Report on *Salmonella* Studies of the Food Safety Consortium

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The meat and meat products research program integrated in the consortium of the University of Arkansas (UA), Iowa State University (ISU), and Kansas State University (KSU) is functioning in safety research predominantly on poultry (UA), pork (ISU), and beef (KSU). Studies on prevention and control of *Salmonella* have been a special emphasis of the Food Safety Consortium.

The goals of the Consortium are to conduct research for:

a. Development of technology for rapid identification of infectious agents and toxins within the processing and distribution chains.

b. Development of a data base and statistical framework to evaluate the extent of potential health risks posed by the contamination of the animal product food chain by infectious agents, chemical/drug residues and toxins.

c. Analysis of the animal product food chain to determine the most effective points at which intervention could occur to control or prevent a microbiological or chemical hazard cycle.

d. Development of techniques to monitor processing and distribution of food products of animal origin to detect potential microbiological or chemical hazards.

e. Development of costs and benefits of risk assessment and interdiction actions in hazard reduction/control.

Summaries of major studies entirely or partly focused on *Salmonella* in the three Consortium Universities are presented here, identified by the principal sites of the research. Reports of all of these studies, including all of the investigators who performed them are available in the annual Food Safety Consortium Reports which are gladly supplied upon request.

*Salmonella* studies will be an increasing priority during 1996-1998. A large study on the epidemiology of *Salmonella* infections in swine at farm level followed through the pork chain to *Salmonella* contamination of pork and pork products at consumer level is in pilot phase at Iowa State University and National Animal Disease Center and will move to full scale in 1996.

A study on hot water rinses on *Salmonella* contaminated swine carcasses will be continued in 1996-1997. Prevalence studies on *Salmonella* as well as other pathogenic bacteria in ground pork from suppliers varying from unfrozen preground product to fresh ground on site product will be continued at Iowa State University.

Beef carcass treatment by steam/vacuum and other methods to reduce *Salmonella* and other pathogens are a major research focus at Kansas State University. Extensive studies on *Salmonella* and other pathogen control both in industry and the laboratory will be focused at the University of Arkansas.

*Salmonella* is the meat borne pathogen of major priority for control at this time. With the Centers for Disease Control and Prevention identifying human salmonellosis in the United States as causing nearly 2 million human illnesses, between 1 and 2 thousand deaths and economic costs between 1 and 2 billion dollars, *Salmonella* control has our attention.
Prevalence of *Salmonella* spp. in Survey Herds

National Animal Disease Center

715 fecal samples collected from 15 swine herds in 15 states (pre-survey study) and 4,977 samples from 100 feedlot cattle herds in 13 states (full survey) were cultured for *Salmonella* spp. in Tetrathionate broth 48 hours, then in Rappaport medium (T48-R); tetrathionate broth 48 hours (T-48); and in GN Hajna broth 24 hours; then in Rappaport medium (GN-R).

<table>
<thead>
<tr>
<th>Species</th>
<th>Tests</th>
<th>No. Tested</th>
<th>Percent Positive for <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>Herds</td>
<td>15</td>
<td>Total 40%</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>715</td>
<td>83% T48-R 93% T-48 78% GN-R 30%</td>
</tr>
<tr>
<td>Cattle</td>
<td>Herds</td>
<td>100</td>
<td>38% T48-R 97% T-48 71% GN-R 55%</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>4,977</td>
<td>5.5% T48-R 77% T-48 50% GN-R 37%</td>
</tr>
</tbody>
</table>

ELISA Tests for *Salmonella* Infection in Swine

Iowa State University

An indirect (antiglobulin) ELISA test developed at Iowa State University, serum ELISA and tissue extract ELISA tests developed in Denmark, and fecal culture have been studied for identification of *Salmonella* infections in experimental and field swine herds.

