RESEARCH REPORT
PORK SAFETY

Title: Vaccination to Prevent Acute Infection by Salmonella in Transport and Lairage Prior to Slaughter - **NPB#02-119**

Investigator: D.L. Harris

Institution: Iowa State University

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Abstract: Two transmission models were utilized to determine if rapid dissemination of Salmonella Typhimurium in pigs could be prevented by either live avirulent vaccines or spray-dried plasma. The 2 models were: (1) intranasal inoculation of a high dose of S. Typhimurium and (2) natural transmission of the organism by contamination of the pen environment with Salmonella infected seeder pigs. These models mimic an important mode of pork contamination which occurs during transport and lairage immediately prior to slaughter.

Avirulent live vaccines for the prevention of disease caused by Salmonella Choleraesuis did not protect pigs against the rapid dissemination of S. Typhimurium in either model. Spray-dried plasma did not protect pigs against S. Typhimurium using model 1. Live avirulent vaccines are likely effective in decreasing the level of Salmonella in tissues at slaughter (based on previously reported field investigations); however, this effect occurs during the finisher phase of production, not during transport and lairage.

Introduction: As the importance of food safety in the pork chain continues to elevate, so does the need to identify and address points of intervention in pork production. While no one critical control point for food safety may supercede the importance of another, it is important to understand all mechanisms of contamination associated with a target organism. One such point is the idea of rapid extraintestinal dissemination (RED) of *Salmonella* spp. in swine.(Loynachan *et al.*, 2001)

In 1995, Fedorka-Cray et al. described Salmonella invasion using esophagotomized pigs in which *Salmonella enterica* subspecies *enterica* serovar Typhimurium was isolated from lymph nodes and cecum three hours after intranasal inoculation.(Fedorka-Cray *et al.*, 1994) In 1999, Vazquez-Torres et al. described a mechanism of Salmonella dissemination in which organisms are transported from the gastrointestinal tract of mice to the bloodstream by CD18-expressing phagocytes.(Vazquez-Torres *et al.*, 1999) All three of these studies suggest that hematogenous spread of Salmonella can occur very quickly and be a source of both gastrointestinal and internal tissue contamination.

In 1987, Morgan et al. described the effect of time in lairage on Salmonella contamination of slaughter pigs.(Morgan, Krautil, and Craven, 1987) They showed that the percentage of pigs carrying Salmonella in their ceca increased directly with the time spent in lairage. Recent work by Hurd et al. has shown that Salmonella infection of pigs occurs in less than 2-3 hours after being placed in pens which previously housed Salmonella infected pigs shedding the organism in their feces.(Hurd *et al.*, 2001) This same group has also shown that pigs at slaughter contain additional serovars not recovered from cohort pigs at the farm of origin. (McKean *et al.*, 2001)

There are various commercially available Salmonella vaccines that have been shown to be efficacious for reducing infection. In 1992, Kramer et al. described an avirulent Salmonella Choleraesuis strain 54 (Sc54, BI-Vetmedica) obtained from repeated neutrophil passage(Roof *et al.*, 1992). This strain was shown to reduce salmonellosis when administered to pigs via drinking water at 10^9 CFU/dose. Letellier et al. showed an increased immune response following Sc54 treatment and suggested that IgA, cellular immunity, and cytokines are involved in reduction of S. Typhimurium in the ileum and mesenteric lymph nodes(Letellier *et al.*, 2000). Another available vaccine is S. Choleraesuis Δcya, Δcrp-cdt (Argus, Intervet Inc.). Studies by Charles et al. in 2000 showed reduced shedding and clinical signs in pigs vaccinated with this strain and subsequently challenged with S. Typhimurium (Charles *et al.*, 2000). Upon necropsy, significantly fewer tissues were positive for S. Typhimurium in vaccinated vs nonvaccinated pigs. Studies by Maes et al. have shown Argus cross protection against other Salmonella serogroups. Vaccinate groups with a higher seropositivity coincided with a significant lower amount of positive lymph nodes at slaughter. (Maes *et al.*, 2001)

It seems likely that increased contamination following lairage and RED are closely related. It is possible that Salmonella-free pigs may become contaminated from the time they leave the farm to the time they are on the rail through direct contact with contaminated trucks, facilities, and/or commingling with infected pigs. Although commercial vaccines have been shown to work in long-term trials, there is no work focusing on acute infection.

