“Immunity Conferred by a Live Salmonella Enteritidis Vaccine against Fowl Typhoid in Laying Hens”

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Introduction

Salmonella Gallinarum with the biotypes Gallinarum and Pullorum causes two septicaemic diseases in poultry: Fowl Typhoid and Pullorum Disease. The former Salmonella Pullorum serovar is not recognised anymore as such. Both biotypes are differentiated by a few biochemical and molecular tests. Mammals may be infected without showing any illness. In contrast to zoonotic Salmonella serovars, Salmonella Gallinarum does not cause any gastroenteric disease in humans transmitted throughout the food chain. Many animals, including men, may become asymptomatic carriers. Vectors such as rodents, flies, darkling beetles and red mites are very important reservoirs and sources of infection. Although Fowl Typhoid has been officially eradicated from North America, Oceania, Japan and many European countries, infection may appear again because wild birds can harbour Salmonella Gallinarum; this has been the case in the United Kingdom in 2006, 2007 and 2011. Fowl Typhoid subsists as an endemic infection in some countries of Central and South America, Africa, the Middle East and several CIS and Asian countries. In some commercial poultry
Pathogenesis of Typhoid *Salmonella*

*Salmonella* is able to pass through the intestinal wall following 3 main alternative routes: (i) *Salmonella* may cross M cells of the Peyer’s patches located near the entrance of the caecum, (ii) through diffuse lymphatic tissue of the gut; (iii) by the apical pole of intestinal cells. Once *Salmonella* reaches the cytoplasm a „*Salmonella* containing vacuole” is produced. Inside this vacuole *Salmonella* Gallinarum replicates and the bacterial cells are transported to the basolateral side of the cell where they are released into the lamina propria. Alternatively *Salmonella* may enter through a dendritic cell, which emits pseudopods between epithelial cells and captures those *Salmonellae* that are located in the lumen.

In the lamina propria *Salmonella* is engulfed by macrophages. In this sub-epithelial location *Salmonella* causes macrophage apoptosis, a process that triggers the inflammation cascade that attracts more phagocytes. Invasion through the brush border and the T junctions of the intercellular space does not cause any damage and therefore is not noticed by the immune system. In addition the lack of flagella by *Salmonella* Gallinarum provides a hiding advantage, as the presence of flagellar proteins (which are highly antigenic) would stimulate the immune response.

In difference to other intracellular pathogens that multiply free in the cytoplasm *Salmonella* induces the formation of intracellular vacuoles. These vacuoles mature in 1 hour. After 3 hours latency *Salmonella* multiplies inside the vacuole. Inside the vacuole *Salmonella* is protected from the action of antibodies, lysoenzymes and from antibiotics that are unable of any intracellular action. Macrophages carry *Salmonella* Gallinarum inside the „*Salmonella* containing vacuole” and disseminate *Salmonella* Gallinarum systemically. Infected macrophages enter by diapedesis into blood vessels and are dragged by the blood stream to the endoplasmic reticulum and reproductive tissues.

Mixed typhoid and paratyphoid infections

After many studies carried out in mice it is known that *Salmonella* growth results in an increase in the number of infected cells with low bacterial numbers in most cells. As *Salmonella* triggers apoptosis the released *Salmonellae* are able to invade neighbouring cells in order to grow the necrotic foci as more and more inflammatory cells are attracted to the site. Some of the released *Salmonellae* invade the blood stream and develop new necrotic foci. Recent research showed that *Salmonella* Gallinarum together with paratyphoid serovars, including *Salmonella* Enteritidis, may concurrently infect the same farm and even the same chicken. Molecular techniques allowed the detection of *Salmonella* Enteritidis in samples that have been taken from Fowl Typhoid diseased laying hens. In fact, when a meticulous study is performed and a number of different samples from the same farm are taken during a long period of time, mixed infections with different serovars are commonly detected.

Both *S. Gallinarum* and *S. Enteritidis* can colonize the reproductive tract

*S. Gallinarum* and *S. Enteritidis* are two phylogenetically related bacteria deriving from a common ancestor bacterium. Both serovars are clonally related and share many common pathogenic factors that allow invasion and egg colonisation. They share the adhesion fimbria SEF14, common D1 O antigens, the same mechanism of infection and intracellular multiplication, and the same lymphokines that allow cross protection among them and the same *Salmonella* plasmid virulence operon. Because of these pathogenic factors that both bacteria share, both serotypes have a tendency to invade the reproductive tissues and are able to colonise the hen’s genital tract. *S. Gallinarum* often causes multiple misshapen ovary follicles, ceasing the production of eggs. On the contrary, hens infected with *S. Enteritidis* usually maintain a normal egg production rate, but eggs are commonly *Salmonella* Enteritidis contaminated.

Diagnostic and monitoring also play an important role

In contrast to zoonotic *Salmonella*, where bacteriological diagnostic methods are applied, serology is used to detect *Salmonella* Gallinarum infected flocks and estimate the prevalence of Fowl Typhoid infection within a flock. The rapid whole blood plate agglutination test can identify positive birds in the farm because agglutination antibodies appear from 3 to more than 10 days after infection. This test is used in eradication programs for chickens but is unreliable in turkeys and ducks due to appearance of false positives. Due to the extended infections with *S. Enteritidis* a high percentage of false positives is usually detected; therefore serology only provides a presumptive diagnosis and requires confirmation by bacteriology or molecular tests before deciding to eliminate the positive birds.

