

Protection conferred by a live *Salmonella enteritidis* vaccine against fowl typhoid

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Salmonella gallinarum with the biotypes gallinarum and pullorum causes two septicaemic diseases in poultry: fowl typhoid (FT) 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 FT 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 UK in 2006-2007-2011.

FT subsists as an endemic infection in some countries from Central and South America, Africa, Middle East, and several CIS and Asian countries. In some commercial poultry operations *Salmonella gallinarum* is found but remains officially unreported, or in fact, the infection may be really unknown when outbreaks take place in backyard flocks.

Birds become chronic carriers passing the disease to their offspring through eggs.

Approximately one third of the eggs are contaminated. Cross contamination in the hatchery is crucial for spreading the disease.

Horizontal transmission usually takes place orally although sometimes occurs by the respiratory route when dry manure is manipulated in the farm and the hen inhales the dust.

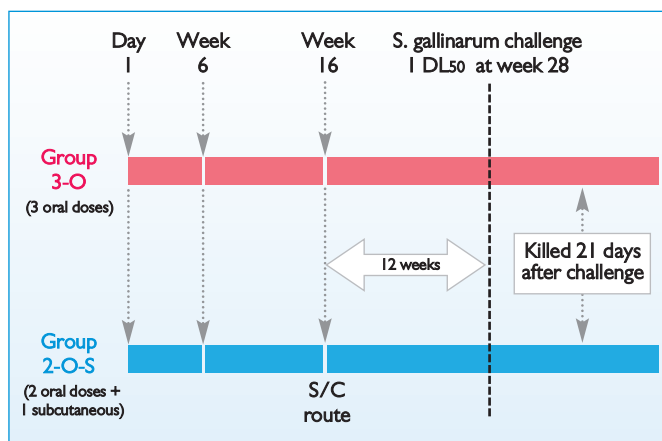


Fig. 1. Illustration of the challenge model.

Environmental contamination (for example feed, water and litter) and cannibalism are significant factors triggering the infection. It has to be taken into account that *Salmonella gallinarum* is able to survive in favourable environments or inside red mites for several months.

Pathogenesis

Salmonella is able to pass through the intestinal wall following three main alternative routes:

- *Salmonella* may cross M cells of the Peyer's patches located near the entrance of the caecum.

- Through diffuse lymphatic tissue of the gut.
- 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 in the lamina propria. Alternatively, salmonella may enter through a dendritic cell, which emits pseudopods between epithelial cells and captures those salmonella 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) 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 one hour.

After three hours latency salmonella multiplies inside the vacuole. Inside the vacuole salmonella is protected from the action of antibodies, lysozymes and from antibiotics that are incapable 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 bloodstream to the reticulum endothelial and reproductive tissues.

Enterohepatic cycle

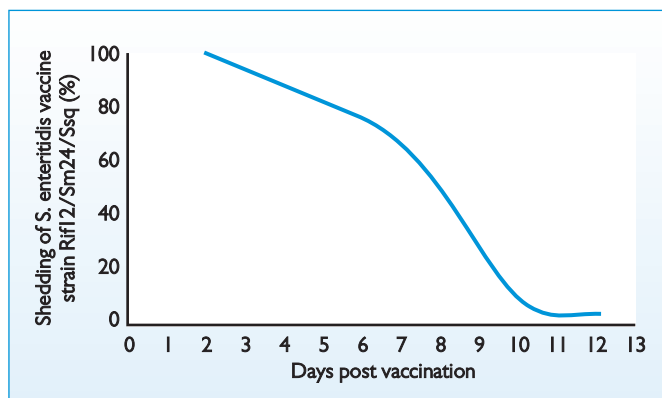
Once salmonella reaches the liver following systemic infection, colonisation of the gall bladder and bile ducts of the liver takes place, in which bacteria replicate extracellularly in the lumen and actively invade the gall bladder epithelium.

Afterwards bacteria replicate inside hepatic epithelial cells and produce the salmonella-containing vacuoles, but, at this stage of infection, do not translocate to the lamina propria and mucosa.

This intracellular infection leads to a local inflammatory response mediated by heterophils with subsequent tissue damage and epithelial desquamation, leading to a massive release

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Fig. 2. Shedding of *S. enteritidis* vaccine strain after vaccination at first day of age with AviPro *Salmonella* Vac E.



Continued from page 19 of salmonella cells and hepatic cellular debris into the lumen. A massive release of cells and salmonella obstruct the bile ducts and release bile into hepatic tissues, which become coloured green.

At this stage, high numbers of salmonellae are secreted together with the bile into the caudal end of the duodenum by the common bile duct. Only then *Salmonella gallinarum* populates the intestine, is excreted in high number and contaminates the environment. The endogenous infection occurs around the fifth day post-infection.

