RESEARCH REPORT

PORK SAFETY

**Title:** Salmonella Abatement in the Pork Chain – **NPB #98-148**

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## I. Abstract

The project focused on the *Salmonella* infection-contamination-infection cycle on swine farms. The results reveal that the salmonella occurrence on swine farms is much more dynamic (prevalence and serotype patterns changing over time) than previously thought. Up to 18 different serotypes can be found on a single farm. The "exchange" of *Salmonella spp*. between infected animals and their contaminated environment plays a key role in determining farm-specific *Salmonella* patterns, which
can and must be identified before implementing intervention measures that are capable of reducing the amount of *Salmonella* carried into the food chain via slaughter hogs.
Subtyping of selected serotypes has shown that serotyping alone does not allow to conclude on infection chains. DNA-fingerprinting methods help to identify infection sources and to develop targeted prevention measures.
Comparing the resistance patterns of *Salmonella* strains isolated from slaughter animals to strains that were isolated from farm environment samples has shown that
antimicrobial resistance in *Salmonella* is wide-spread and not only limited to strains from animals that are potentially treated with antimicrobials.

## II. Introduction

Most research that has been done on studying *Salmonella* infections at the farm level has focused on the animal to animal transmission of the organism. Fecal-oral trans-mission has been well documented, and is the primary means for dissemination of *Salmonella* within a swine herd. However, investigation into identifying the specific sources for the introduction of *Salmonella* into a population and its perpetuation on swine farms have not been completed in detail. Information on the dynamic nature of the *Salmonella* infection-contamination-infection cycle (the horizontal exchange of *Salmonella* between the animals and their corresponding

environment) in swine production is a prerequisite for effective control measures of *Salmonella* at the farm level. Research from the poultry industry in Europe has identified sources for the introduction of *Salmonella* into flocks, despite the complete elimination of *Salmonella* from the starter flock chicks. *Salmonella* prevalence in *Salmonella*-free starter flocks post placement was the same when compared to flocks finished pre-*Salmonella* elimination. This research demonstrated that the contaminated environment was the source for infection of subsequent flocks. *Salmonella* control was only obtained through elimination of the organism from the environment (1,2).

The role of the *Salmonella* contaminated environment for the infection of swine herds has been shown by our research team (3, 4): about 10% of a variety of environmental samples taken on four Minnesota swine farms were salmonella-positive. The importance of the environment has also been shown by a research team from the USDA/ARS and the Iowa State University (5): *Salmonella* elimination from swine was achieved through use of segregated early weaning technologies. Piglets weaned at an early age were reared in a research isolation facility. Two groups were re-established, one continued in isolation, the other was moved to a traditional finishing facility. The animals in the isolation facility remained salmonella-free, those reared in the traditional facility became positive. This research confirms our hypothesis that there are other sources of infection apart from salmonella-shedding animals.

This report presents the result of and conclusions from our investigation into the *Salmonella* exchange between the pigs and their corresponding environment by following one complete production cycle of growing animals from 2 Minnesota swine herds, one with a high (HP) and one with a low (LP) *Salmonella* prevalence in their slaughter pigs. The report also presents the findings and conclusion of subtyping (DNA-fingerprinting and resistance patterns) of selected salmonella strains from the long-term project “Salmonella Abatement in the Pork Chain” that started in 1996 at the College of Veterinary Medicine of the U of MN

## III. Objectives:

1) To identify “salmonella-specific” critical areas and procedures at farm level.
2) To evaluate the use of the Danish mix-ELISA for detecting *Salmonella* infections in swine herds.
3) To apply salmonella subtyping methods for epidemiological analyses of the *Salmonella* strains.

## IV. Procedures

**Objective 1**: Selected, permanently marked pigs on two farms (one farm with a salmonella high-prevalence herd = HP, and one farm with a salmonella low-prevalence herd = LP) were repeatedly sampled: blood and rectal swabs at several times, and ileo-cecal lymph nodes at slaughter from 15 sows and their offspring. Simultaneously, their corresponding environment (feces, dust, feed, water, dirt, etc.) were cultured for *Salmonella*. The environmental samples were categorized as E I, E II and E III;

**E I** - direct contact with the study group animals (i.e. pen surfaces, feeders, water source, gating, feces and other organic matter).

**E II** - indirect contact with the study animals, but in close proximity (water, feed and electrical lines/conduit, surfaces of ventilation and heating equipment, walk-ways within the study group rooms, any in-room gated-off surfaces).

**E III** - indirect contact with the study animals, but outside the rearing environment of the study pigs (organic matter outside the buildings, walk-ways between buildings, feed trucks, offices, etc.).
About 400 pigs were repeatedly sampled for fecal salmonella shedding, 262 lymph nodes and 809 environmental samples were cultivated for *Salmonella*.

**Objective 2**: The about 1000 blood samples taken during the study and the meat pieces collected at slaughter were investigated for *Salmonella* antibodies using the mixed ELISA from NOBL Laboratories.

**Objective 3**: 384 Salmonella strains (28 serotypes from 25 Minnesota swine herds) have been tested for their resistance patterns by the ARS Laboratory in Athens (Dr. Fedorka-Cray). All *S. Typhimurium* strains (about 60) were subtyped via Pulse Field Gel Electrophoresis (PFGE) using the method of CDC that is established at the Minnesota Department of Health (Dr. Jeff Bender).

