

# Assessment of *Salmonella* Enteritidis and *Salmonella* Typhimurium challenge in commercial 84-weeks of age layers vaccinated with a *Salmonella* Enteritidis live vaccine.

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## Introduction

*Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) are the main *Salmonella* causes for foodborne infection in humans.

Vaccination is one of the tools available for broiler breeders and laying hens producers as part of a comprehensive *Salmonella* control plan.

The aim of this study was to assess the level of protection provided by a *Salmonella* Enteritidis live vaccine against SE and ST in 84-week-old vaccinated hens.

## Materials and Methods

Bovans brown commercial layers were vaccinated through the drinking water with a live SE vaccine auxotrophic for adenine and histidine at day-old, 6 weeks, and 13 weeks of age. Hens were tested negative for *Salmonella* official control during rearing and production. At 84-weeks of age, 34 hens were transferred to an experimental farm to be challenged with SE and ST. The birds were divided into three experimental groups: a negative control group (T1), a SE challenge group (T2), and a ST challenge group (T3). Before the *Salmonella* challenge, all animals were individually sampled for *Salmonella* detection. Hens were orally challenged with calibrated SE or ST challenge strains. 48-hours after challenge, *Salmonella* colonization was assessed collecting samples from the spleen and the liver and caecum. *Salmonella* detection was performed using standard microbiological techniques (ISO 6579-1:2017) and *Salmonella* load was done according to Fiorentin et al. (2005). Bird body weight was recorded at different steps of the trial. The challenge dose of  $1.62 \times 10^8$  CFU/mL for SE and  $1.01 \times 10^8$  CFU/mL for ST was administered by oral gavage.

## Results

The cloacal swabs of the hens tested upon arrival at the experimental farm were all negative for any *Salmonella* serovar.

In the SE challenge group (T2), 50% of cecal samples collected 48 hours after challenge tested positive. The SE challenge strain load from the caecum sample was 0.92 Log<sub>10</sub> CFU/g, but no SE was recovered from the liver and spleen samples. In the ST challenge group (T3), no samples tested (caecum, liver and spleen), were positive for the ST challenge strain. In contrast, other authors described the colonization of 88.9 to 97.20% of the livers, 88.9 to 94.4% of the spleens and 22.2 to 80.5% of caecum from different groups of layers challenged with a SE strain (Gast et al., 2019).

Results from this study showed that, commercial layers, at 84 weeks of age, when challenged with field SE and ST, can be protected from ST colonization through 3 vaccinations with a SE live vaccine. In the case of SE, although SE was detected in some caecum samples, *Salmonella* enumeration was sufficiently low to prevent further colonization of internal organs.



Picture 1. Experimental pens



Picture 2. Administration of 1ml of *Salmonella* at  $10^8$  CFU/mL.

Table 1. Study treatment

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Treatment	N° of animals	Strain	Infection titer
T1	10		Negative Control
T2	10	<i>Salmonella</i> Enteritidis	Positive Control with <i>Salmonella</i> challenge at $10^8$ CFU/bird
T3	10	<i>Salmonella</i> Typhimurium	Positive Control with <i>Salmonella</i> challenge at $10^8$ CFU/bird
N° Treatments: 3		Total n° animals: 30	

Table 2. Enumeration of *Salmonella* from viscera samples

Treatment Groups	n	Caecum		Liver		Spleen	
		Mean (Log <sub>10</sub> CFU/g)	% Posit	Mean (Log <sub>10</sub> CFU/g)	%	Mean (Log <sub>10</sub> CFU/g)	%Posit
T1	10	0 <sup>a</sup>	0	0	0	0	0
T2	10	0.92 <sup>b</sup>	50	0	0	0	0
T3	10	0 <sup>a</sup>	0	0	0	0	0
<i>P</i> -value		0.011		-		-	

T1: control group; T2: SE challenge group; T3: ST challenge group.

## Conclusions

This is the longest duration of immunity demonstrated through challenge studies in response to 3 vaccinations with an auxotrophic for adenine and histidine SE vaccine strain answering the field question regarding the protection of older laying flocks over 80 weeks of age.

Regarding ST colonization, none of the samples analyzed tested positive for *Salmonella* detection or enumeration.

Regarding SE colonization, none of the liver and spleen samples tested positive for *Salmonella* detection. In the case of caecum samples, *Salmonella* was detected in 50% of the samples but at a very low concentration. This very low concentration of *Salmonella* in caecum could explain the non-appearance of positive samples in the detection of *Salmonella* in the livers and spleens, demonstrating less colonization in the animal both at the intestinal and systemic levels.

With the study data, it was observed that hens vaccinated with three doses during rearing were protected against ST and SE at least 71 weeks after the third vaccination. These data exceed, by 11 and 8 weeks respectively, the duration of immunity for these *Salmonella* serovars in the vaccine used in this study according to its technical data sheet.