Salmonella immunization confers cross protection without confounding pre-harvest serologic monitoring

Husa, J.*(1), Edler, R.(1), Saltzman, R.(2), Holck, JT.(1), Walter, D.(1)

(1) Boehringer Ingelheim Vetmedica, Inc., Ames, IA, USA. (2) Veterinary Resources, Inc., Ames, IA, USA.

*Husa, J: jhusa@bi-vetmedica.com

Abstract

Food borne Salmonella Typhimurium is a valid concern for the global pork industry. An attenuated oral swine Salmonella Choleraesuis vaccine has proven to be an effective tool for the pre-harvest reduction of carrier rates for multiple Salmonella spp. Serum antibody assays are available to monitor exposure to wild-type Salmonella infection. This clinical study assessed protection induced by an attenuated oral Salmonella Choleraesuis vaccine against challenge infection with S. Typhimurium in swine. A serologic antibody assay was concurrently evaluated for its ability to differentiate vaccinated pigs from those challenged with Salmonella Typhimurium. Vaccination significantly improved clinical scores, pyrexia, and enteric lesion prevalence, while numerically improving average daily weight gain, and group body weight variation in comparison to unvaccinated/challenged pigs. Vaccination, while protecting pigs against disease, did not generate detectable serum antibodies prior to challenge. No vaccinated animals became seropositive prior to challenge, indicating that conventional ELISA tests could be used in vaccinated pigs to monitor wild-type exposure. Following challenge, there was no detectable difference between vaccinated/challenged and non-vaccinated/challenged animals. All strict control pigs remained serum antibody negative. These findings support the use of this vaccine to protect swine against S. Typhimurium, without confounding pre-harvest Salmonella serologic monitoring programs.

Introduction

Salmonella Typhimurium infection in swine reduces growth performance and presents a food safety risk to humans. (Flores et al, 2002. CDC, 2005) A commercial swine vaccine has been shown to reduce the carrier rate of pigs infected with various Salmonella spp including S. Typhimurium. (Neubauer et al, 2005. Kolb et al, 2002. Letellier et al, 2001. Baum et al, 1998.) Various pre-slaughter diagnostic tools can aid in the appropriate implementation of vaccination and

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assessments of the infection status of farms. (Schwartz et al, 2006) Serum antibody tests would be most useful if they did not detect antibodies due to vaccination, while still detecting antibody generated by infection with wild-type Salmonella. This clinical study evaluated heterologous protection and the serum antibody response of pigs vaccinated with attenuated-live S. Choleraesuis vaccine, followed by challenge with virulent S. Typhimurium.

**Materials and Methods**

Sixty weaned pigs, approximately 3 weeks of age, were confirmed to be Salmonella serum antibody and fecal culture negative. They were blocked by weight and sex and randomly assigned to 3 treatment groups (n=20/group, Table 1). Group 1 (Infected Control) pigs were non-vaccinated and then challenged with virulent Salmonella Typhimurium on Day 43. Group 2 pigs (Vaccinates) were vaccinated on Day 0 (Enterisol® SC-54, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO, USA) according to the manufacturer’s label instructions, and then followed by challenge on Day 43. Group 3 (Strict Control) pigs were non-vaccinated and non-challenged. To achieve blinding, the person performing observations or necropsies was not present during treatment administration. Serum samples were collected from all pigs on Days 0, 7, 14, 21, 28, 35, 43, 52, 57, 64 and 70, and tested for anti-Salmonella antibodies (IDEXX HerdChek® Swine Salmonella Antibody Test Kit, IDEXX Laboratories Inc., Westbrook, ME, USA). Rectal temperatures were measured on Days -2, -1, 0, daily from Day 1 through 21, 28, and daily from Day 43 through 58. Individual pig weights were recorded on Days -5, 0, 2, 7, 14, 21, 28, 35, 43, 50, 57, 64, and 71. Clinical observations were recorded on Days -2 through 7, 14, 21, 28, 35, daily from Day 43 through 58, 61, 63, 65, 68, and 70 using a qualitative scoring system. On Day 43, virulent Salmonella Typhimurium was administered intranasally to Groups 1 and 2 as described in previous studies. (Neubauer et al, 2005) On Day 57, half of the pigs in each treatment group were randomly selected for euthanasia. The remaining animals were euthanized on Day 71. Necropsy observations were recorded for all animals. Statistical analysis of pyrexia, average daily gain (ADG), and clinical score data was performed using Two-sample t-test, and enteric lesion statistical analysis used a Fishers Exact Test. Significantly different means were determined using the Tukey-Kramer multiple comparisons method with a confidence level of 95% (JMP v5.1, SAS Institute, Inc., Cary, NC, USA).

