EFFICACY EVALUATION OF *SALMONELLA* ENTERITIDIS SRP VACCINE AGAINST CHALLENGE BY *SALMONELLA* ENTERITIDIS, *S.* TYPHIMURIUM AND *S.* GALLINARUM IN BROWN LAYING HENS.

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Introduction

Currently, *Salmonella enterica* is one of the most important pathogens for industrial poultry due to growing concerns about food safety and public health (GAMA et al., 2003). The paratyphoid *Salmonella* serovars, such as *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (STM) are responsible for poor performance of breeders, decreased egg production in layers, infertility, and mortality (BARROW, 2000), being related to the increase of human foodborne salmonellosis (TAUNAY et al., 1996). In addition to non-typhoidal salmonellosis, poultry production in Brazil and other countries across the world is constantly affected by fowl typhoid outbreaks, a disease caused by the serovar *Salmonella* Gallinarum (SG), Biovar Gallinarum, an avian host-specific bacterium (PENHA FILHO et al., 2017). Fowl typhoid brings losses to the sector due to the mortality of affected birds, including adult birds with a long cycle and, therefore, with greater individual value, such as commercial laying birds and breeding birds (GAST; PORTER, 2020). The control of these bacteria through vaccination is a high priority demand today, both within the scope of the poultry production industry, or by the vaccine industry, the international animal protein trade, retailers, and consumers.

The objective of this study was to evaluate the efficacy of a novel vaccine based on purified siderophore receptors and porin proteins (Vaxxon®SRP®SE), in brown laying hens challenged by SE (*S.* Enteritidis), STM (*S.* Typhimurium) or SG (*S.* Gallinarum).

Materials and Methods

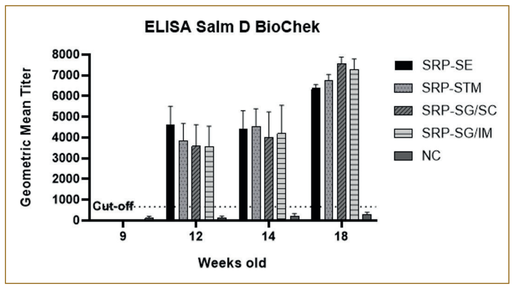
The execution of the experiment was previously approved by the Ethics Committee on the Use of Animals (protocol 008/2021), and all the procedures and animal care were followed. 310-day-old brown laying hens were housed in isolated rooms (biological safety cabins), which have controlled air exhaustion and negative pressure. The birds received appropriate feed, according to the age, and water ad libitum, during the experimental period. The birds were divided into 8 experimental groups: 3 unvaccinated groups, each challenged by SE (PC-SE), STM (PC-STM) and SG (PC-SG), 4 vaccinated groups and challenged by SE (SRP-SE), STM (SRP-STM) and SG (SRP-SG/SC and SRP-SG/IM) and 1 unvaccinated and unchallenged group (NC). Groups SRP-SE, SRP-STM and SRP-SG/SC were vaccinated, subcutaneously, with two Vaxxon®SRP®SE doses at 9 and 14 weeks old and SRP-SG/IM were vaccinated intramuscularly at the same time. Birds were challenged at 18 weeks old with 2 mL of 10^8 CFU/mL inoculum for each *Salmonella* serovar. For PC-SE, SRP-SE, PC-STM and SRP-STM groups, fecal excretion was evaluated through weekly cloacal swabs and systemic infection through bacterial count in the organs and for groups PC-SG, SRP-SG/SC and SRP-SG/IM, clinical signs and mortality were evaluated. Serological evaluation of the vaccinated groups and negative control group was performed through the ELISA Kit Salm D Biochek before the challenge, and the results were statistically analyzed by Two-way ANOVA test (Mixed-model). Mortality and fecal excretion were analyzed by Chi-square test. The One-Way ANOVA with Bonferroni's test was used to analyze bacterial count. A significance level of less than 5% (P<0.05) was adopted for all analyses.

Results and Discussion

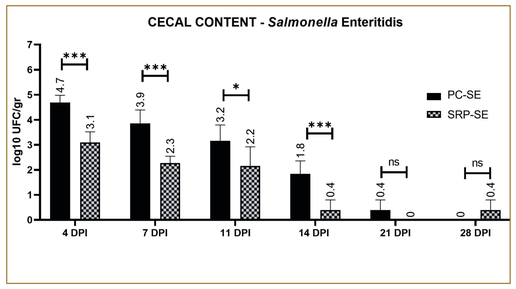
Vaccination seroconverted 100% of high antibody titration before the challenge, as show in graph 01. For hens challenged with SE, bacterial cecal count had a significant (p<0.05) reduction of 1.6, 1.6, 1.0 and 1.4 log10 at 4-, 7-, 11- and 14-days post-inoculation (DPI) in SRP-SE, as show in graph 02. For the groups challenged with STM, a significant reduction of 1.1 and 1.7 log10 in vaccinated group (SRP-STM) was observed at moments 7 and 11 DPI, respectively, as show in graph 03. For the fecal excretion parameter there was also a significant difference between the vaccinated and unvaccinated groups, with a reduction of 48% and 53% of positivity, respectively for birds challenged with SE and STM, in comparison with unvaccinated groups (Table 01). The potential of vaccine protection for SE and STM has been previously characterized with other inactivated and live vaccines, and studies claim that this level of protection reflects epidemiologically in lower rates of food contamination and reduction of outbreaks of human infections by these serovars (ATTERBURY et al., 2009; GROVES et al., 2016).

