An Attenuated *Salmonella gallinarum* Live Vaccine Induces LongTerm Protection Against *Salmonella enteritidis* Challenge in Chickens.

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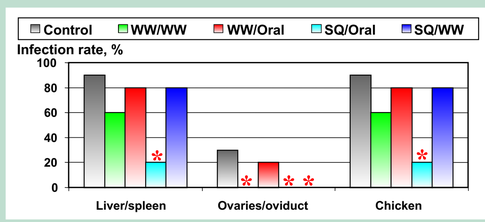
Introduction

The live rough *Salmonella gallinarum* strain, designated 9R, was first described by Smith in 1956 (9). The strain was proved effective and safe as a live vaccine when administered by both the oral and subcutaneous routes (9). Subsequently, other investigators evaluated the vaccine potential of the 9R strain and found it to be efficacious in chickens following challenges with virulent strains of *S. gallinarum, S. pullorum,* and *S. enteritidis* (1-8). The strain was also determined to be stable both *in vitro* and *in vivo* (4,8).

Studies were initiated in our laboratory to evaluate the vaccine potential of the 9R strain in chickens following challenge with virulent *S. enteritidis*. All vaccine used in the studies was derived from a well-characterized Master Seed. An initial experiment (Exp. A) was designed to compare various routes of administration. Chickens vaccinated initially by the subcutaneous route followed by drinking water administration were shown to have a much reduced colonization of the liver, spleen, ovaries/oviduct, and intestines (Figure 1) and a significant reduction in fecal shedding (CFU/g of feces) following challenge with virulent *S. enteritidis* (Figure 2). An additional experiment (Exp. B) confirmed the results of the reduction of colonization of internal tissues (Figure 3).

Based on the above favorable results, a duration of immunity study was performed.

Figure 1. Evaluation of *S. gallinarum* 9R Vaccine against *S.enteritidis* Tissue Colonization (Exp. A)

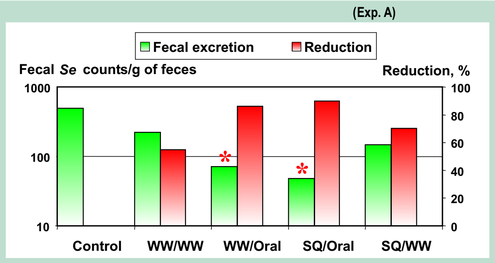


✲ Significantly (P < 0.05) differs from control (ctrl) group.
- 10 birds/group. ~107.0 CFU/dose. 2 vaccinations/bird (at 6 and 10 wks of age).
- Challenged with 108.9 CFU/bird by both IM and oral routes at 14 wks of age.
- Conclusion:

78% protection* by SQ/Oral vaccinations against *Se* infection

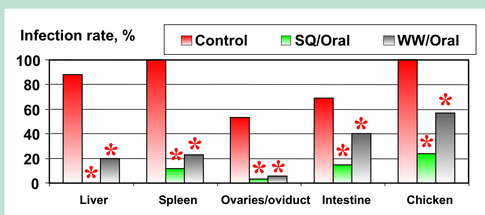
*Preventable fraction = (infectivity of ctrl group - infectivity of vac. group)/infectivity of ctrl group x 100

Figure 2. Evaluation of *S. gallinarum* 9R Vaccine against Fecal Excretion of *S. enteritidis* (Exp. A)



✲ Significantly (P < 0.05) differs from control group.
- 10 birds/group. ~107.0 CFU/dose. 2 vaccinations/bird (at 6 and 10 wks of age).
- Challenged with 108.9 CFU Se/bird by both IM and oral routes at 14 wks of age.
- Fecal samples were taken by swab.

Figure 3. Evaluation of *S. gallinarum* 9R Vaccine against *S.enteritidis* Tissue Colonization (Exp. B)



✲ Significantly (P < 0.05) differs from control (ctrl) group.
- 33-35 birds/group. 107.0 CFU/dose. 2 vaccinations/bird (at 6 and 10 wks of age).
- Challenged with 108.2 CFU/bird by both IM and oral routes at 14 wks of age.
- Conclusion:

77% protection* by SQ/Oral vaccinations against *Se* infection
43% protection* by WW/Oral vaccinations against *Se* infection*

Preventable fraction = (infectivity of ctrl group - infectivity of vac. group)/infectivity of ctrl group x 100

Materials and Methods

Test Animals

Eighty specific pathogen free (SPF) leghorns were randomly divided into two groups of forty.

