The Fight Against Salmonella in Agriculture – Research Perspective
(a view from the trenches)

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The Fight Against *Salmonella* in Agriculture – Research Perspective

*(a view from the trenches)*

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How do we prevent *Salmonella* foodborne illness...?

- Know the enemy
- Understand the scope of the problem
- What is being done about the problem?
- What more can be done?
Salmonella and Foodborne Disease

- Salmonellosis – Non-typhoidal *Salmonella enterica*
- > 2,500 serotypes (~1,700 noted for making animals sick)
- U.S. cases per year:
  - Confirmed ~40,000
  - Estimated ~ 1.4 million

CDC, *Salmonella Annual Summary* - 2009

Laboratory-confirmed *Salmonella* isolates from human sources reported to CDC by age group and sex, 2009

27% of confirmed cases <1 – 4 yrs of age
Salmonella and Foodborne Disease

- Salmonellosis – Non-typhoidal Salmonella enterica
- > 2,500 serotypes (~1,700 noted for making animals sick)
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- Generally self limiting enteritis but can be invasive
  - Hospitalization ~15,000 per year
  - Death ~1% of confirmed cases/yr

- Human health cost ~ $330 million/yr
  - Decreased productivity
  - Medical expense
  
http://www.ers.usda.gov/Data/FoodborneIllness/

- Industry cost ~$100s of millions
  - Product recall
  - Plant closures and clean up
  - Liability costs

CDC, Salmonella Annual Summary - 2009

[Diagram showing laboratory-confirmed Salmonella isolates from human sources reported to CDC by age group and sex, 2009]

- 27% of confirmed cases
  - <1 – 4 yrs of age
- 59% of deaths
  - 65 – 80+ yrs of age
Salmonella and Foodborne Disease

• Primary habitat – animal large intestine / feces
  – “Hearty bug” also survives well in the environment

• 6 serotypes account for >50% human cases
  20 serotypes account for >70% cases
  Are some serotypes more virulent than others?

• Complex etiology
  ~10% cases attributed to outbreaks (OB)
  ~90% sporadic

Many potential sources of Salmonella!
Reservoirs for *Salmonella* and Host Specificity

**CDC Top Serotypes**

Human salmonellosis

- Enteritidis
- Typhimurium
- Newport
- Javiana
- Heidelberg
- Montevideo
- Saint Paul
- Muenchen
- Braenderup
- Infantis
- Thompson
- Agona
- Schwarzengrund
- Bareilly
- Hadar
- Oranienburg

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*Salmonella*

Host specific vs. Host generalist
The Complexity of *Salmonella* Etiology

HHS Healthy People Initiative 2020
Salmonellosis Target:
6.8 cases / 100K people
Presently ~14 cases / 100K

~10-15% of confirmed cases in U.S. attributed to OB linked with contaminated meat consumption

Sporadic Illness ~90%

~40,000 confirmed cases / yr

Salmonellosis Cases by Source 1998 - 2011

Summary of 152 outbreaks representing 12,181 cases of illness – not all inclusive.

http://wwwn.cdc.gov/foodborneoutbreaks/
Post-Harvest Multiple Hurdle Carcass Interventions

FSIS – 1996
Issued Final Rule:
Pathogen
Reduction;
Hazard
Analysis and
Critical
Control
Point
(PR/HACCP)
Systems

2006 – FSIS
Increased those
performance
standards
Efficacy of Multiple Hurdle Carcass Processing Interventions at Reducing *Salmonella* Prevalence

**Beef Processing**

- **Hide** ($n=3040$)
  - Pre-evisceration ($n=3040$)  
  - Post-intervention ($n=3040$)

<table>
<thead>
<tr>
<th>Step</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide</td>
<td>89.6%</td>
</tr>
<tr>
<td>Pre-evisceration</td>
<td>50.2%</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

- ~98% Reduction in *Salmonella* prevalence on carcasses

*Multiple hurdle interventions are very effective for reducing pathogen levels on final carcasses*

Prevalence of Multidrug Resistant *Salmonella* at Harvest

**Beef Processing**

**Hide (n=3040)**

- 6% of all *Salmonella* isolated in this study found to be MDR
  - Consistent subpopulation of any niche
  - ~1.8% Ceftriaxone resistance
  - ~0.1% Nalidixic acid resistance – uncommon

