An Update of Research at the National Animal Disease Center

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Midwest Turkey Producers and Processors
ARS Turkey Research

Animal Production and Protection (82%)

Nutrition/Food Safety/Quality (18%)
Keeping an eye on food safety

U.S. plans testing at turkey plants to reduce cases of salmonella

By PHILIP BRASHER
REGISTER WASHINGTON BUREAU

Washington, D.C. — The government wants to make that turkey drumstick a little safer. The U.S. Agriculture Department plans to start testing for salmonella bacteria in plants that process turkeys.

Food safety

In 1997, the U.S. government set a goal to reduce the number of food-borne illness cases by 50 percent. A look at the progress made:

<table>
<thead>
<tr>
<th></th>
<th>Cases per 100,000 People</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMPYLOBACTER</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>24.6</td>
</tr>
<tr>
<td>2004</td>
<td>12.9</td>
</tr>
<tr>
<td>*2010</td>
<td>12.3</td>
</tr>
</tbody>
</table>

| E. COLI O157     |                          |
| 1997             | 2.1                      |
| 2004             | 0.9                      |
| *2010            | 1.0                      |

| SALMONELLA       |                          |
| 1997             | 13.6                     |
| 2004             | 14.7                     |
| *2010            | 6.8                      |

* Goal

Source: U.S. Centers for Disease Control and Prevention
Turkey
FSIS Nationwide Baseline, 1997

<table>
<thead>
<tr>
<th></th>
<th>CARCASS</th>
<th>GROUND</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. perfringens</em></td>
<td>29.2%</td>
<td>28.1%</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>66.7%</td>
<td>57.3%</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>5.9%</td>
<td>30.5%</td>
</tr>
<tr>
<td><em>C. jejuni/C. coli</em></td>
<td>90.3%</td>
<td>25.4%</td>
</tr>
<tr>
<td><em>E. coli 0157:H7</em></td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>18.6%</td>
<td>49.9%</td>
</tr>
</tbody>
</table>
Salmonella associated human illnesses have remained above public health targets of Centers for Disease Control (CDC)… E. coli O157:H7, Campylobacter and Listeria associated enteritis … have been significantly reduced in recent years and are approaching Healthy People 2010 goals. FSIS and other regulatory agencies have … made reduction of Salmonella a priority. Salmonella outbreaks are primarily associated with poultry, in addition to meat animals and produce. There is a significant need for data to support hazard evaluation and risk assessment of bacterial pathogens from the complex poultry production and processing industry and to provide intervention technologies to help reduce prevalence of Salmonella.
Is holding of pigs at the abattoir a risk factor for Salmonella infection?
8 studies, 1 message

• Studies
  ✓ 1. Clean lairage study
  ✓ 2. APEP - Accel. PRV Eradication Program
  ✓ 3 & 4. Rapid infection experiments
  ✓ 5. Environmental study
  ✓ 6. Cull sow study
  ✓ 7. Short hold and slatted floor study
  ✓ 8. Truck hold study

• Message:
  ✓ Holding pen is a significant risk for Salmonella infection in hogs prior to slaughter.
Where are critical control points within 24 hrs of turkey slaughter?

- On-farm after feed withdrawal?
- Loading on the truck?
- Transport to abattoir (~3 hrs)?
- Holding in the shed (~2 to 6 hrs)?
✓ Does prevalence increase in the final hours pre-slaughter?

✓ When?
FW/Transportation-Salmonella

BROILERS
1980- Increase in *Salmonella* in crated birds (Rigby et al)
1981- FW has no effect on carriage/shedding of *Salmonella* (Rigby et al)
1986- Stress increases peristalsis (Linton et al.)
1993- Increase in bacterial load with longer crating /holding (Renwick)
1995- Transport stress increases pathogens in hogs, poultry (Mulder)
1997- No change in *Salmonella* in ceca without yeast treatment (Line)
1997- Increase in *Salmonella* in crop not ceca (Ramirez)
1999- Increase in *Salmonella* in crop not ceca (Corrier)
2003- No change in *Salmonella* post-transport (Northcutt)

TURKEYS??
Materials & Methods

• 6 commercial turkey operations

• Samples cultured:
  – Barn environment (n=25 swabs)
  – Cage floors after loading (n=100)
  – Cage floors after transport & holding (n=100)
  – Cecum, crop, spleen (n=50 each)
Results
# Summary: time off-feed (hrs)