## V. Results

**Objective 1:** The results of the rectal swab isolation from the study pigs (offspring of the selected sows) of each farm are shown in Table 1. Results are tabulated for sampling at each production stage. A total of 69 pigs out of the 205 original pigs of the HP farm were positive for *Salmonella* at least once during the entire production cycle. 13 pigs were positive on two sampling occasions, and two pigs on three. Only one sample from the LP farm was positive.

Table 1: Rectal swabs of the study pigs

| *Salmonella* fecal swab isolation | HP farm | LP farm |
|---|---|---|
| 14-17 days of age | 0.0% (0/205) | 0.5% (1/195) |
| 35-40 days of age | 19.55% (35/179) | 0.0% (0/166) |
| 70-75 days of age | 26.9% (47/175) | 0.0% (0/165) |
| 120-125 days of age | 1.7% (3/175) | 0.0% (0/162) |
| 167-172 days of age | 0.0% (0/52) | 0.0% (0/107) |
| 181-186 days of age | 0.0% (0/129) | 0.0% (0/31) |
| Total | 33.7% (69/205) | 0.0% (1/195) |

There were 809 environment samples taken in total simultaneously with animal samples from the two study farms. Isolation for *Salmonella* of each is detailed in Table 2.

Table 2: Environmental samples

| Environment sample type | HP Farm | LP Farm |
|---|---|---|
| EI | 8.0% (13/162) | 1.7% (2/120) |
| EII | 17.3% (28/162) | 0.0% (0/120) |
| EIII | 25.0% (35/140) | 2.9% (3/105) |
| Total | 16.4% (76/464) | 1.4% (5/345) |

Cleaning procedures on each farm were investigated by taking post-cleaning environment samples. The results are shown in Table 3.

Table 3: Environmental swabs after cleaning and disinfecting

| Environment | Post-clean HP | Post-clean LP |
|---|---|---|
| EI | 16.25% (13/80) | 0.0% (0/60) |
| EII | 18.75% (15/80) | 0.0% (0/60) |
| EIII | 23.75% (19/80) | 3.3% (2/60) |

The conclusions from these results are: Our preliminary conclusion from our previous 1996/97 NPPC project (Final report December 1997) on **farm-specific *Salmonella* pattern** are confirmed by the results of the 1998/99 NPPC project "Salmonella Abatement in the Pork Chain".

There are at least two areas at farm level that can be identified as "salmonella-

critical": **the cleaning and disinfection procedure** of barns prior to bringing in pigs: at the high-prevalence farm, 28% of the post-wash samples were salmonella positive; at the low-prevalence farm, only 4% of these samples were positive), and **the daily working procedures** seem to have an important influence on the "exchange" of environmental and herd salmonella strains.

**Objective 2:** The majority of the blood and meat juice samples tested for Salmonella antibodies were negative. Only 5 samples from the HP farm and 3 samples from the LP farm were positive. These results of the ELISA testing of the sera and the meat juice samples confirm that the serological testing of herds can only be used as a diagnostic tool at herd level. Furthermore, serological testing makes only sense, if applied to a group of suppliers to identify, which supplying herd out of the group has a comparatively low, medium or high level of contact with *Salmonella spp*. as basis for a coordinated control program. Serological testing provides by no means an accurate estimate of the true prevalence.

**Objective 3:**

**1. Subtyping of *S. Typhimurium* strains by Pulsed Field Gel Electrophoresis (PFGE):** The diversity of *S. Typhimurium* PFGE-subtypes identified on the Minnesota study farms (up to four unique PFGE patterns per farm) shows that several different clones of one *Salmonella* serotype can occur simultaneously on a farm, both in animals (ileocecal lymph nodes) and in diverse environmental material. This finding clearly proves that drawing conclusions on infection sources or chains only based on serovars can easily lead to misconceptions. Using DNA fingerprint techniques will definitively improve our understanding of the on-farm epidemiology of *Salmonella* in swine and pork.

Ninety-two percent of the *S. Typhimurium* strains that were found in the pigs and the environments of the four study farms are common for pigs and humans, which emphasizes the importance of understanding the on-farm epidemiology of zoonotic *Salmonella spp.* as precondition for any reasonable intervention for reducing the introduction of salmonella into the food chain via infected and/or contaminated slaughter hogs.

Compared to *S. Typhimurium* isolates from necropsied pigs (cases from the Veterinary Diagnostic Laboratory of the University of Minnesota), which are to a high percentage multi-resitant PFGE-subtypes: 48% ACST and 24% AKST (5), only 16% of the strains from the study farms were AKST-type strains and none of the 49 isolates belonged to the DT104 group (ACST).

Although there is a tendency for a higher percentage of multi-resistant PFGE-subtypes of *S. Typhimurium* in the animal strains, the numbers are still too small for any conclusion on differences in the susceptibility of animal vs. Environmental isolates, which should be subject of further research.

**2. Sensitivity patterns**: The majority of isolates showed resistance to one or more antibiotics. However, none of the strains was penta-resistant. Resistance to four antibiotics was very rare and occurred only in 8 strains of *S. Typhimurium*, which were isolated from one farm (both from lymph nodes and the environment). The fact that there is no significant difference in the frequency of resistance between strains from lymph nodes (potential treatment of the animals) and strains from the environment, shows that the phenomenon of antimicrobial resistance in *Salmonella spp*. is much more complex than often discussed as direct consequence of the use of antimicrobials in animals, which urgently needs further research.

**References**

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