<table>
<thead>
<tr>
<th>Table 1. Treatment Groups Events Timeline</th>
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<tr>
<td><strong>Day 0</strong></td>
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<td>Group 1 (infected control)</td>
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<tr>
<td>Group 2 (vaccinates)</td>
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<td>Group 3 (strict control)</td>
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V = Vaccination with Enterisol® SC-54
C = Challenge with S. Typhimurium
N₁ = Necropsy one-half of pigs in each treatment group
N₂ = Necropsy remaining pigs in each treatment group

**Results**

The number of days with elevated rectal temperatures following challenge was significantly less for the Vaccinated group than the Infected Control group on 3 of 16 measurement days (P<0.05). Mean clinical observation scores were significantly reduced in the Vaccinates compared to the Infected Controls on 3 of 20 observation days after challenge (P<0.05). Additionally, Vaccinates had numeric improvement of clinical scores compared to Infected Controls on 9 of 20 days, equivalent clinical scores on 7 of 20 days, and numerically higher clinical scores on only a single day.

day. Enteric lesion prevalence at necropsy was significantly reduced in Vaccinates compared to the Infected Controls with 3 of 20, and 9 of 19 pigs respectively showing lesions suggestive of S. Typhimurium infection \((P<0.05)\). Vaccinates tended to have reduced post-challenge variability in average daily weight gain (ADG) (Day 43 through 57) compared to Infected Control pigs, with coefficient of variation (CV) values of 28.1% and 31.4% respectively. This trend continued from Day 57 through Day 71 with CV values of 11.3% and 16.6% respectively (Table 2). Only 1 of 140 serum samples from Vaccinates was ELISA positive from Day 0 through Day 43 (post-vaccination/pre-challenge). This singleton reactor was interpreted as a probable false positive consistent with published diagnostic kit specificity performance (Rossi, Ballagi, 2006) Seroconversion of all Vaccinates and Infected Controls was observed by 9 days following challenge infection (study Day 52). Internal biosecurity measures utilized during the study were validated by the lack of Salmonella seroconversion in all Strict Control pigs (Figure 1).

Table 2: Clinical, Pathologic and Productivity Effects Due to Vaccination

| Days With Rectal Temperature (Pyrexia) Improved Versus Infected Controls: Day 43-57 | Group 1 Infected Controls | Group 2 Vaccinates | P Value |
| ADG: Day 43-57 | NA | 3.16 | 0.001 |
| ADG: Coefficient of Variation: Day 43-57 | 1.07 lbs | 1.35 lbs | 0.018 |
| ADG: Day 57-71 | 31.4% | 26.1% | NA |
| ADG: Coefficient of Variation: Day 57-71 | 1.98 lbs | 2.01 lbs | 0.804 |
| Days With Clinical Scores Improved Versus Infected Controls: Day 44-70 | NA | 3.20 | <0.01 |
| Enteric Lesion Prevalence | 9.19 | 3.20 | 0.04 |

Figure 1. Percent Salmonella Seropositive Pigs

Discussion

Following S. Typhimurium challenge, pigs vaccinated with Enterisol® SC-54 had significant reductions in pyrexia, clinical signs, enteric lesion prevalence and significant improvement in ADG from Day 43 through 57. Vaccinates also demonstrated numeric improvements in ADG from Day 57 through 71 and reduced group weight variation compared to nonvaccinated pigs. These findings confirm the ability of this vaccine to provide heterologous protection. Vaccination did not result in a significant incidence of seroconversion. However, all pigs challenged with virulent Salmonella Typhimurium, regardless of vaccination status, seroconverted within 9 days of challenge. Lack of seroconversion in response to vaccination indicates that this assay is not suitable as a vaccination compliance tool or as an indicator of protective immunity. Rapid seroconversion following infection demonstrates the ability of this assay to differentiate Salmonella-exposed pigs from non-exposed pigs regardless of vaccination status. The findings from this study support the use of this vaccine to clinically protect pigs from heterologous Salmonella infection, while preserving the ability to use the serologic tool to assess exposure status to wild-type Salmonella infection. Other studies have

demonstrated the ability of this vaccine to reduce the carrier rate of multiple Salmonella spp (Neubauer et al, 2005, Kolb et al, 2002, Letellier et al, 2001, Baum et al, 1998), thereby potentially improving the food safety profile of pork from vaccinated pigs. The collective effects reported from this and other referenced studies support broader use of this vaccine, both as a clinical and productivity tool in swine production, as well as a potential pre-harvest food safety improvement measure.

Conclusions

The results of this clinical trial indicate:
- Vaccination of swine with Enterisol® SC-54 provides heterologous protection against Salmonella Typhimurium infection; a common cause of food borne Salmonella illness.
- The IDEXX HerdChek® Swine Salmonella ELISA does not detect a serologic response to Enterisol® SC-54 vaccination, but will detect antibodies to wild-type S. Typhimurium infection regardless of vaccination status. This allows for vaccination without compromising serologic Salmonella monitoring programs using this assay.