<table>
<thead>
<tr>
<th>Flock #</th>
<th>Loading</th>
<th>Transit</th>
<th>Holding</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>2.75</td>
<td>4.0</td>
<td>6.75</td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
<td>3.0</td>
<td>4.38</td>
<td>8.14</td>
</tr>
<tr>
<td>3</td>
<td>0.78</td>
<td>0.265</td>
<td>9.75</td>
<td>10.78</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.75</td>
<td>5.1</td>
<td>6.6</td>
</tr>
<tr>
<td>5</td>
<td>0.73</td>
<td>1.0</td>
<td>4.87</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td>0.92</td>
<td>0.92</td>
<td>5.7</td>
<td>7.62</td>
</tr>
<tr>
<td>Mean</td>
<td>8.2 ± 0.11</td>
<td>1.45 ± 1.1</td>
<td>5.6 ±2.1</td>
<td>7.8 ±1.6</td>
</tr>
<tr>
<td>Range</td>
<td>7.3 – 1.0</td>
<td>0.3 -3.0</td>
<td>4.0 - 9.8</td>
<td>5.6 - 10.8</td>
</tr>
</tbody>
</table>
Cage floors pre-transport

Salmonella prevalence measured in trucks on-farm after loading
Cage floors: Post-transport, holding

Salmonella prevalence measured at plant after transport & holding
Salmonella prevalence measured on-farm, after transport & holding, and at slaughter; by farm

Wesley et al. 2006. J of Food Protection (in press)
Salmonella: Study Design

On-farm (n=30 birds)
- Crop
- Ceca
- Spleen
- Liver/Gall bladder
- Cloacal loop
- Colon

Abattoir (n=30 birds)
- Crop
- Ceca
- Spleen
- Liver/Gall bladder
### S. enterica prevalence: On-farm vs. Abattoir

<table>
<thead>
<tr>
<th></th>
<th>Flock</th>
<th>Cloaca</th>
<th>L Int</th>
<th>Ceca</th>
<th>Crop</th>
<th>Spleen</th>
<th>Liver</th>
<th>Any</th>
<th>Ceca</th>
<th>Crop</th>
<th>Spleen</th>
<th>Liver</th>
<th>Any</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>ND*</td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>ND*</td>
<td>0%</td>
</tr>
<tr>
<td>B</td>
<td>33.3%</td>
<td>53.3%</td>
<td>36.7%</td>
<td>10%</td>
<td>46.7%</td>
<td>10%</td>
<td>83.3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>33.3%</td>
<td>45.5%</td>
<td>73.3%</td>
</tr>
<tr>
<td>C</td>
<td>37.9%</td>
<td>65.5%</td>
<td>96.7%</td>
<td>43.3%</td>
<td>33.3%</td>
<td>6.7%</td>
<td>96.7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>33.3%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>D</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>16.7%</td>
<td>23.3%</td>
</tr>
<tr>
<td>E</td>
<td>0%</td>
<td>0%</td>
<td>6.7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>6.7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>3.3%</td>
<td>3.3%</td>
<td>6.7%</td>
</tr>
<tr>
<td>F</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Overall**

<table>
<thead>
<tr>
<th></th>
<th>22/179</th>
<th>35/179</th>
<th>44/180</th>
<th>17/180</th>
<th>27/180</th>
<th>5/150</th>
<th>60/180</th>
<th>41/180</th>
<th>8/178</th>
<th>16/178</th>
<th>20/142</th>
<th>60/180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.3%</td>
<td>19.6%</td>
<td>24.4%</td>
<td>9.4%</td>
<td>15%</td>
<td>3.3%</td>
<td>33.3%</td>
<td>22.8%</td>
<td>4.5%</td>
<td>9%</td>
<td>14.1%</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

*ND = Not Done.

Is the TURKEY shed = HOG holding pen?
Materials and Methods

- Inoculate 15 birds with “marked” Salmonella
- 24 hours later negative birds into room
- Place groups 6-8 feet apart
- Large fan (56”) between
- Necropsy at 2 and 4 hours after exposure
### Salmonella (+) samples 4 hrs post exposure to contaminated fecal dust

<table>
<thead>
<tr>
<th>Exposure Dose</th>
<th>Nasal Passages</th>
<th>Infra-Orbital Sinuses</th>
<th>Trachea</th>
<th>Lungs</th>
<th>Air Sacs</th>
<th>Ceca</th>
<th>Small Intestine</th>
<th>Spleen</th>
<th># Of Birds With Any Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2x10^9 cfu/gm</td>
<td>4/4</td>
<td>4/4</td>
<td>2/4</td>
<td>4/4</td>
<td>0/4</td>
<td>3/4</td>
<td>4/4</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>2.6x10^7 cfu/gm</td>
<td>2/4</td>
<td>1/4</td>
<td>2/4</td>
<td>4/4</td>
<td>0/4</td>
<td>1/4</td>
<td>3/4</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>2.6x10^5 cfu/gm</td>
<td>0/8</td>
<td>0/8</td>
<td>1/8</td>
<td>3/8</td>
<td>1/8</td>
<td>2/8</td>
<td>4/8</td>
<td>0/8</td>
<td>6/8</td>
</tr>
</tbody>
</table>