Experimental *S. choleraesuis* Infections

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Uninoculated</th>
<th>$10^3$</th>
<th>$10^6$</th>
<th>$10^8$</th>
<th>$10^{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Pigs</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>ELISA assays</td>
<td>All Neg</td>
<td>1 pos</td>
<td>4 pos</td>
<td>All pos</td>
<td>All pos</td>
</tr>
<tr>
<td>Culture assays</td>
<td>All Neg</td>
<td>All Neg</td>
<td>All pos</td>
<td>All pos</td>
<td>-</td>
</tr>
</tbody>
</table>

Field Infection Studies

Danish MIX-ELISA was as effective as culture for identifying finishing units with infected swine.

In a swine herd with history of clinical *Salmonella choleraesuis* infection, the indirect serum ELISA test demonstrated that segregated early weaning reduced the prevalence but did not ensure elimination of infections.
Growth of *Salmonella typhimurium* in Universal Pre-enrichment Media with Supplements

Kansas State University

**Salmonella Generation Time**

Universal pre-enrichment media (UP) 22.9 minutes
UP plus 0.4 units/ml oxyrase 16.5 minutes
UP plus 1.0 μg/ml ferrioxamine E 10.5 minutes
UP plus oxyrases and ferrioxamine E 16.0 minutes

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**Reported Surveys for *Salmonella spp.* Prevalence on Meat**

Iowa State University

<table>
<thead>
<tr>
<th>Carcass</th>
<th>Fresh Meat</th>
<th>Organ Meat</th>
<th>Ground Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary of Reports on Pork</td>
<td>16.2%</td>
<td>14.7%</td>
<td>30.0%</td>
</tr>
<tr>
<td>Summary of Reports on Beef</td>
<td>1.0%</td>
<td>7.8%</td>
<td>-</td>
</tr>
<tr>
<td>Summary of Reports on Poultry</td>
<td>47.4%</td>
<td>41.9%</td>
<td>52.7%</td>
</tr>
<tr>
<td>Three Iowa Plant Study on Pork</td>
<td>0.4-4.4%</td>
<td>0-0.7%</td>
<td>-</td>
</tr>
<tr>
<td>Fourteen Ground Pork Companies</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Food Services of 3 Care Facilities:

Pork products post-thawing | - | 0% of 12 | - | 0% of 6 |
Pork products at end of service | - | 0% of 12 | - | 0% of 6 |

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**Effectiveness of Isolated Weaning and Rearing of Pigs from Three Field Herds**

National Animal Disease Center

Swine herds on three farms were sources of 10-21 day old weaning pigs which were then raised in isolation and monitored by culture for *Salmonella* infections to 6 weeks of age.

<table>
<thead>
<tr>
<th>Farm Trials</th>
<th>Culture Positive Pigs by Trial Number</th>
<th><em>Salmonella spp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1-4</td>
<td>1:0/59 2:19/78 3:15/77 4:0/71</td>
<td><em>S. derby</em></td>
</tr>
<tr>
<td>B 5-9</td>
<td>5:0/7 6:7/74 7:0/20 8:0/21</td>
<td><em>S. mbandaka</em></td>
</tr>
<tr>
<td>C 10-16</td>
<td>10:0/6 11:0/6 12:0/25 13:0/35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>14:0/40 15:0/20 16:0/40</td>
<td>-</td>
</tr>
</tbody>
</table>

52
Effectiveness of Adding Aluminum Sulfate to Broiler Litter to Reduce Pathogens

University of Arkansas

Aluminum sulfate was added to broiler litter in ten farm production units and the broiler chickens were monitored at the farms and slaughter plants for pathogenic bacteria.

Prevalence of *Salmonella* was significantly reduced in treated litter, but at the end of commercial slaughter, no differences were detected on carcasses of broilers raised on untreated litter.

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*Salmonella* Reduction on Poultry Carcasses and Chill Water

University of Arkansas

Freshly butchered chicken carcasses were inoculated with $10^6$ *Salmonella typhimurium*, experimentally treated in chill water and cultured.

Addition of 1% trisodium phosphate, sodium carbonate or sodium bisulfate to chill water reduced *Salmonella* titers by 0.26-0.42 logs on carcasses and by 90-95% in chill water.

Treatment of the carcasses in chill water with these chemicals plus low voltage pulsed electric current reduced *Salmonella* titers by 0.53-1.80 logs on carcasses and below detectable levels in chill water.