Studies with AviPro® *Salmonella* Vac E against Fowl Typhoid

These studies were carried out at INTA Balcarce, Argentina, and published in AVIAN DISEASES 50:280–283, 2006. The laying hens used in these trials belonged to the Lohmann Classic layer line. *Salmonella* free chickens were reared in complete isolation from the 1st day of life under strict isolation and high biosecurity measures. All chickens were caged from the 1st day of life and were individually identified. Vaccinated and non-vaccinated chickens were separately reared. The challenges were carried out in a separate building and after the infection hens were kept in isolators.
Vaccinations. According to the dose recommended by the manufacturer, 0.5 mL containing 100 to 500 million Salmonellae per chicken were orally administered by gavage into the crop. A subcutaneous route was also experimentally tested injecting the same dose behind the neck. Chickens were vaccinated at the first day of life and in the 6th, 16th and 30th week of life.

Challenge strain. Salmonella Gallinarum INTA 91 was used. The virulence was enhanced by subcutaneous inoculations in 18-week-old cocks.

Challenge dose. The 50 percent lethal dose was calculated in pretrials. One lethal dose was set as 0.5 mL containing 20,000 CFU per bird. This lethal dose was orally administered by gavage into the crop. Vaccinated and non-vaccinated laying hens were challenged at 28 and 52 weeks of age.

Experimental design. Each experimental group consisted of 17 hens. The group identified as 3-O was given 3 oral doses. The group identified as 2-O-S was given 2 oral doses and the last dose was administered subcutaneously. A further control group remained non-vaccinated. The 3 groups were challenged in week 28. This challenge was done 12 weeks after the last vaccination. All birds were killed 21 days after challenge.

Figure 1: Illustration of the challenge model with Salmonella Gallinarum strain INTA 91.

Reduction of Salmonella Gallinarum faecal excretion

When 3 oral doses were administered at the 1st day of life and in weeks 6 and 16, the faecal excretion was reduced from 100% in the hens of the non-vaccinated control group to 20% in the hens of the vaccinated group. When the 3rd dose was administered by subcutaneous route, faecal excretion was reduced to 10% in this group.

Figure 3: Percentage of positive samples of S. Gallinarum INTA 91 few days after challenge infection.

Protection against mortality

All except one (out of 16) non-vaccinated hens died whereas all except one (out of 16) orally vaccinated hens survived. No mortality was registered in the group that received 2 oral doses and 1 subcutaneous dose.

Figure 4: Mortality rate 12 weeks after the 3rd dose of AviPro® Salmonella Vac E.


Optimum protection was observed when the hens were challenged in week 28

Shedding of the vaccine strain. After the first vaccination at 1st day of life, the vaccine strain could be recovered from all cloacal swabs up to the 10th day post-vaccination. Thereafter all faecal samples were consistently negative. In contrast after the vaccination boosters given at weeks 6, 16 and 30 of age the vaccine strain could not be isolated anymore.

Figure 2: Shedding of S. Enteritidis vaccine strain after vaccination at 1st day of age with AviPro® Salmonella Vac E.

As a rule Salmonella Gallinarum was isolated from the organs of all dead hens. In contrast, Salmonella Gallinarum could not be recovered from any of the hens that remained alive until their sacrifice at day 21 post-challenge.

No protection was observed when the hens were challenged in week 52

Further studies showed no protection when challenges were carried out either at 22 or 36 weeks after the last vaccine dose. It was demonstrated that protection is related to the time elapsed between the last vaccination and the challenge. Considering the above, a booster vaccination every 12 weeks is strongly recommended.

Conclusions

Fowl Typhoid generates important economic losses for the global poultry industry. *Salmonella Enteritidis* is able to cross immunize against *Salmonella Gallinarum*. Repeated vaccination protects against mortality, organ colonisation and reduces the faecal excretion rate avoiding spread of *Salmonella* in the environment. Protection depends on the time elapsed after the last booster vaccination; hence oral revaccinations in drinking water each 3 months are highly recommended. The vaccine strain Rif12/Sm24/Ssq can be used to design strategies to simultaneously prevent both Typhoid and Paratyphoid infections. If Fowl Typhoid and *Salmonella Enteritidis* cause infections or if Fowl Typhoid is eradicated but *Salmonella Enteritidis* is present, vaccination with a live *S. Enteritidis* vaccine is recommended. Vaccination alone is not enough to control salmonellosis, therefore it should be conceived as part of a holistic concept which also includes hygiene, strict biosecurity measures, diagnostics & monitoring, nutritional management and good farming practices.

Bibliography


AviPro® SALMONELLA VAC E: Lyophilisate for suspension. Statement of the active substance and other ingredients: One dose contains: Live attenuated *Salmonella Enteritidis* bacteria, strain Sm24/Rif12/Ssq, min. 1 x 10⁸ CFU* and max. 6 x 10⁸ CFU*. *CFU = Colony Forming Units. Indications: active immunization of chickens to reduce faecal excretion and colonisation of internal organs with *Salmonella Enteritidis* field strains. Onset of immunity: immunity develops within 14 days of first vaccination: after 15 days the faecal excretion is reduced up to 70 %. Duration of immunity: the immunity lasts until week 60 of life. Vaccination scheme: Chickens (layers and breeders): a single dose at one day of age followed by a second vaccination at 6-8 weeks of age and a third vaccination at 16-18 weeks at least 3 weeks before point of lay. Administration: For administration after resuspension via drinking water. Contraindications: Do not use in sick birds. Do not use in birds in lay and within 3 weeks before onset of lay. Adverse reactions: None known. If you notice any serious effects or other effects not mentioned in this leaflet, please inform your veterinary surgeon. Withdrawal period: meat and offal: 21 days.

To be supplied only on veterinary prescription. Name and address of the manufacturing authorisation holder responsible for batch release and marketing authorisation holder: Lohmann Animal Health GmbH (an Eli Lilly company). Heinz-Lohmann-Str. 4, 27472 Cuxhaven, Germany.