This interval of time is called the incubation period, which is basically the time elapsed since the bird is orally infected until the appearance of FT symptoms.

In typhoid infections the invasion and septicaemia occurs immediately after oral invasion, followed by intestinal colonisation as a result of an endogenous infection when the bird releases salmonellae into the intestinal lumen via the common bile duct.

In contrast, with paratyphoid infections the intestinal colonisation occurs immediately after oral infection and invasion and septicaemia happens at a second stage when salmonellae have previously colonised the gut.

The events in the liver lead to pathognomonic lesions easily recognised during the necropsy. Adult birds suffering acute FT usually have a swollen, friable, often bile stained liver due to the destruction of the epithelial cells of the gallbladder and the bile ducts, which leads to a severe stasis of bile.

After a fortnight the infection becomes chronic with development of round whitish necrotic foci that are included into the hepatic parenchyma.

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 bloodstream and develop new necrotic foci.

An experiment carried out in mice using two differently marked strains of *Salmonella typhimurium* described the mechanism of the necrotic foci development very well.

This experiment demonstrated that two different strains of salmonella may invade the same tissues in an independent way and also explains how mixed infections simul-

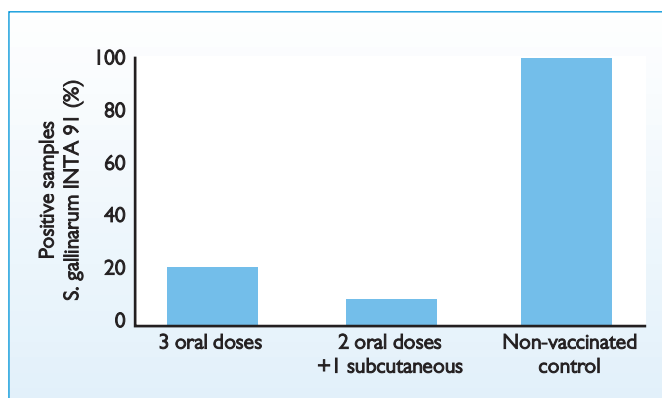


Fig. 3. Percentage of positive samples of *S. gallinarum* INTA 91 two days after challenge infection.

taneously occur in the same animal. 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 FT diseased laying hens.

This may be a common phenomenon that is normally not detected when only standard bacteriology is carried out.

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.

For instance, in a survey carried out in laying hen farms, *Salmonella enteritidis* was isolated together with many other different serovars, including up to five different serovars in the same farm.

As typhoid and paratyphoid infections may occur together at the same farm, in countries with FT it is necessary to simultaneously protect the chickens against both related serovars: *S. gallinarum* and *S. enteritidis*.

The reproductive tract

Salmonella gallinarum and *Salmonella enteritidis* are two phylogenetically related bacteria deriving from a common ancestor bacterium. Both serovars are clonally related and share many common pathogenic factors that allows invasion and egg colonisation.

They share the adhesion fimbria SEF14, common DI 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.

Salmonella gallinarum often causes multiple misshapen ovary follicles, ceasing the production of eggs. On the contrary, hens infected with *Salmonella enteritidis* usually maintain a normal egg production rate, but eggs are commonly *Salmonella enteritidis* contaminated.

Control and eradication

Breeder poultry flocks usually are, in theory, free from infection due to strict official governmental control plans. In contrast, laying hens, particularly in multiple age farms, are more often infected because salmonella persists in the environment or in carrier chickens; hence, new batches of *Salmonella gallinarum* free day-old birds may acquire the infection on the farm.

It has to be considered that all surviving birds remain infected for the duration of their lives and the disease will persist permanently in the farm unless all animals are eliminated, the complete farming area is exposed to a period of fallowing after being emptied and is cleaned and disinfected together with treatment with rodenticides, insecticides and acaricides.

According to the susceptibility of

the bird and its immunity the disease may be unapparent or produce variable mortality, ranging from 0-100%.

High mortality may be triggered by any stress factor as for instance any requirement of high productivity such as an egg production peak, intense reproductive activity or forced moulting.

High economic costs are due to the disposal of dead birds, the constant loss due to culling, the closure of hatcheries and the increased feed and veterinary costs.

In addition, there are important economic costs due to commercial limitations such as loss of sanitary status, for the affected poultry operation, or even for a country as a whole when export products are involved.

Eradication programmes are very costly and require government support. The control of the infection has to be aimed not only to the breeder farms but also to the laying hens and broilers as well.

The lack of government support for the instauration of control plans in laying hen farms is the main cause of the endemic situation that is maintained in many countries.

To calculate the cost of eradication it should be considered the cost of elimination and replacement of infected flocks and decontamination of premises.

Once the disease is eradicated the costs related to a permanent surveillance aimed to avoid re-infection should be considered. Indirect costs related to training and education also need to be taken into account.