Electron microscopic studies showed that electrical treatment disrupted *Salmonella* cytoplasmic membranes and scattered cytoplasm into condensed particles the cells.

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*Salmonella* Reduction on Chicken Carcasses by Combination Treatments

University of Arkansas

In 3 trials, chicken carcasses scalded at 56 and $60^0C$ were each defeathered, then sprayed with $10^7$ CFU *Salmonella typhimurium* and incubated 30 minutes. They were then treated by dipping in 1% trisodium phosphate or/and application of 50 volt 100 Hz electric current and cultured.

Following $56^0 C$ scalding and defeathering, 6.6-7.5 logs *Salmonella* attached per carcass, a mean increased attachment of 0.5 logs following the higher temperature scald.

Treatment with trisodium phosphate reduced *Salmonella* numbers on carcasses scalded at $56^0 C$ by 0.4-0.6 logs and on those scalded at $60^0 C$ by 0.2-1.4 logs, a mean greater reduction of 1.0 logs on poultry treated after the higher temperature scald.

Treatment with trisodium phosphate and electric current reduced *Salmonella* numbers on carcasses scalded at $56^0 C$ by 0.4-0.7 logs and on those scalded at $60^0 C$ by 0.5-1.8 logs, a mean reduction of 0.8 logs on poultry treated after the higher temperatures scald.

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Salmonella Reduction on Poultry Carcasses by Chemical Sprays

University of Arkansas

Freshly butchered chicken carcasses were inoculated with 10⁶ Salmonella typhimurium, experimentally sprayed with food grade chemicals and cultured.

Spraying with 0.1% cetylpyridinium chloride, 1% lactic acid, 5 and 10% trisodium phosphate, and 5 and 10% sodium bisulfate at 30 psi for 30 seconds reduced Salmonella titers by 0.59-1.57 logs.

Increasing spraying time to 90 seconds reduced titers an additional 0.26-2.13 logs, but increasing pressure to 50-120 psi did not significantly further reduce Salmonella titers.

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Salmonella Reduction on Chicken Skin by Cetylpyridinium Chloride

University of Arkansas

Chicken skin and whole carcasses inoculated with 10⁵-10⁷ Salmonella typhimurium were sprayed or dipped in 0.1% cetylpyridinium chloride and cultured.

Spraying contaminated skin for 1-3 minutes at 15-50°C reduced Salmonella titers by 0.9-1.7 logs. Longer chemical contact and higher temperatures were moderately but not always significantly more effective.

Immersion of contaminated skin yielded Salmonella titer reductions of 1.0-1.6 logs, not significantly different from spraying.

Spraying whole inoculated carcasses 30 seconds plus 0-3 minutes contact time reduced Salmonella titers 0.45-0.77 logs.

Immersion of whole inoculated carcasses 1-3 minutes reduced Salmonella titers 2.35-3.84 logs.

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Salmonella Reduction on Commercial Turkey Carcasses by Chemical Treatments

University of Arkansas

Fresh or thawed turkey carcasses treated or not treated at the same processing plants with trisodium phosphate were cultured for Salmonella spp. Freshly butchered turkeys were also chilled in water with 1-3 ppm chlorine dioxide or 25-40 ppm chlorine and cultured for Salmonella spp.

In three experiments, Salmonella was cultured from 65%, 31%, and 5% of untreated turkeys and from 10%, 56%, and 30% respectively of trisodium phosphate treated carcasses.

Prevalence of Salmonella contamination on turkey carcasses was not significantly modified by addition of chlorine dioxide or chlorine to chill water.

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Effectiveness of Decontamination of *Salmonella* on Beef Carcasses

**Kansas State University**

Freshly butchered beef carcasses were contaminated with $10^5$ CFU/cm$^2$ of *Salmonella typhimurium* suspended in cattle feces, treated by single decontamination methods and cultured.