For groups challenged with SG there were a significant reduction in mortality rates (P<0.001), from 3% to 10% compared to 47% of unvaccinated group (PC-SG). Graph 04 shows the difference between groups' survival rates. Other studies that evaluate live vaccine against SG demonstrated similar results (PENHA FILHO et al., 2017). Thus, survival rate reached satisfactory level of protection, considered equal to or greater than 90% for vaccinated hens.

Graph 1:
GMT of the vaccinated groups and negative control group (NC) in the different evaluation times prior to the challenge. Dashed line (--) refers to the cut-off of the ELISA kit.



Graph 2:
Bacterial count in cecal content of SE-challenged birds. *significant statistical difference (p<0.05); ***significant statistical difference (p<0.001); ns- without significant difference between groups.



Graph 3:
Bacterial count in cecal content of STM-challenged birds. *significant statistical difference (p<0.05); ***significant statistical difference (p<0.001); ns- without significant difference between groups.

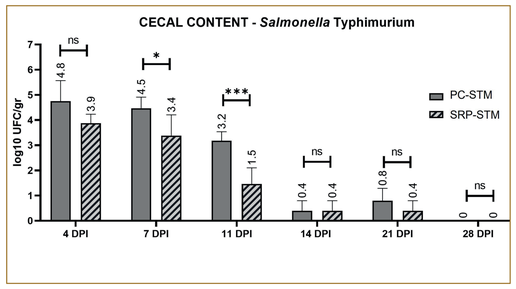
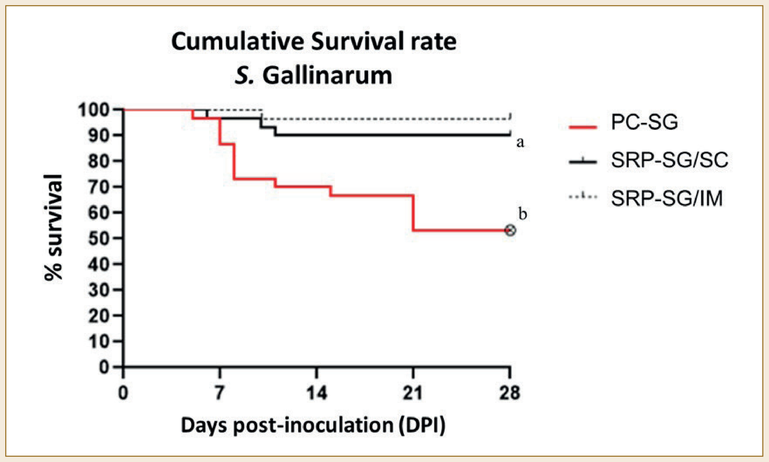


Table 1:
Cloacal swabs results from birds infected with *Salmonella* Enteritidis (PC-SE, SRP-SE) and *Salmonella* Typhimurium (PC-STM, SRP-STM), represented as positive or negative for the challenge strain after plating on selective agar.

DAY	% Positive samples			
	PC-SE	SRP-SE	PC-STM	SRP-STM
4 DPI	53% *a	33% a	**60% a**	**27% b**
7 DPI	20% a	27% a	20% a	13% a
11 DPI	**33% a**	**7% b**	7% a	0% a
14 DPI	7% a	7% a	0% a	0% a
18 DPI	0% a	0% a	0% a	0% a
21 DPI	7% a	0% a	0% a	0% a
25 DPI	7% a	0% a	0% a	0% a
28 DPI	13% a	0% a	0% a	0% a
TOTAL	**18% a**	**9% b**	**11% a**	**5% b**
REDUCTION	**48%**		**53%**	

*Different letters represent significant difference (p<0.05) between vaccinated groups and positive control groups, by Chi-square test.

Graph 4:
Survival rate of brown laying hens in vaccinated (SRP-SG/SC and SRP-SG/IM) and unvaccinated (PC-SG) groups, after the challenge by pathogenic strain of *Salmonella* Gallinarum.
*Different letters represent significant difference (p<0.001) between vaccinated groups and positive control group, by Chi-square test.



Conclusion

- Vaxxon® SRP®SE provided higher antibody titers in ELISA Salm D test, when applied in two doses by subcutaneous or intramuscular route.
- Vaccination with Vaxxon® SRP®SE provided lower fecal shedding and systemic infection in hens experimentally infected with SE and STM.
- Vaxxon® SRP®SE vaccination reduced mortality in hens infected with SG.
- The obtained results suggest the capacity of Vaxxon® SRP®SE vaccine to control salmonellosis in the fields.

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Figure 1:
Macroscopic lesions found in birds of the PC-SG group, with 21 dpi, caused by *Salmonella* Gallinarum. **(A)** Thin birds, with wasted chest musculature and prominent sternum keel; **(B)** immature eggs with ovarian atrophy in adult birds; **(C)** Ovary and oviduct atrophy.