- 16% MDR

**Pre-evisceration (n=3040)**

- 50.2% MDR

**Post-intervention (n=3040)**

- 0.8% MDR

**Predominant Serotypes**

- Newport
- Typhimurium
- Uganda

-Brichta-Harhay et al., (2011) AEM 77:1783-96
Concerns about Drug Resistant *Salmonella* in Ground Beef

- Relatively few outbreaks and recalls, but when they occur they can involve MDR *Salmonella* Newport or Typhimurium

**Newport MDR-AmpC** | **MDR Typhimurium**
--- | ---
2002 47 ill | 2003 58 ill
2007 43 ill | 2009 14 ill
2009 42 ill | 2011 19 ill

- Concern that multidrug resistant (MDR) *Salmonella* are more invasive than susceptible *Salmonella* - *are they?*
**Salmonella Prevalence in Ground Beef**

USMARC Nationwide survey 2007-2008 of *Salmonella* prevalence in ground beef (n=4,136 samples) found **mean prevalence to be 4.2%**  
*Bosilevac et. al., (2009) AEM 75:1892-1900*

- Most commonly observed serotypes: **Montevideo (21%)**, **Anatum (15%)**, **Muenster (8%)**, **Mbandaka(6%)**

- **MDR Salmonella** – 0.6%
  - MDR Typhimurium 0.09%
  - MDR Newport 0.07%

~0.4% of outbreak cases (1998-2011)

No Montevideo outbreaks linked to Ground Beef...
The Future Landscape of the Fight Against *Salmonella* in Agriculture...

MiniLIMS - BioTeam
Sequence, assembly, and annotation data analysis pipeline

USMARC
acquired 2008

USMARC
acquired 2011
Bacterial Pathogen Genome Sequencing

"It is now readily possibly to determine the draft genome sequence of a bacterial pathogen within a two day time span"

Source Tracking - Molecular Epidemiology based on specific patterns of Single Nucleotide Polymorphisms (SNPs) within a pathogens genome sequence
Outbreak occurred
July 2009 – April 2010
273 ill (44 states)
52 hospitalized
0 deaths

Causative agent: S. Montevideo

Highly clonal strains confound epidemiologic investigations

Bosilevac et. al., (2009) AEM 75:1892-1900
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- Performed whole genome sequencing (WGS) of 40 Montevideo Isolates with PFGE patterns matching the OB strain (clinical, environmental, laboratory and food isolates)

- Found a core set of SNPs that defined the outbreak strains – they were even able to determine that one of the “outbreak” cases in California was unrelated

Bosilevac et. al., (2009) AEM 75:1892-1900
Pathogen Source Tracking

Salmonellosis

Sporadic Illness ~90%

Montevideo

Montevideo

Montevideo

Montevideo

Montevideo
Other Future Trends:
Metagenomics & Transcriptomics

**Metagenomics:**
the study of complex mixtures/communities

- Isolate total genomic DNA
- Sequence
- Characterize the “players” in a given microbial niche

**Transcriptomics:**
Direct sequencing of total expressed RNA

- Isolate total expressed RNA
- Convert to cDNA
- Sequence
- Characterize gene expression in a given niche

Scanning electron micrograph (SEM) of bacteria in cattle feces – magnification ~10,000x
Questions that can be asked using genomics technology:

**Metagenomics –**

How do fecal community profiles change with perturbation?
- Antibiotic exposure
- Pathogen colonization

Provides opportunity to discover new probiotics
- Non-pathogens that can displace *Salmonella* in fecal or environmental niche
Questions that can be asked using genomics technology:

Transcriptomics –

How do *Salmonella* respond to carcass processing interventions?

- Characterize the MDR Newport transcriptional profile in response to simulated carcass processing interventions

16% genes showed increased expression:

- Heat shock
- Acid stress
- DNA repair
- Virulence response

Transcriptional profiling of intervention survivors provides information on how to further target these *Salmonella*
Conclusions and Thoughts...

So how do we prevent *Salmonella* foodborne illness...?

- Better source tracking – more accurately identify sources of *Salmonella*
  - SNP based

- Identify opportunities for interventions in Pre-harvest environments
  - Metagenomics
  - Probiotics (competition)

- Increase our understanding of Intervention survivors – Transcriptomics
  - Identify targets for elimination

![Sporadic Illness](pie_chart.png)

![Salmonellosis Cases by Source 1998 - 2011](pie_chart2.png)

Summary of 152 outbreaks representing 12,181 cases of illness – not all inclusive.
Acknowledgments

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