

Evaluation of cross-protection afforded by a *Salmonella Choleraesuis* vaccine against *Salmonella* infections in pigs under field conditions

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Abstract

This field study investigated the efficacy of a *Salmonella Choleraesuis* live vaccine (Argus SC™) to reduce the number of infections with *Salmonella*. Twelve groups of about 380 pigs each were randomly allocated to either vaccination (V) or no vaccination (C). The vaccine was applied orally at 3 and 16 weeks. Forty pigs per group were blood sampled at 3, 10, 16 and 24 weeks to detect possible antibodies against *Salmonella*. The prevalence of *Salmonella* in the lymph nodes was the major variable. In the V groups, only 0.6 % of the lymph nodes was positive, whereas 7.2 % was positive in the C groups ($p < 0.001$). The percentage of seropositive pigs at 24 weeks (cut-off OD >10) was 26 % and 9 % in the V and C groups, respectively ($p < 0.001$). The present study documented that vaccination with a live modified *S. Choleraesuis* vaccine is a useful tool to lower the prevalence of *Salmonella* in swine herds.

Key-words: serology, bacteriology, lymph nodes, vaccination, efficacy

Introduction

Successful control of *Salmonella* at the farm level is not straightforward. There are over 50 different serotypes in swine, *Salmonella* can survive for a long time in the environment, and infected pigs usually become long-term carriers that can shed the bacteria during periods of decreased immunity (Letellier et al., 1999; Schwartz, 1999). Therefore, increasing the immunity of the animal by vaccination may constitute a valuable tool in the control of *Salmonella*. Since *Salmonella* are facultative intracellular bacteria, immunization is best provided with live attenuated

vaccines (Roof et al., 1992). The immunity induced in swine by the use of oral *Salmonella* vaccines as well as the role of cross-protection against multiple serotypes however are not yet fully understood (Kramer et al., 1992; Curtiss et al., 1993; Everest et al., 1995).

A recent study showed that vaccinating nursery pigs with a genetically engineered *Salmonella* Choleraesuis live vaccine (Argus SC™) reduced the shedding and colonisation of *S. Typhimurium* under experimental conditions (Charles et al., 2000). The efficacy of this vaccine to prevent infections with other *Salmonella* serotypes under field conditions is not known. The major aim of the present study was therefore to investigate the efficacy of the vaccine to reduce the prevalence of *Salmonella* in an infected swine farm that was free of *S. Choleraesuis*.

Materials and Methods

Study population

The study was conducted in a swine farm located in Minnesota US. The farm consisted of 2 sow farms located approximately one mile from each other. The piglets were weaned at about 18 days of age. The nursery pigs received dry feed *ad libitum*. Citric acid and antimicrobials (Chlortetracycline, Mecodox™, Tiamulin hydrogen fumarate, Denagard™) were added to the feed for 10 days, starting 6 days after weaning. Tylosin (Tylan®) was added to the feed as a growth-promotant during the entire nursery period, also starting from day 6 after weaning. When the pigs were about 10 weeks old, they were moved to grow-finishing facilities, located 0.2 mile away from one sow farm. Each finishing room consisted of about 380 pigs. The management practices, feeding and housing conditions were identical across all finishing rooms. The pigs were fed a corn-soybean feed *ad libitum* by means of a dry and wet-feeding system that was present in every pen. They received pulse medication with chlortetracycline (Mecodox™) during the grow-finishing period. No acidifiers were applied during the grow-finishing period. All production stages were used all-in/all-out by room. The stand-empty period of the rooms between two close-outs lasted on average 3 to 5 days. During this period, the barns were cleaned and disinfected.

Experimental design

All pigs involved in this study were born from March until June 2000. In total, 12 groups of about 380 pigs each were included. The groups were randomly allocated to either vaccination (n=6) (V) or no vaccination (n=6) (C). The V groups were vaccinated twice with a modified live *S. Choleraesuis* vaccine (Argus SC™, Intervet Inc.). The vaccine was applied orally via the drinking water, and consumed within 4 hours after reconstitution of the lyophilised product. Pigs were vaccinated at 3 weeks and a second time at 16 weeks. No acidifiers nor antimicrobials were applied at least 3 days before and 3 days after each vaccination. Control groups were left untreated.

Blood sampling and serology for *Salmonella*

Forty pigs from each group were selected randomly and successively blood sampled at 22 days (first vaccination), 10 weeks (just before or shortly after moving them to the finishing units), 16 weeks (second vaccination), and within 10 days prior to slaughter. The sera of the blood samples were tested for antibodies against *Salmonella* using the SALMOTYPE® - ELISA (Blaha et al., 1999). OD-values greater than 10 were considered positive.

Slaughterhouse inspection and bacteriology for *Salmonella*

From each group, the ileo-caecal lymph nodes of about 50 pigs were collected for bacteriological testing for *Salmonella*. Therefore, all pigs that had been blood sampled and that reached market weight were selected. To end up with about 50 pigs per group, additional slaughter pigs were selected randomly out of the rest of the group.