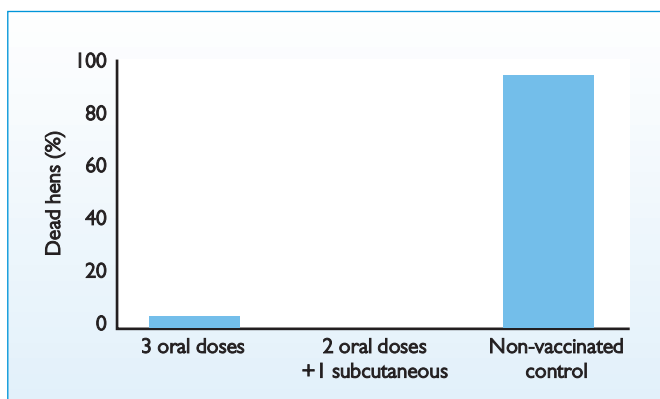
Diagnosis and monitoring

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 FT infection within a flock.

The rapid whole blood plate agglutination test can identify positive birds in the farm because agglutina-

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Fig. 4. Mortality rate 12 weeks after the third dose of AviPro *Salmonella* Vac E.



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tion antibodies appear from three 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 are usually detected; therefore serology only provides a presumptive diagnosis and requires confirmation by bacteriology or molecular tests before deciding to eliminate the positive birds.

Vaccination

Metabolic drift mutants have minus mutations in essential enzymes and metabolic compartments. The alterations of metabolic pathways lead to a longer generation time and to a corresponding reduction in virulence.

The vaccine strain Rif12/Sm24/Ssq is very safe because it has three independent chromosomal mutations, which prevents any reversion to virulence.

The attenuation still ensures invasiveness as well as in vivo propagation for a time period long enough to produce immunity before the vaccine strain is eliminated from the chicken. Therefore this strain elicits mucosal and cellular immunity.

Because the vaccine can be administered at the first day of life, at this age the vaccine strain colonisation is very high and mucosal immunity is intensely produced together with competitive exclusion. At this time the vaccine strain constitutes up to 10% of the intestinal chicken flora; after a maximum of 21 days the vaccine strain is not excreted anymore.

This vaccine strain is unable to survive in the environment avoiding the risk of possible horizontal transmission. In addition, this live vaccine strain can be easily differentiated from wild type strains by simple antibiotic sensibility tests.

During several experiments carried out with the vaccine strain it has

been demonstrated no reversion to virulence or transmission through eggs.

Also, it has been demonstrated that this vaccine reduces faecal excretion of *Salmonella enteritidis*, leading in turn to less contaminated eggs after oviposition.

Likewise, less infected organs means less infection in the reproductive tract, which decreases the production of infected eggs before oviposition. Less contaminated eggs means, of course, fewer chances for an outbreak to occur in humans.

Fowl typhoid studies

These studies were carried out at INTA Balcarce, Argentina. The laying hens used in these trials belonged to the Lohmann Classic layer line. The chickens were colour sexed at the moment of hatching.

Salmonella free chickens were reared in complete isolation from the first day of life under strict isolation and high biosecurity measures.

All chickens were caged from the first day of life and were individually identified with a metallic wing tag.

Vaccinated and non-vaccinated chickens were separately reared. The challenges were carried out in a separate building and after the infection hens were kept within isolators.

● Vaccinations.

According to the dose recommended by the manufacturer, 0.5mL containing 100-500 million salmonellae per chicken were orally administered by gavage or into the crop. A subcutaneous route was also experimentally tested injecting the same oral dose behind the neck. Chickens were vaccinated at the first day of life and at 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 cockerels.

● Challenge dose.

The 50% lethal dose was calculated in pre-trials. One lethal dose was set as 0.5mL 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. Optimum protection was observed when the hens were challenged at week 28.

● Experimental design.

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

● Shedding of the vaccine strain.

After the first vaccination at first day of life, the vaccine strain could be recovered from all cloacal swabs up to the tenth 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.

● Reduction of *S. gallinarum* faecal excretion.

When three oral doses were administered at the first day of life and in weeks six 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 third dose was administered by subcutaneous route, faecal excretion was reduced to 10% in this group.

● Protection against mortality.

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

● Protection against disease.

All diseased hens manifested anorexia, somnolence and depression, but none of them had diarrhoea.

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 days 21 post-challenge. No protection was observed when the hens were challenged at 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 with the time elapsed between the last vaccine dose 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 immunise 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 three months is highly recommended.

The vaccine strain Rif12/Sm24/Ssq can be used to design strategies to simultaneously prevent both typhoid and paratyphoid infections.

If FT and *Salmonella enteritidis* cause infections or if FT 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, diagnostic and monitoring, nutritional management and with good farming practices. ■

References are available
from the author on request