Microtiter detection: *Salmonella*

- Uninoculated 96-deep well plate
- Inoculated with caecal contents (48 hrs)
Transport-Campylobacter

BROILERS

1986- Stress increases peristalsis (Linton)
1995- Fewer Campylobacter when slaughtered on-farm (12.1%) vs. abattoir (56.7%) (Stern)
2001-Increase in Campylobacter/gram post-transport (Whyte)
2001-Crates contaminate broilers (Newell)
2002- Catching and crating increase contamination (Slader)
2003- Feed withdrawal increases Campylobacter on older carcasses (56 days; Northcutt)

TURKEYS

2005-C. jejuni and C. coli shifts associated with transport/holding (Wesley et al 2005)
Materials and Methods
Cloacal swabs on farm ($T_1$, n~120/trial)
Cloacal swabs at abattoir ($T_2$, $n \sim 120$/trial)
Prevalence of *Campylobacter* spp. in five Midwestern turkey flocks as determined by cloacal swabs.

Time 1 = prevalence prior to loading into transport cages (on-farm)
Time 2 = prevalence after transport and holding (at the abattoir)

* Error bars indicate 95% Confidence Interval (precision of prevalence estimate).

* Indicates a significant difference ($P < 0.01$) between Time 1 and Time 2.
Prevalence of *C. jejuni*, *C. coli*, and concurrently positive turkeys

**Time 1**= at the farm  
**Time 2** after transport and holding at the abattoir

Error bars indicate 95% Confidence Interval (precision of prevalence estimate). Error bars with the same letter indicate a significant difference (*P* < 0.01) in *Campylobacter* prevalence between T1 and T2.
Are all *C. coli* alike?

- Differentiate by Pulsed field gel electrophoresis (PFGE)

- *SmaI*
Sma I PFGE of *C. coli*
Wesley et al. 2006. Applied Environmental Microbiology
Campylobacter Diversity

- *C. coli* = 16 patterns/30 isolates
- *C. jejuni* = 9 patterns/50 isolates
- Therefore, *C. coli* more diverse. Why?
Campylobacter: Study Design

On-farm (n=30 birds)
- Crop
- Ceca
- Spleen
- Liver/Gall bladder
- Cloacal loop
- Colon

Abattoir (n=30 birds)
- Crop
- Ceca
- Spleen
- Liver/Gall bladder
## Summary of 6 flocks
(n=360 tukeys)

<table>
<thead>
<tr>
<th></th>
<th>CROP</th>
<th>DUODE NUM</th>
<th>GALL BLD</th>
<th>SPLEEN</th>
<th>ILEUM</th>
<th>CECA</th>
<th>COLON</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FARM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>3*</td>
<td>74.6</td>
<td>0*</td>
<td>0</td>
<td>87.3</td>
<td>64</td>
<td>86.7</td>
</tr>
<tr>
<td>C. coli</td>
<td>1.1</td>
<td>10.67</td>
<td>0</td>
<td>0</td>
<td>18.67</td>
<td>43.89</td>
<td>23.33</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>2.2</td>
<td>72</td>
<td>0*</td>
<td>0</td>
<td>84.7</td>
<td>27.78</td>
<td>78.89</td>
</tr>
<tr>
<td><strong>ABATTOIR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>24*</td>
<td>74.67</td>
<td>14.67*</td>
<td>2.67</td>
<td>92.67</td>
<td>57</td>
<td>80</td>
</tr>
<tr>
<td>C. coli</td>
<td>16.7</td>
<td>14.67</td>
<td>4</td>
<td>0</td>
<td>42</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>12.7</td>
<td>67.33</td>
<td>11.33*</td>
<td>2.67</td>
<td>66.67</td>
<td>24.44</td>
<td>55.56</td>
</tr>
</tbody>
</table>
Conclusions

↑ Campylobacter in crop
↑ Campylobacter in gall bladder
C. coli in cecum
C. jejuni in intestinal tract
Need to speciate and enumerate
Turkeys ≠ Pigs
Campylobacter ≠ Salmonella
Do Feed Withdrawal, Livehaul, and Holding Impact the Prevalence of *Salmonella* and *Campylobacter* in Turkeys?