The lymph nodes were first flamed using 95 % alcohol to decontaminate the surface, placed into a sterile sample bag and disrupted using a hammer to expose the interior. The samples were further processed according to standard procedures, as described elsewhere (Carlson & Blaha, 2001). Briefly, they were incubated with Tetrathionate broth containing iodine for 22-26 hours at 37 °C. After initial incubation, 100 µL of the Tetrathionate broth culture was brought to Rappaport-Vassiliadis R10 broth media, and incubated at 37 °C for 22-26 hours. Ten mL loops were used to strike the Rappaport-Vassiliadis media culture onto XLT-4 agar and Brilliant Green sulfur agar medium plates, and they were incubated for 22-26 hours at 37 °C. *Salmonella* suspect colonies were identified, placed into biochemical test tubes, and incubated for 22-26 hours at 37 °C. Isolates identified as *Salmonella* by the biochemical tests were serotyped using *Salmonella* O- and H-antigen sera.

Statistical analyses

One group of about 380 pigs was the unit of analysis. The proportion of seropositive pigs at different ages and the proportion of slaughter pigs with positive lymph node culture were compared for the V and C groups using Fisher's Exact test. Statistical tests were considered significant at the 95 % confidence level (2-sided). The statistical analyses were performed using SPSS (SPSS 10, SPSS Inc. Illinois 60611, USA, 1999).

Results

The overall number of seropositive pigs was significantly higher in the V groups than in the C groups (Table 1). At 24 weeks, the percentage of seropositive pigs was 26 % and 9 % in the V and C groups, respectively. The proportion of pigs that

was seropositive for *Salmonella* when using the cut-off OD-value > 40, was less than 2 % in both groups.

Only 0.6 % of the lymph nodes at slaughter was positive for *Salmonella* in the V groups, whereas 7.2 % was positive in the C groups ($p < 0.001$) (Table 2). Only 2 V groups had positive pigs, whereas positive pigs were found in 4 out of the 6 C groups. The percentage of positive pigs per group varied from 2 % to 33 %. The main serotypes were *S. Newport* ($n = 9$), *S. Typhimurium* var. Copenhagen ($n = 6$), *S. Mbandaka* ($n = 3$) and *S. Agona* ($n = 1$). Untypeable serotypes were present in 5 samples.

Discussion

This study demonstrated that the live modified *S. Choleraesuis* vaccine was able to significantly reduce the number of infections with *Salmonella* in the selected swine farm. The results corroborate with previous experimental studies which showed that the vaccine reduced the clinical signs following challenge infection with *S. Typhimurium* and the shedding and colonization of *S. Typhimurium* bacteria (Charles et al., 2000). The challenge dose for the infected pigs in the present farm was probably lower than the challenge dose used in the experimental model. Only 7 % of the C pigs was positive at slaughter, and the farm had never experienced a clinical outbreak due to Salmonellosis in the 5 years preceding the study. Thus, even in farms with a low infection pressure, vaccination was able to further reduce the *Salmonella* prevalence. The vaccine was only applied 3 days after weaning to guarantee a sufficient intake of drinking water, since the water consumption is not reliable immediately after weaning (Muirhead & Alexander, 1998). By vaccinating pigs for the first time at about 3 weeks of age, they were able to acquire active immunity before infection.

Less than 2 % of the pigs was positive using the cut-off value OD > 40. Consequently, using the ELISA in this way is not a very sensitive tool to detect *Salmonella* infections, or to assess any serological response following vaccination. When the cut-off value OD > 10 was used, significantly more seropositive pigs were found, and the percentage was higher in the V groups (26 %) than in the C groups (9 %). This indicated that the serological response following vaccination could only be detected using the cut-off value OD > 10, and that even with this low cut-off value, the serological response following vaccination was only minor.

By investigating 12 successive groups, information was obtained concerning the variability of *Salmonella* infection over time within this farm. It appeared that the prevalence varied considerably across the different groups, underscoring the dynamic nature of *Salmonella* infections in swine farms (Davies et al., 1999; Carlson & Blaha, 2001). It is thus important to investigate different groups of pigs within the same farm. No specific reason can be given for the high lymph node prevalence (33 %) in one C group. *S. Newport* was the most prevalent serotype, but *S. Typhimurium* var. Copenhagen, *S. Mbandaka*, *S. Agona* and some untypeable

serotypes were also present in this group. The infection pattern, as evidenced by the serological results, indicated that infections started already in the nursery unit but that most of the pigs became infected during the finishing period. Previous studies suggested that infections occurring after arriving at finishing farms are more important as a source of *Salmonella* in slaughter hogs (Davies et al., 1999, Dahl et al., 1997). The present study demonstrated the presence of multiple serotypes in this farm. This is a common phenomenon in commercial swine operations in the US (Davies et al., 1998; Carlson & Blaha, 2001).

