (IV) Cost Effectiveness of the New Mitigation Approaches for *Salmonella* in Cattle

- NaturSafe has similar anti-*Salmonella* properties in adult cattle.

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(V) Summary of the New Mitigation Approaches for Salmonella in Cattle

- The PSR gene is a cost-effective selection tool for Salmonella-resistant cattle.
- SmartCare and Original XPC are useful antibiotic-free feed and milk replacer additives for combating salmonellosis in pre-weaned dairy calves, while also enhancing production.
- NaturSafe has similar properties and is for use in beef cattle.
- NutriTek is the adult dairy-specific version of the aforementioned products and, given its relatedness to these products, has the same production and anti-pathogen traits.