Objectives:

1. Compare different challenge doses to determine the relationship between extent of infection and challenge dose.
2. Develop a natural challenge model for acute infection using seeder pigs.
3. Determine if experimental pigs vaccinated with live avirulent vaccines are resistant to acute infection of Salmonella when challenged with a virulent Salmonella isolate.
4. Determine if experimental pigs given spray-dried plasma before challenge are resistant to acute Salmonella infection.

Materials and Methods:

Objective 1: Pigs were randomized into three groups; group 1 (3 pigs) challenged with $1x10^8$ cfu of S. Typhimurium χ4232, group 2 (3 pigs) challenged with $1x10^5$ cfu of S. Typhimurium χ4232, and group 3 (2 pigs) of strict negative control. Three hours after intranasal challenge, all pigs were euthanized and necropsied. At necropsy, samples were collected as follows: blood, liver, tonsil, lung, spleen, ceum and colon content. For qualitative analysis of Salmonella, tissues were macerated by a hammer in sample bags and added by BPW broth at the rate of 9ml to 1g of samples. After incubation at 37°C overnight, 100 µl of the BPW culture were transferred into 9ml RV broth and incubated at 42°C overnight. The next day, 100µl of the RV culture were transferred and spread onto XLD agar plate containing 50µg/ml of nalidixic acid, and

incubated 37°C overnight. Suspected Salmonella colonies on XLD plates were further tested with a slide agglutination test with Salmonella O-antisera.

Objective 2: Pigs found to be free of Salmonella by serology, rectal swabs, and pen fecal samples were divided into strict negative, positive exposure, and seeder groups. Four pigs designated as seeder pigs were intranasally inoculated with 6.95 x 10^9 cfu of *Salmonella Typhimurium*. Seeder pigs were allowed to contaminate their environment for 40 hours via fecal shedding at which time the positive control treatment group was placed into the *Salmonella* contaminated. Pen fecal samples were collected prior to introduction of treatment animals into the contaminated environment and after all animals had been necropsied. Three hours post exposure to the *Salmonella* contaminated environment the animals were euthanized and the following tissue samples were aseptically collected S. typhimurium culture: tonsil, mandibular lymph node, thymus, lung, liver, spleen, colon contents, ileocecal lymph node, jejunal lymph node, cecum contents, blood, kidney, muscle, ileum, skin swab, nasal turbinate swab, heart valve swab, and stomach content swab. Culture was performed as for objective 1.

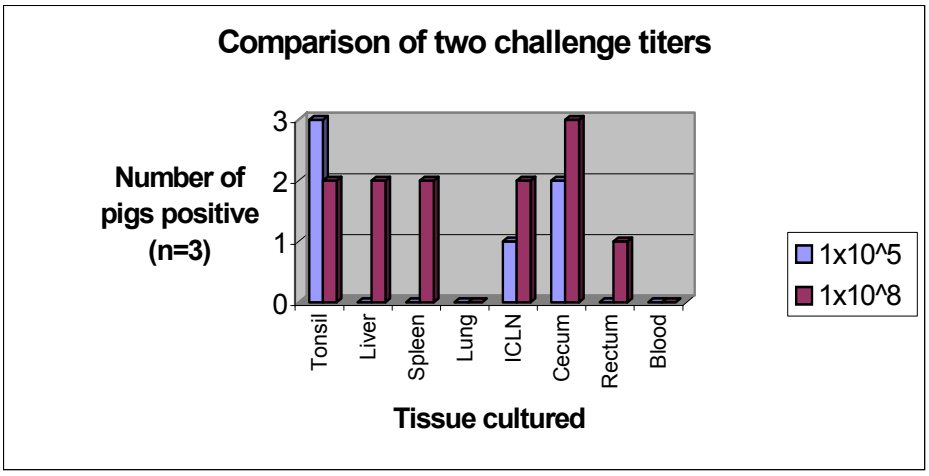
Objective 3: Two different trials were completed to evaluate vaccine. In trial 1, twenty-four pigs were obtained and randomly divided into four groups as follows: group 1 (no vaccination, no challenge), group 2 (vaccinated, no challenge), group 3 (no vaccination, challenged), and group 4 (vaccinated, challenged). Challenge dose was $3x10^9$ and Salmonella culture was as described above. No pigs in any group seroconverted based on the Danish mix-ELISA. All controls were Salmonella negative. Qualitative results for challenged pigs showed no significant difference and are shown below. Quantification of alimentary tissues was not possible in this trial except for tonsils, which showed no significant difference between groups.