In conclusion, the present study documented that vaccination with a live modified *S. Choleraesuis* vaccine is able to provide cross-protection against infections with other *Salmonella* serotypes under field conditions. The results reinforce the potential use of *Salmonella* vaccination as a management tool complementary to known intervention measures to lower the prevalence of *Salmonella* in swine herds. Further studies in farms with different management practices and with different infection patterns should be conducted to confirm the present results.

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References

1. Blaha, Th., J. Gabert, Th. Kramer, C. Weber (1999): Testing the proficiency of the German Test Kit "SALMOTYPE® -ELISA" to identify *Salmonella* antibodies in porcine sera and meat juices in USA diagnostic laboratories. In: Proc. 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork. August 5-7, Washington DC, USA, 24-25.
2. Carlson, A., Th. Blaha (2001): In-herd prevalence of *Salmonella* in 25 selected Minnesota swine farms. *Swine Health Prod.* 9, 7-10.
3. Charles, S., A. Abraham, E. Trigo, G. Jones, T. Settje (2000): Reduced shedding and clinical signs of *Salmonella* Typhimurium in nursery pigs vaccinated with a *Salmonella* Choleraesuis vaccine. *Swine Health Prod.* 8, 107-112.
4. Curtiss, R., S. Kelly, O. Hassan (1993): Live oral avirulent *Salmonella* vaccines. *Vet. Microbiol.* 37, 397-405.
5. Dahl, J., A. Wingstrand, B. Nielsen, D. Baggesen (1997): Elimination of *Salmonella* Typhimurium infection by strategic movement of pigs. *Vet. Rec.* 140, 679-681.
6. Davies, P., F. Bovee, J. Funk, M. Morrow, F. Jones, J. Deen (1998): Isolation of *Salmonella* serotypes from feces of pigs in a multiple-site production system. *J. Am. Med. Assoc.* 212, 1925-1929.
7. Davies, P., J. Funk, M. Morrow (1999): Fecal shedding of *Salmonella* by a cohort of finishing pigs in North Carolina. *Swine Health Prod.* 7, 231-234.

8. Everest, P., P. Griffiths, G. Dougan (1995): Live *Salmonella* vaccines as a route towards oral immunization. *Biologicals* 23, 119-124.
9. Kramer, T., M. Roof, R. Matheson (1992): Safety and efficacy of an attenuated strain of *Salmonella Choleraesuis* for vaccination of swine. *Am. J. Vet. Res.* 53, 444-448.
10. Letellier, A., S. Messier, J. Paré, J. Ménard, S. Quessy (1999): Distribution of *Salmonella* in swine herds in Québec. *Vet. Microbiol.* 67, 299-306.
11. Muirhead, M., T. Alexander (1998): Managing pig health and the treatment of disease. 5M Enterprises Ltd. Sheffield UK, 285-346.
12. Roof, M., J. Roth, T. Kramer (1992): Porcine Salmonellosis: characterization, immunity and potential vaccines. *Comp. Contin. Educ. Pract. Vet.* 14, 411-424.
13. Schwartz, K. (1999): Salmonellosis. In: Straw B., Mengeling W., D'Allaire S., Taylor D. (Eds.). *Diseases of Swine* 8th Edition, Ames Iowa. Iowa State University Press, 535-551.

Table 1. The percentage of pigs seropositive for *Salmonella* at different ages

Group	Number of seropositive pigs (n = 40)							
	3 weeks		10 weeks ^a		16 weeks ^a		24 weeks ^a	
	V	C	V	C	V	C	V	C
1	5	0	15	0	15	5	26	10
2	6	6	4	5	4	0	14	4
3	0	0	0	1	0	3	1	3
4	0	0	4	0	10	3	13	0
5	0	1	0	1	0	0	1	3
6	0	0	0	0	1	1	2	0
Overall	11/240	7/240	23/235	7/233	30/228	12/227	57/216	20/221
1 (%)	(4.6%)	(2.9%)	(9.8%)	(3.0 %)	(13.2 %)	(5.3 %)	(26.4 %)	(9.0 %)

^a There was a significant difference at 10, 16 and 24 weeks ($p < 0.01$)

Table 2. The percentage of pigs with *Salmonella* in the ileo-caecal lymph nodes

Group	The number (%) of positive pigs			
	V		C	
1	0/41	(0 %)	17/52	(33 %)
2	0/60	(0 %)	1/48	(2 %)
3	0/69	(0 %)	0/58	(0 %)
4	1/57	(2 %)	4/54	(7 %)
5	1/70	(1 %)	0/64	(0 %)
6	0/37	(0 %)	1/55	(2 %)
Overall (%)	2/334	(0.6 %) ^a	23/321	(7.2 %) ^b

^{a, b} $p < 0.001$