Vaccine and Vaccinations

An attenuated *S. gallinarum* strain 9R live vaccine, derived from a well-characterized Master Seed, was used at 10^7 CFU/dose. At 6 weeks of age, one of the groups of birds received a 0.2 ml subcutaneous vaccination and four weeks later the same group of birds received an oral booster vaccination in the drinking water. The remaining forty birds remained as unvaccinated controls.

Challenge

Six months post second vaccination all eighty birds were challenged both orally and intramuscularly with 10^6 CFU of a virulent strain of *S. enteritidis*.

Tissue Colonization of *S. enteritidis*

At seven days post challenge, all of the birds were euthanized and the liver, spleen, ovaries, oviduct and cecal tonsils were aseptically removed . The ovaries and oviduct were pooled and the cecal tonsils were cut into small pieces (5mm). The liver and spleen from each bird was homogenized separately. Approximately 0.1 g of each individual sample was placed into 4.5 ml of TT broth, and immediately 10-fold dilutions were prepared in TT broth and incubated. A loopful of the TT broth culture from each dilution was then streaked onto a Xylose-Lysine-Tergitol 4 (XLT-4) agar plate supplemented with novobiocin (20 g/ml). After plate incubation, the plates displaying *S. enteritidis (*black) colonies were confirmed by biochemical and serological testing. Chickens and their individual tissues were scored as positive or negative for reisolation of *S. enteritidis*. Positive cultures were quantitated by correlation to the highest 10-fold dilution resulting in a positive *S. enteritidis* colony.

Evaluation

Differences in the reisolation rates and quantities of *S. enteritidis* between vaccinates and controls were expressed as "preventable fractions" calculated by the formula:

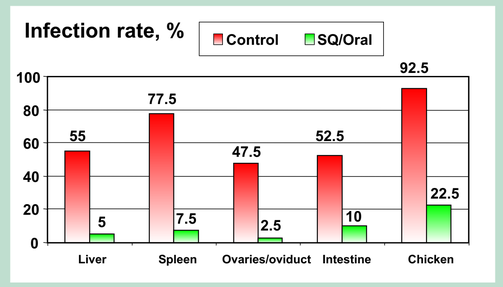
$$\frac{\text{(reisolation rate/Se counts of the control group - reisolation rate/Se counts of the vaccinate group)}}{\text{(reisolation rate/Se counts of the control group)}} \times 100$$

The results were analyzed by the Chi-square test.

Results

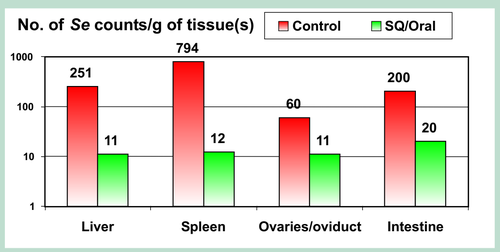
Two doses of the *S. gallinarum* 9R strain, administered to chickens at the minimum dose level (10^7 CFU/dose) by the subcutaneous and oral routes, respectively, were effective in significantly (P<0.001) reducing the reisolation rates (preventable fractions) of virulent *S. enteritidis* in tissues (liver, spleen, ovaries/oviduct and intestine) following challenge. The interval between the second vaccine dose and administration of the challenge was six months. Numbers of *S. enteritidis* were also significantly reduced in the tissues of vaccinated chickens (P<0.05).

Figure 4. *S. enteritidis* Infection Rate in Chickens*: Six Month Duration of Immunity Study (Exp. C)



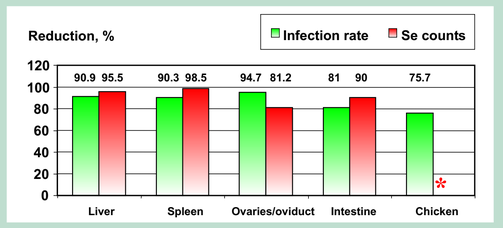
· Significantly (P < 0.05) differs from control group.

Figure 5. *S. enteritidis* Counts in Tissues*:Six Month Duration of Immunity Study (Exp. C)



* Significantly (P < 0.05) differs from control group.

Figure 6. Reductions of *S. enteritidis* Infection Rate and Countsin Chickens: Six Month Duration of Immunity Study (Exp. C)



* Significantly (P < 0.05) differs from control group.
✲ Not applicable.
- % reduction of *Se* infection rate = (% infected birds of control group - % infected birds ofvaccinate group)/% infected birds of control group x 100
- % reduction of *Se* counts = (counts of control group - counts of vaccinate group)/counts of controlgroup x 100

Conclusion

This study demonstrated that the *S. gallinarum* 9R strain, administered at a minimum dose level (below field dose) was capable of inducing a protective six-month duration of immunity in vaccinated chickens following a severe challenge with *S. enteritidis*.

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