In trial 2, the natural exposure model using seeder pigs as described above was used. Thirty-eight pigs were randomly divided into group 1 (no vaccination, no exposure), group 2 (seeder pigs), group 3 (non-vaccinated, exposed), and group 4 (vaccinated, exposed). Both groups 3 and 4 were divided in half so that 7 pigs were necropsied at 3 hours post exposure and 7 at 6 hours post exposure. Vaccination was intranasal with an avirulent S. Choleraesuis strain. Seeders were challenged with 5.7 x 10^9 cfu of virulent S. Typhimuium and allowed to contaminate the pen for 36 hours before introduction of groups 3 and 4. Salmonella culture following necropsy was as above.

Objective 4: Twenty five pigs were divided into three groups as follows: group 1 (no plasma, no challenge), group 2 (no plasma, challenged), and group 3 (plasma, challenged). Pigs were offered either normal water for control groups while the solutein group received water mixed with spray-dried plasma according to manufacturer's instructions. Challenge titer was $3x10^9$ cfu and Salmonella culture was as above.

Results:

Objective 1: Pigs challenged with the lower dose had fewer organs Salmonella culture positive after three hours, most notably those of non-alimentary origin.



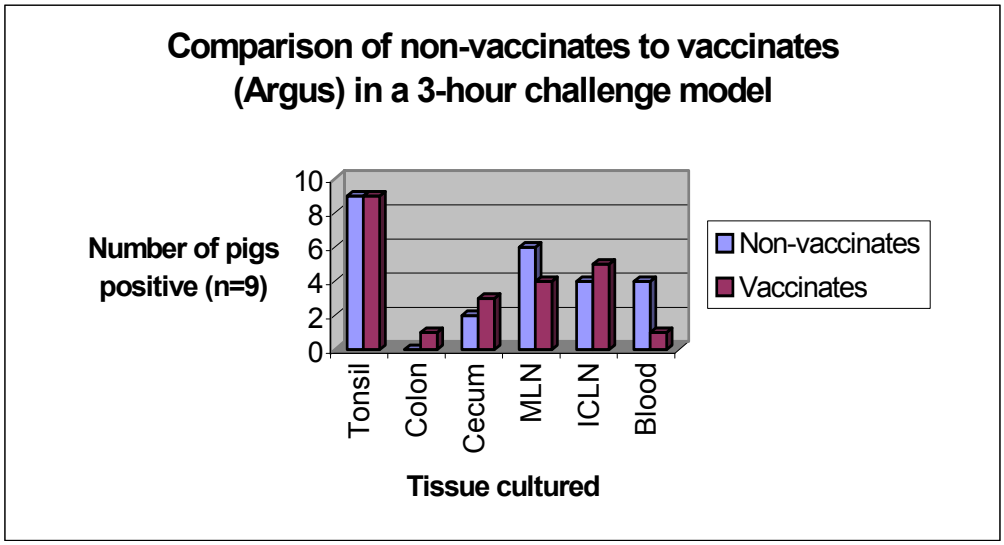
Objective 2: Seeder pigs were found to shed, on average, 4.78 X 10^4 Salmonella Typhimurium per gram of feces 20 hours after intranasal inoculation. Pigs were found to be acutely infected with *Salmonella* within three hours of exposure to a *Salmonella* contaminated environment (see table 1).

Table 1. Acute *Salmonella* Infection of Alimentary and Non-alimentary tissues

Tissue	Positive Controls (n=8)
Tonsil	8
average Salmonella isolated	7.7 X 10^2
MLN	2
Thymus	1
Lung	2
Liver	1
Spleen	2
ICLN	1
Muscle	5
Kidney	0
Jejunal Ln.	0
Ileum	8
Colon contents	2
average Salmonella isolated	4.4 X 10^2
Cecum contents	8
average Salmonella isolated	1.4 X 10^4
Stomach content swab	4
Skin swab	7
Heart valve swab	0
Nasal swab	7
Blood	0
% of all *Salmonella* positive tissues	40
% positive alimentary tissues	75
% positive non-alimentary tissues	27

Objective 3: For trial 1, qualitative results for challenged pigs showed no significant difference and are shown below in Figure 2. Quantification of alimentary tissues was not possible in this trial except for tonsils, which showed no significant difference between groups.