<table>
<thead>
<tr>
<th>Decontamination Treatment</th>
<th>Mean Titer Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife trimming of visible contamination</td>
<td>2.5 logs</td>
</tr>
<tr>
<td>35°C water wash</td>
<td>1.3 logs</td>
</tr>
<tr>
<td>Vacuum followed by 54°C water spot cleaning</td>
<td>3.3 logs</td>
</tr>
<tr>
<td>15 seconds steam</td>
<td>3.4 logs</td>
</tr>
</tbody>
</table>

Combination Decontamination of *Salmonella* on Beef Carcasses

**Kansas State University**

Freshly butchered beef carcasses were contaminated with $10^5$ CFU/cm$^2$ of *Salmonella typhimurium* suspended in cattle feces, treated by combination decontamination methods and cultured.

<table>
<thead>
<tr>
<th>Decontamination Treatment</th>
<th>Mean Titer Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife trimmed; 35°C water wash</td>
<td>4.9 logs</td>
</tr>
<tr>
<td>Knife trimmed; 35°C water wash; 15 seconds steam</td>
<td>4.6 logs</td>
</tr>
<tr>
<td>35°C water wash; 15 seconds steam</td>
<td>4.4 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash</td>
<td>3.5 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash; 15 sec. steam</td>
<td>3.8 logs</td>
</tr>
<tr>
<td>Knife trimmed; 35°C water wash; 2% lactic acid spray; 15 sec. steam</td>
<td>5.1 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash; 5-15 sec. steam</td>
<td>4.2-5.0 logs</td>
</tr>
</tbody>
</table>

Studies on a Newly Recognized Bacteriocin from *Bacillus subtilis*

**University of Arkansas**

A bacteriocin elaborated by a strain of *Bacillus subtilis* cultured from a Chinese fermented food was tested for bacterial inhibitory activity in vitro.

The partially purified bacteriocin was broadly inhibitory to *Salmonella* spp. and a selected group of both Gram negative and Gram positive pathogenic bacteria.

The bacteriocin was heat stable, active over a broad pH range, but was inactivated by peptidases and lipase.
Effectiveness of Irradiation on *Salmonella* Contaminated Pork

**Iowa State University**

Low dose (0.75-0.90 kGy) and medium dose (1.8-2.0 kGy) irradiation was applied to pork chops and sliced ham inoculated with 5-6 logs of *Salmonella typhimurium* and then held at 7°C for 7 days and at 25°C for 2 more days with cultures at 0, 7, and 9 days.

Both low dose and medium dose irradiation were effective in immediate reductions in culturable *Salmonella*, by at least 2 logs at low dose and at least 4 logs at medium dose.

Irradiation at levels tested (≤2.0 kGy) did not kill *Salmonella*, permitting resumption of growth when subjected to incubation temperature after one week storage.

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Acid and Thermal Resistance of Acid Adapted *Salmonella* spp.

**Iowa State University**

*Salmonella typhimurium* and *S. typhimurium* ATCC14028, *S. dublin*, and *S. heidelberg* adapted to growth at pH5.0 were inoculated on beef rounds, which were then tested for lactic acid rinse tolerance at 1.5 and 3.0%, and for thermosterance at 23 and 55°C.

Acid rinses were significantly more effective in inactivating acid adapted than the parent strains of *Salmonella typhimurium* ATCC14028, *S. dublin*, and *S. heidelberg* of bovine origin and equally effective against *Salmonella typhimurium* of bovine origin.

Acid adaptation significantly decreased the tolerance of *Salmonella typhimurium* and *S. dublin* of bovine origin to 55°C heat.

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Epidemiology of Salmonellosis in Children

**University of Arkansas**

90 children under 4 years of age with acute salmonellosis admitted to Arkansas Children’s Hospital were studied.

54% of the patients were under one year old, 27% were one year old, 11% were 2 years old and 8% were 3 years old.

Five clusters involved 12 patients, 10 of *Salmonella newport* and two of *S. typhimurium*. Common foods were not a factor in these clusters.

Pulsed field gel electrophoresis of genomic DNA was studied for fingerprinting *Salmonella* isolates obtained from the Arkansas State Health Department. 22 isolates of *S. typhimurium* and 29 isolates of *S. newport* did not support identification of common sources but 17 isolates of *S. meunster* were identified to a common barbequed beef source.