*Salmonella*---NO!
Quantitate?
Genotype?

*Campylobacter*--YES!
Quantitate?
Genotype?
Substantial microbial community changes in the intestine of the pre-adolescent turkey

Alexandra Scupham
Cecal Feces Time-courses (CFT)

- Two time-courses (2004, 2005)
- Day-of-hatch males
- Diurnal lighting (12L:12D)
- Weekly sampling of cecal feces (CF)
- Total DNA analysis
  - ARISA
  - Library sequencing
  - Real time PCR
Bacterial ARISA
Campylobacter coli glyA copies/gram cecal contents

- Weeks 9
- Weeks 11
- Weeks 12
- Weeks 14
• Cecal microbiota change throughout life; stabilize towards puberty
• Bacterial species richness does not change significantly over the last 10 weeks
• Microbial communities respond to host signals
• Perturbation of non-climax communities allows colonization by pathogens
• *B. uniformis* dominate the microbiota after an environmental perturbation at week 11; may drive establishment of the adult climax community.
Transcriptional profiling of the porcine response to *Salmonella*

National Animal Disease Center
Shawn Bearson PhD, Jolita Uthe DVM

Beltsville Agricultural Research Center
Joan Lunney PhD, Atabak Royae Phd
Dan Kuhar

Iowa State University
Christopher Tuggle PhD, YanFang Wang PhD,
Dan Nettleton PhD, Jack Dekkers PhD,
Long Qu
Objective

Identify transcriptional differences in the porcine response to experimental inoculation with *Salmonella enterica* serovars Choleraesuis (narrow host range) and Typhimurium (broad host range)
Three Functional Genomic Analyses

- Suppression Subtractive Hybridization
- Real-time PCR of a panel of immune-related genes
- DNA microarray analysis
Typhimurium Choleraesuis

8 24 48

hours p.i. vs non-infected pigs

Down-regulated in ST- pigs; up-regulated in SC-pigs

Co-suppressed genes

Down-regulated in ST- pigs; up-regulated in SC-pigs

Co-induced genes

Up-regulated in ST- pigs; down-regulated in SC-pigs
Number of genes differentially-expressed during *Salmonella* infections

- **1400** genes down-regulated
- **1200** genes up-regulated

- **p < 0.01**
- **Fold change > 2.0**
- **FDR ranges from 0.04 - 0.26**

*Compared to the non-infected pigs*
Real-time PCR of 22 Immune-related Genes

Typhimurium

- IFNG
- IL12A
- IL12B
- IL18
- INDO
- IRF1
- CIITA
- SOCS1
- STAT1
- TNF
- IL4
- IL13
- IL25
- IFNA
- IL1B
- IL6
- IL8
- CSF2
- SLC11A1
- TLR4
- IL10
- TGFB1
- RPL32

Th1 response

Th2 response

Innate/inflammation

T cell-regulation

Housekeeping

Choleraesuis

- IFNG
- IL12A
- IL12B
- IL18
- INDO
- IRF1
- CIITA
- SOCS1
- STAT1
- TNF
- IL4
- IL13
- IL25
- IFNA
- IL1B
- IL6
- IL8
- CSF2
- SLC11A1
- TLR4
- IL10
- TGFB1
- RPL32

*Significant compared to non-infected control pigs
Real-time PCR analysis of several NFκB-regulated genes confirms the differences in transcriptional induction between the *Salmonella*-infected swine observed in the microarray study.
Fewer NFκB-regulated genes identified as transcriptionally activated in Typhimurium- versus Choleraesuis-infected pigs

<table>
<thead>
<tr>
<th>Typhimurium-infected pigs</th>
<th>Choleraesuis-infected pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hpi</td>
<td></td>
</tr>
<tr>
<td>14 proteins</td>
<td>23 proteins</td>
</tr>
<tr>
<td>48 hpi</td>
<td></td>
</tr>
<tr>
<td>5 proteins</td>
<td>62 proteins</td>
</tr>
</tbody>
</table>
Are similar studies possible in turkeys in response to 

*Salmonella*???

*Campylobacter*???
Help Wanted!
Materials and Methods
Campylobacter jejuni

CASES: 1,963,141

HOSPITALIZATIONS: 10,539

DEATHS: 99

Mead et al. 1999. Emerging Infectious Diseases 5:607-625
Typhimurium vs Choleraesuis (swine)

Clinical


Histological


Genetics