Figure 2



Results for Trial 2 are presented in Table 2.

Table 2

Vaccination at 3 hours vs. Vaccination at 6 hours

Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	Diff (P<0.05)	Diff (more after 6hr)	
MLN			No diff
ICLN			No diff
Blood			No diff (P=0.1431)

Non-vaccination at 6 hours vs. Vaccination at 3 hours

Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	Diff (P<0.05)	Diff (more in non-v)	
MLN			No diff
ICLN			No diff
Blood			No diff

Non-vaccination at 6 hours vs. Vaccination at 6 hour

Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	Diff (P<0.05)	Diff (more in vac)	
MLN			No diff
ICLN			No diff
Blood			P=0.0513 that there is a negative association between the groups.

Non-vaccination at 3 hours vs. Vaccination at 6 hour

Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	Diff (P<0.05)	Diff (more in vacc)	
MLN			No diff
ICLN			No diff
Blood			P=0.0513 that there is a negative association between the groups.

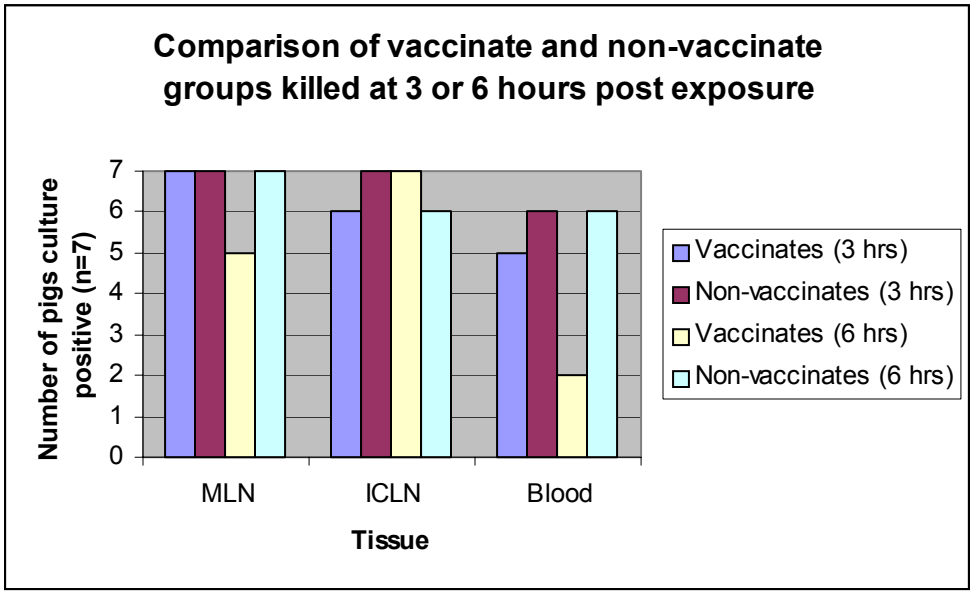
Non-vaccination at 3 hours vs. Vaccination at 3 hour

Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	No diff	No diff	
MLN			No diff
ICLN			No diff
Blood			No diff

Non-vaccination at 3 hours vs. Non-vaccination at 6 hour

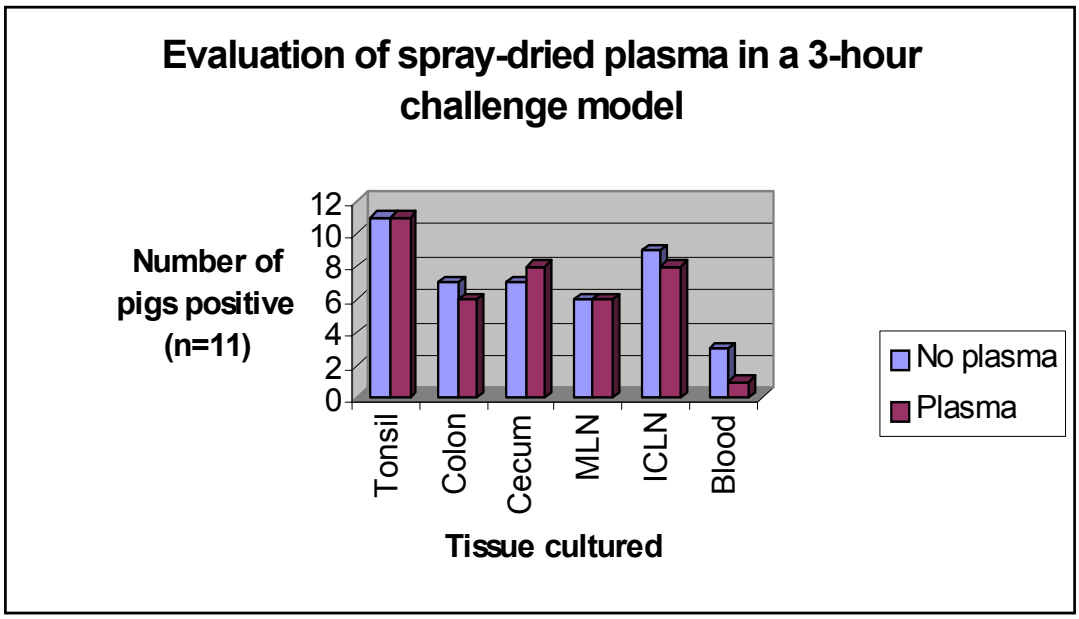
Tissue	T test (quant)	Wilcoxon (quant)	Fisher exact (qual)
Tonsil	No diff	No diff	
Ileum	No diff	No diff	
Cecum	P=0.0507	No diff at alpha 0.025 for one tailed; 0.05 for two tailed; but difference at alpha 0.05 one tailed and alpha 0.10 two tailed (6 hours more Sal)	
MLN			No diff
ICLN			No diff
Blood			No diff

Figure 3.



Objective 4: No significant difference between groups was seen for quantitative or qualitative culture. Qualitative data is shown in Table 3.

Table 3: Comparison of acute Salmonella infection of pigs with or without spray-dried plasma



Discussion: Salmonella Typhimurium disseminates rapidly to the intestinal tract and non-alimentary tissues of pigs within hours after exposure either by intranasal inoculation or via contaminated pen environment. Currently available Salmonella Choleraesuis live avirulent vaccines are efficacious for the prevention of disease caused by S. Choleraesuis. This research was conducted to determine if these same vaccines could prevent infection by Salmonella Typhimurium, an important human foodborne pathogen present in pigs pre-harvest. Two transmission models were utilized to determine if rapid dissemination of Salmonella Typhimurium in pigs could be prevented by either live avirulent vaccines or spray-dried plasma. The 2 models were: (1) intranasal inoculation of a high dose of S. Typhimurium and (2) natural transmission of the organism by contamination of the pen environment with Salmonella infected seeder pigs. These models mimic an important mode of pork contamination which occurs during transport and lairage immediately prior to slaughter.

Avirulent live vaccines for the prevention of disease caused by Salmonella Choleraesuis did not protect pigs against the rapid dissemination of S. Typhimurium in either model. Spray-dried plasma did not protect pigs against S. Typhimurium using model 1. Live avirulent vaccines are likely effective in decreasing the level of Salmonella in tissues at slaughter (based on previously reported field investigations); however, this effect occurs during the finisher phase of production, not during transport and lairage.

Lay Interpretation: Currently available Salmonella Choleraesuis live avirulent vaccines are efficacious for the prevention of disease caused by S. Choleraesuis. This research was conducted to determine if these same vaccines could prevent infection by Salmonella Typhimurium, an important human foodborne pathogen present in pigs pre-harvest. These results indicate that S. Choleraesuis avirulent vaccines do not prevent

the rapid dissemination of S. Typhimurium when pigs are challenged either with a high dose (intranasal) or a low dose (natural challenge via pen contamination) of the organism. It is very likely these avirulent vaccines do decrease the levels of Salmonella in pigs at slaughter but this protection occurs when pigs are exposed to Salmonella during the finishing phase of production, not during transport and lairage. Treatment of pigs with spray-dried plasma does not prevent the rapid dissemination of S. Typhimurium.

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